The effect of inositol-hexakisphosphate (phytate) on urinary risk factors for calcium oxalate urolithiasis in South African population groups with different kidney stone risk profiles: theoretical modelling, *in vitro* crystallisation experiments and *in vivo* human studies.

Saajidah Fakier

BSc (Hons) UCT

Thesis presented for the Degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

Faculty of Science

University of Cape Town

August 2015

Acknowledgements

I would like to thank the following individuals and organizations who have contributed to the present thesis:

My supervisor, **Professor. Allen Rodgers** for his excellent supervision and for his tremendous contribution towards shaping this thesis,

My Co-supervisor, **Professor Graham Jackson** for his expertise on solution equilibria and potentiometry and for his contribution towards Chapters 2 and 3,

Dr. Shameez Allie-Hamdulay for her assistance with JESS,

Professor Felix Grases, **Dr. Antónia Costa-Bauzá** and **Adrian Rodriguez** for hosting me in their laboratory at the University of Balearic Islands, Spain

Professor Neil Ravenscroft for his advice on anion-exchange resins,

Dr. Tracy Craig (Chemical Engineering Department, UCT) for allowing me to run experiments in her laboratory on a Coulter Counter,

Dr. Ahmed Hammouda for his technical assistance with potentiometric titrations,

Mrs. Diane Pinnock for chemical analysis of urine samples,

Dr. Dawn Webber for her encouraging words and for believing in my potential,

Members of the Kidney Stone Research Laboratory, **Dr. Dalielah Jappie-Mohamed**, **Dr. Takalani Theka** and **Ms. Mpho Mokoena**

My friends, **Ziningi Charity Madonsela**, **Muneebah Adams**, **Mishqah Martin** and **Allistair Mokoena** for their moral support,

My Family, **Mom**, **Dad**, **Mustaqeem**, **Ouma** and **Sharfaa**, shukran for helping me through the challenges of this degree,

Finally, **the financial assistance of the National Research Foundation (NRF) towards this research is acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.** Thanks are also extended toward the University of Cape Town and the Oppenheimer Memorial Trust (OMT) for financial support.

Publications and Conference Proceedings

F. Grases, A. Costa- Bauza, S. Fakier, A. Rodriguez. A novel metal-dye system for urinary phytate detection at micro-molar levels in rats. *Anal Methods* 2013; **5**:3016

S. Fakier, A. L. Rodgers, D. Webber. Urinary phytate (IP6) excretion in South African stone-prone and stone-free population groups relative to its dietary intake. Urolithiasis, *Proceeding from the 2nd Experts in Stone Disease conference*, Cape Town, South Africa, 10- 13 December 2014.

Overseas Studentships

University of Balearic Islands, Spain (September and October 2012)

Renal Lithiasis Laboratory

Supervisor: Prof. Felix Grases

Abstract

The effect of inositol-hexakisphosphate (phytate) on urinary risk factors for calcium oxalate urolithiasis in South African population groups with different kidney stone risk profiles: theoretical modelling, *in vitro* crystallisation experiments and *in vivo* human studies.

Saajidah Fakier

August 2015

The principal aims of this thesis were to establish whether soluble calcium-phytate complexes inhibit calcium oxalate crystallisation and whether a higher dietary intake of phytate in South African black subjects compared to white subjects may contribute to the relative rarity of urolithiasis in this group.

Potentiometric titrations were conducted to determine thermodynamic binding constants of soluble calcium-phytate complexes. Binding constants of seven complexes were identified. These were included in the data base of the Joint Experts Speciation System computer program to model the effect of phytate on the urinary supersaturation of calcium salts. Physiological concentrations of phytate failed to decrease ionized calcium and hence the urinary supersaturation of calcium salts.

These theoretical predictions were then tested in an *in vitro* model. Calcium oxalate crystallisation experiments were conducted in simple salt solutions, artificial urine and real urine of the respective groups. The following parameters were measured: ionized calcium; calcium oxalate metastable limit; calcium oxalate particle volume-size distribution; calcium oxalate crystal nucleation, aggregation and growth kinetics. Deposited crystals were examined by scanning electron microscopy. The results confirmed those of the theoretical modelling. Furthermore, the results demonstrated that the inhibitory capacity of phytate is of a kinetic nature rather than a thermodynamic one. Phytate inhibited calcium oxalate crystal aggregation and the inhibition of calcium oxalate crystal growth was found to be independent of the physiological concentration of phytate.

In vivo studies were conducted in which phytate-deficient, phytate-rich diets and a phytate supplement were administered in healthy black and white male volunteers. The baseline intake of phytate was assessed using food frequency questionnaires; urinary phytate was determined using a novel assay; biochemical and physiochemical urinary risk factors were measured. The black group had a significantly higher baseline intake of phytate culminating in a significantly higher urinary phytate excretion. No significant difference in urinary crystallisation kinetics was observed as being due to phytate *per se*.

The findings of this thesis contribute to the pool of knowledge on urolithiasis and provide insight on the relative rarity of this disease in South Africa's black population.

List of Abbreviations

$\%I_A$……..……percentage inhibition of aggregation

$\%I_N$.....percentage inhibition of nucleation

AU……..Artificial urine

BPU……...Pooled urine of black subjects

CaOx……..Calcium oxalate

CaP……..Calcium phosphate

CME…….....Crystal matrix extract

COD…….……Calcium oxalate dihydrate

COM…….…….Calcium oxalate monohydrate

COT…….……Calcium oxalate trihydrate

E°……..Standard electrode potential

Ecell……Electrochemical cell

Eg……Glass electrode

Ej…….…….Salt bridge

EMF……..……Electromotive force

Eref…….……Reference electrode

F……Faraday's constant

GAGs……Glycosaminoglycans

GLEE……..Glass electrode evaluation

HAP…….Hydroxylapatite phosphate

HMW……...…High molecular weight

HySS…….……..Hyperquad Simulation and Speciation

IH…….……Idiopathic hypercalciuria

IP6…….……Phytate

ISE-Ca^{2+}……Calcium ion-selective electrode

ISE-H^+……Hydronium ion-selective electrode

ITC……Isothermal titration calorimetry

JESS……Joint Experts Speciation System

KSRL……Kidney Stone Research Laboratory

LMW……Low Molecular Weight

log……Logarithm

MSL……Metasable limit

NC……Nephrocalcin

OPN……Osteopontin

Phy……Phytate

PSD……Particle size distribution

PTH……Parathyroid hormone

PU……Pooled urine

R……Universal molar gas constant

RS……Relative supersaturation

s……slope

SE……Standard error

SEM……Scanning electron microscopy

SS……Supersaturation

T……Temperature

tCaP……tribasic calcium phosphate

THP……Tam-horsfall protein

TRI……Tiselius risk index

UPTF1……Urinary prothrombin fragment 1

WPU……Pooled urine of white subjects

z……number of electrons

List of Units

μM……micromolar

g……grams

$g.mol^{-1}$……grams per mole

hr……hour

M……moles per litre

mg……milligram

min^{-1}……per minute

mins……minutes

ml……millilitre

mM……millimolar

mV……millivolts

nm……nanometer

rpm……revolutions per minute

s^{-1}……per second

Table of Contents

List of Tables

List of Figures

Chapter 1 : General introduction

Kidney stone disease, also referred to as urolithiasis, renal stone disease and nephrolithiasis, dates back to centuries ago.[1, 2] This disease is multifactorial and is governed by urinary risk factors, dietary factors as well as epidemiological & etiological factors, all of which will be described in greater detail in the subsequent paragraphs. The occurrence of kidney stone disease in South Africa is of interest in the context of the work presented in this thesis because its prevalence in the black population is less than 1 % whereas 15 % of the white population are prone to this disease.[3-5] Thus, the coexistence of a stone-prone and a relatively stone-free population group provides added interest to research involving this disease.

Composition of stones

Essential body fluids are supersaturated with slightly soluble materials that can crystallise.[6] These crystals can be deposited in the body and is known as mineralization or calcification, since calcium is one of the major minerals that can be deposited in the human body.[6] Examples of calcification under physiological conditions are tooth and bone formation.[7, 8] The aforementioned processes are well-defined and limited, unlike pathological crystallisation of salts in the urinary tract which may lead to the formation of kidney stones. Different types of crystals are precipitated in different parts of the nephron.[9, 10] The most common types of crystals observed in the urine are as follows: calcium oxalate (CaOx), calcium phosphate (CaP), uric acid and struvite.[11-14] Kidney stones are composed of these crystalline materials and often contain a mixture of several types with one or two that are predominant.

Calcium Oxalate stones

Approximately 80 % of kidney stones contain calcium, of which most are calcium oxalate (CaOx).[15-17] This type of crystal has three different forms namely CaOx monohydrate (COM), CaOx dihydrate (COD) and CaOx trihydrate (COT).[18] COM is most common in stones whereas the latter does not occur in stones. This is because COT is not thermodynamically stable and is thus transformed into COM and COD. Stones of this type may also contain CaP.[19, 20]

Calcium Phosphate stones

2- 20% of kidney stones are CaP stones.[21, 22] These stone types are present as apatite (also reported as carbonate apatite) or calcium hydrogen phosphate (brushite).[23, 24] The frequency of brushite stones is less than apatite.[21, 22] Stones of this type may be found in combination

with CaOx or struvite but never with uric acid as the latter precipitates at a much lower pH.[25, 26]

Uric Acid Stones

Approximately 8% of stones are uric acid stones.[23] This type of stone forms when urine is supersaturated with uric acid, an acidic urine pH (less than 5.5) or a combination of the two.[23, 27] Abnormal purine metabolism also causes uric acid stone formation.[23, 27]

Struvite

1% of stones are struvite stones, also known as "triple phosphate" and magnesium-ammonium-phosphate-carbonate-apatite.[23, 27] These stones are formed in the presence of urease-producing bacteria (eg. *Proteus mirabilis*) due to urinary tract infections and is more common in females than in males.[28, 29]

The mechanism of stone formation

Crystals in urine, known as crystalluria, is common to both stone formers and healthy people.[30-32] This process involves two physicochemical facets namely a thermodynamic and a kinetic process.[33-35] The former aspect includes urinary supersaturation which in turn leads to the nucleation of smaller crystals and the latter refers to the rates of crystal nucleation, growth, aggregation and phase transformation. The use of human tissue biopsies and intraoperative imaging led to the identification of three pathways for kidney stone formation.[36-38] These pathways include overgrowth on interstitial apatite plaques (Randall's plaque theory[39-42]), growth on plugs of dilated ducts of Bellini (BD) and free solution crystallisation.[36-38] These concepts are described in detail in the paragraphs which follow.

Supersaturation

Supersaturation is the driving force for crystallisation and is the free energy necessary for this physicochemical process.[33, 43] Supersaturation can be expressed by the following equation $\Delta G = RT\ln(A_i/A_0)$ where R is the gas constant, T is the temperature and A_i & A_0 refers to the activity product of the salt at specific conditions and at equilibrium, respectively.[33] The ratio of A_i to A_0 is known as the relative supersaturation (RS). When this ratio is greater than 1, the urine is said to be supersaturated.[23, 33] Although supersaturation is essential for the formation of crystals which may lead to the subsequent formation of stones, it is not always sufficient to differentiate between stone formers and healthy people.[43-46]

Nucleation

Nucleation is defined as the initial step in the formation of a solid phase from a supersaturated solution.[47, 48] This crystallisation process will occur when the RS is greater than 1 and when the metastable limit (MSL, defined as the ability of a solution to resist spontaneous crystallisation[6, 49, 50]) is surpassed. Two types of nucleation processes exist

namely homogeneous and heterogeneous nucleation.[6, 47] Homogeneous nucleation is spontaneous and occurs in a pure solution (i.e no foreign surfaces) at relatively low RS values.[6, 43, 47] Homogenous nucleation is very unlikely to occur in urine since this medium is a complex solution containing many foreign surfaces which may serve as a platform for the formation of new crystals. The latter this is known as heterogeneous nucleation which occurs at RS values lower than that of a pure solution.[6, 47]

Growth

Nucleation may lead to crystal growth when a nucleus of a critical size has been established and when the RS remains above 1.[6, 43, 48] Crystal growth is an intricate process involving the adsorption of new substances into the crystal lattice of existing crystals.[6, 43, 48] These crystals may then adhere to each other to form larger particles via a process known as aggregation[6, 48] which is described below.

Aggregation

This mechanism is also referred to as agglomeration and involves the adhesion of particles in solution to form larger particles as mentioned above. Of the three essential processes that are required for stone formation, crystal aggregation is considered the most important mechanism in the context of kidney stone formation.[6, 33] This is because the rate of aggregation is rapid relative to nucleation and growth and may lead to large crystalline particles in renal tubules.[6, 33]

Overgrowth on interstitial apatite plaque (Randall's plaque theory)

This theory is defined as interstitial papillary and medullary deposits of apatite (plaque) which may serve as a nidus of a relatively large concretion.[39-42] This pathway of stone formation has been postulated by Randall in 1937 where he examined several kidneys at post-mortem and found plaque in 20% of the kidneys, some of which had stones attached to them.[39] More recently, endoscopic mapping of kidneys in CaOx stone formers revealed large amounts of Randall's plaque which were prominent in CaOx stone formers with hypercalciuria.[51, 52] Another study confirmed that plaque is composed of apatite and that its origin is in the basement membrane of the thin limb of Henle's loop in idiopathic CaOx stone formers (ICSF) only.[53] Other studies have also demonstrated that Randall's plaque is common in ICSF.[54-58] These studies showed that stones of ICSF were attached to Randall's plaque[56-58]; the plaque surface area on kidneys was significantly higher in ICSF than in controls[54] and that a correlation between the mean plaque surface area and the number of stone events exists[55].

Growth on plugs from dilated ducts of Bellini

This pathway is common in brushite and primary hyperthyroid stone formers.[36-38] Apatite deposits are found in ducts of Bellini (BD) and inner medullary collecting ducts (IMCD) of

these stone formers.[36-38] These deposits “plug” the ducts leading to dilation of the ducts. [36-38] Stones may grow from plugs which protrude from the dilated ducts into the urinary space. [36-38] CaP stones were found to grow via this pathway.[36, 37]

Free solution crystallisation

This pathway refers to kidney stones which are free in the renal pelvis and were never attached. [36, 37] Stones which form via this pathway are composed of cystine. [36, 37] The latter is poorly soluble and supersaturated in the urine in BD which leads to stone formation.[36]

Risk factors associated with stone formation

There are several risk factors associated with CaOx stone formation. These may be divided into urinary risk factors, dietary habits and epidemiological and etiological aspects.

Urinary Risk Factors

Urinary calcium

An excessively high urinary excretion of calcium (greater than 250 mg/ 24 hr in women and 300 mg/ 24 hr in men) is known as hypercalciuria.[59-61] Hypercalciuria is defined as a risk factor for calcium stone formation as it increases the urinary supersaturation of CaOx and CaP salts.[59, 62, 63] In 30 – 50 % of calcium stone formers, hypercalciuria is often accompanied by normal serum calcium levels and in the absence of known causes of increased urinary calcium such as hyperthyroidism, cancer, sarcoidosis and an increase in vitamin D intake.[59, 64, 65] This is referred to as primary or idiopathic hypercalciura (IH). Three mechanisms for IH have been classified, namely *absorptive hypercalciuria*, *resorptive hypercalciuria* and *renal leak hypercalciuria*.[59, 61, 66]

Absorptive hypercalciuria is the most common mechanism and is associated with elevated levels of 1,25-hydroxyvitamin D_3.[59, 67-69] The latter molecule (as well as the parathyroid hormone (PTH)) regulates the synthesis and activity of transporters responsible for the translocation of calcium from the intestine, kidney and bone during calcium homeostasis. Thus, elevated levels of 1,25-hydroxyvitamin D_3 results in an increase in the absorption and subsequent excretion of urinary calcium.

Resorptive hypercalciuria is caused by an increase in bone turnover which occurs due to an overproduction of PTH.[70, 71] As mentioned above, this hormone is responsible for the regulation of calcium homeostasis.

Renal leak hypercalciuria occurs due to an abnormality in renal tubular calcium reabsorption.[59, 66]

Increased urinary calcium excretion is also correlated to an increase in dietary calcium and dietary sodium intake.[49, 72-75]

Urinary oxalate

An elevated urinary oxalate excretion of greater than 45 mg/ 24 hr is classified as hyperoxaluria.[63, 64] Urinary calcium was previously regarded as the most important risk factor for CaOx kidney stone formation.[76-78] However, urinary oxalate is now regarded as the limiting risk factor since it has a greater effect on the urinary saturation of CaOx.[79, 80] A correlation between urinary oxalate and stone disease has been reported.[81, 82] These studies have shown that stone formers have a higher urinary oxalate concentration than healthy controls. Increased levels of urinary oxalate may be caused by a high dietary intake thereof[16, 83], hyperabsorption[84], increased endogenous production[84] as well as a deficiency of oxalate-degrading bacteria.[16, 85-87]

Urinary Uric Acid

An abnormally high excretion of uric acid (greater than 800 mg/ 24 hr in men and 750 mg/ 24 hr in females) is known as hyperuricosuria.[64, 88, 89] This poses a risk for the formation, growth and aggregation of calcium oxalate crystals.[89, 90] High urinary concentrations of uric acid were found in a third of CaOx stone formers which was postulated to be as a result of the over consumption of dietary protein or the endogenous overproduction of uric acid.[91] Other researchers have hypothesized that the relationship between uric acid and CaOx may be as a result of the “salting-out” effect, whereby uric acid acts as a catalyst for CaOx crystal aggregation.[92] Allopurinol therapy has been recommended for patients with high urinary uric acid.[93] More recently an *in vitro* study has demonstrated that theobromine inhibits uric acid crystal nucleation and growth suggesting its potential therapeutic application in patients with uric acid nephrolithiasis.[94]

Urinary pH

Urinary pH is also a risk factor for the crystallisation of CaOx, CaPs as well as uric acid.[25, 49, 95-97] Urine with an alkaline pH is less susceptible to the formation of CaOx crystallisation; phosphate and citrate are ionised at this pH which may then bind calcium thus decreasing the saturation of CaOx.[25, 95, 96] However, an increased complexation of calcium and phosphate at an alkaline pH (pH > 6) may increase the risk of CaP crystallisation if hypercalciuria is present.[25, 96, 98] Patients undergoing therapeutic alkalinisation to reduce CaOx formation should therefore be monitored for the risk of CaP formation. At a pH less than 5.5, uric acid crystallisation occurs.[95, 99] Thus, in order to decrease the risk of the formation of the aforementioned salts, it is recommended to maintain a urinary pH between 6 and 7.[16, 97, 100]

Other urinary risk factors include that of a decrease in the urinary excretion of inhibitors such as citrate and magnesium. These are described later on page 11.

Dietary risk factors

Dietary oxalate

Research has shown that urinary oxalate excretion is correlated to its dietary intake.[83, 101-104] The amount of dietary oxalate that is excreted in the urine ranges from 10-20 %[105] to 40-53 %.[83, 106] Studies have also shown that an increase in dietary oxalate ingestion with a concomitant decrease in dietary calcium intake, increased the excretion of urinary oxalate.[83 77, 107-110] As dietary oxalate is directly related to its urinary excretion, CaOx stone formers are advised to reduce their intake of oxalate rich foods to less than 100 mg/ day.[25] These foods include beetroot, chocolates, parsley, rhubarb, spinach, strawberries, tea and wheat bran.[83, 101, 104, 105, 111]

Dietary calcium

As urinary calcium was thought to be the most important risk factor for CaOx stone formation, stone formers were advised to decrease their intake of this mineral.[112-114] However, recent studies have discovered that this practice is inappropriate and potentially harmful.[49, 74, 115-117] A study in which a large cohort of men was investigated after decreasing the ingestion of dietary calcium over a period of 4 years showed that 8.7 % of the participants developed stones.[115] Another study showed that the restriction of dietary calcium increased the relative supersaturation of CaOx.[49] A decrease in the intake of dietary calcium therefore has an inverse effect on the formation of CaOx; this is because less calcium is available for the binding of oxalate in the gut which in turn leads to an increase in the absorption of oxalate and its subsequent excretion in urine.[16] As mentioned previously, increasing urinary oxalate is a risk factor for CaOx stone formation. Patients are therefore advised to consume 800 – 1200 mg of calcium per day.[83, 118]

Dietary Protein

Dietary protein has also been shown to influence CaOx urolithiasis. Epidemiological studies have shown a correlation between the incidence of urolithiasis and the consumption of animal protein (purine-rich).[115, 119-122] Studies investigating the effect of dietary protein on urinary risk factors associated with CaOx urolithiasis showed that urinary calcium and uric acid increased whereas urinary citrate decreased when a diet with a high proportion of meat was consumed.[115, 123-127] The mechanism of action for the increase in urinary calcium after animal protein consumption is as a result of an increase in endogenous acid production.[128-130] This leads to metabolic acidosis which increases calcium reabsorption from the bone leading

to an increase in urinary calcium excretion. An animal protein intake of 0.8- 1.2 g/ kg body weight per day is therefore recommended for kidney stone patients.[118, 131]

Dietary sodium

Research has shown that dietary sodium intake is directly proportional to urinary calcium excretion.[72-74, 132, 133] A recent study showed that urinary calcium decreased in idiopathic hypercalciuric stone formers (n= 210) when a diet low in sodium was consumed.[134] Thus, CaOx stone formers are advised to maintain a sodium intake of 2-3 g per day.[25, 134, 135]

Dietary fatty acids

Studies have shown that CaOx stone formers have an abnormal renal phospholipid composition which results in hyperoxaluria and hypercalciuria.[136-138] A higher concentration of arachidonic acid (AA, a polyunsaturated omega-6 fatty acid) in the membrane phospholipids is present in stone formers.[136-138] This fatty acid facilitates intestinal calcium absorption, hypercalciuria and alters the urinary excretion of prostaglandin 2 (PGE2, a phospholipid metabolite).[137, 139] AA and PGE2 can be reduced by essential fatty acid supplementation. These essential fatty acids contains eicosapentaenoic and docosahexaenoic polyunsaturated acids (omega-3 fatty acids) which increases the cell membrane's fluidity, flexibility and permeability to the regulation of ion transport such as calcium, sodium and potassium and to the transmembrane exchange of substances like oxalate.[136, 140] Research has confirmed that fatty-acid supplementation reduces calcium, oxalate and PGE2 in urine of CaOx stone formers.[139, 141-143] CaOx stone formers are therefore advised to consume omega-3 fatty acids daily.[139, 141, 143] Sources of this fatty acid are fish, fish oil, flax seed oil, walnut oil and it can also be consumed in supplemental form.

Fluid intake

One of the most common dietary measures for the prevention of stone formation is to maintain dilute urine by adequate fluid intake; this reduces the supersaturation of stone-froing salts.

Although "fluid intake" usually refers to water intake, other beverages such as lemon juice,[144-147] cranberry juice,[148] orange juice,[146] grape juice and apple juice[145] were also studied in the context of urolithiasis. These beverages were however administered not only to increase urinary volume but to increase the urinary concentration of inhibitors such as citrate as well as to increase urinary pH. While some of these beverages increased urinary citrate and pH (lemon, cranberry and orange juice) others (grape and apple juice), have increased the risk of kidney stone formation. Thus, caution should be exercised with regard to the type of beverage used to increase urinary volume. Beverages such as cola and hot chocolate are also not advised due to their high oxalate content.[104, 149] Notwithstanding these adverse

effects of beverages, the intake of water as source of fluid intake has been shown to decrease the risk of stone formation in studies consisting of both stone formers and healthy individuals.[117, 150-155] Stone formers are therefore advised to drink water as a source of fluid intake between 2.5- 3 L per day to maintain a urinary volume of 2 L. [156-158]

Epidemiological and etiological risk factors

Climate and geographical distribution

The risk of CaOx urolithiasis is higher in the western hemisphere where the prevalence is 5-9 % in Europe, 12% in Canada and 13-15 % in the USA than in the eastern hemisphere 1-5 %.[27] However, a higher risk is present in Saudi Arabia (20.1%).[27] The higher incidence in Arabia is due to socio-economic factors, local dietary intake habits which is high in oxalate as well as environmental factors such as the hot climate.[159] Hot climates contribute to dehydration and decreased urinary volume, increased urine osmolality, and increased concentration of calcium and oxalate.[160, 161] A recent study in which the 24 hr urine compositions of 50 marines were monitored before mobilization to the desert, after 30 days in the desert and 2 weeks after returning from the desert was conducted.[162] This study showed that the daily urine output decreased significantly after being in the desert. The urinary excretion of calcium, uric acid, sodium, magnesium and potassium as well as pH decreased and small changes were observed for citrate and oxalate in this study. The researchers hypothesized that the kidneys preserved water and electrolytes while in the desert; however, the relative supersaturation of uric acid and sodium urate increased significantly which indicates an increased risk for stone formation when exposed to desert conditions. In Saudi Arabia, a correlation between stone incidence and both temperature and atmospheric pressure has been reported.[163] Two recent studies have also demonstrated that an increase in stone incidence with global warming is evident; showing that temperature is correlated to stone incidence.[164, 165]

Occupation

Research has shown that an increase in risk factors associated with urolithiasis is associated with certain occupations.[166, 167] This is most common in sedentary occupations such as aviation pilots or truck drivers[167-169] whereas individuals doing manual work such as farming and forestry have a lower risk of forming stones.[170] A recent study also demonstrated that workers in the steel industry who are exposed to hot temperatures have an increased risk for kidney stone formation.[171] Hypocitraturia and low urine volumes were observed in this study which are risk factors for CaOx urolithiasis.[171]

Gender

Epidemiological studies have shown that the prevalence of urolithiasis in males is higher than in females with a ratio of 2:1.[120, 172] Studies also showed that males have a higher urinary excretion of oxalate.[173, 174] These differences may be attributed to sex hormones in which oestrogen is thought to play a protective role in females by lowering the urinary supersaturation of stone-forming salts.[173, 175] Testosterone, in males, on the other hand may increase urinary oxalate resulting in an increased risk for kidney stone formation.[176, 177]

Race

The relationship between race and the occurrence of CaOx urolithiasis has been investigated for many years.[4, 178, 179] These studies were conducted to identify clinical and/or epidemiological factors within each group that will account for the difference in the occurrence of stone formation between the groups.[180] Differences in the occurrence of stone formation between two race groups within the same country have been observed.[16] For example, in the US the occurrence of idiopathic stone disease is more prevalent in whites than in blacks.[181-183] Similarly in South Africa the prevalence of CaOx kidney stone disease in whites is 15 % whereas less than 1% of the black population are prone to this disease.[3, 4] Some studies showed that certain urine parameters may account for the difference in the racial prevalence of this disease.[4, 5, 182, 184, 185] Several studies have been done to elucidate the rarity of CaOx urolithiasis in South African blacks and will be discussed in greater detail towards the end of this chapter.

Modulators of stone formation

Crystals formed in the urine are normally excreted harmlessly in non-stone formers.[31, 186] Other mechanisms that may also lead to stone formation such as Randall's plaques remain as such in non-stone formers.[51] These phenomena indicate that urine contains certain substances that modulate the mechanisms essential for stone formation i.e. supersaturation, nucleation, growth and aggregation.[187] There are two types of modulators: high molecular weight (HMW) modulators such as proteins and glycosaminoglycans (GAGs) and low molecular weight (LMW) compounds such as citrate and pyrophosphate.[187] These modulators influence crystal formation and retention by direct interaction with the crystal or indirectly by influencing the urinary environment.[187] It should be noted that these modulators may either inhibit or promote the mechanisms associated with kidney stone formation. As CaOx is the major type of kidney stone, the modulators associated with this type will be described below.

High molecular weight modulators

GAGs

GAGs are polyanionic compounds composed of repeating disaccharide units that consist of N-acetylated or sulphated glucosamine or galactosamine amino sugar and a glucuronic acid N-free monosaccharide.[188] Urinary GAGs include heparan sulfate (HS), chondroitin sulphate A, B and C (CS-A, CS-B, CS-C), dermatan sulphate (DS), keratan sulphate (KS) and hyaluronic acid (HA).[189-191] These GAGs have been shown to inhibit certain mechanisms of CaOx crystallisation due to their ability to bind to CaOx crystal surfaces.[192] *In vitro* studies showed that CS is able to inhibit crystal aggregation[193-195] and growth[193]. HS was also reported to inhibit crystal growth and aggregation.[196] However, the inhibitory effects in *in vitro* experiments do not always reflect the effects *in vivo*. An *in vivo* study showed that CS promotes nucleation and aggregation[197] whereas another showed that HS promotes CaOx crystal nucleation (which may be seen as favourable since urinary supersaturation is decreased) and inhibits aggregation.[198] Furthermore, some studies investigating the excretion of GAGs in stone-formers showed that the concentration is significantly less when compared to healthy individuals[88, 199, 200] whereas others found no significant difference[201-203]. These differences may however be as a result of different techniques used for the quantification of GAGs as well as different patient selection criteria employed. Another study showed that recurrent CaOx stone formers excreted fewer GAGs than non-stone formers.[204] Thus, increasing the excretion of GAGs may enhance the inhibitory activity of urine.

Proteins

Proteins are excreted in urine in relatively small quantities at concentrations less than 150 mg per day in normal urine.[205] Tamm-Horsfall protein (THP), nephrocalcin (NC), osteopontin (OPN) and urinary prothrombin fragment 1 (UPTF1) have been studied extensively and are believed to play an inhibitory role in CaOx stone formation.[205, 206] THP is the most abundant protein found in urine at concentrations between 20-200 mg/ day. *In vitro* studies on this protein in undiluted, ultra-filtered urine showed that it inhibits CaOx crystal aggregation due to steric hindrance as opposed to adsorption to the crystal surface.[198] This protein does not show any major inhibitory activity with respect to CaOx crystal growth or nucleation.[205] NC on the other hand was shown to inhibit CaOx crystal growth[207], nucleation[208] and aggregation[209, 210]. OPN was also shown to inhibit CaOx crystal nucleation in supersaturated solutions[208, 211] as well as aggregation[212]. This protein is also capable of modulating the adhesion of COM crystals to renal epithelium cells[208] thus preventing their retention and subsequent formation of stones. UPTF1 was also reported to be a powerful inhibitor of CaOx crystal growth and aggregation in human urine.[213]

Low molecular weight inhibitors

Pyrophosphate

Pyrophosphate, found at concentrations between 15- 100 μM in urine, was the first urinary crystallisation inhibitor discovered.[214] This LMW compound was shown to inhibit CaOx crystal nucleation[215], growth and aggregation[216-218] due to its ability to irreversibly bind to the CaOx crystal surface. It was also shown to inhibit the formation of calcium phosphates.[219, 220] Furthermore, it also favours the more preferential form of CaOx i.e. COD[221] which is less retained on the renal epithelium and also more common in normal urine than in urine of stone formers.[222] The effect of pyrophosphate in undiluted urine however, is not comparable to that found in inorganic media. For example, pyrophosphate had no effect on the amount of oxalate required to induce CaOx crystal nucleation and aggregation in urine.[206] The effect of pyrophosphate on CaOx crystallisation under physiological conditions is therefore difficult to elucidate.

The role of pyrophosphate in kidney stone disease was also investigated by comparing its concentration in urine of normal individuals and stone formers. Although some studies showed that the urinary excretion of pyrophosphate in stone formers was significantly less than in healthy individuals[223, 224], others showed that there was no significant difference.[225, 226] However, even though no significant difference in the urinary excretion of pyrophosphate is observed between stone formers and non-stone formers, it is possible that increasing the excretion of pyrophosphate may increase the inhibitory activity of urine.[187]

The oral administration of orthophosphate was shown to increase the excretion of pyrophosphate however, this therapy was not proven effective in preventing stone formation.[227] Potassium phosphate (Urophos-K) also increased the excretion of pyrophosphate.[228] This treatment increased urinary citrate and inhibited crystal aggregation; it also decreased the nucleation of brushite. The effect of Urophos-K on the inhibition of crystal growth has not however been shown.

Citrate

In vitro studies in aqueous media showed that citrate is able to inhibit CaOx crystal nucleation[215, 229, 230], growth[193, 216, 231] and aggregation[193, 232]. It was also shown to inhibit CaOx deposition in undiluted[233] and concentrated[234] urine. The inhibitory activity of this low molecular weight compound is due to its ability to chelate calcium as well as its ability to bind to the CaOx crystal surface. Furthermore, *in vivo* studies have shown that hypocitraturia is common in stone formers[235-240] and that, upon the administration of alkali therapy, the inhibition of crystal aggregation was increased[241-243]. Clinical trials have investigated the efficacy of several citrate-containing compounds such as potassium citrate[244-246], calcium

citrate[247, 248] and sodium-citrate-bicarbonate-tartrate[249]. These studies showed a decrease in the risk factors associated with urolithiasis and as such citrate has become an accepted form of stone therapy.

Magnesium

Magnesium is able to form ion complexes with oxalate which accounts for its inhibitory effect on CaOx crystallisation.[17] *In vitro* experiments, in inorganic aqueous solutions as the reaction medium, demonstrated magnesium's ability to inhibit CaOx crystal nucleation[250], growth[215] and aggregation.[193, 251] Another mechanism by which it may inhibit CaOx crystallisation is by binding to the surface of the crystals and becoming incorporated into the crystal lattice.[252] On the other hand, other studies showed that magnesium has no effect on CaOx crystal nucleation[215], growth[216-218] and combined growth and aggregation.[253] *In vitro* studies in urine showed that magnesium inhibits growth[254, 255] and increases the CaOx MSL but has no effect on aggregation.[254]

Phytate is another LMW inhibitor of CaOx urolithiasis and is described below.

Phytate

Phytate, also known as myo-inositol-hexakisphosphate, IP6 or phytic acid (in its fully protonated form), is of plant origin where it serves as a storage form of phosphorus and minerals and has a molecular weight of 660 $g.mol^{-1}$.[256-258] It is mainly found in whole-grains, legumes, oil seeds and nuts.[259] This molecule is the central focus of the current thesis and as such will be discussed in detail in the paragraphs that follow and will henceforth be referred to as IP6.

Structure of phytate

IP6 consists of a myo-inositol ring and 6 phosphate groups hence the name "myo-inositol-hexakisphosphate".[256, 258, 260, 261] The prefix "myo" refers to the configuration of the 6 hydroxyl groups on the inositol ring in which 5 may be in the axial position and 1 in the equatorial position (5axial / 1 equatorial) or alternatively with 5 in the equatorial position and 1 in the axial position (5 equatorial/ 1 axial). Each phosphate group is esterified to the inositol ring. Conformational studies on the structure of IP6 demonstrated that the 5 axial/ 1 equatorial conformer is predominant at physiological pH (6- 7) as shown in Figure 1.1.

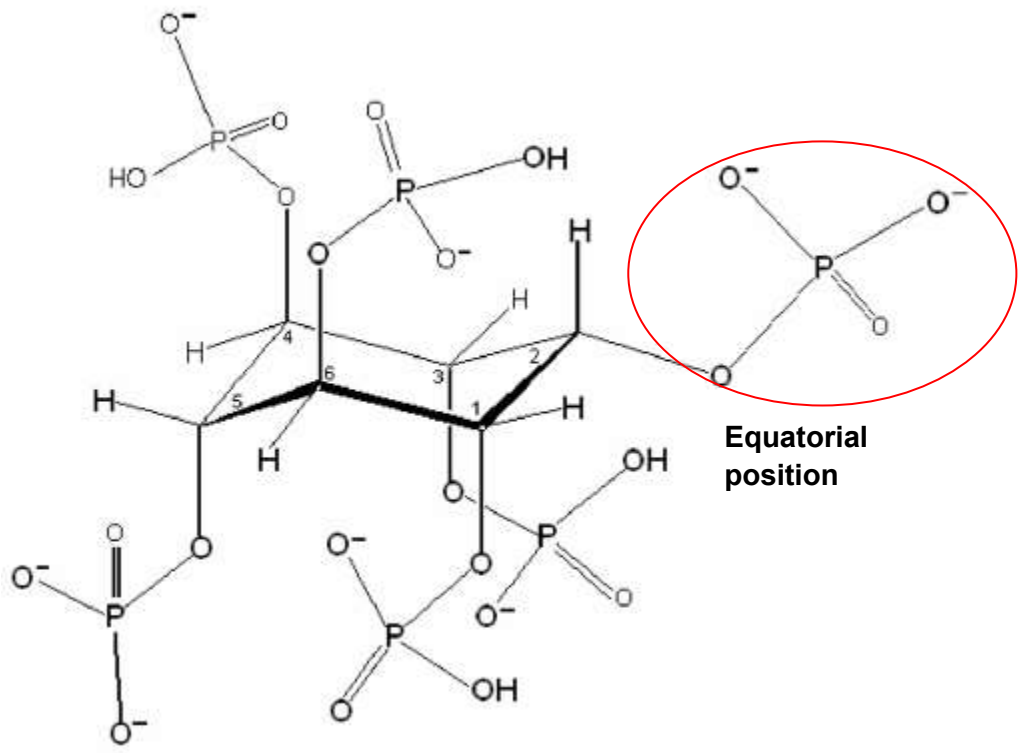


Figure 1.1 Structure of IP6 at physiological pH (6-7)[259]. Conformation: 5 axial/ 1 equatorial.

Bioavailabity of phytate in living organisms

One of the first reports indicating the absorption of IP6 was demonstrated in 1980 whereby radiolabelled IP6 was administered to rats and recovered in blood, organs, bones and urine.[262] A later study also administered radiolabelled IP6 to rats and found a large amount of radioactivity in the liver, kidneys and trace amounts in blood and urine.[263] A more recent study in which IP6 was administered to rats for 7 days showed that both plasma and urinary IP6 was significantly higher than in the control rats.[264] Furthermore, the IP6 levels in organs such as the brain and kidney was also higher than in the control rats.[264] Similar results were also observed in other studies indicating that dietary IP6 increases the concentration of this substance in plasma, urine and organs of rats.[265-267]

In humans, a study investigating the pharmacokinetic profile of IP6 was conducted.[268] This study consisted of 7 individuals and the administration of 3 different doses of IP6 between 400- 3200 mg/ day was investigated. All IP6 rich foods such as whole-grains, rice and legumes were restricted for 15 days. This study demonstrated that both plasma and urinary IP6 decreased within 15 days of an IP6 deficient diet. Furthermore, the same urinary excretion profile of IP6 was observed for the 3 different doses of IP6 leading the authors to speculate that urinary IP6 excretion reaches a plateau which cannot be exceeded by an

excess intake of dietary IP6. Other studies also demonstrated the correlation between dietary IP6 and its bioavailability in humans.[265, 269]

The above indicates that the bioavailability of IP6 in both humans and rats is dependent on its dietary intake to maintain its normal concentrations in plasma, urine and tissues.

Nutrient or antinutrient

As mentioned above, IP6 has six phosphate groups (each with hydroxyl moieties) giving it a large negative charge under physiological conditions.[258] This molecule is therefore able to chelate ions such as Zn^{2+}, Fe^{2+}, Mg^{2+} and Ca^{2+}.[258] Therefore, during gastro-intestinal passage, IP6 may inhibit the absorption of these essential trace elements and minerals (when a mineral- deficient diet is consumed with an IP6- rich diet) leading to deficiencies of these minerals in the organism.[270-272] IP6 was therefore regarded as an antinutrient for many decades. As a result, research has been done to remove IP6 from food by proper processing to improve the bioavailability of essential trace elements and minerals.[259] This consequently led to a decrease in the intake of IP6 in more affluent societies. However, in the last 2 decades, beneficial properties of IP6 have been reported. These properties include antioxidant[273] and anticancer activities[274], positive effects on blood glucose and blood cholesterol[275, 276] as well as the inhibition of CaOx and CaP crystallisation and the prevention of urolithiasis.[277]

Phytate and urolithiasis

This molecule's ability to chelate multivalent cations such as Ca^{2+} is of relevance in CaOx urolithiasis. The administration of IP6 as a prophylactic treatment of urolithiasis dates back to 1958 where high doses of IP6 as the sodium salt (8.8 g/ day) was used to treat stone-formers with hypercalciuria.[278] This study demonstrated a significant decrease in urinary calcium which occurred due to the formation of insoluble calcium-phytate complexes in the intestinal tract culminating in a decrease in calcium absorption.[278] Other studies also showed that the consumption of rice bran[279] and unprocessed bran[280] (source of IP6) decreased the urinary excretion of calcium in patients with idiopathic hypercalciuria. More recently, however, research has been focused on the administration of lower doses of IP6 with the aim of increasing its urinary excretion to increase the urine's inhibitory activity towards CaOx and CaP crystallisation as cited in a review article by Schlemmer et al.[259] Both i*n vitro* as well as *in vivo* experiments in animals and humans have been conducted. Research in this field has been conducted principally by Grases and his co-workers (*University of Balearic Islands, Spain*). The outcome of these studies are described below.

In vitro studies showed that IP6 has the ability to inhibit the crystallisation of both CaOx and CaP. The effect of IP6 on the early stages of COM crystallisation on the urothelium was

studied. [281] This was investigated by adding synthetic urine containing different concentrations of IP6 onto three different inert substrates, namely wax, CaP and CaP-mucin to determine its effect on the heterogeneous nucleation of COM. It was found that IP6 inhibited the nucleation of COM on all three substrates at a concentration as low as 7.15 x 10^{-7} M. The effect of IP6 on the development of COM crystals have also been studied on living substrates such as the urothelium of the pig urinary bladder.[282, 283] These studies showed that IP6 prevented the development of COM and CaP deposits. Recent studies have also investigated the *in vitro* effect of IP6 on crystallisation kinetics in artificial urine.[220, 284-287] These studies showed that IP6 inhibited the nucleation of brushite at concentrations as low as 1.21 x 10^{-5} M and hydroxyapatite at a concentration of 3x10^{-6} M[220]; inhibited the growth rate of COM crystals on hydroxyapatite crystal surfaces at a concentration of 2.27x10^{-6} M under normocalciuric conditions and at 9.09x 10^{-6} M under hypercalciuric conditions[284]; inhibited the growth rate of artificial stones in the presence of 2.5x10^{-6} M IP6[285] and inhibited the regrowth of COM[286] and COD fragments of post extracorporeal shock wave lithotripsy (ESWL)[287] in the presence of 3.03 x 10^{-6} – 9.09 x 10^{-6} M IP6.

***In vivo* studies in rats** showed that the number of calcifications on kidneys in lithogenic rats was reduced when the rats were treated with IP6 (1.08 x 10^{-4} M).[288] Similar results were obtained in another study in which rats were treated with vitamin D to induce the calcification of renal tissues.[284] This study showed that a significant reduction in calcium deposits was observed when rats were treated with IP6.[284] This was confirmed in another study in which rats fed with a purified diet, free of IP6, were more susceptible to kidney calcifications than those fed with a diet containing 1% Na-IP6.[289] Urine analysis of these rats revealed that those fed with IP6 had a higher excretion of this substance than those consuming the purified diet.[289] Furthermore, the concentration of both calcium and phosphorous in the kidneys of the control group of rats (fed with the purified diet) were greater than in the kidneys of the rats fed with 1% Na-IP6.[289] Previous studies also demonstrated a decrease in urinary calcium excretion, in lithogenic rats, after the administration of IP6.[279, 290] More recently, a study investigated the effect of four different salt forms of IP6 , namely calcium-magnesium-IP6, magnesium-potassium-IP6, sodium-IP6 and potassium-IP6 on the urinary excretion of calcium in lithogenic rats.[291] Results of this study showed that calcium-magnesium IP6 had no effect on the excretion of urinary calcium whereas the other three salts decreased the amount of calcium significantly and that potassium- IP6 was the most effective.[291]

***In vivo* studies in humans** demonstrated that the normal urinary concentration of IP6 oscillates between 0.757- 4.54 x 10^{-6} M as cited in a review article by Schlemmer et al.[259] Furthermore, the urinary excretion of IP6 was found to be significantly lower in stone formers than in normal individuals.[265] An in vivo study investigated the effect of IP6 ingestion in CaOx

stone formers.[292] In this study, a lithogenic test (a test developed by Grases et al to evaluate crystallisation propensity in urine[293]) was performed prior to and post administration of 120 mg of IP6 for 15 days (n= 12 CaOx stone formers).[292] This study had a control group consisting of 13 CaOx stone formers. After 15 days of IP6 ingestion, the lithogenic test was negative for 50 % of the stone formers whereas only 7 % of the control group tested negative. This study demonstrated that an increase in urinary IP6 has the potential to reduce the risk of CaOx stone formation.

In summary, both *in vitro* and *in vivo* experiments have demonstrated the inhibitory capacity of IP6 towards CaOx and CaP crystallisation.

CaOx urolithiasis in South Africa

As mentioned earlier, the occurrence of kidney stone disease in the black population is relatively rare (1 %) whereas its occurrence in the white population (%15) is comparable to other western countries.[3-5] It was hypothesised that various factors, including urine composition[4, 5, 294-298], renal handling of lithogenic and antilithogenic substances[16, 295, 296, 299-301] as well as dietary habits[5, 294-298, 302] may account for the rarity of CaOx urolithiasis in the former group.

Several differences in urinary risk factors associated with CaOx urolithiasis were observed between the two groups. Urinary calcium[4, 5, 294-296, 298, 299, 302], phosphate[4, 5, 295, 296, 299, 302] and urate[296, 299, 302] was found to be lower in the black group. However, all were within the normal range. Furthermore, although a lower urinary concentration of these risk factors may decrease the risk of kidney stone formation, it cannot account for the absolute rarity of the disease in this group. Other differences such as a higher urinary oxalate[295, 297] and lower urinary citrate[4, 294, 297, 302] and magnesium[298, 302] excretion in the black group are counterintuitive as it suggests that the black group has a higher risk of forming CaOx kidney stones compared to the white group. Other urinary parameters such as sodium have been reported to be higher in the black group[4, 5, 294] whereas inconsistent or no differences have been found in urinary pH[4, 295]. The rarity of CaOx urolithiasis in the black population can therefore not be attributed to the traditional urinary risk factors associated with this disease.

The composition of urinary macromolecules (proteins and GAGs) in the respective groups has also been investigated. A study investigating the inhibitory activity of crystal matrix extract (CME) isolated from urine of black and white males showed that CaOx crystal nucleation was inhibited in artificial urine and that the inhibitory activity of CME derived from the urine of blacks was greater.[303] It was speculated that the inhibitory activity was due to a protein called UPTF1. A follow up study investigating the effect of UPTF1 (in real urine) isolated from urine of black and white subjects, showed that it promoted nucleation.[304] In the

same study, CaOx crystal size was decreased in the presence of UPTF1 and its inhibitory activity was shown to be synergistically dependent on urine composition.[304] The inhibitory activity of UPTF1 was greater in its endogenous urine. Furthermore, the inhibitory activity of UPTF1 in the urine of black males was superior, suggesting that its conformation may be influenced by the urine composition.[304] Structural analysis showed that UPTF1 derived from urine of black individuals have more ɣ-carboxyglutamic acid and sialic acid which may influence its modulatory capacity.[305]

Other studies included the investigation of albumin[306, 307], bikunin[308, 309] and THP[310, 311]. Albumin isolated from urine of black individuals inhibited CaOx crystal growth and aggregation to a greater extent compared to those isolated from urine of white individuals.[306] The greater inhibitory activity of the albumin isolated from the urine of the former group may be due to the presence of higher negative charges on this molecule compared to albumin isolated from urine of white individuals.[307]

Bikunin isolated from urine of the two groups showed that CaOx crystal growth and nucleation was inhibited in the presence of this substance.[308] Bikunin derived from urine of black individuals inhibited CaOx crystal aggregation to a greater extent compared to the bikunin isolated from the urine of white individuals.[308]

THP isolated from the urine of black subjects demonstrated a greater inhibitory capacity of CaOx crystal aggregation compared to THP isolated from the urine of white subjects.[310, 311] Furthermore, the THP derived from the urine of the former group inhibited CaOx crystal growth in its endogenous urine as well as in the urine of white subjects.[310, 311] In contrast, THP derived from the urine of white subjects promoted CaOx crystal growth in its endogenous urine and showed inhibition in the urine of black subjects.[310, 311] It was speculated that the difference in inhibitory activity may be due to structural differences such as a higher molecular weight, more cysteine and glycine, and less alanine, valine, leucine and phenylalanine in the THP of the black group.[297]

The role of GAGS was also investigated.[312] Chondroitin sulphate and dermatan sulphate were found to be higher in urine of black individuals.[312] Crystallisation experiments demonstrated that GAGs from the respective race groups promoted CaOx crystal nucleation and inhibited aggregation in both artificial and real urine.[312] Furthermore, GAGs isolated from the urine of black subjects inhibited CaOx crystal aggregation and growth in real urine to a greater extent than those isolated from the urine of white subjects.[312] This suggested that GAGs may play a protective role against kidney stone formation in the black group.

In addition to the investigation and comparison of urinary parameters between the South African black and white population groups, dietary studies have also been conducted. These studies showed that dietary sodium intake is greater in the black group compared to the white group.[5, 294] This correlates with the higher urinary sodium found in the former group.[4, 5, 294] However, despite the well-known fact that an increase in dietary sodium leads to an increase in urinary calcium, the latter is lower in the black group as stated earlier. Dietary calcium was reported to be lower in the black group which may account for its lower urinary excretion in this group, however this does not explain the lower incidence of CaOx urolithiasis in this group.[297, 298, 302] Furthermore, animal protein was also found to be lower in the black group which is consistent with the lower incidence of CaOx urolithiasis in the black group as animal protein is associated with an increase in the risk of this disease.[295, 297, 298, 302] Interestingly, dietary oxalate was found to be higher in the black group.[297, 298, 302] This is counterintuitive as an increase in dietary oxalate is a risk factor for CaOx urolithiasis. Similarly, dietary magnesium[297, 298, 302] and vitamin B6[295, 297] were found to be lower in the black group even though these two dietary factors may reduce the risk of CaOx urolithiasis.

Extensive research on the renal response to dietary and supplemental challenges has also been conducted. These studies included the investigation of a high dietary oxalate/low calcium challenge[295], low calcium[296], high oxalate[296], lacto vegetarian[296], vitamin C supplementation[296] and vitamin E supplementation[298] amongst others. The results of these studies demonstrated that a different renal response to the respective challenges were evident between the two groups. Some of these studies will be described in the paragraphs below.

A study investigating the effect of a diet rich in oxalate (510 mg) and poor in calcium (318 – 334 mg) was conducted.[295] Healthy South African black and white males (11 per group) were enrolled in the study. This study showed that urinary oxalate increased significantly in the white group (by 57 %) whereas no change was observed in the black group. The researchers proposed that lower oxalate absorption rates in the black group may be present.

Interestingly, when a low calcium (400 mg) diet was administered in another study, urinary oxalate increased significantly in the black group whereas no difference was observed in the white group.[296] The researchers speculated that the black individuals may be more sensitive to the proposed mechanism whereby a diet low in calcium leads to an increase in the urinary excretion of oxalate[107] and a consequent increase in the risk of CaOx stone formation. In the same study, a high oxalate diet (510 mg) was administered.[296] Surprisingly, the urinary oxalate did not increase in either group relative to their baseline excretions. In the white group, urinary potassium, pH and the RS of brushite increased whereas urinary citrate

increased in the black group. The researches acknowledged that the increase in both pH and citrate are favourable as it decreases the risk of stone formation.[96, 241, 313] The increase in urinary potassium in the white group was also seen as a protective mechanism for the inhibition of CaOx crystallisation in response to the oxalate challenge.[314] However, the researchers postulated that since the black group are relatively immune to stone formation, the citrate response in this group to the high oxalate protocol may be a more effective protective mechanism than the increased urinary pH and potassium in the white group.

The investigation of high dietary calcium (915 mg) and a calcium supplement (915mg) was also investigated.[299] This study showed that both dietary calcium and a calcium supplement did not significantly alter any urinary biochemical or physicochemical risk factors in the black group. However, in the white group, urinary potassium increased significantly and the RS of brushite decreased significantly after a high calcium diet was administered. The (Tiselius Risk Index) TRI and the RS of CaOx decreased and the CaOx MSL increased after ingestion of the calcium supplement. These changes were regarded as favourable and in agreement with the hypothesis that a diet rich in calcium decreases the risk of CaOx stone formation.[49, 115]

The effect of vitamin C (1000 mg), high dietary sodium chloride (15 -18 g) and a lacto-vegetarian diet on the urinary risk factors associated with CaOx urolithiasis in the respective groups was also investigated.[296] After the vitamin C challenge, the TRI increased in the black group whereas no changes were observed in the white group. Urinary chloride increased in both groups whereas sodium increased in the black group only after the ingestion of high dietary sodium; surprisingly, the urinary calcium excretion did not increase in either group. Urinary pH increased significantly in the black group only after a lacto-vegetarian diet was followed.

Another study investigated the effect of the following supplements: vitamin B6 (100mg), L-glutamine (2000mg) and L-cysteine (500mg) on the urinary risk factors for CaOx urolithiasis.[299] In the white group, the vitamin B6 challenge significantly decreased urinary calcium, urinary phosphate and the RS of brushite; L-glutamine significantly decreased the RS of CaOx; and L-cysteine decreased urinary calcium. No significant changes were observed in the black group.

Other supplements such as vitamin E have also been investigated. A study showed that the ingestion of this supplement by the respective groups significantly increased plasma vitamin E in the white group as well as urinary citrate in both groups.[298] The increase in urinary citrate was however greater in the white group. The researchers speculated that the increase in plasma vitamin E in the white group only, may be indicative of different absorption rates of

the vitamin between the respective groups or that the mechanism whereby it is packaged into chylomicrons[315], which are secreted into the systematic circulation, may be suppressed in the black group. The greater increase in urinary citrate in the white group was speculated to be as a result of the inhibition of lipogenesis of AA by vitamin E[316, 317], leading to an increase in urinary citrate, which may occur to a lesser extent in the black group.

Finally, a dietary survey conducted at Groote Schuur Hospital (South Africa) during 1971 - 1979 revealed that the most important difference between the black and white population groups seemed to be the type of phosphorus (P) ingested.[318] The main source of P in the white group was inorganic P derived from animal sources. In the black group, inorganic P was comparable to that of the white group. However, in addition to inorganic P, the black group ingested a considerable amount of organic P derived from maize, beans and brown bread in the form of IP6. Modlin hypothesised that the significantly higher intake of IP6 in the black group compared to the white group may manifest itself in a higher urinary excretion which may contribute to the relative rarity of renal lithiasis in the former group.

More recently, a pilot study investigating the effect of dietary IP6 on urinary risk factors associated with CaOx urolithiasis was conducted in healthy black and white South African subjects in the Kidney Stone Research Laboratory (KSRL) at the University of Cape Town.[319] However, this study revealed counterintuitive findings whereby the black group excreted a significantly lower amount of urinary IP6 than the white group while on their unrestricted diets. In addition, the renal response to an IP6 dietary challenge was investigated in the aforementioned study. This dietary intervention revealed that IP6 excretion increased significantly in the black group after IP6 administration, whereas no change was observed in the white group. The researchers suggested that the renal handling of dietary IP6 may be different in the two groups.

The hypotheses by Modlin as well as the findings of the latter study are of interest in the present PhD project.

Hypotheses and objectives

In the aforementioned study, quantification of urinary phytate was determined using an indirect colorimetric assay in which the amount of phosphate per IP6 molecule was detected.[319] The reliability of the method therefore depends on adequate separation of inorganic phosphate and IP6 during sample preparation. However, the sample preparation used in this study did not adequately separate inorganic phosphate. As such the results and conclusions presented in that study remain questionable. Before any firm conclusions can be drawn, urinary IP6 needs to be determined by a much more reliable and robust method. Indeed, this constitutes one of the objectives of the present study. Furthermore, the notion of urinary phytate being an inhibitor of calcium stone formation deserves interrogation. This aspect constitutes a second objective. Finally, the observation of a much higher dietary intake of IP6 in South African black individuals compared to their white counterparts, and the relative rarity of stone disease in the former group, warrant investigation.

The hypotheses of the present thesis are as follows:

1. Exogenously derived urinary IP6 is a powerful inhibitor of calcium renal stone formation by virtue of its ability to form calcium- phytate complexes.
2. The relative rarity of renal stone formation in South Africa's black population compared to the white population can be partly attributed to the much higher dietary intake of IP6 in this group.

The overall aim of the work described in this thesis is to test the rigour of the above two hypotheses.

In order to achieve this overall aim, the following objectives were defined:

1. Determine the calcium-phytate thermodynamic formation constants using potentiometry.
2. Use the aforementioned constants to model the effect of urinary IP6 on the concentrations of ionized calcium and the supersaturation of calcium salts in urine.
3. Test the practical application of a new method for the determination of urinary IP6 in an animal model with a view of applying the method in human urine.
4. Test the predictions of the modelling performed in objective 2 by investigating the *in vitro* effect of IP6 on CaOx crystallisation mechanisms in artificial urine and real urine from healthy black and white South African subjects.
5. Assess the dietary intake of IP6 and other nutrients in healthy black and white subjects using semi-quantitative food frequency questionnaires.

6. Determine the baseline concentration of urinary IP6 in the groups identified in objective 5 above.
7. Determine the concentration of IP6 and other lithogenic and anti-lithogenic urinary components in each group after the administration of an IP6- deficient and IP6- rich diets.
8. Determine the urinary physicochemical and biochemical risk factors associated with CaOx crystallisation following the dietary protocols described in objective 7 above.

Chapter 2 : Potentiometric determination of calcium-phytate equilibrium constants

Introduction

Since previous studies demonstrated that phytate inhibits the crystallisation of calcium salts *in vitro* at concentrations similar to those which occur in urine[284, 285, 320], theoretical modelling of its effects on CaOx crystallisation processes could provide insight on the mechanisms by which this occurs. Modelling can be achieved by using the speciation program JESS (Joint Expert Speciation System).[321, 322] The latter has previously been used by the Kidney Stone Research Lab (KSRL) at the University of Cape Town, South Africa, in several studies to investigate the urinary speciation of calcium and other complexes in the context of urolithiasis.[323-327] One of these studies revealed that a calcium-citrate-phosphate species, previously unreported in urolithiasis research, accounted for a significant percentage of the complexation of calcium in urine.[323] More recently, the potential of malate (a polycarboxylic anion) as a chelator of free calcium was investigated.[324] Modelling of this urinary component showed that it had no effect on the supersaturation of CaOx and CaP salts in urine at physiological concentrations.[324] In another recent study the supersaturation of urinary salts were determined in urine of enteric hyperoxaluric patients, healthy subjects and stone formers.[325] This study revealed that the supersaturation of CaOx was substantially higher in patients with enteric hyperoxaluria than in healthy individuals and stone formers despite their lower calcium excretions. The JESS modelling performed in this study suggested that calcium supplementation can help to reduce stone risk in patients with enteric hyperoxaluria.[325] Other modelling studies by the KSRL have also shown that urinary sulfate could theoretically reduce the risk of CaOx and CaP stone formation[326] and that precursors of CaOx and CaP should be taken into account when investigating stone forming mechanisms.[327]

JESS contains a database of thermodynamic constants for over 12 000 chemical complexes which can be used for modelling chemical speciation at different pH values, temperatures and ionic strengths (IS). However, the database does not contain formation constants for phytate complexes. Moreover, a review of the literature reveals that only three quantitative studies on the formation constants of soluble alkaline earth metal complexes with phytate have been conducted and that large discrepancies in the magnitudes of these constants exist.[328-330] Two of these studies used ISE-H^+ potentiometry to investigate the binding of Ca^{2+} to phytate.[328, 329] One of these identified only 3 mononuclear species[328] whereas the other identified 3 binuclear species and 4 trinuclear species in addition to 3 mononuclear species.[329] The magnitude of the formation constants in the former study was several orders

higher than the latter study. The third study used ISE-Ca^{2+} potentiometry to investigate the binding of Ca^{2+} to phytate.[330] This study reported conditional formation constants of two soluble Ca-phytate complexes, one of which was mononuclear and one of which was binuclear.[330] The author of JESS, Peter M May (*Murdoch University, Murdoch Western Australia*), also indicated (in private correspondence) that the inclusion of literature phytate-complex equilibrium constants in the JESS database resulted in unsatisfactory results due to poorly defined thermodynamic constants of these complexes. Furthermore, the IS dependence of all formation constants in the JESS data base seemed to be acute when phytate was included in the data base, possibly due to the highly negative charge of the latter molecule. Thus, in light of all of the above, the determination of reliable formation constants (at an IS similar to that of the urine model) was necessary in the present project so that modelling of phytate-calcium complexes could be undertaken.

Theory: equilibrium reactions

Stability constants, also referred to as formation constants, are equilibrium constants and are a measure of the strength of interaction between two or more components in solution equilibria.[331] A formation constant, denoted by "K", is associated with all equilibrium reactions.[331] For example, consider the protonation of a ligand described by the following equation:

$$L + H \leftrightarrow LH \quad (1)$$

where L is the ligand and H is the proton. The thermodynamic protonation constant may be expressed as:

$$^{T}K_{H} = \frac{\{LH\}}{\{L\}\{H\}} \quad (2)$$

where {LH}, {L} and {H} are the respective activities of a single protonated ligand, ligand and proton; respectively. The activity is related to the concentration by the following equation:

$$\{X\} = \Upsilon_{x}[X] \quad (3)$$

where {X} is the activity of the species X (a ligand, proton or metal ion), Υ_x is the activity coefficient of species X and [X] is the concentration of X. Equation (2) can therefore be written as follows:

$$^{T}K_{H} = \frac{(\Upsilon_{LH}[LH])}{(\Upsilon_{L}[L])(\Upsilon_{H}[H])} \quad (4)$$

Equation (4) may be re-arranged as:

$$^{T}K_{H} = \left[\frac{\Upsilon_{LH}}{\Upsilon_{L}\Upsilon_{H}}\right]\left[\frac{[LH]}{[L][H]}\right] \quad (5)$$

When measuring the equilibrium constant, a medium containing a background electrolyte at a constant IS is used. At a constant IS:

$$\left[\frac{\Upsilon_{LH}}{\Upsilon_{L}\Upsilon_{H}}\right] = \text{constant} \quad (6)$$

Equation (5) may therefore be expressed as:

$$K_{H} = \frac{[LH]}{[L][H]} \quad (7)$$

where ${}^{T}K_{H}$ is now the concentration equilibrium constant. For a ligand with 2 protonation constants, K_1 and K_2, the protonation constants may be expressed in a stepwise manner:

$$L + H \leftrightarrow LH \qquad K_1 = \frac{[LH]}{[L]\,[H]} \qquad (8)$$

$$LH + H \leftrightarrow LH_2 \qquad K_2 = \frac{[LH_2]}{[LH]\,[H]} \qquad (9)$$

Thus, for a ligand with n protonation constants the formation constant may be written as:

$$K_n = \frac{[LH_n]}{[LH_{n-1}]\,[H]} \qquad (10)$$

The formation constant for the interaction between a metal (M) and a ligand may be expressed in a similar series of equations:

$$M + L \leftrightarrow ML \qquad K_{ML} = \frac{[ML]}{[M]\,[L]} \qquad (11)$$

$$ML + L \leftrightarrow ML_2 \qquad K_{ML2} = \frac{[ML_2]}{[ML]\,[L]} \qquad (12)$$

Therefore,

$$K_{MLn} = \frac{[ML_n]}{[ML]\,[L]} \qquad (13)$$

The cumulative formation constant (β) for protonation of a ligand with n protons may be calculated as follows:

$$\beta_{1n} = {}^{T}K_{11} \times {}^{T}K_{12} \times {}^{T}K_{13} \ldots\ldots K_{1n} = \frac{[LH_n]}{[L]\,[H]^n} \qquad (14)$$

Similarly, the cumulative β for a reaction between M and L may be expressed as:

$$\beta_{MLn} = {}^{T}K_{ML1} \times {}^{T}K_{ML2} \times {}^{T}K_{ML3} \ldots\ldots K_{MLn} = \frac{[ML_n]}{[ML]\,[L]^n} \qquad (15)$$

In the present study, the ligand of interest is phytate which consists of a 6-membered ring with 6 phosphates and 12 dissociable protons over a wide pH range.[332-335] The equilibrium

equation for the protonation of the ligand (phytate) in the present study is therefore expressed as follows:

$$H^{+1} + H_{n-1}Phy^{-(12-n+1)} \leftrightarrow H_nPhy^{-(12-n)} \qquad K_{1n} = \frac{[PhyH_n]}{[PhyH_{n-1}]\,[H]} \qquad (16)$$

Since phytate is cyclic and contains 6 phosphate moieties, more than one M is able is able to bind per ligand. Thus equation (13) cannot be applied for the complexation between Ca^{2+} and phytate in the present study. The equilibrium equation used for complexation between Ca^{2+} and phytate in the present study is therefore written as:

$$_mCa^{2+} + H_nPhy^{-(12-n)} = Ca_mPhyH_n{}^{-(12-2m-n)} \qquad K_{mn} = \frac{[Ca_mPhyH_n]}{[Ca_m]\,[H_nPhy]} \qquad (17)$$

Methods

Experimental techniques used for the determination of formation constants include isothermal titration calorimetry (ITC) and potentiometric titrations. Briefly, the former technique measures the heat that is released or absorbed when binding occurs between two substances.[336-338] Heat is measured by a calorimeter during the gradual titration of one substance to another. Analysis of the reaction heat as a function of concentration provides the formation constants as well as the reaction stoichiometry, enthalpy and entropy. [336-338] This technique is generally used for the study of macromolecules.[336, 339, 340] A potentiometric titration on the other hand is the measurement of the change in potential (EMF) as a titration progresses at a fixed temperature and IS.[331, 341] This technique is commonly used to study acid-base reactions, redox reactions and complex formation reactions. [331, 341] Potentiometric measurements are performed using ion-selective electrodes (ISE) including the hydronium selective electrode (ISE-H^+, also referred to as a glass electrode) and the calcium selective electrode (ISE-Ca^{2+}, made of a polymeric substance). These electrodes are by definition sensitive to the change in concentration of a certain analyte i.e. H^+ and Ca^{2+}; respectively. The resulting potential as well as the volume at each titration point is used to determine the binding constants using a mathematical algorithm. This technique is recommended for the study of low-molecular weight (LMW) compounds.[341] Since phytate is regarded as a LMW compound, potentiometry was selected for the determination of calcium-phytate formation constants in the present study.

When using glass electrode potentiometry, several systematic errors need to be minimized. These include temperature, electrode calibration, control of CO_2 contamination, water quality and preparation and standardization of acid, base, ligand and metal solutions. The methods used in the present study to minimize these systematic errors are described below. Experimental details on the titrations conducted to determine protonation constants of the ligand (phytate) as well as the metal-ligand (Ca-phytate) formation constants are also described.

Instrumentation

A Ω *Metrohm* potentiometer (*848 Titrino plus*) fitted with a glass electrode with an internal Ag/ AgCl reference electrode, automatic titrator (burette; Ω *Metrohm*) and titration vessel with a magnetic stirrer (Ω *Metrohm 801*) was used in the present study for potentiometric titrations. The estimated accuracy of the EMF and titrant volume was ± 0.1 mV and 0.003 ml; respectively. Temperature was kept constant at 25 ± 0.1 °C by means of a circulating "water jacket" around the titration vessel in an air-conditioned laboratory.

Calibration of glass electrode

As mentioned earlier, changes in potential, also known as the electromotive force (EMF) are measured in potentiometry. This technique is used when an end-point in an acid-base titration is difficult to determine; for example in a very dilute solution or when a suitable indicator is not available.

In potentiometry, ion selective electrodes are used as the "indicator" together with a reference electrode and a sensitive potentiometer (a device that measures the EMF). These electrodes include that of ISE-H^+ and ISE-Ca^{2+} as mentioned earlier. Electrodes are immersed in a solution containing an analyte. This experimental setup is referred to as an "electrochemical cell" (E_{cell}). E_{cell} for ISE-H^+ (glass electrode) potentiometry may be depicted as follows[342]:

Reference electrode | salt bridge | analyte solution | glass electrode

E_{ref} E_j E_g

where each electrode possesses a half-reaction. The reference electrode has a half-reaction with an accurately known potential and is not affected by the concentration of the analyte or any other ions in the solution whereas E_g depends on the activity of H^+ in the solution. E_j is the electrode junction potential that develops at liquid junctions at each end of the salt bridge and should remain constant at constant IS and temperature. The measured/ observed EMF, E_{cell}, is therefore as follows:

$$E_{cell} = E_{ref} + E_j + E_g \qquad (18)$$

Since E_g is dependent on the activity of H^+, E_{cell} may be written in terms of the Nernst equation (an equation which shows how potential varies with the activity of an analyte) as follows at constant IS:

$$E_{cell} = E° + \frac{2.30\ RT}{z\ F} \log [H^+] \qquad (19)$$

where E° is the standard electrode potential, R is the universal molar gas constant (8.314 $J.K^{-1}mol^{-1}$), T is the temperature in Kelvin, z is the number of electrons and F is Faraday's constant (96 485 C mol^{-1}). A plot of E_{cell} vs log $[H^+]$ yields a graph with an intercept corresponding to E° and a slope (s) of 2.303RT/zF. For the glass electrode, z =1. The slope

of such an electrode should therefore be equal to 59.14 mV at 298 K. Equation (19) may be simplified as follows:

$$E_{cell} = E° + s \log [H^+] \quad (20)$$

In order to minimize systematic errors when carrying out potentiometric titrations the electrode should be calibrated regularly as described below.

Electrode calibration was performed in the present study by titrating a strong base (0.1 M NaOH) against a strong acid (0.01 M HCl) at 25 ± 0.1 °C. A typical acid-base titration plot as obtained in the present study is shown in Figure 2.1 for the titration of a strong base against a strong acid. This titration curve may be divided into three regions as indicated. The acid region indicates the amount of unreacted acid in the flask at the beginning of the titration at which the measured EMF is positive. The end point region indicates the region at which one drop of base causes a drastic change in the measured EMF. The equivalence point (point at which the number of moles of base is equal to the number of moles of acid) is found within the end point region. Lastly, the alkaline region indicates the region at which an excess amount of base is present and the measured EMF is negative.

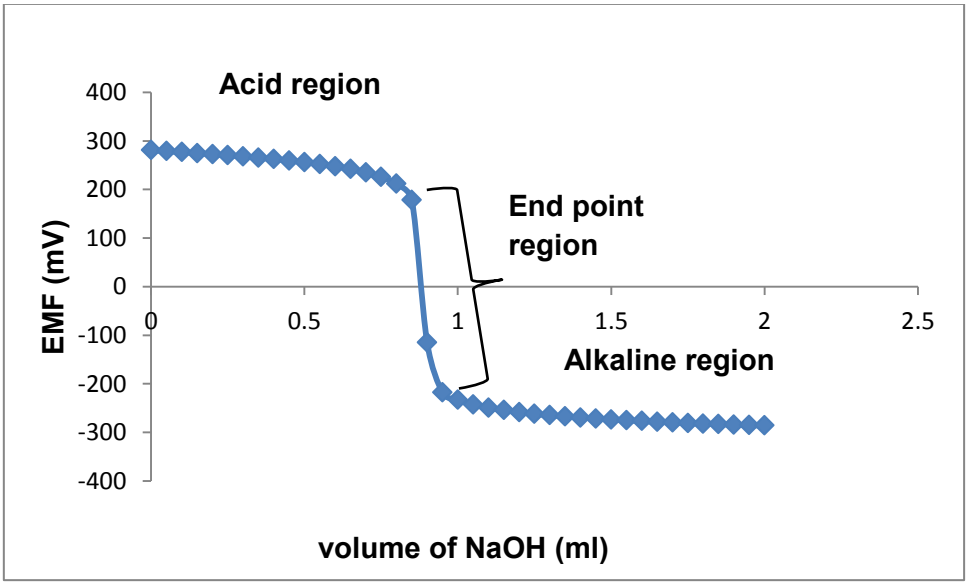


Figure 2.1 A typical EMF vs volume of NaOH plot depicting an acid-base titration as obtained in the present study.

The electrode was then calibrated using the above data in a computer program called GLEE (glass electrode evaluation) which was designed for the calibration of a glass electrode.[343] This computer program provides the pseudo-Nernstian standard electrode potential (E°) and slope (s) of the electrode which is obtained from a plot of EMF vs pH as shown in Figure 2.2. Data points used for the calibration of the electrode is between pH values of 2.5- 4.0 and 10.7- 11.3. These ranges are chosen for the following reasons:

1. The measured pH deviates from linearity at very low pH of an acid-base titration.
2. Calculation of the hydrogen ion concentration is subject to large error in the end-point region since the pH rises very sharply from pH 3.5 to 10 for the addition of one drop of base. Furthermore, the presence of carbonate impurity can affect the data up to pH ≈ 10.7. Data within the region of 4.0 to 10.7 is therefore omitted.
3. At high pH values, errors due to the liquid junction potential exist therefore data above pH 11.3 is omitted.

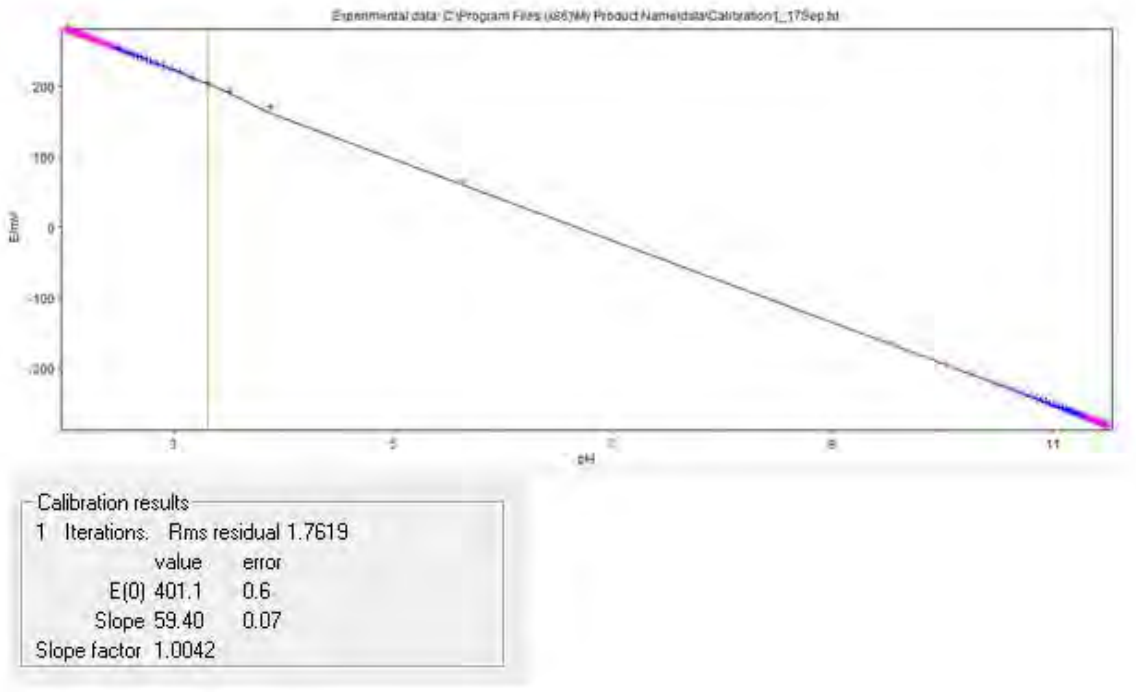


Figure 2.2 A typical EMF vs pH plot for the calibration of glass electrode using GLEE[343] as obtained in the present study. Pink points indicate data points which are omitted in the calibration.

CO_2 contamination

Titrations were conducted under a stream of purified nitrogen gas (an inert gas) to prevent CO_2 contamination. The level of carbonic acid contamination also referred to as the level of protolytic impurity was checked by using the aforementioned acid-base potentiometric data in

GLEE[343] to obtain a "Gran" plot as shown in Figure 2.3. A Gran plot is constructed by plotting the data obtained from the acid region and alkaline region; these results in two slopes which should meet at a point corresponding the end point of a titration. An indication of CO_2 contamination is when the two slopes do not have the same end point. GLEE[343] calculates the percentage difference in the two end points and is reported as the "protolytic impurity level" as shown in Figure 2.3. A percentage difference of ≤ 1 % is regarded as favourable.

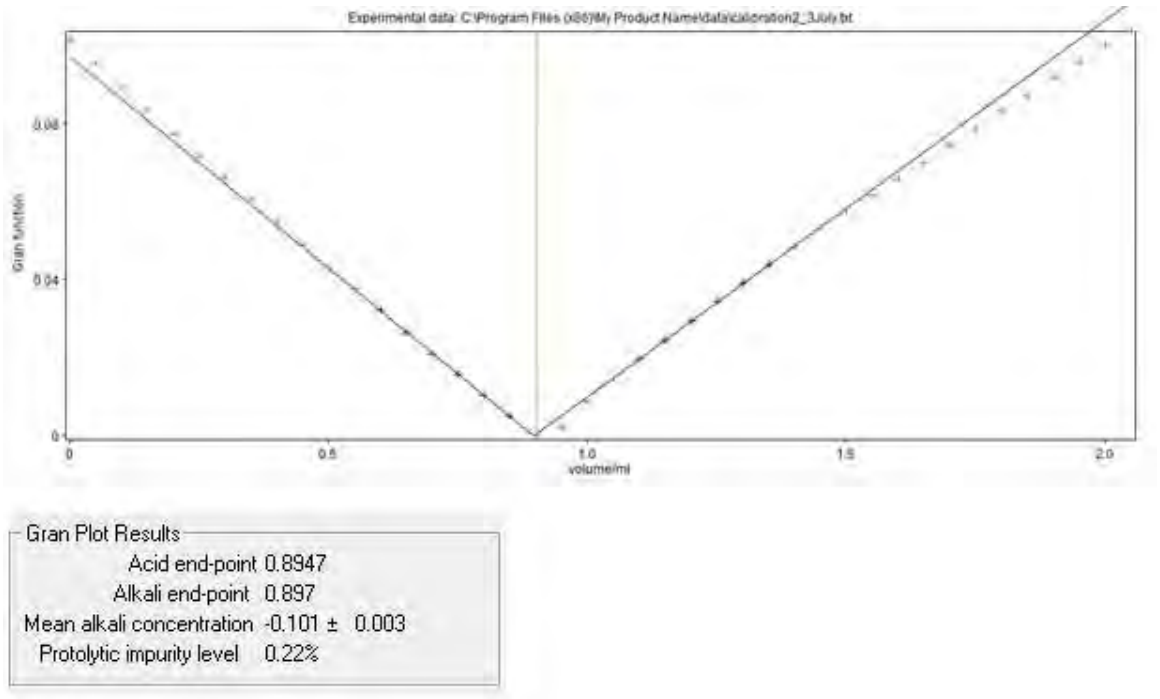


Figure 2.3 A typical Gran plot constructed in GLEE[343] as obtained in the present study for the determination of CO_2 contamination. Pink points indicate data points which were excluded from the calculation.

Preparation of solutions

Millipore water was boiled and cooled to room temperature for the removal of CO_2. This water was used for the preparation of all solutions in the present study. **NaOH and HCl solutions** were prepared from ampoules of NaOH (*1.09959-Trisol, Merck*) and HCl (*1.09970-Trisol, Merck*). NaOH (0.1 M) was standardized using potassium hydrogen phthalate (KHP, a "primary standard"). HCl (0.01 M and 5 mM) was standardized using the standardized NaOH.[344] **Phytic acid stock solution** (10 mM) was prepared by weighing the dipotassium salt (*Sigma Aldrich*) and dissolving it in H_2O followed by the removal of the potassium by a strong cationic exchange resin (*Dowex 50 x 8- 200 mesh, H^+ form, Sigma*). The absence of potassium was checked by flame atomic absorption spectroscopy (AAS).

Calcium (5 mM) solution was prepared in 5 mM HCl and standardized using EDTA in a complexometric titration.[345, 346] Eriochrome blackT was used as an indicator in this titration in which the end point is signalled by a colour change from red to blue. All standard solutions were prepared in A-grade glassware (volumetric flasks) and the IS was kept constant at 0.15 M by the addition of NaCl (*Sigma Aldrich*). This IS was chosen as it mimics the physiological concentration of urine.[347, 348]

Ligand protonation and metal-ligand titrations

Potentiometric data for the protonation of phytate was conducted by titrating 0.1 M NaOH against a volume of 15 ml phytate at concentrations between 1.003- 1.019 mM. For metal-ligand titrations, 0.1 M NaOH was titrated against 25 ml of a solution containing both phytate and Ca at metal: ligand concentration ratios of 1:1, 2:1, 3:1 and 4:1; titrations were stopped when precipitation occurred. All titrations were carried out with gentle stirring under inert conditions to avoid CO_2 contamination.

Calculations

The program HyperQuad[349] was used to refine potentiometric data obtained for phytate protonation and complexation between Ca and phytate. This program uses least-squares refinement. A comprehensive account of the mathematical equations used in the refinement algorithm can be found in Gans 1985.[350] Briefly, the method of refinement is based on 5 assumptions as follows:

1. Each chemical species in the solution equilibria has a formation constant associated with it and is expressed as a concentration quotient as shown in the equations presented earlier under “Theory”.
2. The electrodes used obey a pseudo-Nernstian equation as shown in equation (20).
3. Systematic errors such as electrode calibration, sample weighings and dilutions, standardization of reagents, temperature variance and water quality have been minimized.
4. The “independent” variable is not subject to error and error in the “dependent” variable assumes a Guassian distribution. The “independent” and “dependent” variables are defined below.
5. A model of the equilibrium system has been hypothesized and is representative of the experimental observations.

The dependent variable in the refinement algorithm is the measured EMF. The independent variable is the titre volume. Since the independent variable is the titre volume, the EMF in the region of the end point is subject to large error and is significantly affected by any error in the titre volume; a standard propagation formula is used to calculate the error in the measured EMF and is as follows:

$$\sigma^2 = \sigma_E^2 + (\delta E/\delta V)^2 \sigma_V^2 \qquad (22)$$

where σ^2 is the calculated variance of the measurement, σ_E^2 is the error of the electrode, σ_V^2 is the error in the titre volume and δE/δV is the slope of the titration curve. Weights are assigned at each titration point and are inversely proportional to the variance in equation (19). Thereby accommodating for the error in measurement near the end point where δE/δV is large. Weights are calculated by estimating δE/δV at each point using a method of unit weights which is essential for batch titration data. These weights are then used to estimate the agreement between the theoretical model and experimental data and are calculated from the following equation:

$$\sigma = \sum_i \left[\frac{W_i r^2}{m-n} \right]^{1/2} \qquad (23)$$

Where m is the number of data points and n is the number of parameters. σ is the calculated agreement between the theoretical model and experimental data and should in principle approach a value of 1 if the assumptions in 1 -5 are fulfilled and σ_E and σ_V were assigned correctly.

The general strategy used in the present study for the refinement of formation constants is as follows:

1. Measured potentiometric data including the titre volume and measured EMF, the measured standard electrode potential and Nernstian- slope, are inserted into HyperQuad.
2. A theoretical model of the equilibrium system is hypothesized, including the estimated formation constants (based on literature values) for each predicted species.
3. Formation constants may be refined by selecting the “refinement” option. Formation constants may also be kept “constant” or “ignored” in a model before starting the refinement iteration.
4. If the refinement is successful i.e. “convergence” is obtained between the experimental and theoretical model then the calculated formation constants for each species are accepted. However, if refinement is not successful, a new model is hypothesized. This process is known as “model selection”.
5. The model with the lowest σ value and no ill-defined formation constants is chosen as the best model. Formation constants are ill-defined if its standard deviation is greater than 33 % of its value or is negative. Standard deviations of ill-defined constants are

flagged as “excessive” or “negative” in HyperQuad[349] and the refinement iteration is then stopped (at this point a new model is selected).

6. The species distribution is then plotted in HySS[351] (a computer program) using the formation constants obtained in 5.

Results and discussion

Calibration of glass electrode

The glass electrode was calibrated by titrating 0.1 M NaOH against 10 mM HCl at a constant IS of 0.15 M (NaCl) and temperature of 25 °C. The resulting potentiometric data was used in GLEE[343] for the determination of the Nernstian slope, standard electrode potential (E°), pKw as well as the protolytic impurity. The results of n = 8 titrations are reported in Table 2.1. A range together with the median is reported for each parameter. Plots of the raw data are included in Appendix 2. An acid-base titration was conducted on each day before commencing titrations with the ligand to ensure that a Nernstian slope within the range below was obtained.

Table 2.1 Calibration of glass electrode at t= 25 °C. The range and median of n= 8 titrations are reported.

Nernstian-slope	E°	pKw	Protolytic impurity level (%)
58.6 - 59.4 (59.0)	393.5 - 403.2 (397.9)	13.67 - 13.72 (13.67)	0.22- 1.45 (0.68)

The accurate determination of formation constants using potentiometry is dependent on reducing systematic errors as mentioned on page 33. These include the calibration of the electrode and the removal of CO_2 contamination. In the present study the calibration of the glass electrode was successful, giving a median value of 59.0 for the Nernstian- slope. This indicates that the electrode's functionality was excellent. As the glass electrode is sensitive to the presence of H^+ ions, the control of CO_2 contamination is important to prevent the formation of carbonate which affects the concentration of H^+ in solution. The prevention of CO_2 contamination in the present study was successful as indicated by the level of protolytic impurity (median value = 0.68%). These systematic errors were therefore minimized in the present study.

Protonation of phytic acid

The protonation of phytate was studied within the pH range of 2 -11 using a glass electrode. This pH range was chosen as the protonation of phytate occurs over a wide pH range as reported in previous studies. [332-335]

A replicate of 5 titrations (total of 609 data points) were conducted to study the protonation of phytate. The resulting data was used in HyperQuad[349] to determine the formation constants of $H_nPhy^{(12-n)-}$. A hypothetical model with 1≤ n ≤ 8 was proposed for the protonation of the former species and log β's were estimated from a previous study by Stefano et al.

Results are reported in Table 2.2 which summarises values for log β, the mean experimental log K's and literature log K's. log β of the first two protonation constants was kept constant in the model during refinement in the present study and will be justified below. The agreement between the hypothesized model and the experimental data is indicated by the parameter "σ" which was equal to 14 as shown in Table 2.2. This result will be discussed later. In the present study, the magnitude of the first three protonation constants were close to one another followed by a decrease in log K for protonation constants 4- 8.

Table 2.2 Experimental and literature protonation constants of Phytate. IS = 0.15 M, t = 25 °C.

Species (model)	logβ	σ	log K_{exp}	log K_{lit}[335]
H_1Phy^{-11}	9.41	14	9.41	9.41
H_2Phy^{-10}	19.08		9.67	9.67
H_3Phy^{-9}	28.49		9.41 ± 0.05	9.33
H_4Phy^{-8}	36.44		7.95 ± 0.09	7.97
H_5Phy^{-7}	42.75		6.31 ± 0.13	6.35
H_6Phy^{-6}	48.06		5.31 ± 0.14	5.10
H_7Phy^{-5}	51.28		3.22 ± 0.15	2.75
H_8Phy^{-4}	53.73		2.4 ± 0.14	n.o

log β is the logarithm of the sum of the overall protonation constants, σ is an estimate of the fit between the theoretical and experimental data. log K refers to the following reaction: $H^+ + H_{n-1}Phy^{(12-n)-} = H_nPhy^{(12-n)-}$. n.o = not observed.

The agreement of the theoretical model and the experimental data is shown in Figure 2.4 for the protonation of phytate. Plots of all other titrations conducted for the determination of phytate protonation constants are included in Appendix 2. The speciation distribution of $H_nPhy^{(12-n)-}$ is shown in Figure 2.5.

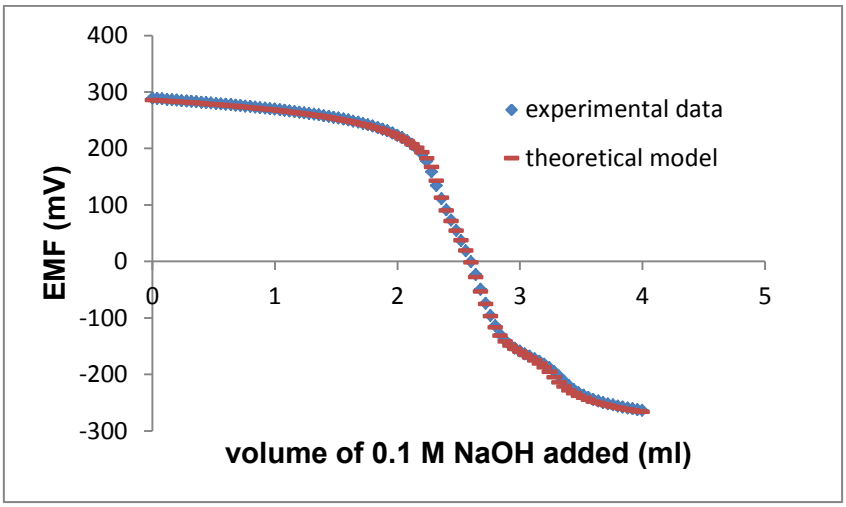


Figure 2.4 A typical plot of EMF vs volume of NaOH added for the titration of NaOH (0.1 M) against phytate (0.001 M). Experimental curve is shown in blue and theoretical in red. IS = 0.15 M (NaCl); t = 25 °C

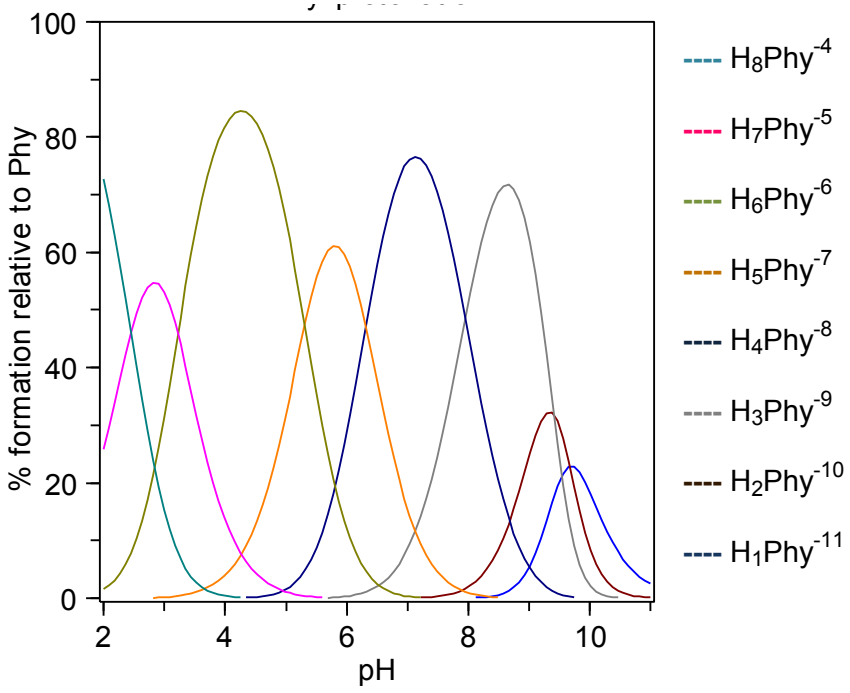


Figure 2.5 Species distribution of $H_nPhy^{(12-i)-}$ (0.001 M) in the pH range of 2-11 using HySS[351]. t = 25 °C; IS = 0.15 M (NaCl)

Comment

In order to determine stability constants of any metal-ligand complex, protonation constants are required as a first step as the acid-base equilibria of a ligand affects its interactions with other ions and ligands. Extensive research has been conducted on the acid-base properties of phytate.[332-335, 352-354] However, the stability constants for phytate protonation differ amongst laboratories. This is mainly due to different experimental conditions which have been used in which both the medium (containing a background electrolyte) and IS significantly affect the value of the determined stability constants.[352-354] Most quantitative studies on the protonation of phytate in alkali media have reported only 7 protonation constants (over a pH range of 2 – 11) as the first 5 occurs at a pH less than 2.[335, 352-354] However, others have reported up to 8 and 12 protonation constants.[333, 334] Eight protonation constants were identified in the present study over a pH range of 2- 11 as mentioned above.

During the "model selection" process in the present study, difficulty in obtaining convergence between the experimental data and the theoretical model was encountered. It was found that this difficulty occurred due to the unique acid-base properties of phytate in which previous studies have reported that the first 3 protonation constants are of similar magnitude and shows a peculiar trend in alkali media.[334, 335, 352, 355] These studies have demonstrated that in a KCl medium the following trend in log K occurs for the first 3 protonation constants: $\log K_1 > \log K_3 > \log K_2$ and in a NaCl medium: $\log K_2 > \log K_1 > \log K_3$.[335, 352] This peculiar trend is due to strong interactions between phytate and alkali metals. When NaCl is the background electrolyte, the inversion in strength of the first two protonation constants at an IS < 0.6 M is mainly due to the formation of Na_5H_2Phy which has two hydrogen bridges.[334, 355] In light of the aforementioned, the first two protonation constants were kept constant during refinement in the present study. Of the remaining constants which were refined in the present study, H_3Phy – H_7Phy were comparable to the study by Stefano et al in which calculated protonation constants at an IS of 0.15 M were reported (as shown in Table 2.2).[335] The eighth protonation constant in the present study is comparable to a previous study in which $NaClO_4$ (IS = 0.15 M) was used as the background electrolyte ($\log K_8 = 1.98$).[334]

Calcium-phytate complexation

The formation of Ca-phytate complexes was investigated over a pH range of 3-7. No significant complexation occurred at pH < 3 and precipitation occurred at pH > 7. The following number of titrations were conducted at each metal:ligand molar ratio: 1:1 (5 titrations), 2:1 (3 titrations), 3:1 (3 titrations) and 4:1 (2 titrations). A total number of 515 experimental data points were used in HyperQuad[349] for the determination of formation constants of $Ca_mPhyH_n^{-(12-2m-n)}$. A model with 1≤ m ≤3 and 1≤ n ≤6 was hypothesized for the former species and log β was estimated from literature values reported by Crea et al[329] and Torres et al.[328]

Seven Ca-phytate species (4 mononuclear and 3 binuclear) were identified using HyperQuad[349] as shown in Table 2.3. The hypothetical model used in the present study differed to the literature. Furthermore, a comparison of the formation constants identified in the present study to those in the literature is not in agreement. These findings will be discussed below.

Table 2.3 Experimental and literature formation constants of $Ca_mPhyH_n^{-(12-2m-n)}$

Species (model)	logβ	σ	log K_{exp} (NaCl, IS = 0.15 M; t = 25 °C)	log K_{lit}[329] (NaCl, IS = 0.25 M; t = 25 °C)	log K_{lit}[328] ($NaClO_4$, IS = 0.15 M t = 37 °C)
$CaPhyH_3^{-7}$	36.22	12	7.73 ± 0.05	3.42	-
$CaPhyH_4^{-6}$	42.69		6.25 ± 0.05	2.06	8.3
$CaPhyH_5^{-5}$	48.23		5.48 ± 0.05	2.11	8.4
$CaPhyH_6^{-4}$	52.31		4.25 ± 0.05	-	7.4
Ca_2PhyH^{-7}	26.01		16.6 ± 0.25	-	-
$Ca_2PhyH_2^{-6}$	32.59		13.51 ± 0.21	-	-
$Ca_2PhyH_3^{-5}$	39.27		10.78 ± 0.09	6.47	-
$Ca_2PhyH_4^{-4}$	n.o.		-	5.24	-
$Ca_2PhyH_5^{-3}$	n.o		-	4.09	-
$Ca_3PhyH_2^{-4}$	n.o		-	11.23	-
$Ca_3PhyH_3^{-3}$	n.o		-	9.41	-
$Ca_3PhyH_4^{-2}$	n.o		-	7.35	-
$Ca_3PhyH_5^{-1}$	n.o		-	6.31	-

log β is the sum of the overall formation constants. n.o. (not observed), σ is the estimate of agreement between the theoretical model and experimental data. log K refers to the following reaction: $M_m^{2+} + H_nPhy^{-(12-n)} = M_mPhyH_n^{-(12-2m-n)}$

The agreement between the theoretical model used in the present study and the experimental data is shown in Figure 2.6 for a titration conducted at a Ca:Phytate molar ratio of 1:1. Plots of all other titrations are shown in Appendix 2.

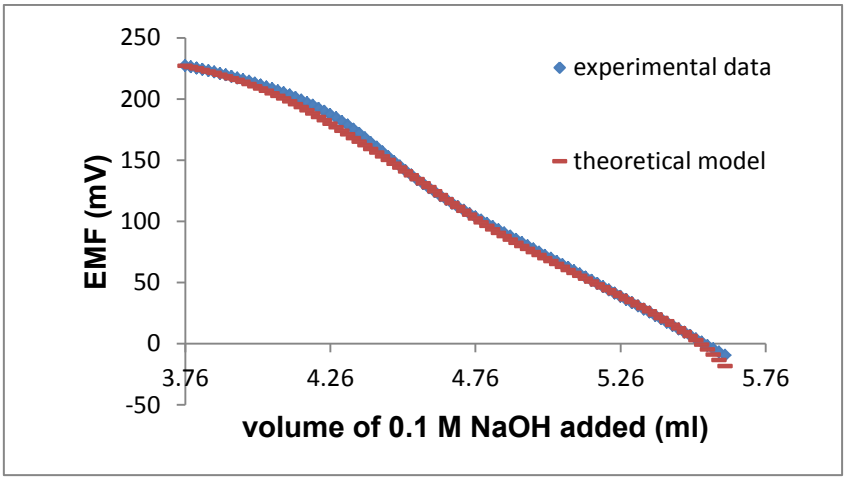


Figure 2.6 A typical EMF vs volume of NaOH added for the titration of NaOH (0.1 M) against a solution containing Ca: 2 mmol.L^{-1} and phytate: 2 mmol.L^{-1}. IS = 0.15 M, t = 25 °C. Experimental curve is shown in blue and theoretical in red.

The percentage distribution of each species relative to phytate at a Ca:Phy molar ratio of 1:1 is shown in Figure 2.7. Only mononuclear species were present at this ratio with maximum percentages of 65. $CaPhyH_5^{-5}$ was present over the entire pH range investigated with a maximum at pH = 4.8.

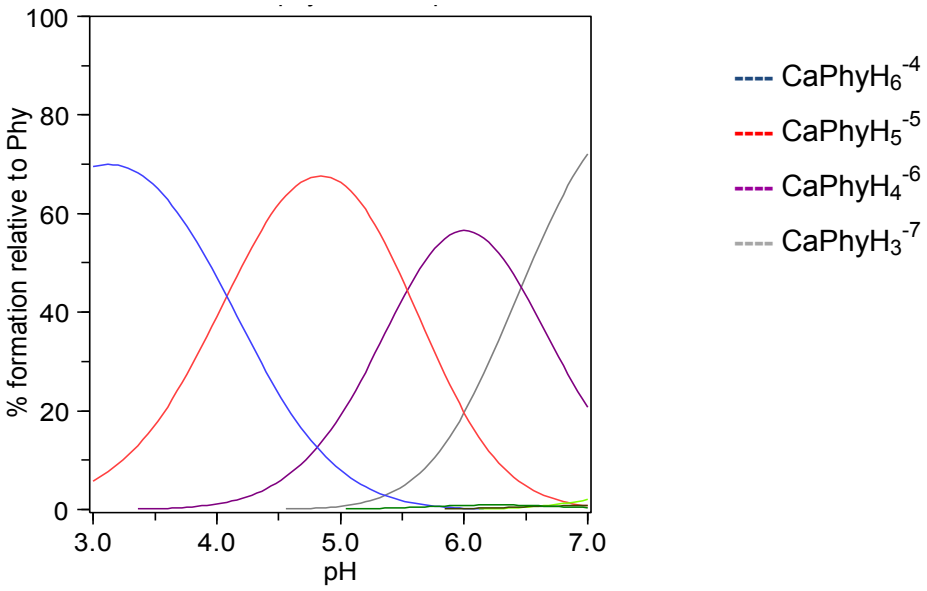


Figure 2.7 Species distribution of $Ca_mPhyH_n^{-(12-2m-n)}$ at a Ca:Phy molar ration of 1:1. Ca: 2 mmol.L^{-1}, phytate: 2 mmol.L^{-1}, IS = 0.15 M (NaCl), t =25 °C

The percentage distribution of species at a Ca:Phytate molar ratio of 2:1 is shown in Figure 2.8. The formation of binuclear species was present at this metal: ligand ratio, however mononuclear species were still predominant.

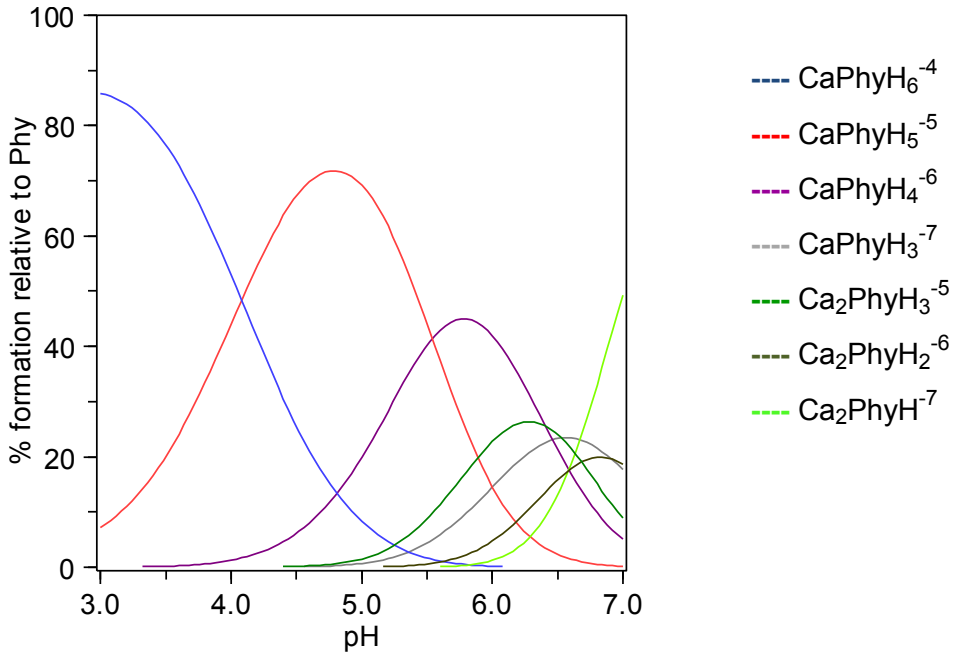


Figure 2.8 Species distribution of $Ca_mPhyH_n^{-(12-2m-n)}$ at a Ca:Phy molar ration of 2:1. Ca: 4 mmol.L^{-1}, phytate: 2mmol.L^{-1}, IS = 0.15 M (NaCl), t =25 °C

Species distribution plots at Ca:Phytate molar ratios of 3:1 and 4:1 are also shown in Figure 2.9 and Figure 2.10. The formation of trinuclear species was not found at the conditions used in the present study.

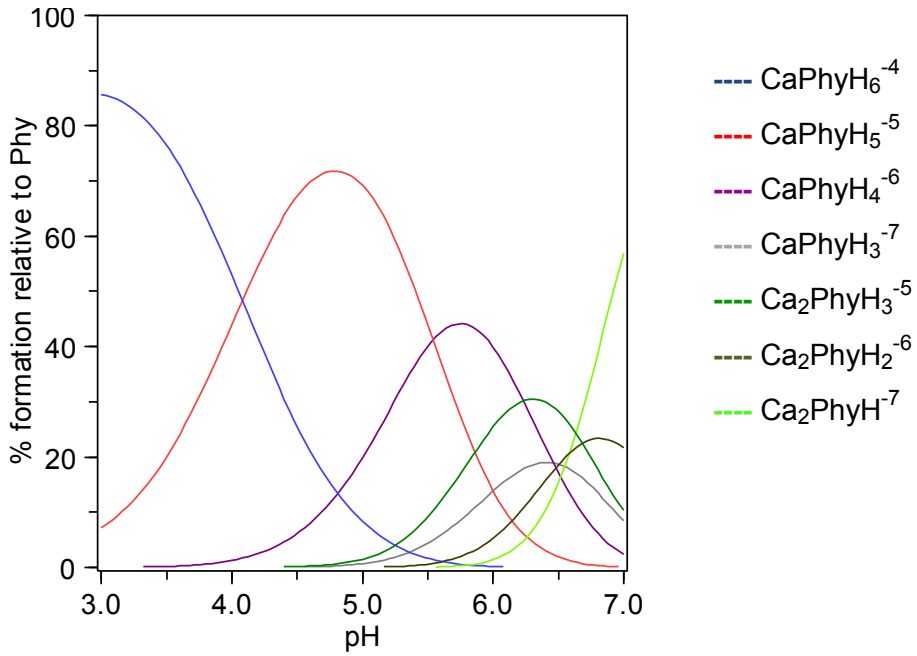


Figure 2.9 Species distribution of $Ca_mPhyH_n^{-(12-2m-n)}$ at a molar ration of 3:1. Ca: 3 mmol.L ^{1}, phytate: 1 mmol.L^{-1}, IS = 0.15 M (NaCl), t =25 °C

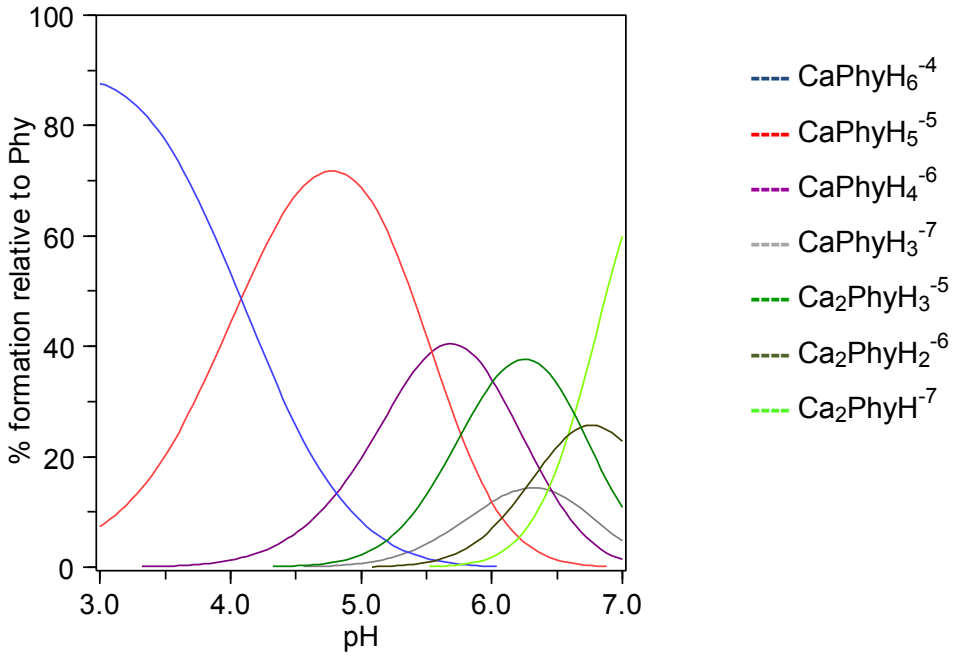


Figure 2.10 Species distribution of $Ca_mPhyH_n^{-(12-2m-n)}$ at a molar ration of 4:1. Ca: 4 mmol.L^{-1}, phytate: 1 mmol.L^{-1}, IS = 0.15 M (NaCl), t =25 °C

Comment

As stated earlier, only a few studies have been conducted for the determination of the stability constants of $Ca_mPhyH_n^{-(12-2m-n)}$. A comprehensive study by Crea et al investigated the formation constants of complexes formed between calcium and phytate at molar ratios of 1:1, 3:1 and 4:1 at IS between 0.1 and 0.75 M (NaCl) using glass electrode potentiometry.[329] These conditions are comparable to the conditions used in the present study. The study by Crea et al[329] reported formation constants for 10 $Ca_mPhyH_n^{-(12-2m-n)}$ complexes of which 3 were mononuclear ($CaPhyH_5^{-5}$, $CaPhyH_4^{-6}$, $CaPhyH_3^{-7}$), 3 were binuclear ($Ca_2PhyH_5^{-3}$, $Ca_2PhyH_4^{-4}$, $Ca_2PhyH_3^{-5}$) and 4 were trinuclear ($Ca_3PhyH_2^{-4}$, $Ca_3PhyH_3^{-3}$, $Ca_3PhyH_4^{-2}$, $Ca_3PhyH_5^{-1}$).[329] In the present study, formation constants of only 7 species were identified, 4 of which were in common with the former study as shown above (Table 2.3). The constants of the present study were however several orders of magnitude higher than those of Crea et al.[329] The reason for the discrepancy between results of the present study and those of Crea et al[329] is due to differences in the hypothesized model for the Ca-phytate equilibrium system. The model of Crea et al[329] consisted of trinuclear species in addition to mono- and binuclear species whereas trinuclear species were not included in the model of the present study. This would undoubtedly affect the stability constants of other species.

As a check of the credibility of the results obtained in the present study, the model of the equilibrium system and the stability constants of Crea et al[329] was used in HyperQuad[349] as a hypothetical model for the experimental data obtained in the present study. No convergence was obtained between the model of Crea et al[329] and the experimental data obtained in the present study as shown below in Figure 2.11. This can be compared to the excellent agreement between the theoretical model used in the present study and the experimental data. The hypothesized model for the equilibrium system in the present study is therefore accurate.

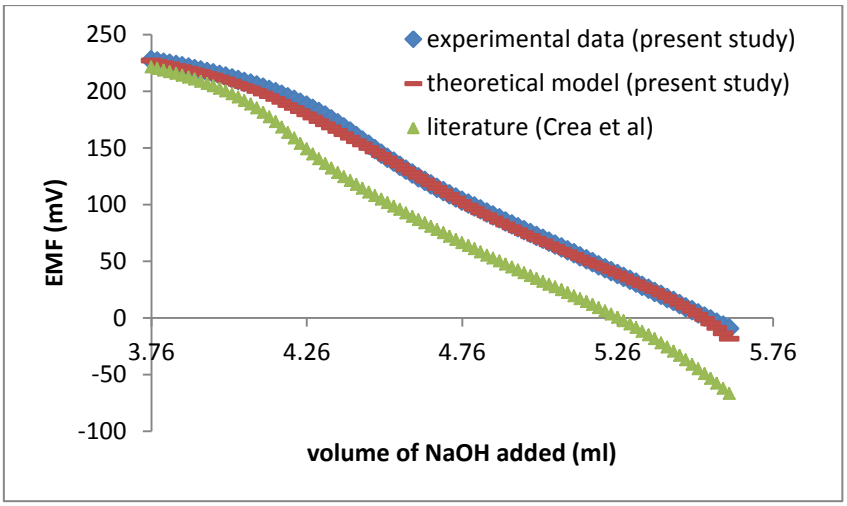


Figure 2.11 A comparison of the equilibrium model used in the present study to those of Crea et al.[329] Ca: 2 mmol.L^{-1}, Phy: 2 mmol.L^{1}; IS = 0.15 M, t = 25 °C

In another study by Torres et al, formation constants of $Ca_mPhyH_n^{-(12-2m-n)}$ complexes were investigated in $NaClO_4$ at Ca:Phy ratios of 1:1 and 1:5 and a temperature of 37 °C.[328] Of course in this study only mononuclear species were identified ($CaPhyH_6^{-4}$, $CaPhyH_5^{-5}$, $CaPhyH_4^{-6}$) due to the presence of an excess of ligand as opposed to an excess of metal. The magnitude of these stability constants is comparable to those of the present study.

Conclusion

Comparison of the formation constants for $Ca_mPhyH_n^{-(12-2m-n)}$ complexes obtained in the present study to those of Crea et al[329] and Torres et al[328] highlights the challenge in obtaining accurate stability constants of alkaline-earth metal- phytate complexes as well as the necessity to experimentally determine these constants under conditions similar to those in urine.

It should be borne in mind that the use of accurately determined formation constants in JESS is essential for the reliable determination of chemical speciation in urine or any other system. As mentioned earlier, an indication of the reliability of formation constants obtained using HyperQuad[349] is based on the σ value (an estimation of the fit between the theoretical model and experimental data) as well as the standard deviation of the respective log K values. In the present study a σ value of 14 and 12 was obtained for the respective models used in the present study. In an ideal case σ would be equal to 1 if assumptions 1-5 have been fulfilled as mentioned on page 33. The values obtained in the present study, although > 1, were the smallest values obtained for this parameter during the "model selection" process. Furthermore, no ill-defined formation constants were obtained as the standard deviations of

the log K values obtained in the present study for $H_nPhy^{(12-n)-}$ and $Ca_mPhyH_n^{-(12-2m-n)}$ complexes were within acceptable limits i.e. less than 33 % of its corresponding log K and no negative log K values were obtained. Both of these parameters are of course affected by the accuracy of instrumentation used as well as the control of CO_2 contamination. These factors were minimized as stated above. In addition to this, the protonation constants of phytate obtained in the present study were comparable to literature values in which the experimental conditions were similar. All of the above lends confidence to the results obtained in the present study.

The formation constants of both $H_nPhy^{(12-n)-}$ and $Ca_mPhyH_n^{-(12-2m-n)}$ complexes obtained in the present study were therefore used in the JESS program to model Ca-phytate speciation in urine. This is reported in the next chapter.

Chapter 3 : Effect of phytate on chemical speciation and supersaturation of stone-forming salts in urine of South African black and white subjects: theoretical modelling

Introduction

"Chemical speciation of an element" refers to its distribution amongst defined chemical species in a particular system.[341] The term "chemical species" is defined as a specific form of an element: isotopic composition, electronic or oxidation state, or complex or molecular structure.[341] Chemical speciation is of importance in a wide range of applications in analytical chemistry, geochemistry, toxicology and environmental chemistry.[356, 357] In environmental studies chemical speciation is essential for determining the biological availability and toxicity of trace constituents as well as the geochemical behaviour of chemical species.[356] As mentioned in the previous chapter chemical speciation has also provided insight in the field of urolithiasis. In this context chemical speciation is useful for the determination of the speciation of calcium and other elements which in turn contribute to the supersaturation of stone-forming salts. For example, the distribution of total calcium in urine can be at different concentrations of aqueous or solid CaOx, CaP, calcium- citrate and other calcium-containing complexes. If most of the calcium is complexed to citrate (or some other anionic ligand in urine), then less of it will be available for binding to free oxalate (or phosphate). As a consequence, the supersaturation (SS) of CaOx and CaP would be lower, thereby decreasing the risk of crystallisation of these substances in urine.

Both chemical speciation and the concomitant RS of urine can be assessed using the software program JESS.[321, 322] As mentioned in the previous chapter JESS contains a substantial database of thermodynamic constants for multiple binary, tertiary and quaternary complexes which may form at equilibrium in urine. This data base has now been expanded by inclusion of the thermodynamic binding constants for phytate protonation and calcium-phytate complexes, which were determined experimentally as part of the present PhD project and which were described in the previous chapter.

As mentioned in Chapter 1 of this thesis, the molecular structure of phytate contains 6 phosphate groups and has a high negative charge at physiological pH. Therefore, in principle, phytate is able to complex calcium in urine thereby contributing to the modulation of CaOx crystallisation. The extent to which this occurs depends on the chemical composition of each individual urine sample. Since the incidence of stone formation is different in South Africa's black and white population groups, the influence of phytate may differ in the two groups.

The hypotheses that phytate is able to exert thermodynamic effects on CaOx and CaP crystallisation in urine and that such effects may be different in black and white South Africans were tested in the present project. The study is described in this chapter.

Methods

Urine data and the theoretical model

Individual baseline urine compositions of healthy South African black (n = 18) and white (n =16) males were used as input data for the JESS theoretical model. The urinary parameters which were used for each sample are listed in Table 3.1. Values of each urine parameter for each urine sample are included in Appendix 3.1. These urine parameters were determined as described in Chapter 6. Equilibrium constants and hence species formation are dependent on temperature and ionic strength (IS).[327, 358] Accordingly, the temperature for the model was set at 37°C (physiological temperature). IS was calculated by JESS for each urine sample.

Table 3.1 Urinary parameters used in the JESS theoretical model.

Urinary parameters	
pH	Citrate (mol/L)
Calcium (mol/L)	Phosphate (mol/L)
Magnesium (mol/L)	Chloride (mol/L)
Sodium (mol/L)	Urate (mol/L)
Potassium (mol/L)	Phytate (mol/L)
Oxalate (mol/L)	

Modelling urinary saturation and speciation

The physiological concentration of phytate in urine is between 0.5- 3 mg/l corresponding to 0.76 – 4.45 µM.[259] The effect of three physiological concentrations of phytate within in this range, on the SS of stone-forming salts was modelled in each urine sample. These concentrations were as follows: 0.76 µM, 2.27 µM and 4.45 µM. In addition, the effect of two non-physiological concentrations of phytate i.e. 30 µM and 1.50 mM phytate was also studied. These concentrations were chosen as they had been used in an *in vitro* study by Saw et al which will be discussed later. [285]

The RS of the following stone-forming salts were calculated for each urine sample at each modelled concentration of phytate: COM, COD, COT, CaOx, brushite, hydroxylapatite (HAP), octacalcium phosphate (OCP), tribasic calcium phosphate (tCaP), uric acid and sodium urate (Na-urate). The supersaturation of the latter two urinary salts were determined because of potential effects due to possible changes in IS (this will be discussed later). This was

followed by determining the speciation of calcium, phytate, oxalate, magnesium, phosphate and citrate in each urine sample. JESS has a maximum output of 30 species per element. However, only the 15 most predominant species per element were determined in the present thesis.

Statistical analysis

Statistical analyses were performed using GraphPad InStat3 for intra- and inter-group comparisons of urinary saturation and speciation of elements. Mean values were compared using paired t-tests for intra-group comparisons and unpaired t-tests for inter-group comparisons. Repeated measures analysis of variance (ANOVA) followed by a post-hoc Dunnett test was performed for multiple comparisons (3 or more groups) where applicable. A p-value < 0.05 was considered as statistically significant.

Results

Urinary supersaturation

The urinary RS of stone-forming salts at each concentration of phytate are shown in Table 3.2 and Table 3.3 for black and white subjects; respectively. Mean values and the standard error of the mean (SE) are reported. Raw data are reported in Appendix 3.2. Repeated ANOVA followed by a post-hoc Dunnett test in which comparisons were made to the lowest physiological concentration i.e. 0.76 µM was done. In both groups there were no statistical differences in the RS values of all salts at phytate concentrations of 2.27 µM, 4.45 µM and 30 µM relative to the lowest concentration of 0.76 µM. However, RS values of all salts were significantly lower at the highest phytate concentration of 1.50 mM in both groups.

Table 3.2 Effect of phytate on the RS of urinary salts in black subjects (n =18).

	Mean ± SE					p value			
RS	**0.76 µM phytate (A)**	**2.27 µM phytate (B)**	**4.45 µM phytate (C)**	**30 µM phyate (D)**	**1.50 mM phytate (E)**	**A vs B**	**A vs C**	**A vs D**	**A vs E**
COM	3.92 ± 0.72	3.91 ± 0.72	3.90 ± 0.72	3.79 ± 0.71	2.10 ± 0.43	>0.05	>0.05	>0.05	<0.01*
COD	1.47 ± 0.27	1.47 ± 0.27	1.46 ± 0.27	1.42 ± 0.27	0.79 ± 0.16	>0.05	>0.05	>0.05	<0.01*
COT	2.10 ± 0.37	2.00 ± 0.37	1.99 ± 0.37	1.94 ± 0.36	1.08 ± 0.22	>0.05	>0.05	>0.05	<0.01*
CaOx	4.45 ± 0.85	4.53 ± 0.83	4.52 ± 0.83	4.40 ± 0.82	2.44 ± 0.49	>0.05	>0.05	>0.05	<0.01*
Brushite	1.66 ± 0.18	1.66 ± 0.18	1.65 ± 0.18	1.60 ± 0.18	0.96 ± 0.17	>0.05	>0.05	>0.05	<0.05*
HAP	3.83E+11 ± 2.53E+11	3.74E+11 ± 2.47E+11	3.61E+11 ± 2.38E+11	2.35E+11 ± 1.52E+11	5.99E+09 ± 3.80E+09	>0.05	>0.05	>0.05	<0.05*
OCP	4109 ± 2525	4043 ± 3943	3943 ± 2415	2958 ± 1772	250 ± 150	>0.05	>0.05	>0.05	<0.05*
tCaP	283 ± 158	280 ± 156	275 ± 153	217 ± 119	28 ± 15	>0.05	>0.05	>0.05	<0.05*
Uric acid	1.09 ± 0.29	1.09 ± 0.29	1.09 ± 0.29	1.09 ± 0.29	1.04 ± 0.28	>0.05	>0.05	>0.05	<0.01*
Na-Urate	5.19 ± 0.91	5.19 ± 0.91	5.19 ± 0.91	5.18 ± 0.91	4.91 ± 0.88	>0.05	>0.05	>0.05	<0.01*

*Indicates statistical significance.

Table 3.3 Effect of phytate on the RS of urinary salts in white subjects (n= 16).

	Mean ± SE					p-value			
RS	**0.76 µM Phy (A)**	**2.27 µM Phy (B)**	**4.45 µM Phy (C)**	**30 µM Phy (D)**	**1.50 mM Phy (E)**	**A vs B**	**A vs C**	**A vs D**	**A vs E**
COM	2.82 ± 0.53	2.82 ± 0.53	2.81 ± 0.53	2.75 ± 0.52	1.62 ± 0.28	>0.05	>0.05	>0.05	<0.01*
COD	1.06 ± 0.20	1.06 ± 0.20	1.06 ± 0.20	1.03 ± 0.20	0.61 ± 0.11	>0.05	>0.05	>0.05	<0.01*
COT	1.50 ± 0.27	1.50 ± 0.27	1.44 ± 0.27	1.41 ± 0.27	0.83 ± 0.14	>0.05	>0.05	>0.05	<0.01*
CaOx	3.28 ± 0.62	3.21 ± 0.63	3.26 ± 0.61	3.19 ± 0.61	1.87 ± 0.33	>0.05	>0.05	>0.05	<0.01*
Brushite	1.41 ± 0.19	1.41 ± 0.19	1.41 ± 0.19	1.38 ± 0.19	0.87 ± 0.12	>0.05	>0.05	>0.05	<0.01*
HAP	1.57E+07 ± 8.69E+06	1.55E+07 ± 8.57E+06	1.53E+07 ± 8.44E+06	1.31E+07 ± 7.10E+06	2.89E+06 ± 1.41E+06	>0.05	>0.05	>0.05	<0.05*
OCP	62 ± 25	62 ± 25	63 ± 26	58 ± 24	16 ± 8	>0.05	>0.05	>0.05	<0.01*
tCaP	3.20 ± 1.01	3.19 ± 1.01	3.17 ± 1.01	2.64 ± 0.85	1.15 ± 0.41	>0.05	>0.05	>0.05	<0.01*
Uric acid	1.54 ± 0.59	1.54 ± 0.59	1.54 ± 0.59	1.60 ± 0.59	3.48 ± 2.00	>0.05	>0.05	>0.05	<0.01*
Na-Urate	6.42 ± 1.34	6.42 ± 1.39	6.42 ± 1.39	6.42 ± 1.39	6.12 ± 1.34	>0.05	>0.05	>0.05	<0.01*

*Indicates statistical significance.

Inter-group comparisons of the RS of stone-forming salts at each phytate concentration were also determined. No statistically significant differences were observed between the two groups. p values for each comparison are reported in Appendix 3.3.

Speciation

Ionized calcium (Ca^{2+})

Since the hypothesis being tested here is that phytate binds with calcium to reduce the concentration of Ca^{2+}, the concentration of this species at each concentration of phytate was compared. Results are shown in Figure 3.1 and Figure 3.2 for black and white subjects, respectively. In both groups, no significant differences in the concentration of Ca^{2+} were observed at physiological phytate concentrations or at the hypothetically elevated concentration of 30 µM. However, the concentration of Ca^{2+} was significantly lower at a concentration of 1.50 mM phytate relative to all other concentrations of phytate ($p < 0.01$).

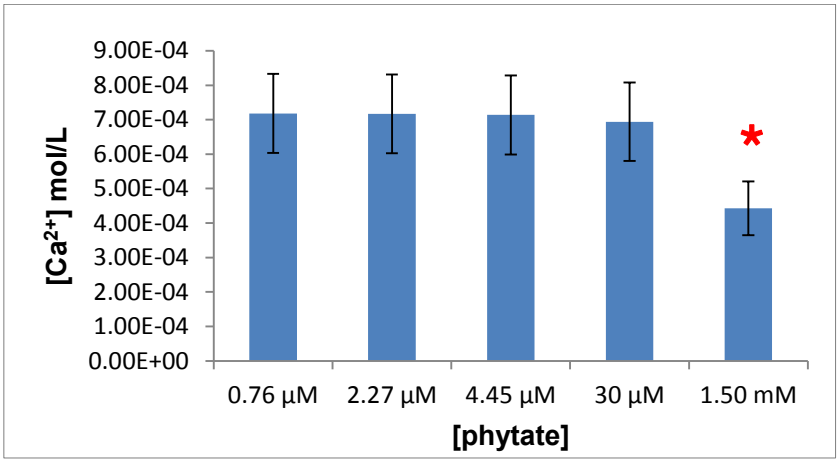


Figure 3.1 Calculated concentration of ionized calcium in urine of black subjects at different concentrations of phytate. Error bars indicate standard error of the mean. *Significantly different to all other concentrations ($p < 0.01$).

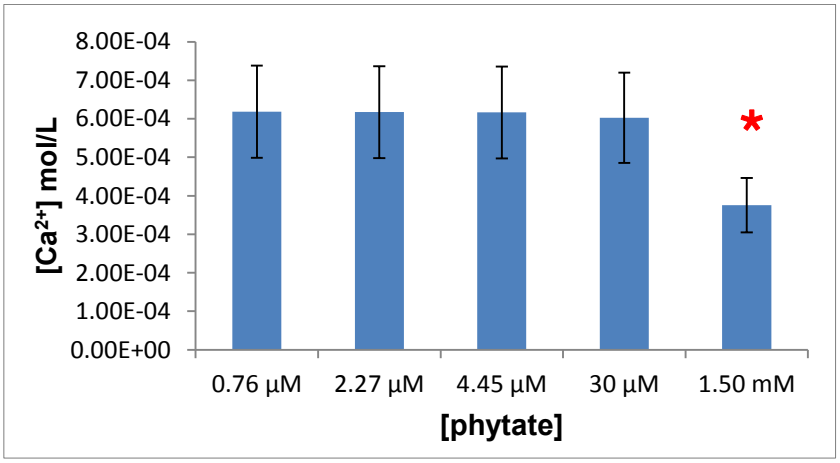


Figure 3.2 Calculated concentration of ionized calcium in urine of white subjects at different concentrations of phytate. Error bars indicated standard error of the mean. *Significantly different to all other concentrations. ($p < 0.01$)

In order to gain insight into the effects of phytate on urinary speciation at a phytate concentration of 1.50 mM, comparisons were made with the speciation at the lowest physiological concentration of phytate (0.76 μM). Mean values and the standard error of the mean (SE) are shown in bar-graphs, while actual values are given in tables. All raw data are reported in Appendix 3.2.

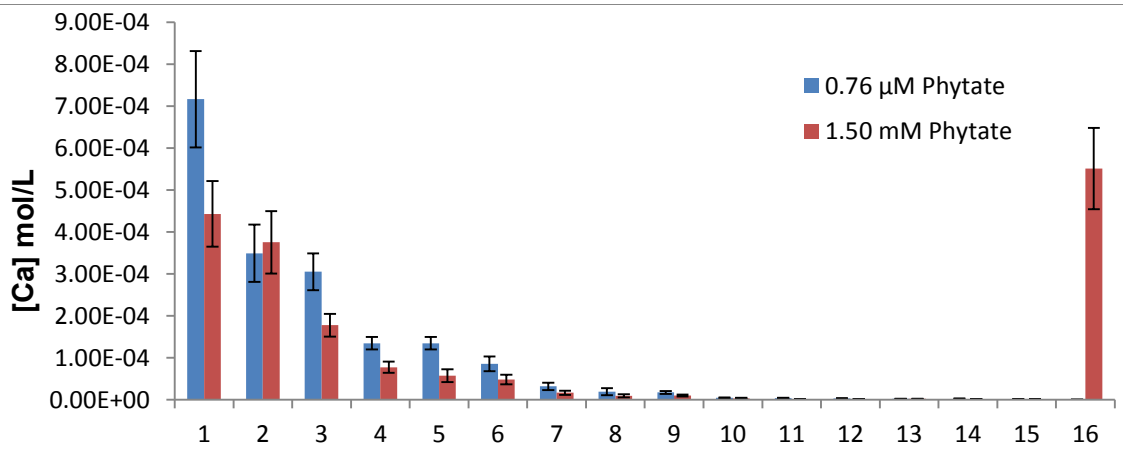
It should be noted that in the speciation bar graph representations of speciation (as well as in corresponding tables with actual values) in the paragraphs that follow, the concentrations which are indicated are those of the element (calcium and magnesium) or ligand (oxalate, citrate, phosphate and phytate) in each species.

Calcium speciation

The mean concentration of calcium in each of the calcium species at a concentration of 0.76 μM and 1.50 mM phytate is shown in Figure 3.3 and Figure 3.4 for the black and white group, respectively, while actual values for each species for both groups are given in Table 3.4. Individual values of each species for each urine sample are reported in Appendix 3.2.

As has been demonstrated above, the concentration of Ca^{2+} is significantly decreased when the concentration of phytate is raised to 1.50 mM in urine of both black and white subjects. This effect is accounted for by the highly significant increase in the formation of Ca_2HPHy^{-7} (species number 16 in Figure 3.3 and Figure 3.4) which utilizes a larger percentage of the available Ca^{2+}. The significant decrease in the concentration of Ca^{2+} is accompanied by a concomitantly significant decrease in the formation of all other calcium complexes in both groups except for that of $CaCitPO_4^{-4}$ (species number 2 in Figure 3.3 and Figure 3.4) which increased significantly in both groups. This latter observation is surprising and will be discussed later in the discussion.

Intergroup comparisons of the calcium speciation was also determined. No statistical difference was observed. p values are reported in Appendix 3.3.

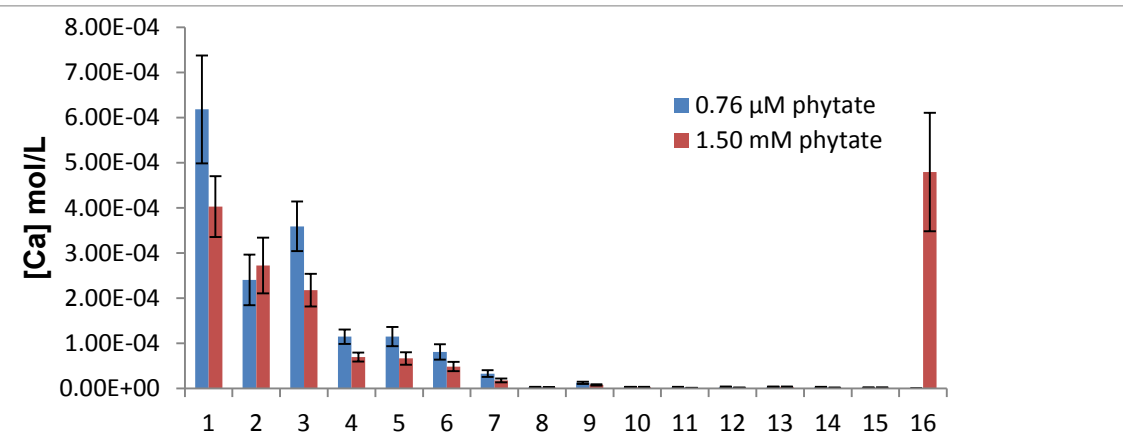


1. Ca^{+2} **2.** $CaCitPO_4^{-4}$ **3.** $CaCit^{-1}$ **4.** $CaHPO_4$ **5.** $CaH_3(PO_4)_2^{-1}$ **6.** $CaCl^{+1}$

7. $CaH_2PO_4^{+1}$ **8.** $CaPO_4^{-1}$ **9.** CaOx **10.** $CaH_2(PO_4)_2^{-2}$ **11.** Ca_2Ox^{+2} **12.** CaHCit

13. $CaCit_2^{-4}$ **14.** $CaH_2Cit_2^{-2}$ **15.** $CaHCitPO_4^{-3}$ **16.** Ca_2HPhy^{-7}

Figure 3.3 Calcium speciation in urine of black subjects (n= 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. Ca^{+2} **2.** $CaCitPO_4^{-4}$ **3.** $CaCit^{-1}$ **4.** $CaHPO_4$ **5.** $CaH_3(PO_4)_2^{-1}$ **6.** $CaCl^{+1}$

7. $CaH_2PO_4^{+1}$ **8.** $CaPO_4^{-1}$ **9.** CaOx **10.** $CaH_2(PO_4)_2^{-2}$ **11.** Ca_2Ox^{+2} **12.** CaHCit

13. $CaCit_2^{-4}$ **14.** $CaH_2Cit_2^{-2}$ **15.** $CaHCitPO_4^{-3}$ **16.** Ca_2HPhy^{-7}

Figure 3.4 Calcium speciation in urine of white subjects (n= 16) in the presence of 0.76 µM and 1.50 mM phytate.

Table 3.4 Calcium speciation‡ in urine of black and white subjects at a concentration of 0.76 µM and 1.50 mM phytate.

Species	Black subjects (n= 18)		p- value	White subjects (n= 16)		p value
	[Ca] ($x10^{-6}$ mol/L) Mean ± SE			[Ca] ($x10^{-6}$ mol/L) Mean ± SE		
	0.76 µM phytate	1.50 mM phytate		0.76 µM phytate	1.50 mM phytate	
Ca^{+2}	716 ± 115	443 ± 78	0.0001*	618 ± 119	403 ± 68	0.009*
$CaCitPO_4^{-4}$	349 ± 68	375 ± 74	0.02*	241 ± 56	272 ± 62	0.0006*
$CaCit^{-1}$	305 ± 44	178 ± 27	<0.0001*	359 ± 55	218 ± 36	<0.0001*
$CaHPO_4$	135 ± 15	78 ± 13	<0.0001*	115 ± 16	69 ± 10	0.0005*
$CaH_3(PO_4)_2^{-1}$	135 ± 15	58 ± 15	<0.0001*	115 ± 21	67 ± 14	0.0003*
$CaCl^{+1}$	86 ± 17	48 ± 11	<0.0001*	81 ± 17	49 ± 10	0.003*
$CaH_2PO_4^{+1}$	32 ± 9	17 ± 5	0.002*	33 ± 8	18 ± 4	0.002*
$CaPO_4^{-1}$	19 ± 8	10 ± 4	0.06	3.26 ± 0.80	2.33 ± 0.60	0.004*
CaOx	18 ± 3	10 ± 2	<0.0001*	13 ± 2	7.76 ± 1.39	0.002*
$CaH_2(PO_4)_2^{-2}$	4.61 ± 1.06	3.73 ± 1.05	0.0002*	3.38 ± 0.63	2.87 ± 0.63	0.03*
Ca_2Ox^{+2}	3.87 ± 1.26	1.45 ± 0.50	0.01*	2.59 ± 0.92	0.95 ± 0.35	0.03*
CaHCit	3.02 ± 0.92	1.39 ± 0.46	0.004*	3.39 ± 0.80	1.58 ± 0.41	0.0008*
$CaCit_2^{-4}$	2.39 ± 0.46	2.39 ± 0.48	0.85	3.65 ± 0.77	3.79 ± 0.85	0.16
$CaH_2Cit_2^{-2}$	2.24 ± 0.90	1.31 ± 0.57	0.014*	2.81 ± 0.83	1.59 ± 0.53	0.002*
$CaHCitPO_4^{-3}$	1.80 ± 0.33	1.55 ± 0.33	<0.0001*	1.96 ± 0.25	1.74 ± 0.26	0.004*
Ca_2HPhy^{-7}	1.19 ± 0.86	551 ± 97	<0.0001*	0.94 ± 0.12	479 ± 131	0.002*

‡Expressed as the concentration of calcium in each calcium-species. *Indicates statistical significance.

Phytate speciation

The mean concentration of phytate in each phytate-species is shown in Figure 3.5 and Figure 3.6 for the black and white group, respectively, while actual values for each species for both groups are given in Table 3.5. Individual values of each species for each urine sample are reported in Appendix 3.2. These results confirm a significant increase in the formation of Ca_2HPhy^{-7} at a concentration of 1.50 mM phytate in both groups. However, not all of the phytate binds with calcium to form Ca_2HPhy^{-7} at the latter concentration of phytate; instead a larger amount of phytate exists as the mono-protonated $HPhy^{-11}$. The percentage distribution of phytate in urine in the presence of 0.76 µM and 1.50 mM phytate is shown in Figure 3.7 and Figure 3.8 for both groups. Interestingly, it is seen that at the lower concentration of phytate the species Ca_2HPhy^{-7} dominates while at the higher concentration of phytate, $HPhy^{-11}$ is the major species. This will be discussed later.

Inter-group comparisons of phytate speciation showed no statistical significance between the two groups. p values are reported in Appendix 3.3.

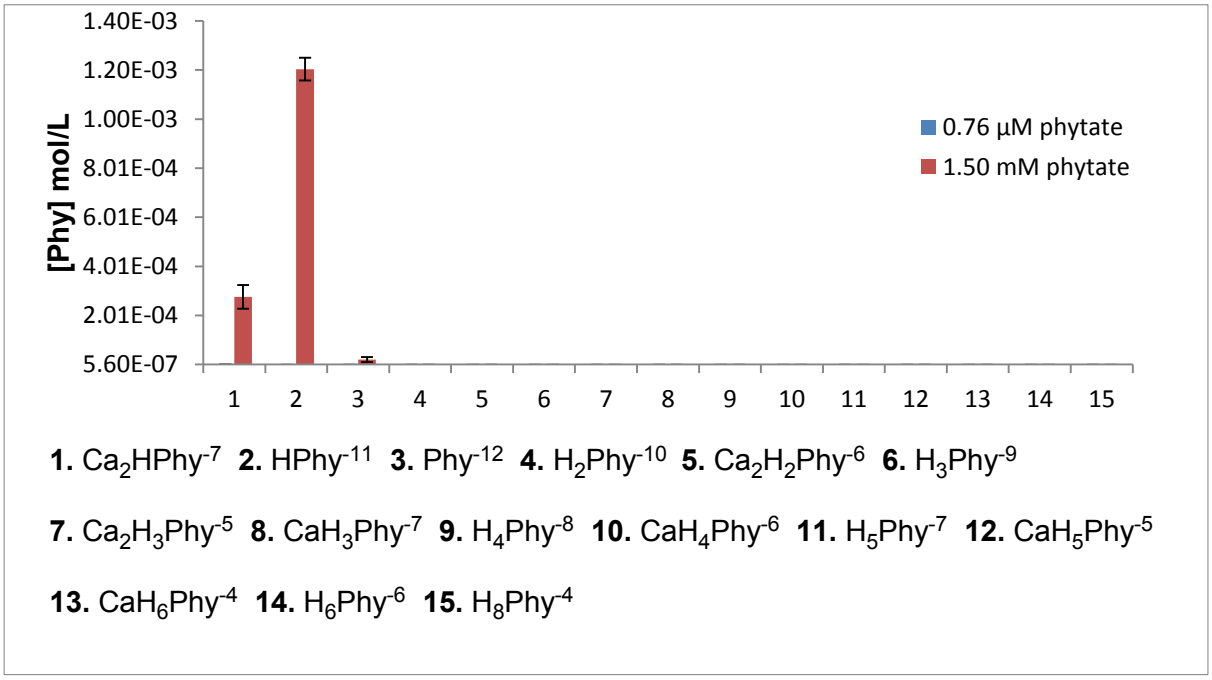
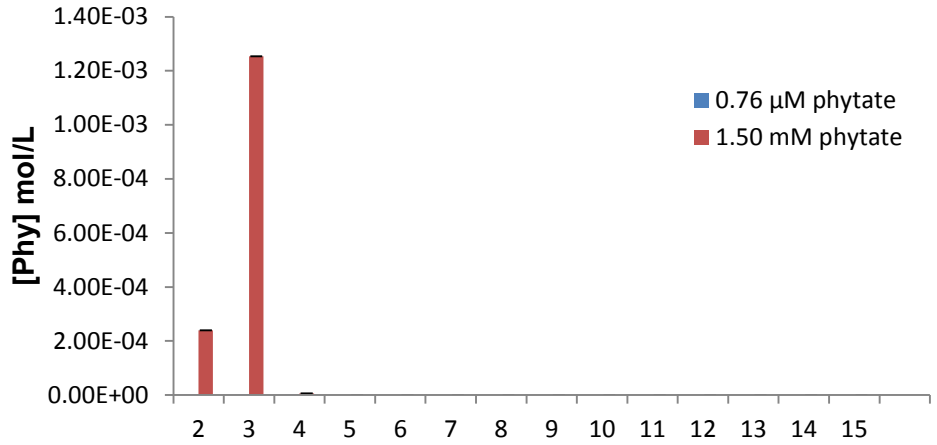


Figure 3.5 Phytate speciation in urine of black subjects (n= 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. Ca_2HPhy^{-7} **2.** $HPhy^{-11}$ **3.** Phy^{-12} **4.** H_2Phy^{-10} **5.** $Ca_2H_2Phy^{-6}$ **6.** H_3Phy^{-9}

7. $Ca_2H_3Phy^{-5}$ **8.** CaH_3Phy^{-7} **9.** H_4Phy^{-8} **10.** CaH_4Phy^{-6} **11.** H_5Phy^{-7} **12.** CaH_5Phy^{-5}

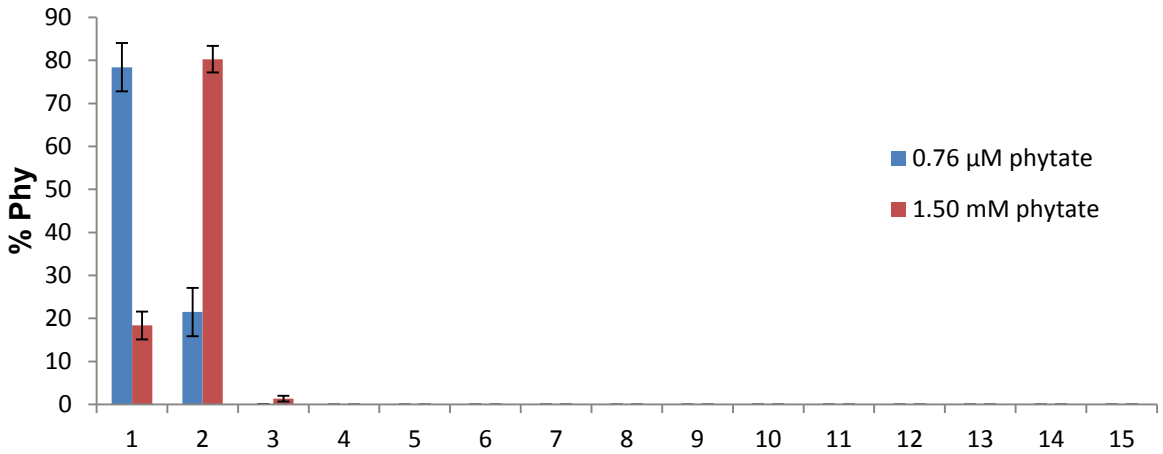
13. CaH_6Phy^{-4} **14.** H_6Phy^{-6} **15.** H_8Phy^{-4}

Figure 3.6 Phytate speciation in urine of white subjects (n= 16) in the presence of 0.76 µM and 1.50 mM phytate.

Table 3.5 Phytate speciation‡ in the urine of black and white subjects in the presence of 0.76 µM and 1.50 mM phytate.

Species	Black subjects (n= 18)		P value	White subjects (n =16)		p value
	[Phytate] x (10^{-7} mol/L) Mean ± SE			[Phytate] x (10^{-7} mol/L) Mean ± SE		
	0.76 µM phytate	1.50 mM phytate		0.76 µM phytate	1.50 mM phytate	
Ca_2HPhy^{-7}	5.93 ±0.45	2756 ± 484	< 0.0001*	4.72 ± 0.62	2396 ± 656	0.002*
$HPhy^{-11}$	1.63 ± 0.43	12040 ± 461	< 0.0001*	2.98 ± 0.71	12540 ± 645	<0.0001*
Phy^{-12}	0	202 ± 11	<0.0001*	0	62 ±27	<0.0001*
H_2Phy^{-10}	0	0	n.d	0	0	n.d
$Ca_2H_2Phy^{-6}$	0	0	n.d	0	0	n.d
H_3Phy^{-9}	0	0	n.d	0	0	n.d
$Ca_2H_3Phy^{-5}$	0	0	n.d	0	0	n.d
CaH_3Phy^{-7}	0	0	n.d	0	0	n.d
H_4Phy^{-8}	0	0	n.d	0	0	n.d
CaH_4Phy^{-6}	0	0	n.d	0	0	n.d
H_5Phy^{-7}	0	0	n.d	0	0	n.d
CaH_5Phy^{-5}	0	0	n.d	0	0	n.d
CaH_6Phy^{-4}	0	0	n.d	0	0	n.d
H_6Phy^{-6}	0	0	n.d	0	0	n.d
H_8Phy^{-4}	0	0	n.d	0	0	n.d

‡Expressed as the concentration of phytate in each phytate-species. *Indicates statistical significance. “n.d” = not determined as the concentration of these species are negligible.

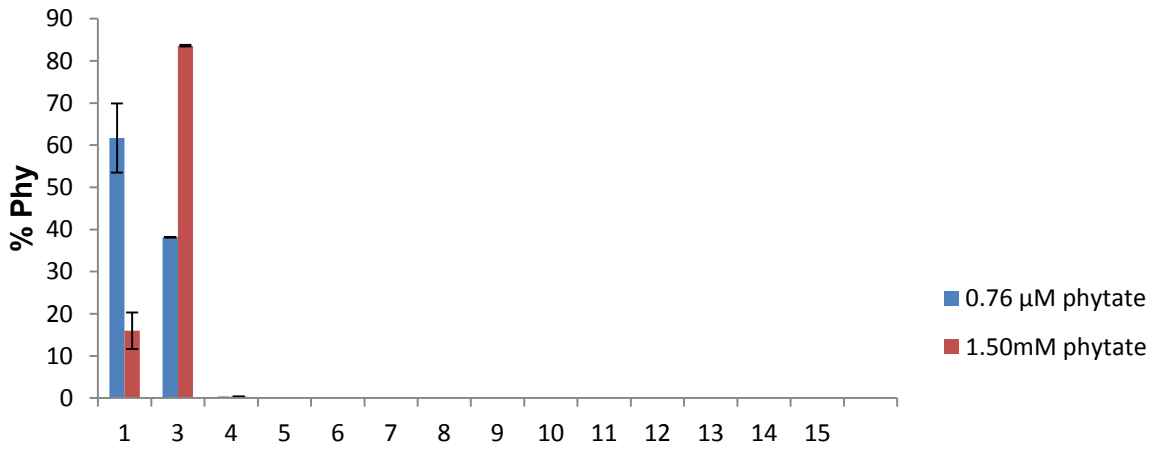


1. Ca_2HPhy^{-7} **2.** $HPhy^{-11}$ **3.** Phy^{-12} **4.** H_2Phy^{-10} **5.** $Ca_2H_2Phy^{-6}$ **6.** H_3Phy^{-9}

7. $Ca_2H_3Phy^{-5}$ **8.** CaH_3Phy^{-7} **9.** H_4Phy^{-8} **10.** CaH_4Phy^{-6} **11.** H_5Phy^{-7} **12.** CaH_5Phy^{-5}

13. CaH_6Phy^{-4} **14.** H_6Phy^{-6} **15.** H_8Phy^{-4}

Figure 3.7 Percentage distribution of phytate in urine of black subjects (n = 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. Ca_2HPhy^{-7} **2.** $HPhy^{-11}$ **3.** Phy^{-12} **4.** H_2Phy^{-10} **5.** $Ca_2H_2Phy^{-6}$ **6.** H_3Phy^{-9}

7. $Ca_2H_3Phy^{-5}$ **8.** CaH_3Phy^{-7} **9.** H_4Phy^{-8} **10.** CaH_4Phy^{-6} **11.** H_5Phy^{-7} **12.** CaH_5Phy^{-5}

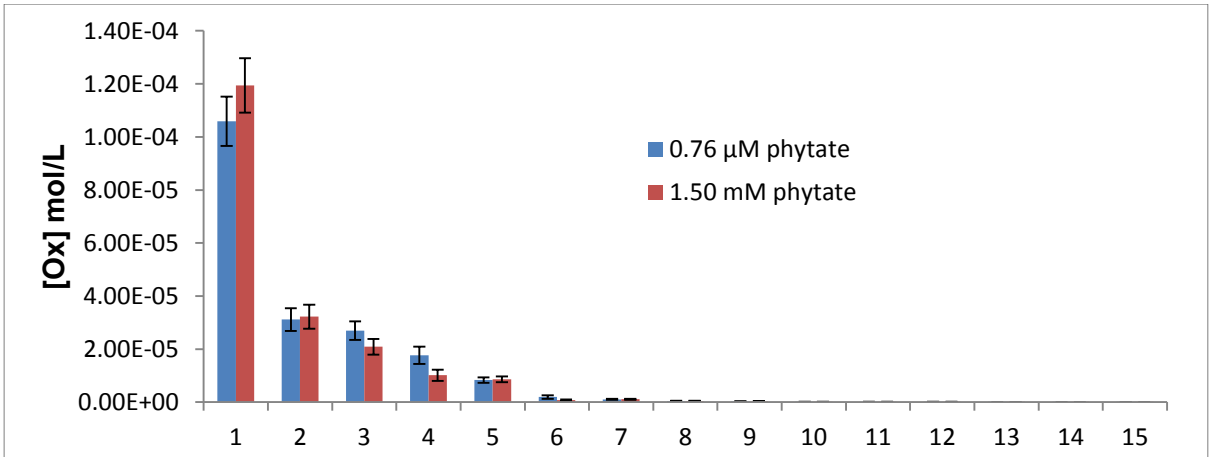
13. CaH_6Phy^{-4} **14.** H_6Phy^{-6} **15.** H_8Phy^{-4}

Figure 3.8 Percentage distribution of phytate in urine of white subjects (n= 16) in the presence of 0.76 µM and 1.50 mM phytate.

Oxalate speciation

The mean concentration of oxalate in each oxalate-species is shown in Figure 3.9 and Figure 3.10 for the black and white groups, respectively, while actual values for each species are reported in Table 3.6. Individual values of each species for each urine sample are reported in Appendix 3.2. Interestingly, the concentration of ionized oxalate (Ox^{-2}) increased significantly at the elevated phytate concentration in both groups. This effect can be attributed to concomitant decreases in the formation of the complexes MgOx and CaOx (species number 3 and 4 in Figure 3.9 and Figure 3.10) in both groups of subjects which thereby release unbound Ox^{-2} and raise its concentration. The decrease in the concentration of CaOx is due to the decrease in the concentration of Ca^{2+} caused by complexation of phytate (Figure 3.3 and Figure 3.4) while the decrease in the formation of MgOx is surprising and will be discussed later.

Inter-group comparisons of the distribution of oxalate showed no statistical significant difference between the two groups. p values are reported in Appendix 3.3.

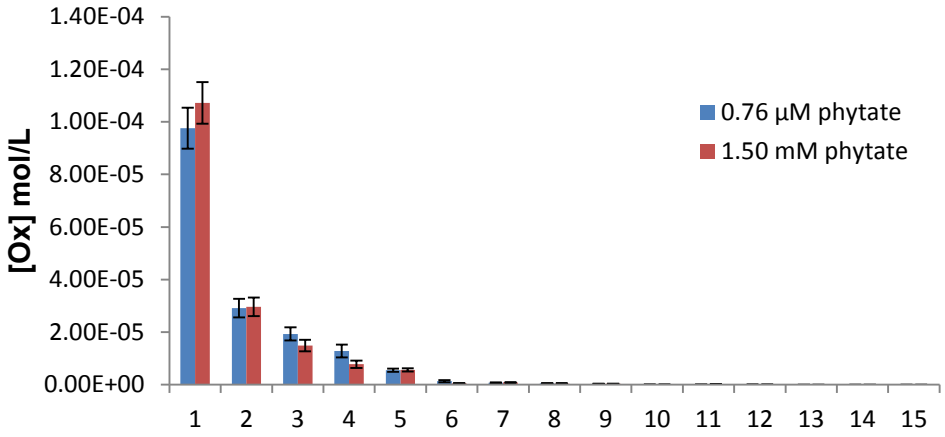


1. Ox^{-2} **2.** $NaOx^{-1}$ **3.** MgOx **4.** CaOx **5.** KOx^{-1} **6.** Ca_2Ox^{+2} **7.** $MgHOxPO_4^{-2}$

8. HOx^{-1} **9.** $MgOxCit^{-3}$ **10.** $CaHOxPO_4^{-2}$ **11.** Mg_2Ox^{+2} **12.** $MgOx_2^{-2}$ **13.** $CaOx_2^{-2}$

14. $CaOxCit^{-3}$ **15.** $CaHOxCit^{-2}$

Figure 3.9 Oxalate speciation in urine of black subjects (n = 18) in the presence of 0.76 µM and 1.5mM phytate.



1. Ox^{-2} **2.** $NaOx^{-1}$ **3.** MgOx **4.** CaOx **5.** KOx^{-1} **6.** Ca_2Ox^{+2} **7.** $MgHOxPO_4^{-2}$

8. HOx^{-1} **9.** $MgOxCit^{-3}$ **10.** $CaHOxPO_4^{-2}$ **11.** Mg_2Ox^{+2} **12.** $MgOx_2^{-2}$ **13.** $CaOx_2^{-2}$

14. $CaOxCit^{-3}$ **15.** $CaHOxCit^{-2}$

Figure 3.10 Oxalate speciation in urine of white subjects (n = 16) in the presence of 0.76 µM and 1.50 mM phyatate.

Table 3.6 Oxalate speciation‡ in urine of black and white subjects in the presence of 0.76 µM and 1.5mM phytate.

Species	Black (n= 18)			White (n= 16)		
	[Ox] (x 10^{-8} mol/L) Mean ± SE		p value	[Ox] (x 10^{-8} mol/L) Mean ± SE		p value
	0.76 µM phytate	1.50 mM phytate		0.76 µM phytate	1.50 mM phytate	
Ox^{-2}	10590 ± 926	11940 ± 1022	<0.0001*	9757 ± 778	10720 ± 796	<0.0001*
$NaOx^{-1}$	3119 ± 429	3223 ± 452	0.04*	2912 ± 348	2961 ± 349	0.16
MgOx	2699 ± 351	2087 ± 298	0.0001*	1930 ± 253	1490 ± 219	<0.0001*
CaOx	1768 ± 329	1016 ± 210	<0.0001*	1279 ± 239	776 ± 139	0.002*
KOx^{-1}	833 ± 104	862 ± 109	0.02*	549 ± 61	562 ± 64	0.11
Ca_2Ox^{+2}	193 ± 63	72 ± 25	0.01*	130 ± 46	47 ± 17	0.03*
$MgHOxPO_4^{-2}$	104 ± 20	110 ± 21	0.007*	75 ± 9	81 ± 10	0.0005*
HOx^{-1}	46 ± 13	46 ± 13	0.99	43 ± 7	41 ± 7	0.08
$MgOxCit^{-3}$	24 ± 4	31 ± 6	0.004*	28 ± 3	33 ± 4	0.008*
$CaHOxPO_4^{-2}$	18 ± 4	13 ± 3	<0.0001*	13 ± 2	10 ± 2	0.002*
Mg_2Ox^{+2}	15 ± 3	12 ± 3	<0.0001*	10 ± 2	7.39 ± 2.03	0.0001*
$MgOx_2^{-2}$	13 ± 3	14 ± 3	0.31	7.97 ± 1.09	7.99 ± 1.19	0.92
$CaOx_2^{-2}$	5.87 ± 1.67	3.95 ± 1.20	0.003*	3.41 ± 0.70	2.39 ± 0.52	0.002*
$CaOxCit^{-3}$	4.50 ± 0.95	3.48 ± 0.77	0.0002*	4.46 ± 0.90	3.60 ± 0.80	<0.0001*
$CaHOxCit^{-2}$	4.33 ± 1.65	2.80 ± 1.12	0.01*	3.89 ± 1.03	2.59 ± 0.78	0.0006*

‡Expressed as the concentration of oxalate in each oxalate-species. *Indicates statistical significance.

Magnesium speciation

Magnesium speciation is shown in Figure 3.11 and Figure 3.12 for the respective groups while actual values for each species are reported in Table 3.7 for both groups. Individual values for each species for each urine sample are reported in Appendix 3.2. The formation of several magnesium species changed significantly in the presence of 1.50 mM phytate. A significant increase in Mg^{2+} is observed with a significant decrease in MgOx in both groups. This is counterintuitive since an increase in the former species (as well as the increase in Ox^{2-} as shown above) should result in an increase in MgOx. This will be discussed later.

Inter-group comparisons of magnesium speciation showed that the formation of $MgCit_2^{-4}$ was significantly lower in the black than the white group in the presence of both 0.76 µM (p= 0.03) and 1.50 mM (p= 0.02) phytate. This result is due to a lower citrate concentration in the black than the white group (1.47 ± 0.14 vs 1.77 ± 0.12 mmol/L; p = 0.13) albeit not statistically significant.

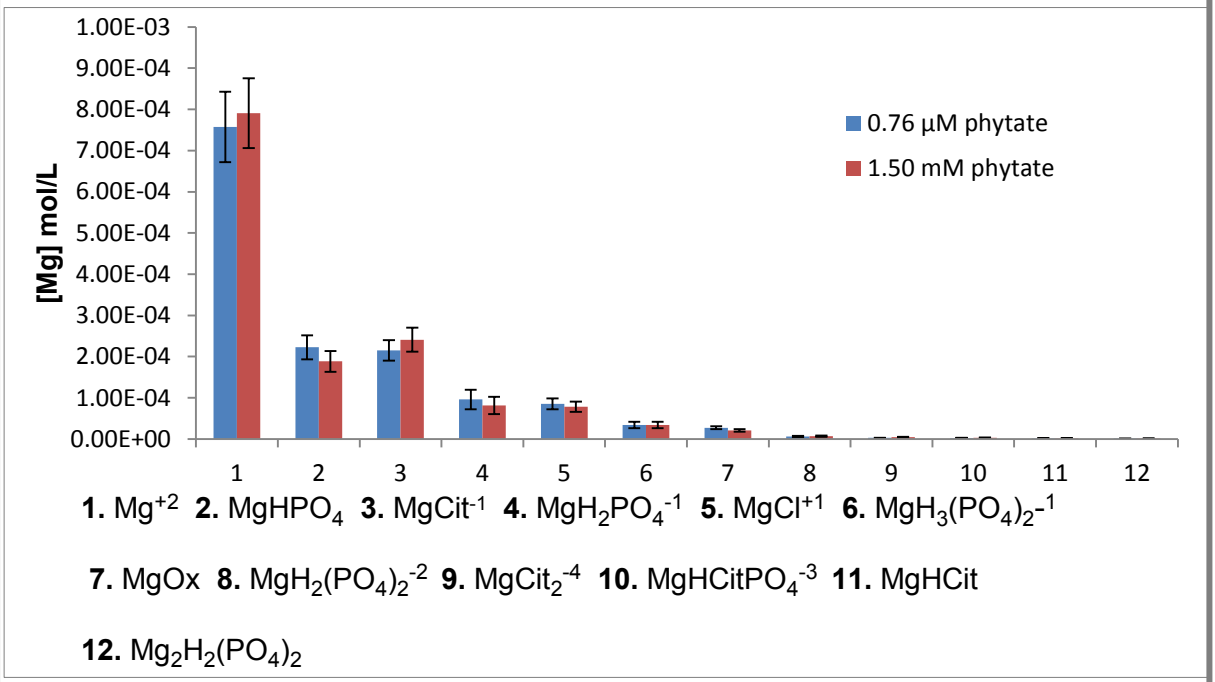
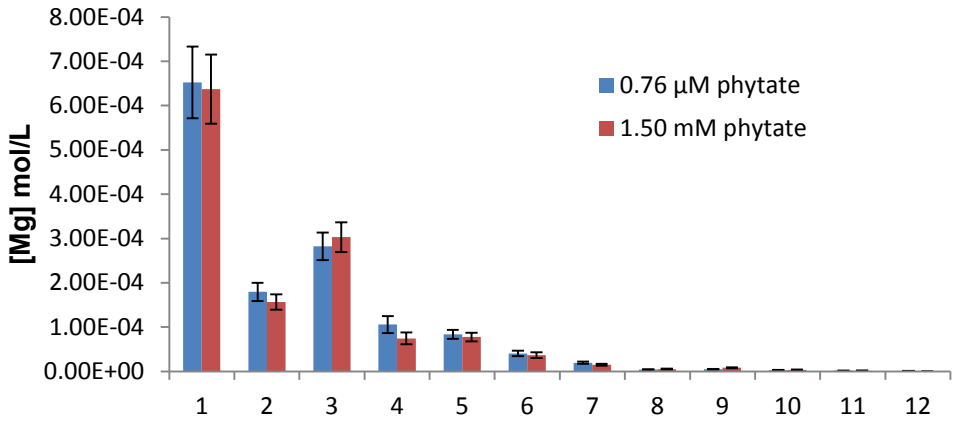


Figure 3.11 Magnesium speciation in urine of black subjects (n= 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. Mg^{+2} **2.** $MgHPO_4$ **3.** $MgCit^{-1}$ **4.** $MgH_2PO_4^{-1}$ **5.** $MgCl^{+1}$ **6.** $MgH_3(PO_4)_2^{-1}$

7. MgOx **8.** $MgH_2(PO_4)_2^{-2}$ **9.** $MgCit_2^{-4}$ **10.** $MgHCitPO_4^{-3}$ **11.** MgHCit

12. $Mg_2H_2(PO_4)_2$

Figure 3.12 Magnesium speciation in urine of white subjects (n =16) in the presence of 0.76 µM and 1.50 mM phytate.

Table 3.7 Magnesium speciation‡ in urine of black and white subjects in the presence of 0.76 μM and 1.50 mM phytate.

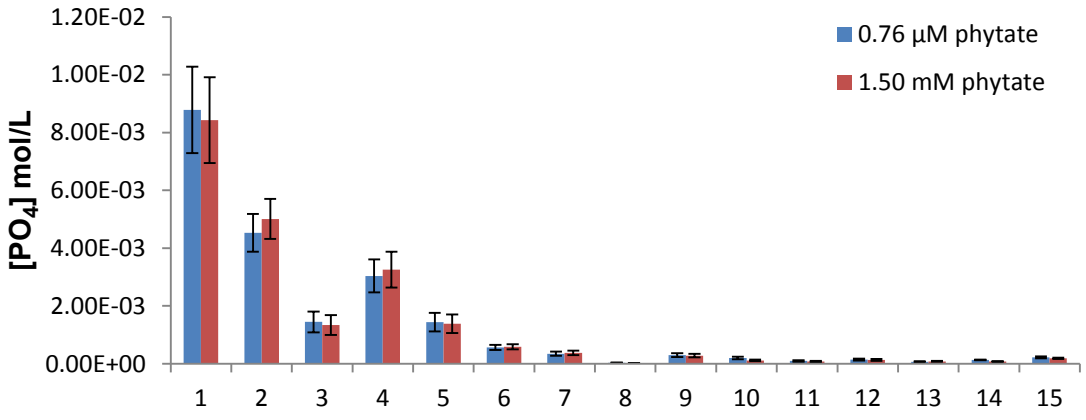
Species	Black subjects (n =18)			White subjects (n =16)		
	[Mg] (x 10^{-7} mol/L) Mean ± SE		P value	[Mg] (x 10^{-7} mol/L) Mean ± SE		p value
	0.76 μM phytate	1.50 mM phytate		0.76 μM phytate	1.50 mM phytate	
Mg^{+2}	7574 ± 854	7911 ± 848	0.0005*	6521 ± 809	6375 ± 780	0.69
$MgHPO_4$	2224 ± 293	1884 ± 252	<0.0001*	1796 ± 205	1567 ± 175	<0.0001*
$MgCitric^{-1}$	2152 ± 250	2407 ± 291	0.0013*	2823 ± 309	3030 ± 337	0.046*
$MgH_2PO_4^{+1}$	960 ± 237	812 ± 210	<0.0001*	1057 ± 191	744 ± 133	0.07
$MgCl^{+1}$	854 ± 134	781 ± 126	<0.0001*	837 ± 103	776 ± 96	0.0006*
$MgH_3(PO_4)_2^{-1}$	339 ± 76	338 ± 80	0.89	404 ± 62	368 ± 63	0.35
MgOx	270 ± 35	209 ± 30	<0.0001*	193 ± 25	148 ± 22	<0.0001*
$MgH_2(PO_4)_2^{-2}$	60 ± 14	67 ± 16	0.01*	42 ± 8	50 ± 9	0.0003*
$MgCit_2^{-4}$	26 ± 4¥	46 ± 7¥	<0.0001*	48 ± 8¥	82 ± 13¥	<0.0001*
$MgHCitPO_4^{-3}$	22 ± 4	28 ± 5	<0.0001*	27 ± 4	35 ± 4	0.0001*
MgHCit	16 ± 4	14 ± 4	0.0001*	19 ± 4	14 ± 3	0.04*
$Mg_2H_2(PO_4)_2$	13 ± 3	10 ± 2	0.001*	7.84 ± 1.50	8.87 ± 1.90	0.002*

‡Expressed as the concentration of magnesium in each magnesium- species. *Statistically significant. ¥indicates statistical significant difference between groups.

Phosphate speciation

The mean concentration of phosphate in each phosphate-species is shown in Figure 3.13 and Figure 3.14 for the black and white groups, respectively, while actual values for each species are reported in Table 3.8 for both groups. Individual values for each species of each urine sample are reported in Appendix 3.2. Of interest is the significant decrease in the formation of $H_2PO_4^{-1}$ (species number 1 in Figure 3.13 and Figure 3.14) in the presence of 1.50 mM phytate in both groups. This is accompanied by a significant increase in HPO_4^{-2} (species number 2 in Figure 3.13 and Figure 3.14) which forms upon the deprotonation of the former species. HPO_4^{-2} is a precursor for the formation of CaP salts. Thus, its significant increase may lead to an increase in the SS of CaP salts. However, the converse occurred (Table 3.2 and Table 3.3) because the increase in HPO_4^{-2} is exceeded by a greater decrease in Ca^{2+} (Table 3.4 and Table 3.5), culminating in a decrease in the SS of CaP salts.

No statistical difference in the speciation of phosphate was observed between the groups (p values are reported in Appendix 3.3).

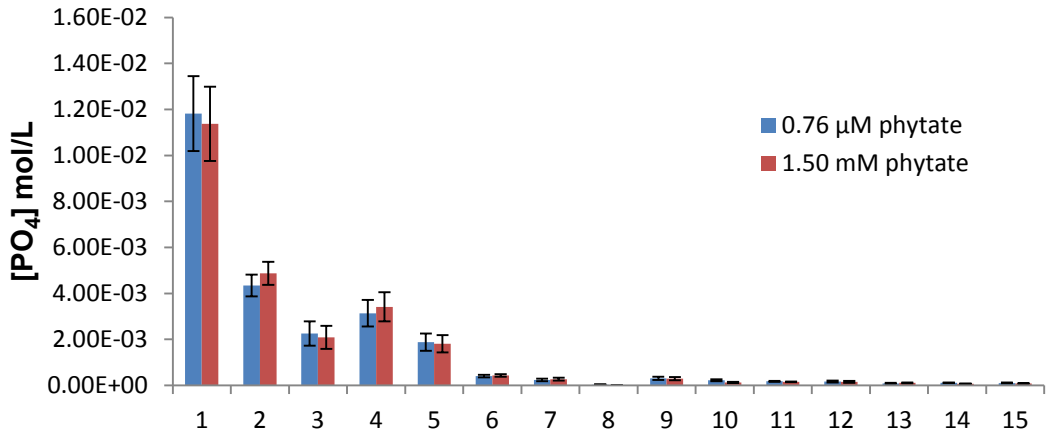


1. $H_2PO_4^{-1}$ **2.** HPO_4^{-2} **3.** $H_4(PO_4)_2^{-2}$ **4.** $HNaPO_4^{-1}$ **5.** H_2NaPO_4 **6.** $HKPO_4^{-1}$

7. $CaCitPO_4^{-4}$ **8.** $CaH_2PO_4^{+1}$ **9.** H_2KPO_4 **10.** $CaH_3(PO_4)_2^{-1}$ **11.** $MgH_2PO_4^{+1}$

12. HNa_2PO_4 **13.** $H_3(PO_4)_2^{-3}$ **14.** $CaHPO_4^{+1}$ **15.** $MgHPO_4$

Figure 3.13 Phosphate speciation in urine of black subjects (n= 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. $H_2PO_4^{-1}$ **2.** HPO_4^{-2} **3.** $H_4(PO_4)_2^{-2}$ **4.** $HNaPO_4^{-1}$ **5.** H_2NaPO_4 **6.** $HKPO_4^{-1}$

7. $CaCitPO_4^{-4}$ **8.** $CaH_2PO_4^{+1}$ **9.** H_2KPO_4 **10.** $CaH_3(PO_4)_2^{-1}$ **11.** $MgH_2PO_4^{+1}$

12. HNa_2PO_4 **13.** $H_3(PO_4)_2^{-3}$ **14.** $CaHPO_4^{+1}$ **15.** $MgHPO_4$

Figure 3.14 Phosphate speciation in urine of white subjects (n= 16) in the presence of 0.76 µM and 1.50 mM phytate.

Table 3.8 Phosphate speciation‡ in urine of black and white subjects in the presence of 0.76 μM and 1.50 mM phytate.

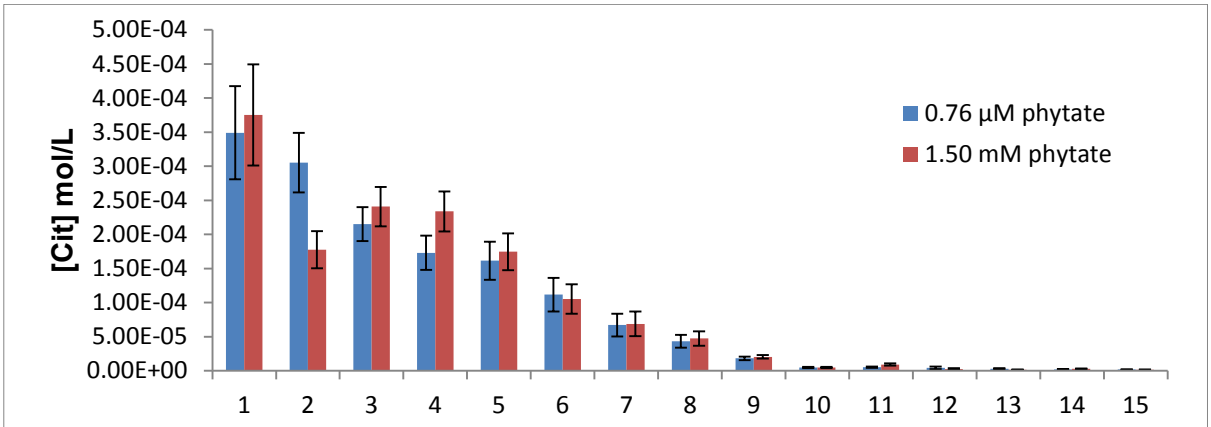
Species	Black subjects (n= 18)		P value	White subjects (n= 16)		p value
	$[PO_4]$ ($\times 10^{-5}$ mol/L) Mean ± SE			$[PO_4]$ ($\times 10^{-5}$ mol/L) Mean ± SE		
	0.76 μM phytate	1.50 mM phytate		0.76 μM phytate	1.50 mM phytate	
$H_2PO_4^{-1}$	879 ± 150	843 ± 148	0.0001*	1182 ± 163	1138 ± 161	<0.0001*
HPO_4^{-2}	453 ± 65	501 ± 69	<0.0001*	434 ± 48	487 ± 50	<0.0001*
$H_4(PO_4)_2^{-2}$	145 ± 36	134 ± 34	0.002*	225 ± 53	209 ± 50	0.001*
$HNaPO_4^{-1}$	304 ± 57	325 ± 62	0.001*	314 ± 58	342 ± 63	0.0003*
H_2NaPO_4	144 ± 32	139 ± 32	0.0002*	188 ± 38	181 ± 38	<0.0001*
$HKPO_4^{-1}$	56 ± 9	58 ± 9	0.03*	41 ± 5	43 ± 6	0.0004*
$CaCitPO_4^{-4}$	35 ± 7	38 ± 7	0.01*	24 ± 6	27 ± 6	0.0006*
$CaH_2PO_4^{+1}$	3.20 ± 0.89	1.70 ± 0.51	0.002*	3.30 ± 0.75	1.79 ± 0.43	0.002*
H_2KPO_4	30 ± 6	28 ± 6	<0.0001*	31 ± 7	29 ± 7	<0.0001*
$CaH_3(PO_4)_2^{-1}$	20 ± 4	12 ± 3	<0.0001*	23 ± 4	13 ± 3	0.0003*
$MgH_2PO_4^{+1}$	10 ± 2	8.12 ± 2.10	<0.0001*	11 ± 2	8.86 ± 1.66	0.0002*
HNa_2PO_4	14 ± 4	13 ± 3	<0.0001*	17 ± 5	16 ± 4	0.0004*
$H_3(PO_4)_2^{-3}$	6.86 ± 1.65	8.04 ± 1.85	0.0004*	9.06 ± 1.67	11 ± 2	0.0002*
$CaHPO_4^{+1}$	13 ± 1	7.77 ± 1.33	<0.0001*	11 ± 2	6.95 ± 1.00	0.0005*
$MgHPO_4$	22 ± 3	19 ± 3	<0.0001*	18 ± 2	16 ± 2	<0.0001*

‡Expressed as the concentration of phosphate in each phosphate-species. *Indicates statistical significance.

Citrate speciation

The mean concentration of citrate in each citrate-species is shown in Figure 3.15 and Figure **3.16** for the black and white groups, respectively, while actual values for each species are reported in Table 3.9 for both groups. Individual values for each species of each urine sample are reported in Appendix 3.2. Of interest is the significant increase in unbound citrate (Cit^{-3}, species number 4 in Figure 3.15 and Figure 3.16) in both groups. This increase is accounted for by a significant decrease in the formation of $CaCit^{-1}$ (species number 2 in Figure 3.15 and Figure 3.16). The latter is due to a significant increase in the formation of Ca_2HPhy^{-7} which utilizes a large amount of Ca^{2+}.

Inter-group comparisons showed that the concentration of Cit^{-3} was lower in the black group than the white group in the presence of 0.76 µM phytate, tending toward statistical significance (p= 0.05) and was significantly lower in the presence of 1.50 mM phytate (p =0.01). This result is due to a lower urinary citrate concentration in the black than the white group as mentioned previously. These comparisons are reported in Appendix 3.1 and Appendix 3.3.

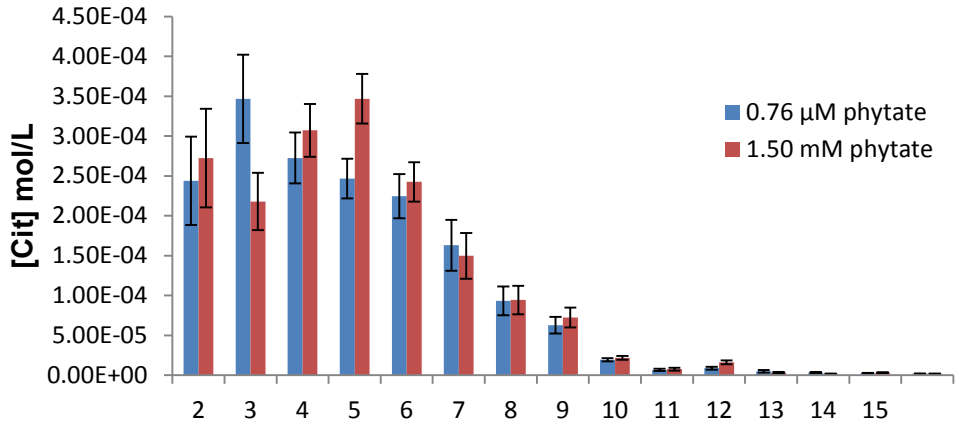


1. $CaCitPO_4^{-4}$ **2.** $CaCit^{-1}$ **3.** $MgCit^{-1}$ **4.** Cit^{-3} **5.** $NaCit^{-2}$ **6.** Na_2Cit^{-1} **7.** $HNaCit^{-1}$

8. $HCit^{-2}$ **9.** $KCit^{-2}$ **10.** $CaCit_2^{-4}$ **11.** $MgCit_2^{-4}$ **12.** $CaH_2Cit_2^{-2}$ **13.** CaHCit **14.** $MgHCitPO_4^{-3}$

15. $CaHCitPO_4^{-3}$

Figure 3.15 Citrate speciation in urine of black subjects (n = 18) in the presence of 0.76 µM and 1.50 mM phytate.



1. $CaCitPO_4^{-4}$ **2.** $CaCit^{-1}$ **3.** $MgCit^{-1}$ **4.** Cit^{-3} **5.** $NaCit^{-2}$ **6.** Na_2Cit^{-1} **7.** $HNaCit^{-1}$

8. $HCit^{-2}$ **9.** $KCit^{-2}$ **10.** $CaCit_2^{-4}$ **11.** $MgCit_2^{-4}$ **12.** $CaH_2Cit_2^{-2}$ **13.** CaHCit

14. $MgHCitPO_4^{-3}$ **15.** $CaHCitPO_4^{-3}$

Figure 3.16 Citrate speciation in urine of white subjects (n= 16) in the presence of 0.76 µM and 1.50 mM phytate.

Table 3.9 Citrate speciation‡ in urine of black and white subjects in the presence of 0.76 µM and 1.50 mM phytate.

Species	Black subjects (n= 18)			White subjects (n= 16)		
	[Cit] (x 10^{-6} mol/L) Mean ± SE		P value	[Cit] (x 10^{-6} mol/L) Mean ± SE		p value
	0.76 µM phytate	1.50 mM phytate		0.76 µM phytate	1.50 mM phytate	
$CaCitPO_4^{-4}$	349 ± 68	375 ± 74	0.02*	244 ± 55	272 ± 62	0.004*
$CaCit^{-1}$	305 ± 44	178 ± 27	<0.0001*	347 ± 55	218 ± 36	0.0004*
$MgCit^{-1}$	215 ± 25	241 ± 29	0.001*	273 ± 32	307 ± 33	0.01*
Cit^{-3}	173 ± 25¥	234 ± 29¥	<0.0001*	247 ± 25¥	347 ± 31¥	0.002*
$NaCit^{-2}$	162 ± 28	175 ± 27	0.02*	225 ± 28	243 ± 25	0.06
Na_2Cit^{-1}	112 ± 25	105 ± 22	0.13	163 ± 32	150 ± 29	0.02*
$HNaCit^{-1}$	67 ± 17	69 ± 18	0.43	93 ± 18	94 ± 18	0.81
$HCit^{-2}$	43 ± 9	47 ± 11	0.09	63 ± 10	72 ± 13	0.20
$KCit^{-2}$	18 ± 3	20 ± 3	0.01*	19 ± 2	22 ± 2	0.06
$CaCit_2^{-4}$	4.78 ± 0.92	4.78 ± 0.95	0.86	6.70 ± 1.52	7.58 ± 1.71	0.21
$MgCit_2^{-4}$	5.22 ± 0.90	9.29 ± 1.44	<0.0001*	8.84 ± 1.71	16 ± 3	0.0001*
$CaH_2Cit_2^{-2}$	4.48 ± 1.80	2.62 ± 1.14	0.01*	5.04 ± 1.64	3.17 ± 1.06	0.02*
$CaHCit$	3.02 ± 0.92	1.39 ± 0.46	0.004*	3.26 ± 0.81	1.58 ± 0.41	0.002*
$MgHCitPO_4^{-3}$	2.25 ± 0.44	2.78 ± 0.48	<0.0001*	2.73 ± 0.34	3.37 ± 0.38	0.0002*
$CaHCitPO_4^{-3}$	1.80 ± 0.33	1.55 ± 0.33	<0.0001*	2.00 ± 0.25	1.74 ± 0.26	0.002*

‡Expressed as the percentage of citrate in each citrate-species. *Indicates intra-group statistical significance. ¥indicates statistical significant difference between groups.

Discussion

The hypothesis under investigation in the study described in this chapter was that urinary phytate modulates both CaOx and CaP crystallisation in a thermodynamic manner by virtue of the ability of its 6 phosphate groups to bind calcium, thus decreasing the SS of CaOx and CaP salts. Since the results of this theoretical study showed that the concentration of urinary Ca^{2+} and the concomitant RS values of Ca-containing salts were unaffected at physiological concentrations of phytate the aforementioned hypothesis is rebutted.

The failure of phytate to significantly decrease the concentration of Ca^{2+} at physiological concentrations of this ligand in the urine models used in the present study can be explained as follows. Firstly, although phytate contains 6 phosphate moieties with a highly negative charge at physiological pH, making it a favourable candidate for the binding of Ca^{2+}, a maximum of only two Ca^{2+} ions can bind per mole of phytate as reported in the preceding chapter. As such, the notion of phytate being a strong chelator of Ca^{2+} in urine because of its 6 potential binding sites is exaggerated. Secondly, the question of whether the formation of the solitary Ca_2HPhy^{-7} (as observed in the present study in urine of both groups) is able to significantly reduce the concentration of Ca^{2+}, clearly depends on the physiological urinary concentration of $PhyH^{-11}$ and how it compares with the concentration of calcium. The physiological concentration of urinary phytate and hence $PhyH^{-11}$ is 1000 times smaller than that of calcium (micro-molar vs milli-molar). It is therefore not surprising that the physiological concentrations of phytate as well as the much higher non-physiological concentration of 30 µM had no effect on the concentration of Ca^{2+} and the concomitant SS values of CaOx and CaP salts in the present study. This argument is supported by the observed significant decrease in Ca^{2+} and the SS of CaOx and CaP salts at a phytate concentration of 1.50 mM (due to the formation of Ca_2HPhy^{-7}) in the present study. This occurred since the magnitude of this phytate concentration is comparable to that of urinary calcium.

Three surprising findings have been highlighted in the present study at the hypothetically elevated concentration of phytate (1.50 mM) relative to the lower physiological concentration of this ligand (0.76 uM). These were the significant increase in $CaCitPO_4^{-4}$, the significant decrease in MgOx and the significant decrease in the SS of Na-Urate and uric acid. The first can be explained in terms of the concentrations of Ca^{2+}, Cit^{-3} and HPO_4^{-2} as these are precursors for the formation of the complex species $CaCitPO_4^{-4}$. Since a large amount of Ca^{2+} is utilized by $PhyH^{-11}$ in the present study culminating in a significant increase in both Cit^{-3} and HPO_4^{-3} it is speculated that the increase in the concentration of the latter two

species compensated for the decrease in Ca^{2+} and contributed to the increase in $CaCitPO_4^{-4}$.

On the other hand, the decrease in MgOx with an increase in Mg^{2+} and Ox^{2-} is counterintuitive and suggests that a decrease in its formation constant has occurred. Similarly the decrease in the SS of uric acid and Na-urate indicates that its solubility increased. As mentioned earlier, factors which affect both the magnitude of formation constants and the SS include pH, temperature and IS. Since the first two were fixed in the present study it is speculated that a significant change in the latter may have occurred due to the large negative charge of phytate. Indeed, statistical comparisons of the IS at a concentration of 1.50 mM phytate relative to 0.76 µM phytate showed a significant increase in the IS as shown in below in Table 3.10.

Table 3.10 Effect of 0.76 µM and 1.50 mM phytate on the IS of urine from black and white subjects. Mean ± SE.

Black subjects (n= 18)			White subjects (n= 16)		
0.76 µM	**1.50 mM**	**P- value**	**0.76 µM**	**1.50 mM**	**p value**
0.13 ± 0.05	0.22 ± 0.05	<0.0001*	0.15 ± 0.01	0.23 ± 0.01	<0.0001*

*Indicates statistical significance.

In general, an increase in IS results in a decrease in the magnitude of stability constants and an increase in solubility.[359-361] Thus, the significant decrease in MgOx (with a concomitant increase in Mg^{2+} and Ox^{2-}) is explained by the significant increase in IS in the present study. Similarly the decrease in the SS of uric acid and Na-urate is due to the increase in the IS.

It should be noted that other monovalent and divalent cations such as Na^+, K^+ and Mg^{2+} may also bind to phytate to form a wide variety of binary, ternary and quaternary complexes such as $Na_2H_5Phy^{-5}$, $Na_3H_4Phy^{-5}$, $Na_4H_6Phy^{-2}$, $K_2H_{10}Phy$, $Mg_2H_2Phy^{-6}$, $Mg_3H_5Phy^{-5}$ and MgH_3Phy^{-7}.[332] The determination of stability constants for such species was however not undertaken in the present thesis and was therefore not included in the JESS model. Thus, a significant percentage of the mono-protonated species $HPhy^{-11}$ is observed at a concentration of 1.50 mM phytate which may otherwise have been complexed to other metal ions. In any case, the inclusion of these formation constants would have had a negligible effect on the RS of CaOx and CaP salts under physiological conditions as the concentrations of these species would be negligible due to the low physiological concentration of phytate as mentioned above.

In this study, it was also hypothesized that urinary phytate may have different effects in the urine of black subjects compared to their white counterparts due to differences in their urinary composition. However, the modelled effects of phytate in the present study were similar between the respective groups. This indicates that it is unlikely that dietary (and hence urinary) phytate can account for the differences in stone modulation in the two groups.

It is clear that urinary phytate does not modulate CaOx and CaP crystallization in a thermodynamic manner due to the low concentration of this ligand relative to calcium and other urinary components. Thus, if it is indeed an inhibitor of urinary calcium crystallization as has been suggested by some authors, it must do so via a kinetic process. Indeed, previous in vitro studies showed that phytate concentrations between 0.75 μM and 2 μM reduced the rate of CaOx crystal nucleation and growth in artificial urine. [320, 362] More recently, a study by Saw et al showed that the growth rate of artificial stones decreased significantly in artificial urine containing urinary macromolecules (UMM) in the presence of 2.5 μM phytate and that the concentration of Ca^{2+} was not affected at this concentration of phytate.[285]

The study by Saw et al[285] also demonstrated that a phytate concentration of 30 μM decreased the crystallization rate in artificial urine (without the addition of UMM) and that mill-molar (as opposed to micro-molar) concentrations of phytate were required to decrease the concentration Ca^{2+}. This result led the authors to conclude that the inhibitory effect of phytate is not via a decrease in the supersaturation of CaOx.[285] Interestingly, a concentration of 30 μM phytate also decreased the crystallization rate in human urine (although not to the same extent as in the former medium). However, the effect of milli-molar concentrations of phytate on the concentration of Ca^{2+} was negligible. This is in contrast to the theoretically predicted result for 1.50 mM phytate in the present study. This may be due to thermodynamic effects of urinary macromolecules in real urine which cannot be modelled without stability constants involving the formation of Ca-UMM complexes.

Recognizing that theoretical modelling of thermodynamic factors cannot include or address every possible condition which prevails in solution, and that the modelling of kinetic factors is difficult (and beyond the scope of the present study), the next phase of the overall project shifted to an *in vitro* model in which the effects of phytate on both of these factors were investigated in artificial urine and in the urine of black and white subjects. These studies are described in Chapter 5.

Chapter 4 : Method for the determination of phytate in urine

Introduction

The determination of IP6 in biological samples such as urine is challenging since its concentration is extremely low (micromolar) and the sample matrix is complexed.[266, 363] Methods developed for the determination of this substance in urine require sophisticated instrumentation such as HPLC[364] and ICP[365, 366] as well as laborious sample preparation including hydrolysis, evaporation and digestion.[363] These methods can therefore not be routinely applied in all analytical laboratories.

As mentioned in Chapter 1, IP6 is composed of an inositol and 6 phosphate moieties. Most methods for the quantification of IP6 in urine are therefore based on properties of the inositol[364, 367] or phosphate moiety[365, 366, 368]. Both approaches require hydrolysis of the IP6 molecule. On the other hand, determination of IP6 in foods without the hydrolysis of the molecule has been successfully carried out.[259] The latter methods are based on the indirect colorimetric detection of IP6 since the molecule has no absorption bands in the UV-visible light range and no reagent interacts directly with it to form a coloured compound.[259] These methods exploit the high binding affinity of the phosphate groups to polyvalent cations such as Fe^{3+}, Y^{3+} and Cu^{2+}.[259] A metal- dye system is used containing one of these metals. IP6 binds to the metal resulting in a decrease in the concentration of the metal-dye compound and a subsequent decrease in the absorbance. An example of a metal-dye system is Wade's reagent in which the coloured compound is Fe(III)-sulfosalicylic acid.[369] In the presence of IP6, its characteristic pink colour decreases upon complexation of the metal to IP6. Methods applied in food analysis are however not sensitive enough to detect IP6 in biological samples in the absence of significant preconcentration.

These challenges have recently been addressed by Costa-Bauza and Grases et al[363] who developed a method that can be used for routine analysis without the use of sophisticated instrumentation and hydrolysis of the molecule. This method ("Method 1") involved the purification and preconcentration of IP6 using solid phase extraction (SPE) prior to colorimetric detection. The colorimetric assay used Fe(III)-thiocyanate as the metal-dye system with a detection limit of 0.055 µM. Subsequent to this method, another was developed ("Method 2") for the detection of IP6 in urine of rats using an alternative metal-dye system.[370] This metal-dye system consisted of Al(III)- xylenol orange. Method 2 was slightly modified for the detection of IP6 in human urine[371] and will be discussed later in the present chapter.

During the validation process and assessment of the practical application of Method 2, a dietary intervention was conducted by the present researcher in a rat study in the Laboratory of Renal Lithiasis at the University of Balearic Islands, Spain, as part of a student exchange program. The aim was for the present researcher to receive expert training in all aspects of this method (as well as Method 1) in the laboratory in which it was developed, and to ultimately apply it in a project for a PhD thesis, involving urine from human subjects in the Kidney Stone Research Laboratory (KSRL) at the University of Cape Town. However, because of the very high cost of the resin (*AG 1-x8 chloride form, 200 – 400 mesh; BIO RAD*) used in both methods, an alternative resin had to be sourced and used. The efficacy of the alternative resin in both methods therefore had to be tested prior to its use in the KSRL.

The objectives of this chapter were therefore to:

1. Test the practical application of Method 2 by investigating whether it is able to detect a change in IP6 concentration in the urine of rats in relation to the dietary intake of this substance.
2. Determine the efficacy of an alternative resin for possible use in both methods by testing its capacity for removing interfering substances, its reproducibility and the percentage recovery it achieves.
3. Selection of the “best” method for IP6 analysis in the KSRL by comparison of the two methods with respect to removal of interfering substances, reproducibility and percentage recovery.

Methods

Experiments described in the present chapter were conducted at the University of Cape Town in the KSRL and at the University of Balearic Islands, Spain in the Renal Lithiasis Laboratory. Experiments conducted in the latter laboratory are denoted by the following symbol "‡".

The protocols for both methods of urinary IP6 pre-concentration and quantification in each method are first described. This is followed by a description of the testing of the practical application of Method 2. Finally the investigation of the efficacy of an alternative resin for use in Method 1 and Method 2 is described.

Preconcentration of phytate (method 1)

The method described by Costa-Bauza et al[363] was used for sample preparation and preconcentration of IP6. Briefly, 20 ml of urine was set to a pH of 3 (using 1 M HCl) and was diluted with 20 ml of distilled water. This was transferred to a 150 ml beaker containing 0.5 g of an anion exchange resin without previous conditioning and mechanically shaken for 15 mins at a speed of 160 rpm (*Labcon; Johannesburg*). The resin mixture was then transferred to an empty 20 ml SPE tube (*Sigma Aldrich*), fitted with a frit, to separate the urine mixture from the resin. Care was taken not to let the resin run dry. The resin was then washed with 120 ml HCl (50 mM) in a stepwise manner by adding approximately 20 ml aliquots of HCl with manual stirring using a glass rod. The resin was then washed with 5 ml distilled water. Finally, IP6 was eluted with 4 ml of 2 M NaCl. This was also done in a stepwise manner by adding 1 ml aliquots of NaCl and mechanically shaken for 5 mins at 180 rpm. The final eluate (4 ml) was homogenized by vortex-mixing and used for quantification as described in the following paragraph.

Quantification of phytate (method 1)

IP6 was quantified using a colorimetric assay.[363] A reagent containing Fe-SCN was prepared daily by mixing equal volumes of two solutions (S1 and S2). S1 was a mixture of 8 ml KSCN (5 M) and 2 ml glycine/HCl buffer (0.5 M, pH 2). S2 was a mixture of 5 ml $Fe(NO_3)_3$ (5 mM) in HNO_3 (5 mM), 2.5 ml $NaClO_3$ (0.4 M) and 5 ml sodium persulfate (75 mM).

IP6 standards in the range of 0 – 25 µM were prepared from dipotassium phytic acid (Sigma Aldrich) in NaCl (2 M). The assay was conducted by adding 0.5 ml of reagent to 3 ml of the eluate or standard. Absorbance was measured at 460 nm (*Spectronic Unicam; Helios gamma*) 5 min after mixing. IP6 was determined from the calibration curve. A typical calibration curve obtained in the present study is shown Figure 4.1. A schematic diagram which summarises Method 1 is shown in Figure 4.2.

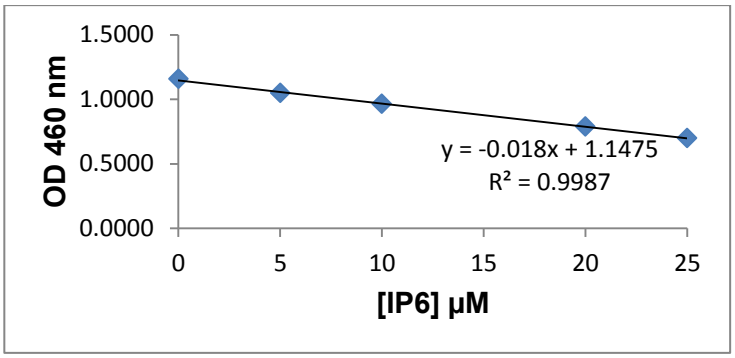


Figure 4.1 A typical plot of absorbance vs IP6 concentration depicting a calibration curve for the determination of IP6 (Method 1)

A. Adsorption step

1. 20 ml urine adjust to pH 3
2. Add 20 ml H_2O
3. Transfer to 150 ml beaker containing 0.5 g anion exchange resin
4. Stir at 160 rpm for 15 mins
5. Transfer to empty 20 ml SPE tube

B. Wash and elution steps

1. Let urine elute
2. Wash resin with 120 ml HCl 50 mM
3. Wash resin with 5 ml H_2O
4. Elute IP6 with 4 x 1 ml NaCl 2M

C. Colorimetric detection

1. Homogenize eluate by vortex-mixing
2. Pipette 3 ml eluate/ standard into test tubes
3. Add 0.5 ml reagent
4. Vortex-mix
5. Measure absorbance at 460 nm after exactly 5 mins

D. Calculate urinary IP6 concentration

Figure 4.2 Schematic diagram (adapted from Grases et al[370]) for the detection of urinary IP6 using Method 1.

Preconcentration of phytate (method 2)

This method was originally developed for the determination of IP6 in the urine of rats.[370] A 10 ml aliquot of urine was mixed with an equal volume of 10 mM EDTA (ehtylenediaminetetraacetic acid) and diluted with 20 ml of distilled water. The pH of the resulting mixture was adjusted to 6 (using 1 M HCl or 1 M NaOH depending on the initial pH) and this was then transferred to a 50 ml corning tube and centrifuged at 3000 rpm for 15 mins. The supernatant was then acidified to a pH of 3 (1 M HCl) and was quantitatively transferred to a 150 ml beaker containing 0.25 g of an anion exchange resin without previous conditioning. Each mixture was stirred at 160 rpm for 15 mins using an orbital stirrer to facilitate adsorption of phytate to the resin. These mixtures were then carefully transferred to an empty SPE tube fitted with a frit to separate the urine mixture from the resin. The resin was washed with 300 ml HCl (100 mM) followed by washing it twice with 5 ml aliquots of distilled water. During washing, the resin was stirred manually with a glass rod. IP6 was then eluted with 2 M NaCl in 5 steps using 0.5 ml of NaCl each time. The total volume (2.5 ml) was then homogenized by vortex mixing prior to phytate quantification.

This method was slightly modified for the determination of IP6 in human urine.[371] The same procedure was followed except dilution of the urine with distilled water was not necessary (as human urine is not as concentrated as the urine of rats) and a volume of 100 ml HCl (50 mM) was used for washing of the resin.

Quantification of phytate (method 2)

Phytate was determined colorimetrically using aluminium and xylenol orange. The following solutions (S1 and S2) were freshly prepared. S1 consisted of 4mM $Al(NO_3)_3.9H_2O$ in an acetic acid/acetate buffer (1.5 M, pH= 5.2) and S2 contained 4mM Xylenol Orange (XO) in distilled water. IP6 standards with a concentration of 0 – 14 µM were prepared using phytic acid dipotassium salt (*Sigma, Aldrich*) in NaCl (2 M).

The assay was conducted by adding 0.5 ml of S1 to 2.5 ml of the eluate or standard and vortex-mixed. After 30 seconds, 0.5 ml of S2 was added. The mixture was then allowed to stand for 45 mins after which absorbance readings were taken at 560 nm. The concentration of IP6 in each eluate was determined from a calibration curve. An example of a typical calibration curve obtained in the present study is shown in Figure 4.3. A schematic diagram which summarises Method 2 is shown in Figure 4.4.

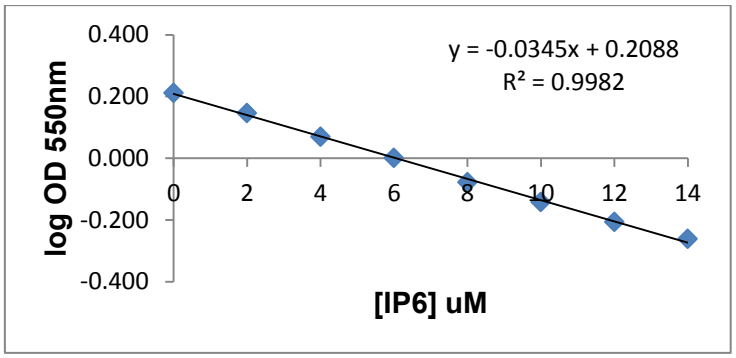


Figure 4.3 A typical plot of the logarithm of absorbance vs IP6 concentration depicting a calibration curve for IP6 determination (Method 2)

A. Chelation of Ca &

1. Add 10 ml EDTA 10 mM to 10 ml urine
2. Add 20 ml H_2O (not required for human urine)
3. Adjust to pH 6
4. Centrifuge at 3000 rpm for 10 mins

B. Adsorption step

1. Adjust supernatant to pH 3
2. Transfer to 150 ml beaker containing 0.2 anion exchange resin
3. Stir at 160 rpm for 15 mins
4. Transfer to an empty 20 ml SPE tube

C. Wash and elution

1. Let urine elute
2. Wash resin with 300 ml HCl, 100 mM (100 ml HCl, 50 mM for human urine)
3. Wash resin with 2 x 5 ml H_2O
4. Elute IP6 with 5 x 0.5 ml NaCl 2 M

D. Colorimetric detection

1. Homogenize eluate by vortex-mixing
2. Add 0.5 ml of S1 to eluate (2.5 ml) or standard
3. Vortex-mix and wait 30 s
4. Add 0.5 ml of S2
5. Vortex mix and wait 45 mins

E. Calculate urinary IP6

Figure 4.4 schematic diagram (adapted from Grases et al[370]) for the determination of IP6 in urine of rats and humans using Method 2.

Assessment of the practical application of Method 2: Animal study ‡

All aspects involved in the development of Method 2 had been previously performed by members of the Renal Lithiasis Laboratory in Spain prior to the present researcher's studentship. The practical application of the method on the other hand was tested by the present researcher. As mentioned in Chapter 1, previous studies in both animals and rats have demonstrated that urinary IP6 decreased when its dietary intake was restricted and increased when it was administered. Testing the practical application of Method 2 was therefore undertaken by conducting a dietary intervention in a rat study as described below.

Three male Wistar rats (28- 30 months old) and 2 female Wistar rats (11 months old) from Harlan Ibérica S.A (Barcelona, Spain) were used in this study. Rats were housed (1 or 2 rats) per cage at a temperature of 18 – 23 °C and a relative humidity of 50 % with a 12 h light-dark cycle. They were fed a purified diet containing undetectable amounts of IP6 (*AIN-76A Harland Tekland, Madison, WI, USA*) for a duration of 15 days. This was followed by the administration of a standard non-purified diet containing 1 % IP6 (*UAR A03; Panlab S. L.; Barcelona, Spain*) for another 17 days. Diets and tap water were fed *ad libitum*. The composition of each diet is shown in Table 4.1.

Table 4.1 Composition of AIN-76A purified diet and UAR-A03 standard non-purified diet.

	AIN-76A (g. kg^{-1} dry mass)	UAR-A03 (g. kg^{-1} dry mass)
Protein	203	267
Carbohydrate	650	565
Lipid	50	57
Cellulose	50	45
Ash	47	66
Calcium	3.91	9.90
Magnesium	0.47	1.85
Zinc	0.028	0.045
Phosphorus[a]	7.20	5.30
IP6	undetectable	9.0

[a]Excludes phosphorus from IP6.

24 hr urine samples were collected by housing the rats in metabolic cages (*Tecniplast; Gazzada; S. A. R. L.; Buggagiate, Italy*). Urine was collected on day 0 which served as the baseline sample and periodically over the course of the study to determine urinary IP6 in relation to its dietary intake.

The procedures used in this experiment were applied according to the Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Efficacy of an alternative resin

The efficacy of an alternative, more affordable, resin (*Dowex 1 x 8 chloride form, 200- 400 mesh; Sigma Aldrich*) for use in both Method 1 and Method 2 was tested in the KSRL. Since the purification of IP6 (in both methods) is based on its adsorption to an anionic exchange resin, a study involving the removal of interfering anions such as oxalate, sulfate and phosphate which occur in urine was undertaken in the present project. Artificial urine (AU) was used for this purpose. The reproducibility of both methods using the alternative resin was determined by calculating the co-efficient of variation (CV) of intra-day replicates (n= 5). The percentage recovery was determined by the addition of 1 and 2 µM IP6 to the AU. Finally, both methods were used for the determination of IP6 and its percentage recovery in human urine.

Artificial urine

A stock solution of AU was prepared from 320 mM NaCl, 50 mM NaH_2PO_4, 6.52 mM $MgCl_2$, 164.2 mM KCl, 4.34 mM K_3Citrate, 43.8 mM $(NH_4)_2SO_4$ and 7.0 mM NH_4Cl and adjusted to a pH of 6 (using HCl 1M).[372] Standard solutions of calcium ($CaCl_2$ 120 mM) and oxalate (Na_2Ox 50 mM) were prepared separately. 16.7 ml $CaCl_2$ and 3 ml Na_2Ox was added to 480.3 ml of the AU stock solution and diluted to a volume of 1000 ml using milliQ water. The final concentration of calcium and oxalate was therefore 2.0 mM and 0.15 mM; these concentrations are within the range of urinary concentrations routinely determined in the KSRL during the past 5 years. The composition of AU is shown Table 4.2.

AU was kept at 4°C for up to 1 week and was discarded upon precipitation of salts and/or bacterial growth. All solutions were prepared in Millipore water with a resistivity of 18 mΩ.

Table 4.2 The composition of artificial urine.

Species	Concentration (mM)
Na^+	185.15
Cl^-	252.86
K^+	88.61
PO_4^{2-}	25
NH_4^+	47.3
SO_4^{2-}	21.9
Mg^{2+}	3.26
Cit	2.17
Ca^{2+}	2.0
Ox^{2-}	0.15

Real urine

24 hr urine samples were provided by 7 healthy males (while on their free diets) between the age of 18 – 25 years from the student cohort at the University of Cape Town. Each male provided a minimum of 1 x 24 hr sample; a total of 14 urine samples was analysed in the present study. Samples were collected in sterilised plastic bottles without any preservative. These were tested for bacteria and blood and were discarded if tested positive. All IP6 analysis was performed on the day that urine samples were received.

Calculations and statistical analysis

The reproducibility of both methods was determined by calculating the CV (expressed as a percentage). This was calculated using the following equation:

CV= (standard deviation/ average) x 100

Paired t-tests were performed for the comparison of urinary IP6 detected in the same urine sample using Method 1 vs Method 2. The software program GraphPad Instat 3 was used for this purpose.

Results

Assessment of the practical application of Method 2: animal study ‡

The mean urinary excretion profile of IP6 in rats in relation to its dietary intake is shown in Figure 4.5. All raw data are reported in Appendix 4.1. A noticeable decrease in urinary IP6 was observed after 6 days of consuming the phytate-deficient AIN- 76A diet and reached values that were close to zero within 15 days. When a standard diet containing 1 % IP6 was administered, urinary IP6 started to increase after 4 days and reached maximum values within 15 days. The same trend was observed in both male and female rats, irrespective of age. The reason for the decrease in urinary IP6 in male rats after day 30 is not obvious.

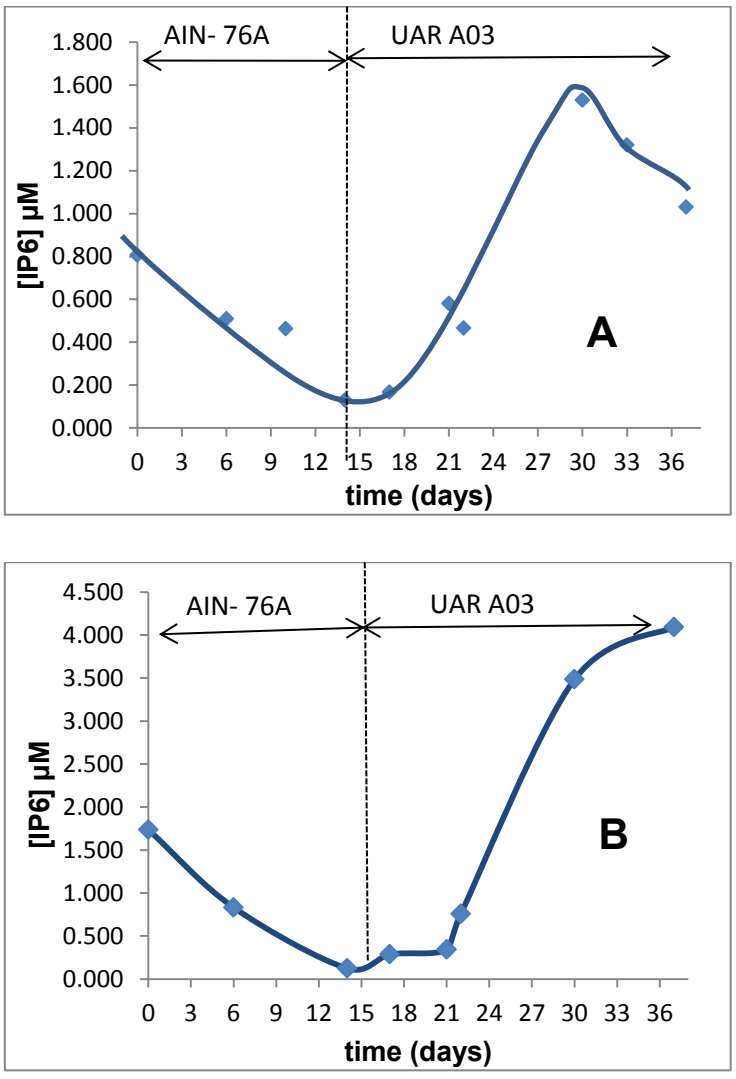


Figure 4.5 Urinary IP6 excretion in relation to its dietary intake over a period of 37 days in male (A) and female (B) Wistar rats.

Efficacy of an alternative resin

The mean results obtained for the removal of interfering ions as well as the reproducibility and percentage recovery of IP6 in AU is shown in Table 4.3 for both methods. All raw data are reported in Appendix 4.2. Consideration of the mean value and its SE for the assay of IP6 in AU without the addition of IP6 indicates that it's close to zero for both Method 1 and Method 2 (0.11 ± 0.02 µM and 0.30 ± 0.18 µM, respectively). This indicates that the removal of interfering ions was efficient. The CV was small indicating that reproducibility of both methods is good. However, the percentage recovery for Method 2 was found to be lower than for Method 1.

Table 4.3 Study of interferences, reproducibility and percentage recovery of IP6 in artificial urine.

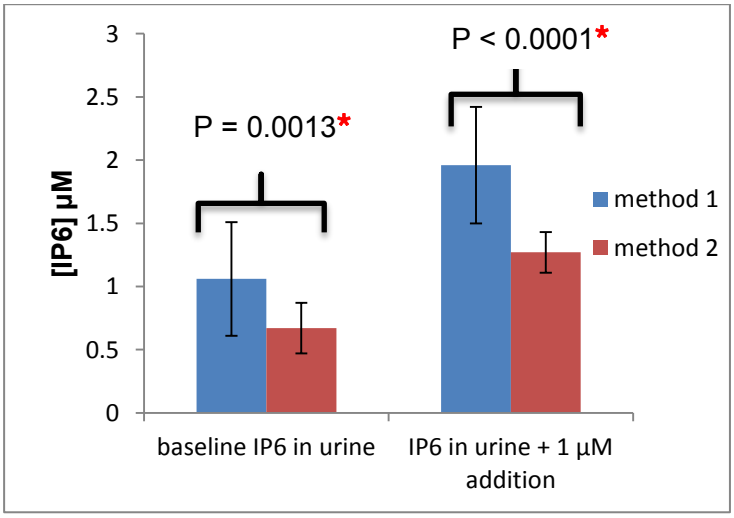
	IP6 added (µM)	IP6 detected ± SD (µM)	CV (%)	% recovery
Method 1				
AU (n =5)	0	0.11 ± 0.02	baseline	
AU (n= 5)	1	1.17 ± 0.09	7	106
AU (n = 5)	2	1.84 ± 0.13	7	87
Method 2				
AU (n =5)	0	0.30 ± 0.18	baseline	
AU (n= 5)	1	0.81 ± 0.11	14	51
AU (n = 5)	2	1.42 ± 0.09	7	56

The determination of IP6 as well as its percentage recovery in real urine samples, using both methods, is shown in Table 4.4. Each determination was performed in duplicate per urine sample; mean values are reported in Table 4.4 . For simplicity the standard error of the mean has been omitted in this table but is included in Appendix 4.2 (raw data).

Table 4.4 Detection and percentage recovery of IP6 in real urine.

	IP6 detected (μM)					
	Method 1			Method 2		
Addition of IP6 (μM)	0	1	% recovery	0	1	% recovery
Urine 1	1.79	2.71	92	0.62	1.36	73
Urine 2	1.61	2.64	103	0.90	1.52	63
Urine 3	1.41	2.22	81	0.77	1.30	53
Urine 4	0.40	1.27	87	0.36	1.25	88
Urine 5	1.31	2.12	82	0.66	1.37	72
Urine 6	0.84	1.58	75	0.79	1.32	53
Urine 7	0.24	1.09	85	0.48	1.06	59
Urine 8	1.37	2.13	76	1.08	1.55	46
Urine 9	1.38	2.13	74	0.80	1.30	50
Urine 10	0.65	1.64	99	0.67	1.24	58
Urine 11	1.23	2.06	83	0.71	1.28	57
Urine 12	0.81	1.68	87	0.43	0.99	56
Urine 13	0.98	2.14	116	0.66	1.27	61
Urine 14	0.89	1.96	106	0.40	1.04	64

Statistical comparisons of the above data are shown in Figure 4.6. Mean values, the standard error, p values as well as the percentage recovery is shown. These comparisons indicated that the baseline IP6 detected in urine using Method 2 was significantly lower compared to Method 1 ($p = 0.0013$). Furthermore, the concentration of IP6 detected in urine after the addition of 1 μM IP6 was also significantly lower for Method 2 ($p < 0.0001$), culminating in a lower % recovery.



Mean % recovery:

Method 1: 89 ± 3

Method 2: 61 ± 3

Figure 4.6 Statistical comparison of IP6 detected in urine using Method 1 and Method 2. Error bars indicate the standard error of the mean. *Indicates statistical significance.

Comment

The colorimetric assay used in Method 1 is based on a decrease in the concentration of Fe(III)-SCN upon the complexation of Fe to IP6. This assay is however compromised by traces of reducing substances which remain after purification of samples which reduce Fe(III) to Fe(II) and results in a false positive for the detection of IP6. To overcome this limitation, Method 2 was developed by Costa-Bauza[370] and colleagues (as mentioned earlier in this Chapter) in which Al(III)-xylenol orange was used as an alternative metal-dye system. Aluminium has 3 oxidation states i.e +1, +2 and +3. However, the latter oxidation state is most common. This metal is therefore not susceptible to reduction by traces of reducing substances in the purified sample hence overcoming the aforementioned limitation when using Fe. In addition to this modification, the purification and preconcentration of IP6 via SPE has also been modified by Costa-Bauza[370] and colleagues by using EDTA in the sample preparation. This was done to complex metals such as calcium and magnesium, subsequently preventing the loss of IP6 during an intermediate step of the purification process as the latter is able to bind to these metals. The lower percentage recovery in Method 2 (using the alternative resin, Dowex 1x 8) was therefore surprising as EDTA was used to increase the recovery of IP6. However, EDTA has four carboxylic acid groups with pKa's between 0 – 2.66. It is therefore possible that some of the un-chelated EDTA adsorbed to the resin (Dowex 1x 8) due to its high negative charge. This has been shown for other anion exchange resins in which the purification of proteins was studied.[373] The adsorption of EDTA therefore resulted in the reduction of the resin capacity in the present study and the subsequent loss of IP6 during the washing step which led to low percentage recoveries.

Conclusions

Proof of the practical application of Method 2 (using the AG 1x8 resin) was demonstrated in this study since it was able to detect a decrease in urinary IP6 concentrations when its dietary intake was restricted as well as a gradual increase thereof when dietary IP6 was administered to rats. However, assessment of the more affordable, alternative resin (Dowex 1x8) for utilization in both Method 1 and Method 2 showed that this resin was unsuitable for the latter method due to a reduction in the resin's capacity as described above. Method 1 was therefore chosen for IP6 urine analysis in subsequent studies undertaken in the present thesis in the KSRL.

Chapter 5 : *In vitro* study of the effect of phytate on calcium oxalate crystallisation

Introduction

The crystallisation propensity of urine may be used as an evaluation of the risk to form kidney stones.[374] Thus, *in vitro* crystallisation experiments in both inorganic media and real urine remain of fundamental importance in urolithiasis research.[50, 374, 375] Different methods have been developed to mimic physiological aspects of the renal system as well as to differentiate between processes of crystallisation such as nucleation, aggregation and growth.[50] These methods may be classified by the type of process used, the analytical technique or by the supersaturation profile.[50] The crystallisation experiments may be either batch (discontinuous), continuous, seeded or unseeded.[50] Many of these experiments may be conducted using sophisticated instrumentation or by using simple and affordable methods (depending on the equipment that's available). However, inter-procedural comparisons may not necessarily be the same.[376] It is therefore important to use caution when making inter-procedural comparisons and the same method should be used for quantitative comparisons.[377]

In vitro experiments do however have certain limitations regarding the imitation of physiological conditions e.g. simple experiments in inorganic media in which the urine flow dynamics are not modelled.[374, 378] It should also be noted that results obtained in inorganic media do not necessarily reflect those of real urine as the latter is a complex medium containing many components which may act as inhibitors and/ or promoters of crystallisation[375, 379] Nevertheless, experiments in inorganic media provide useful insight into the effect of individual urinary components in isolation on crystallisation mechanisms.[374]

In the present thesis, theoretical modelling showed that phytate (IP6) does not affect the concentration of ionized calcium (Ca^{2+}) nor the supersaturation of urine with respect to CaOx and CaP under physiological conditions. The objectives of the present chapter were therefore as follows:

1. To test experimentally the theoretical predictions described earlier in this thesis (Chapter 4), using *in vitro* experiments in which the concentration of Ca^{2+} in artificial and real urine is determined in the presence of physiological and non-physiological concentrations of IP6.

2. To determine and compare the effects (if any) of IP6 on crystallisation propensity in artificial urine and in urine of South African black and white subjects by measuring

CaOx MSLs, CaOx crystallisation rates, CaOx crystal size distributions and CaOx crystal morphologies.

3. To investigate the effect of IP6 on specific CaOx kinetic crystallisation mechanisms (nucleation, growth and aggregation).

Methods

Experimental approach

Various experiments were conducted in AU and in pooled urine of black (BPU) and white (WPU) subjects. These are summarised in Table 5.1. These experiments were conducted to achieve the first two objectives of the present study as outlined in the introduction to this chapter.

Experiments conducted to achieve the third objective of the present study as outlined in the introduction to this chapter, namely to determine the effect of IP6 on specific crystallisation mechanisms, were conducted in metastable CaOx solutions. These are summarised in Table 5.2.

Table 5.1 Summary of various experiments conducted in artificial and pooled urine of black and white subjects to achieve objectives 1 and 2.

	$[Ca^{2+}]$	n	CaOx MSL	n	Crystallisation kinetics	n	PSD	n	SEM	$n^{‡}$
AU + 0 µM IP6 (**AU0**)	√	6	√	3	√	3	√	3	√	2
AU + 0.757 µM IP6 (**AU1**)	√	3	√	3	√	3	√	3	√	2
AU + 2.27 µM IP6 (**AU2**)	√	3	√	3	√	3	√	3	√	2
AU + 4.45 µM IP6 (**AU3**)	√	3	√	3	√	3	√	3	√	2
AU + 15 µM IP6 (**AU4**)	n.d*	_	√	3	n.d**	_	n.d**	_	n.d**	_
BPU	√	3	√	3	√	3	n.d***	_	√	2
BPU + 2.27 µM (**BPU2**)	√	3	√	3	√	3			√	2
BPU + 4.45 µM (**BPU3**)	√	3	√	3	√	3			√	2
BPU + 15 µM (**BPU4**)	√	3	√	3	√	3			√	2
BPU + 30 µM (**BPU5**)	√	3	√	3	√	3			√	2
WPU	√	3	√	3	√	3	n.d***	_	√	2
WPU + 2.27 µM (**WPU2**)	√	3	√	3	√	3			√	2
WPU + 4.45 µM (**WPU3**)	√	3	√	3	√	3			√	2
WPU + 15 µM (**WPU4**)	√	3	√	3	n.d**	_			n.d**	_
WPU + 30 µM (**WPU5**)	√	3	√	3	n.d**	_			n.d**	_

Abbreviations shown in parentheses are used throughout the chapter. Concentrations of IP6 in BPU2- 5 and WPU2- 5 are the total concentrations. Concentrations of 15 µM and 30 µM IP6 were chosen based on a previous study.[285]

n: number of experiments conducted. $n^{‡}$: number of SEM stubs.

*not determined; no attempt made.

**not determined; excessively high MSL thus crystallisation could not be induced.

***not determined; unavailability of a Coulter Counter at the time at which experiments were conducted.

Table 5.2 Summary of various experiments conducted in unseeded and seeded metastable CaOx solutions to achieve objective 3.

metastable solution of CaOx + Test substance‡	Crystal Nucleation & Aggregation assay	n	Crystal Growth assay	n
Metastable CaOx solution + 0 µM IP6 (control)	√	18	√	15
Metastable CaOx solution + 0.757 µM IP6	√	6	√	6
Metastable CaOx solution + 2.27 µM IP6	√	6	√	6
Metastable CaOx solution + 4.45 µM IP6	√	5	√	6
Metastable CaOx solution (control)	√	6	n.d*	–
Metastable CaOx solution + AU0	√	7		
Metastable CaOx solution + AU1	√	5		
Metastable CaOx solution + AU2	√	4		
Metastable CaOx solution + AU3	√	5		
Metastable CaOx solution (control)	√	3	√	6
Metastable CaOx solution + PU	√	6	√	6
Metastable CaOx solution + (PU + 2.27 µM IP6; **PU2**)	√	6	√	6
Metastable CaOx solution + (PU + 4.45 µM IP6; **PU3**)	n.d*	–	√	6

Abbreviations shown in parentheses will be used throughout the chapter. Concentrations of IP6 in PU2 and PU3 indicates the total concentration.

‡In cases where urine and urine dosed with various concentrations of IP6 were used as the test substance, the following volume ratios were used in the respective assays: nucleation and aggregation assay- **1 ml** $CaCl_2$: **200 µl** urine : **1 ml** K_2Ox and in the growth assay- **2 ml** CaOx solution : **200 µl** urine. These ratios were used to obtain a sufficient difference in the rate of each crystallisation mechanism relative to the control.[210]

*not determined; malfunction of instrument at the time at which experiments were conducted.

Phytic acid

Stock solutions of phytate (1 mM and 10 mM) were prepared by dissolving phytic acid dipotassium salt (*Sigma*) in milliQ water. These were used for standard additions in experiments which will be described below.

Preparation of artificial urine

Artificial urine was prepared as described in Chapter 4. The composition of this medium is shown in Table 4.2.

Real urine collection and treatment

24 hr urine samples were collected by healthy black (n= 6) and white (n= 6) South African males from the University of Cape Town. The latter was used in objective 3 of the present study. Each subject provided one 24 hr sample. These 24 hr samples were collected in plastic bottles without any preservative. Urine samples were tested for the presence of blood and infection (*Combur 10 test strip, Boehringer Manheim, Manheim Germany*) and were discarded if tests were positive. All samples were filtered through a 0.75 µm filter paper followed by a 0.45 µm nitrocellulose filter paper prior to all crystallisation experiments to remove cellular debris.

For objective 1 and 2 of the present study, urine samples were pooled according to race to eliminate the effect of intra-racial urinary differences with respect to composition.[304, 305] Each pool was constituted by mixing equal volumes (300 ml) of the 6 urine samples in accordance with a previous study on the optimum number of samples required for a statistically representative pool.[380] The endogenous amount of IP6 in pooled urine of black subjects (BPU) and white subjects (WPU) was determined, using the method described in Chapter 4, prior to standard additions of IP6 (Appendix 5.5). The urine composition of each pool was determined (as described in Chapter 6) and is shown in Table 5.3 together with the SS of CaOx, brushite and urate (calculated using JESS[321, 322]).

Table 5.3 Urine composition and the relative supersaturation of salts in pooled urine samples of black and white subjects.

Parameter	BPU	WPU
pH	6.13	6.24
Citrate (x 10^{-3} mol/L)	2.01	1.67
Oxalate (x 10^{-4} mol/L)	1.95	1.56
Calcium (x 10^{-3} mol/L)	1.93	1.68
Magnesium (x 10^{-3} mol/L)	1.13	1.27
Sodium (x 10^{-1} mol/L)	1.53	1.51
Potassium (x 10^{-2} mol/L)	2.22	1.91
Urate (x 10^{-3} mol/L)	3.92	3.40
Phosphate (x 10^{-2} mol/L)	3.23	3.03
Chloride (x 10^{-1} mol/L)	1.38	1.31
Phytate (x 10^{-6} mol/L)	1.00	1.10
RS CaOx	4.05	2.90
RS Brushite	1.97	1.96
RS Urate	1.49	1.04

For objective 3 of the present study, an additional set of 24 hr urine samples was collected by healthy males (n =4), irrespective of race. These were pooled (to eliminate the effect of inter-urinary differences with regard to composition) and treated as described above. The urine composition and SS of salts in this pooled urine (PU) is shown in Table 5.4.

Table 5.4 Urine composition and the relative supersaturation of pooled urine used for objective 3 of the present study.

Parameter	PU
pH	6.36
Citrate (x 10^{-3} mol/L)	1.61
Oxalate (x 10^{-4} mol/L)	1.40
Calcium (x 10^{-3} mol/L)	1.84
Magnesium (x 10^{-3} mol/L)	1.13
Sodium (x 10^{-2} mol/L)	9.65
Potassium (x 10^{-2} mol/L)	3.15
Urate (x 10^{-3} mol/L)	2.36
Phosphate (x 10^{-2} mol/L)	1.52
Chloride (x 10^{-1} mol/L)	1.38
Phytate (x 10^{-6} mol/L)	0.708
RS CaOx	3.59
RS Brushite	1.67
RS Urate	0.56

Objectives 1 & 2: Ca^{2+} and crystallisation propensity

Determination of ionized calcium

Ca^{2+} was determined using a calcium ion selective electrode (*Photometer, Metrohm, Swiss*). The electrode was calibrated regularly, to ensure that the Nernstian equation was obeyed, using six calcium standards in the range of 0.1 – 50 mM. A typical calibration plot obtained in the present study is shown in Figure 5.1. Ca^{2+} was measured in samples at 37 °C.

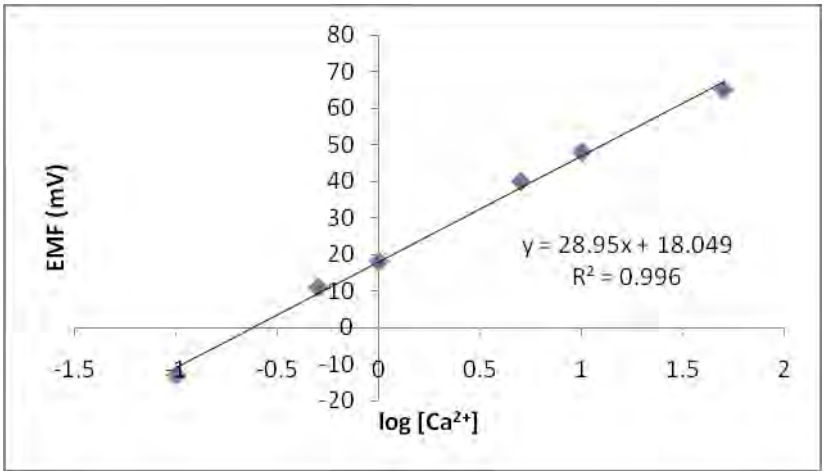


Figure 5.1 Calibration curve used to determine ionized calcium in urine samples.

CaOx metastable limit

The CaOx metastable limit (MSL) is a measure of the ability of a solution to resist spontaneous crystallisation.[50] In whole urine this can be used as an indicator of the urine's heterogeneous nucleation capacity.[50] The protocol described by Ryall was used to determine the CaOx MSL of a particular solution[233] but a spectrophotometer (*Spectronic Unicam, Helios gamma*) instead of a Coulter Counter was used to follow crystallization by measuring the change in turbidity at 620 nm.

In this method, 10 ml aliquots (x 13) of each sample were incubated at 37°C. Each of these was then dosed with progressively increasing concentrations of sodium oxalate (Na_2Ox) to obtain final concentrations of oxalate between 0.15 – 1.95 mM. Dosing was performed at 1 minute intervals. After an incubation period of 30 minutes per aliquot, the absorbance was measured in a glass cuvette. The MSL was determined by plotting the absorbance versus Na_2Ox concentration. The concentration at which a sudden increase in absorbance occurred was taken as the MSL. A typical plot as obtained in the present thesis is shown in Figure 5.2.

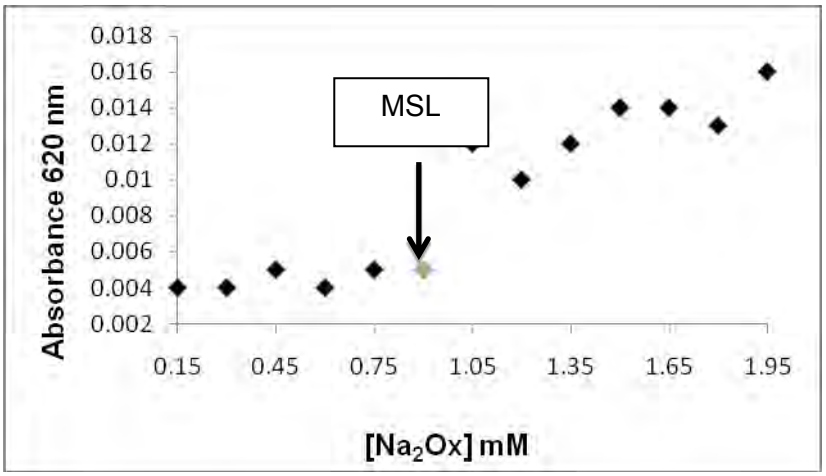


Figure 5.2 A typical plot of absorbance vs concentration of Na_2Ox from which the CaOx MSL of a solution is determined.

CaOx Crystallisation kinetics

Crystallisation kinetics were determined in samples by adding Na_2Ox (500 µl per 50 ml sample) at a concentration of 0.15 mM above the previously determined MSL (thus inducing crystallisation) and measuring the change in turbidity at 620 nm over a certain time period as described below. In cases where crystallisation failed to be initiated after dosing with 0.15mM Na_2Ox, a higher dosing concentration was used. Samples (50 ml) were incubated in soda-lime bottles in a shaking water bath (100 rpm; *Labcon Johannesburg*) at 37 °C. The absorbance at 620nm was measured at time zero and every 15 minutes over a period of 2 hrs. A typical kinetics plot as obtained in the present thesis is shown in Figure 5.3. The rate of CaOx crystallisation was determined from the slope of the curve with increasing absorbance.

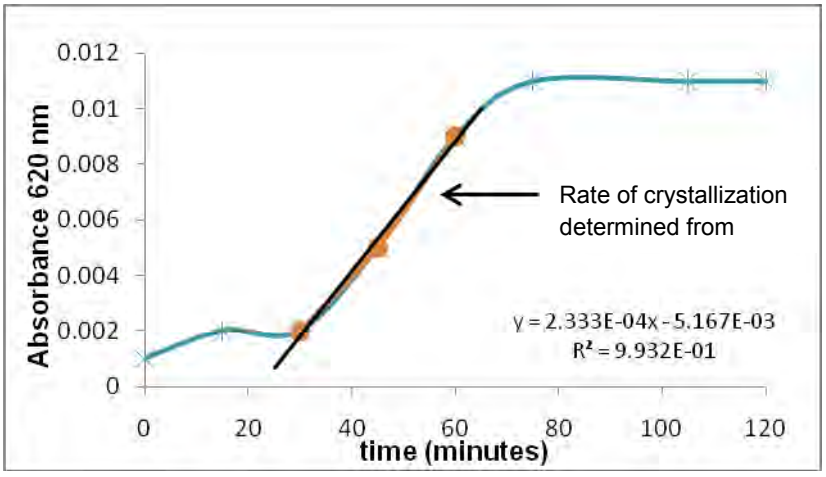


Figure 5.3 A typical plot of absorbance vs time which depicts CaOx crystallisation kinetics.

Particle size distribution

CaOx crystallisation was induced in samples as described above for the crystallisation kinetics experiments and were incubated at 37 °C in a shaking water bath (100 rpm; *Labcon, Johannesburg*) for a duration of 2 hrs. The mean particle size and total volume of the precipitated particles were measured using a Coulter Counter (*Multisizer III, Coulter Electron, England*). Graphs of particle volume vs particle size were plotted. A typical graph as obtained in the present thesis is shown in Figure 5.4. The mode of the curve was regarded as the average size of particles precipitated in a particular solution.

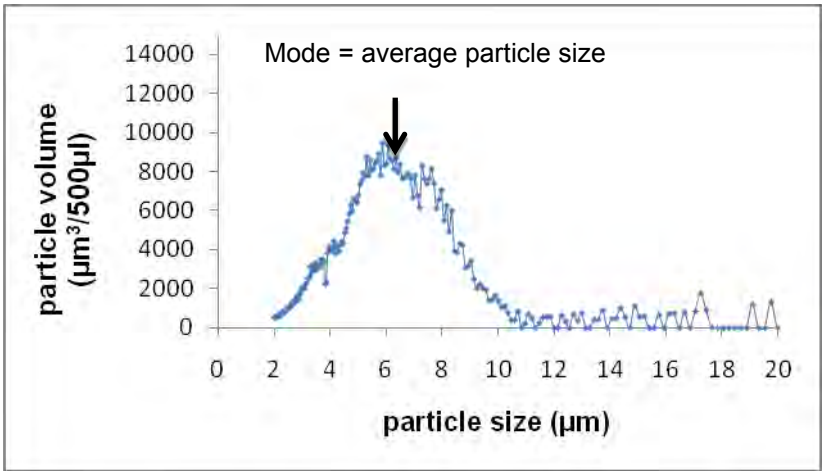


Figure 5.4 A typical particle volume- size distribution plot as obtained in the present study.

Scanning electron microscopy

At the end of each kinetics experiment (i.e after 2 hrs) as described above, 2 ml of each sample was filtered through a 0.22 µm filter paper (*Merck Millipore*). Filter papers were dried at room temperature and glued onto aluminium stubs for the analysis of crystals. Each stub was coated with gold palladium and crystals were viewed using a *Nova NanoSEM 230* scanning electron microscope operating at a landing energy of 5.00 keV, spot size of 2 and working distance of 5.5 - 6.1 mm. Stubs were viewed at magnifications of x 1000 to obtain an overview of the entire surface and at x 5000 to record typical deposits.

Objective 3: specific crystallisation mechanisms

Nucleation and aggregation

These crystallisation characteristics were investigated as described in a spectroscopic assay which was developed by Hess et al.[381] This crystallisation experiment is a simple batch/ discontinuous process in which the change in particle number in a metastable supersaturated solution of CaOx is followed continuously by measuring a change in turbidity

as a function of time. The assay was designed for the investigation of the effects of individual urinary components in isolation, on these crystallisation mechanisms.

The metastable supersaturated solution of CaOx was prepared by mixing two solutions as described below.

Stock solutions of calcium (8.5 mM $CaCl_2$, 200 mm NaCl, 10 mM NaAc, pH 5.7) and oxalate (1.0 mM $K_2C_2O_4$, 200 mM NaCl, 10mM NaAc, pH 5.7) were prepared in distilled water, filtered (0.22 µM) daily before use and incubated in a water bath at 37°C. 1 ml of the calcium solution was transferred to a glass cuvette followed by the addition of a test substance (IP6, artificial urine, artificial urine dosed with various concentrations of IP6, pooled urine, pooled urine dosed with various concentrations of IP6). The volume of the test substance was varied to achieve various desired concentrations as described on page 92. Oxalate (1 ml) was added last to induce crystallisation. Absorbance was measured at 620 nm using a UV-Vis spectrophotometer (*AnalytiKJena, Germany*) fitted with a thermostatted mantle (37°C) and magnetic stirrer (500 rpm) for up to 1800s.

The rate of nucleation was measured from the slope of the linear part of the graph with increasing absorbance; aggregation was measured from the slope of the linear part of the graph with decreasing absorbance. The percentage inhibition of CaOx nucleation (% I_N) and aggregation (% I_A) was calculated from the following equation:

$$\textbf{\% Inhibition} = 100 - [100 \times (S_T/S_C)]$$

where S_T and S_C are the slope of the test mixture and the control; respectively. A typical plot as obtained in the present thesis is shown in Figure 5.5.

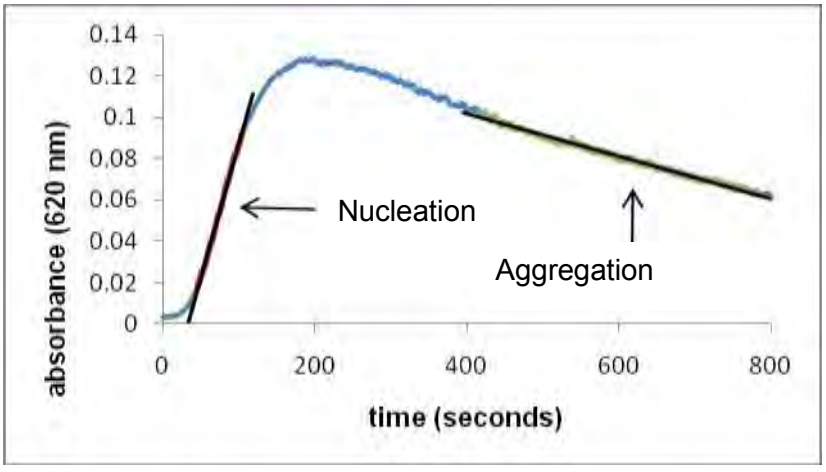


Figure 5.5 A typical plot of absorbance vs time which depicts simultaneous nucleation and aggregation.

Growth

Crystal growth was monitored using a modified seeded crystal growth method.[382] Similar to the above experiment, this study was designed to investigate the effect of individual urinary components in isolation on this crystallisation process. In this method crystal growth is measured by the decay of supersaturation which is measured indirectly by the change in turbidity. A metastable CaOx solution (0. 5 mM $CaCl_2$ and 0.5 mM Na_2Ox) was prepared in a tris(hydroxyl-methyl)-aminomethane (Tris) buffer (10 mM Tris HCl, pH 7.2, 90 mM NaCl). [382] The mixture was stirred at room temperature for the duration of the experiment. In addition, a crystal slurry (16 mg/ml) was prepared from COM crystals in the same tris buffer and was stirred overnight to allow for equilibration. These COM crystals were prepared using the method described by Pak et al[383]. Crystal type was confirmed by powder X-ray diffraction.[384] An aliquot of the metastable CaOx solution (2 ml) was transferred to a quartz cuvette in a UV-VIS spectrophotometer (*AnalytiKJena, Germany*) fitted to a thermostatted mantle (37°C) and magnetic stirrer (500 rpm). The test substance (IP6, artificial urine, artificial urine dosed with various concentrations of IP6, pooled urine, pooled urine dosed with various concentrations of IP6) was then added to achieve various desired concentrations as described on page 92. Finally, the COM slurry (50 µl) was added last to induce crystal growth. Absorbance was measured at 214 nm for 400-600 s.

The percentage inhibition of CaOx growth (% I_G) was calculated from the following equation:

$$\% \text{ Inhibition} = [(S_C - S_T)/ S_C] \times 100$$

where S_C and S_T represent the slope of the control and the test mixture; respectively. A typical plot obtained in the present thesis is shown in Figure 5.6.

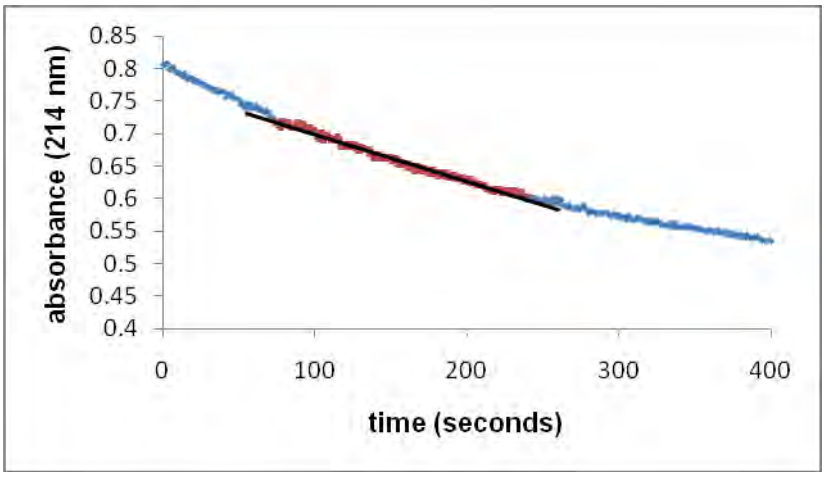


Figure 5.6 A typical plot of absorbance vs time from which the rate of CaOx crystal growth is determined.

Statistical analysis

Data were statistically analysed using GraphPad Instat3. One-way analysis of variance (ANOVA) was performed for multiple comparisons (3 or more groups) followed by a post-hoc Tukey- Kramer test. Unpaired t-tests were performed for inter-group comparisons. A p value < 0.05 was regarded as statistically significant.

Results

Ca^{2+} and crystallisation propensity

Artificial urine

Concentration of Ca^{2+}

The effect of IP6 on the mean concentration of Ca^{2+} in AU is shown in Figure 5.7. Actual values are reported in Table 5.5. All raw data are in Appendix 5.1. No significant change in the concentration of Ca^{2+} was observed.

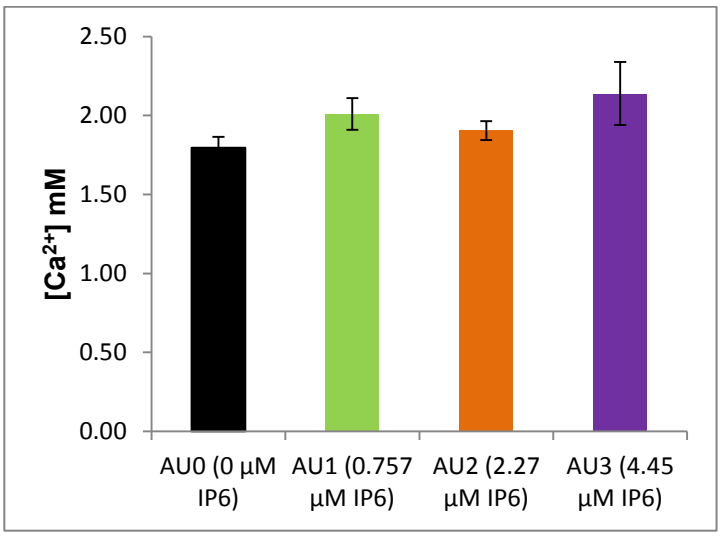


Figure 5.7 Effect of IP6 on free calcium in AU. Error bars indicate standard error of the mean.

Table 5.5 Concentration of Ca^{2+} in AU and in AU dosed with IP6. Mean ± SE.

sample	[Ca2+] mM
AU0 (0 µM IP6)	1.80 ± 0.07
AU1 (0.757 µM IP6)	2.01 ± 0.10
AU2 (2.27 µM IP6)	1.90 ± 0.05
AU3 (4.45 µM IP6)	2.24 ± 0.20

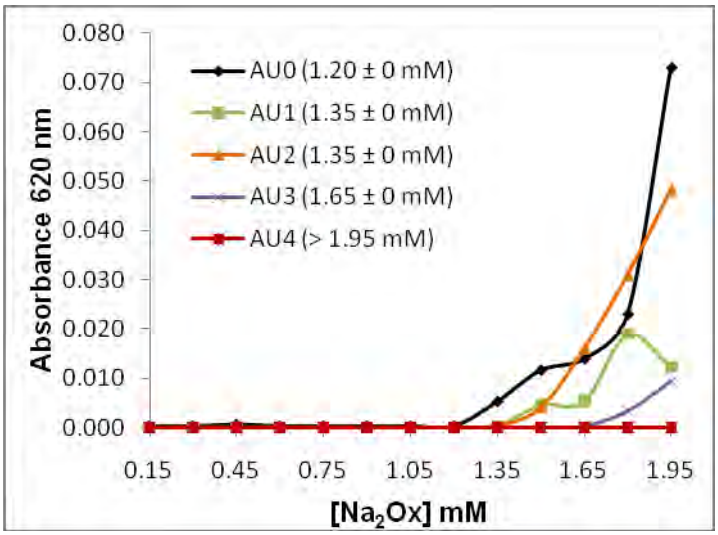
CaOx MSL

The mean CaOx MSL of AU and AU dosed with 4 different concentrations of IP6 are shown in Figure 5.8. The CaOx MSL of each replicate was identical and therefore had a standard deviation of zero; thus statistical analysis could not be performed. Instead, the percentage difference with respect to the control was calculated as follows:

$$[MSL_T - MSL_c / \text{average}] \times 100$$

where MSL_T and MSL_C refer to the MSL of the test and the control urine; "average" refers to the average value of the two MSLs. In the present study a % difference in the MSL of more than 30 % was regarded as "substantial". This arbitrary definition is based on five previously published studies in which percentage differences of 26, 37, 59, 80 and 86 were found to be statistically significant.[249, 296, 302, 305, 306]

These results showed that the CaOx MSL was not affected at the two lower physiological concentrations of IP6. A substantial increase in the MSL was however observed at the higher physiological (4.45 µM) and non-physiological concentrations of IP6 (15 µM). Raw data are included in Appendix 5.2.



Comparisons (% difference):

AU0 vs AU1: 12 %

AU0 vs AU2: 12 %

AU0 vs AU3: 32 %

AU0 vs AU4: > 48%

Key :

AU0 : 0 µM IP6

AU1 : 0.757 µM IP6

AU2 : 2.27 µM IP6

AU3 : 4.45 µM IP6

AU4 : 15 µM IP6

Figure 5.8 Mean change in absorbance vs [Na_2Ox] in AU indicating the CaOx MSL in the presence of IP6. The mean CaOx MSLs ± SE are shown in parentheses in the figure legend.

CaOx Crystallization Kinetics

As mentioned in the methods section, crystallisation kinetics were determined by the addition of Na_2Ox. However, preliminary experiments in AU with the addition of Na_2Ox at 0.15 mM higher than the MSL failed to cause any increase in crystallisation; thus a concentration of 0.3 mM above the MSL was used.

The kinetics plots in Figure 5.9 show the mean change in absorbance upon crystallisation with respect to time in AU and AU dosed with 3 different concentrations of IP6. Each experiment was performed in triplicate. All raw data are reported in Appendix 5.2. The decrease in the absorbance which occurs at 90 minutes in two of the plots (AU0 and AU2) is due to crystals settling below the path of the UV beam.

The mean rate of crystallisation was determined from the slope of each curve between 30 and 75 mins, and is shown in Figure 5.10. Actual values are reported in Table 5.6.

The rate of crystallisation decreased significantly in the presence of all concentrations of IP6 relative to AU0 ($p < 0.001$). The decrease in the rate of crystallisation was concentration dependent and showed the following trend:

AU0 > AU1 > AU2 = AU3

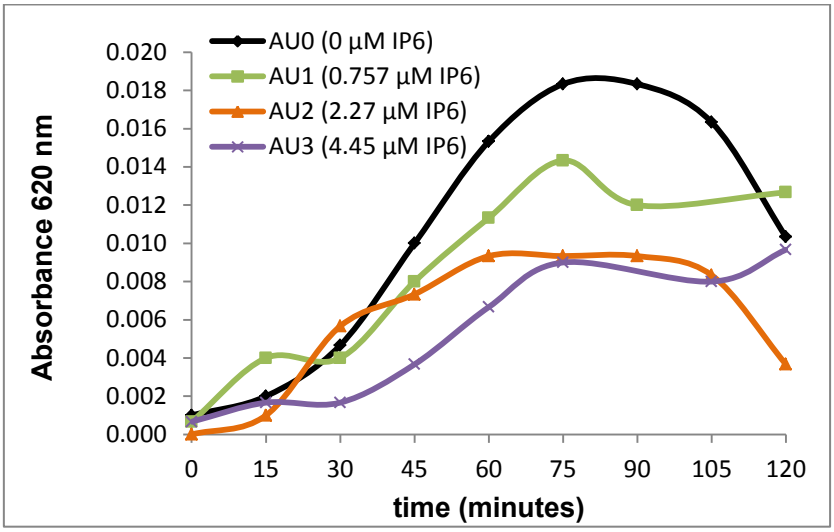
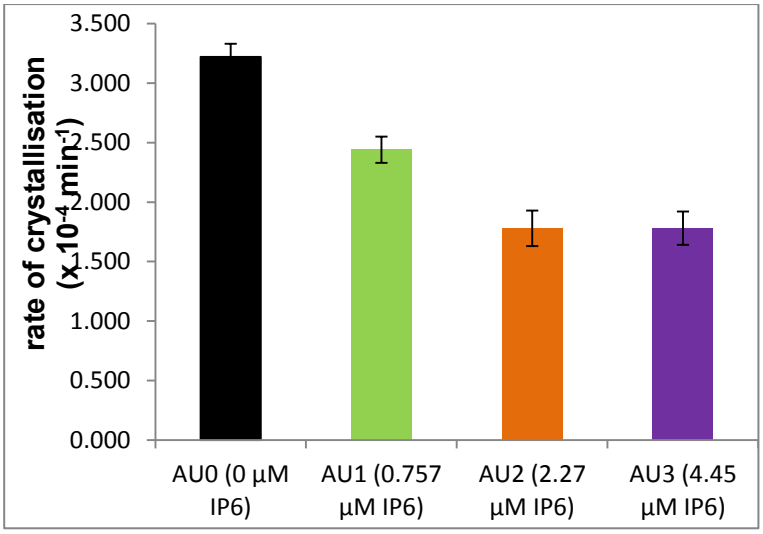


Figure 5.9 Mean change in absorbance vs time in AU indicating CaOx crystallisation kinetics in AU in the presence of IP6.



Comparisons:

AU0 vs AU1 : P < 0.05*

AU0 vs AU2: P < 0.001*

AU0 vs AU3: P < 0.001*

AU1 vs AU2: P < 0.05*

AU1 vs AU3: P < 0.05*

AU2 vs AU3: P > 0.05

Figure 5.10 Effect of IP6 on the rate of CaOx crystallisation in AU. Error bars indicate the standard error of the mean *Indicates statistical significance.

Table 5.6 Rate of crystallisation in AU and AU dosed with IP6. Mean ± SE.

Sample	Rate of crystallisation ($x\ 10^{-4}\ min^{-1}$)
AU0 (0 µM IP6)	3.22 ± 0.11
AU1 (0.757 µM IP6)	2.44 ± 0.11
AU2 (2.27 µM IP6)	1.78 ± 0.15
AU3 (4.45 µM IP6)	1.78 ± 0.14

Particle Size Distributions

The mean PSD of particles in AU in the presence of different concentrations of IP6 are shown in Figure 5.11. The mean particle size of crystals obtained from the mode of each plot is shown in Table 5.7. Each experiment was performed in triplicate. Plots of all raw data are shown in Appendix 5.3. The addition of 4.45 µM IP6 (AU3) decreased the particle size significantly relative to AU0 ($p < 0.01$).

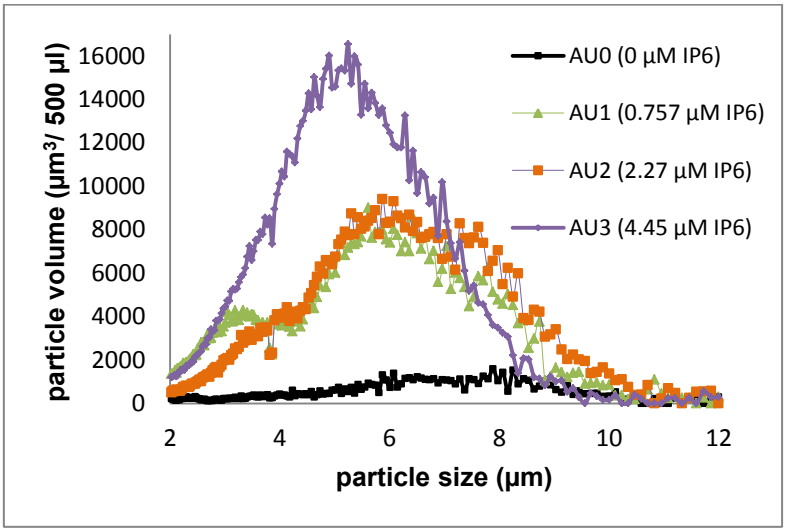


Figure 5.11 Effect of IP6 on the particle size distribution in AU.

Table 5.7 Effect of IP6 on the average particle size in AU. Mean ± SE.

	Particle size (µm)	P-value (comparison relative to AU0)
AU0 (0 µM IP6)	7.15 ± 0.12	--
AU1 (0.757 µM IP6)	6.65 ± 0.40	> 0.05
AU2 (2.27 µM IP6)	6.81 ± 0.23	> 0.05
AU3 (4.45 µM IP6)	5.26 ± 0.16	< 0.01*

*Indicates statistical significance.

Scanning Electron Microscopy

Electron micrographs of crystals deposited in AU and AU dosed with different concentrations of IP6 were recorded at the same magnification and are shown in Figure 5.12. The total surface-area of each stub was examined and electron micrographs of typical deposits with respect to morphology, size and aggregation were recorded. Several single, oval-shaped COM crystals (approximate size: 8 µm) were predominant in AU control (Figure 5.12 a). A few small aggregates of tiny COM crystals (approximate size: 4 µm) were observed in the presence of larger COM crystals (approximate size: 10 µm) in AU1 (Figure 5.12 b). Very few crystals were observed in AU2; these included a few small, bipyramidal COD crystals (approximate size: 4 µm) (Figure 5.12 c). Several small aggregated COD crystals in the presence of larger single COD crystals (approximate size: 8 µm) were observed in AU3 (Figure 5.12 d) no COM crystals were observed.

To summarise, the overall trend observed in AU is that the hydration of the CaOx crystal changed from COM to COD with increasing IP6 concentration.

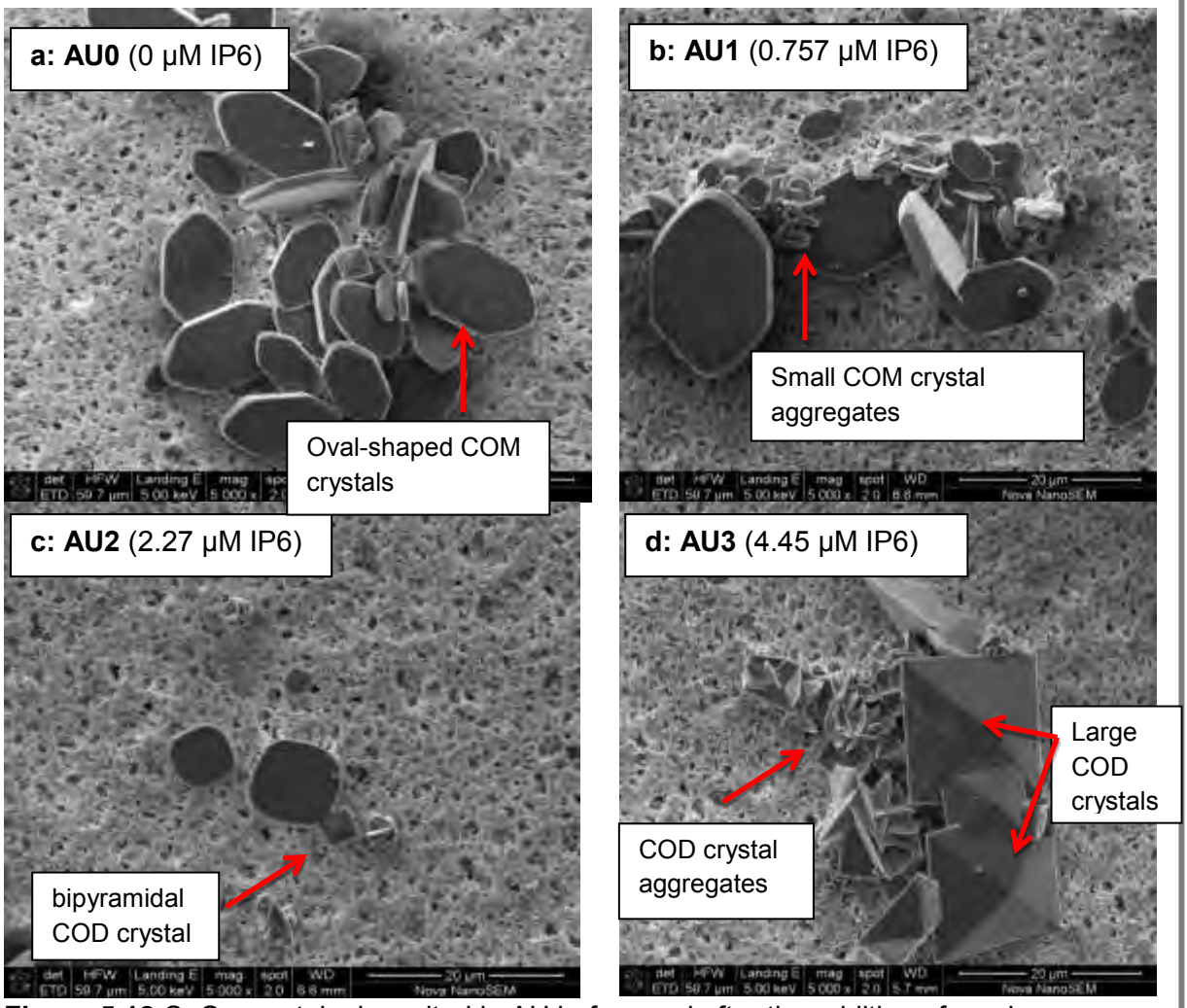
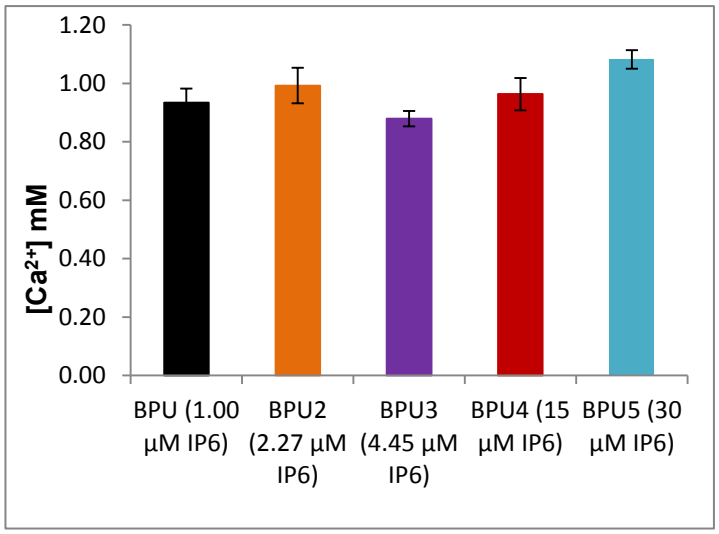


Figure 5.12 CaOx crystals deposited in AU before and after the addition of varying concentrations of IP6.

Real urine of black and white subjects

Concentration of Ca^{2+}

The effect of IP6 on the mean Ca^{2+} in pooled urine of black subjects is shown in Figure 5.13. Actual values are reported in Table 5.8. Each experiment was performed in triplicate. Raw data are reported in Appendix 5.4. No significant change in Ca^{2+} was observed.



Comparisons:

BPU vs BPU2: $p > 0.05$

BPU vs BPU3: $p > 0.05$

BPU vs BPU4: $p > 0.05$

BPU vs BPU5: $p > 0.05$

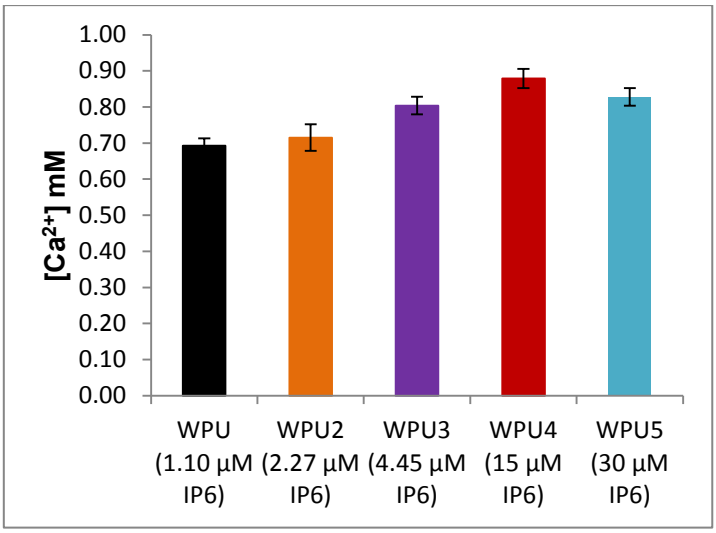
Figure 5.13 Effect of IP6 on the concentration of Ca^{2+} in pooled urine of black subjects. Error bars indicate standard error of the mean.

Table 5.8 Concentration of Ca^{2+} in BPU and BPU dosed with IP6. Mean ± SE.

sample	$[Ca^{2+}]$ mM
BPU (1.00 µM IP6)	0.93 ± 0.05
BPU2 (2.27 µM IP6)	0.99 ± 0.06
BPU3 (4.45 µM IP6)	0.88 ± 0.03
BPU4 (15 µM IP6)	0.96 ± 0.06
BPU5 (30 µM IP6)	1.08 ± 0.03

Figure 5.14 shows the effect of IP6 on the mean concentration of Ca^{2+} in pooled urine of white subjects. Actual values are reported in Table 5.9. Each experiment was performed in triplicate and raw data are reported in Appendix 5.4. Similar to the experiments in urine of black subjects, no change in Ca^{2+} was observed.

Intergroup comparisons are reported later on page 114.



Comparisons:

WPU vs WPU2: $p > 0.05$

WPU vs WPU3: $p > 0.05$

WPU vs WPU4: $p > 0.05$

WPU vs WPU5: $p > 0.05$

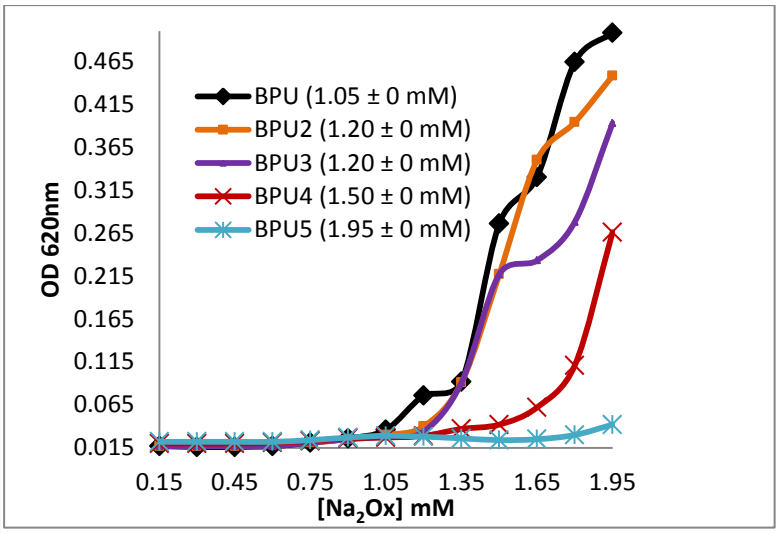
Figure 5.14 Effect of IP6 on the concentration of Ca^{2+} in pooled urine of white subjects. Error bars indicate standard error of the mean.

Table 5.9 Concentration of Ca^{2+} in WPU and WPU dosed with IP6. Mean ± SE.

sample	[Ca2+] mM
WPU (1.10 µM IP6)	0.69 ± 0.02
WPU2 (2.27 µM IP6)	0.72 ± 0.04
WPU3 (4.45 µM IP6)	0.80 ± 0.02
WPU4 (15 µM IP6)	0.88 ± 0.03
WPU5 (30 µM IP6)	0.83 ± 0.02

CaOx MSL

The effect of IP6 on the mean CaOx MSL in BPU is shown in Figure 5.15. Each experiment was performed in triplicate. All raw data are reported in Appendix 5.5. The percentage difference, relative to the baseline sample, is also reported. It is seen that the physiological concentrations of IP6 had no affect the CaOx MSL. However, MSL values increased substantially at the non-physiological concentrations of 15 µM and 30 µM IP6.



Comparisons (% difference):

BPU vs BPU2: 13 %

BPU vs BPU3: 13 %

BPU vs BPU4: 35 %

BPU vs BPU5: 60 %

Key :

BPU : control

BPU2 : 2.27 µM IP6

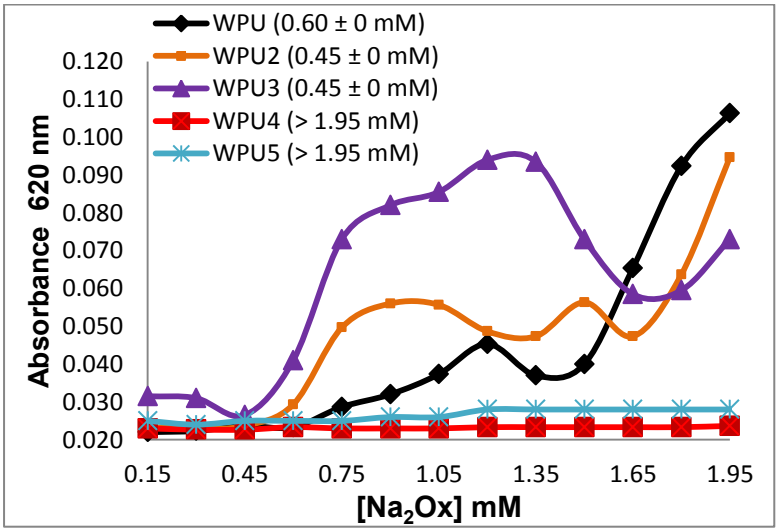
BPU3 : 4.45 µM IP6

BPU4 : 15 µM IP6

BPU5 : 30 µM IP6

Figure 5.15 Mean change in absorbance vs [Na_2Ox] in BPU indicating the CaOx MSL in the presence of IP6. The mean CaOx MSLs ± SE are shown in parentheses in the figure legend.

Figure 5.16 shows the effect of IP6 on the mean CaOx MSL in WPU. Each experiment was performed in triplicate. Similar to the black males, the physiological concentrations of IP6 did not affect the CaOx MSL whereas the higher concentrations increased it substantially.



Comparisons (% difference):

WPU vs WPU2: -29 %

WPU vs WPU3: -29 %

WPU vs WPU4: > 53 %

WPU vs WPU5: > 53 %

Key :

WPU : control

WPU2 : 2.27 µM IP6

WPU3 : 4.45 µM IP6

WPU4 : 15 µM IP6

WPU5 : 30 µM IP6

Figure 5.16 Mean change in absorbance vs [Na_2Ox] in WPU indicating the CaOx MSL in the presence of IP6. The mean CaOx MSLs ± SE are shown in parentheses in the figure legend.

Intergroup comparisons are reported later on page 114.

CaOx Crystallization Kinetics

Figure 5.17 shows the effect of IP6 on the mean change in absorbance with respect to time upon CaOx crystallisation in BPU. Each experiment was performed in triplicate. Raw data are reported in Appendix 5.5.

The rate of crystallisation was determined from the slope between 30 and 75 mins and is shown in Figure 5.18 together with statistical comparisons. Actual values are reported in Table 5.10.

These results indicate that IP6 decreased the rate of CaOx crystallisation significantly as follows: **BPU = BPU2>> BPU3 > BPU4 > BPU5.**

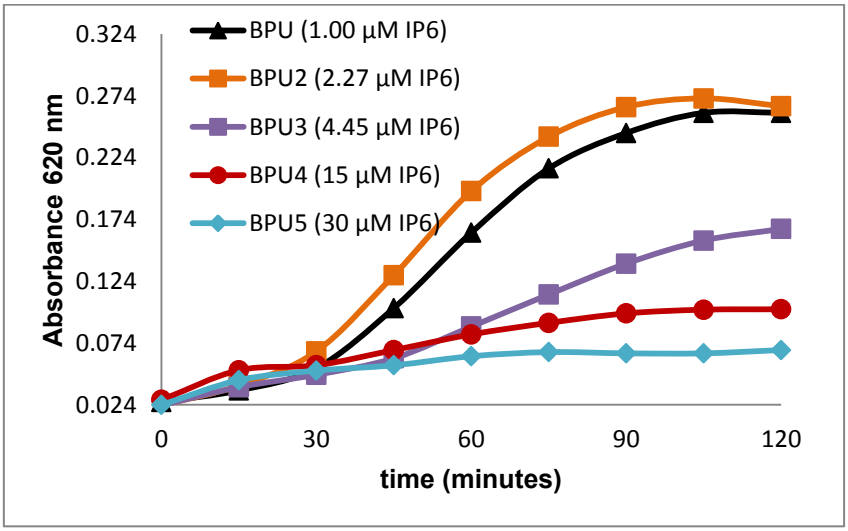
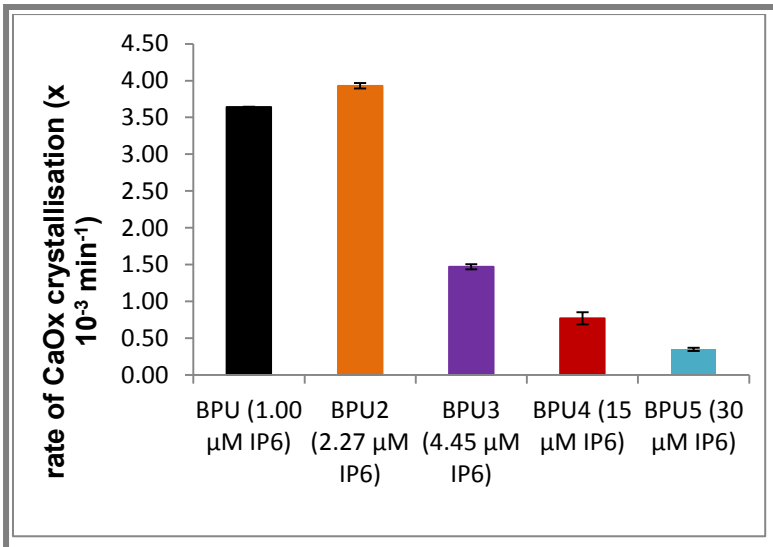


Figure 5.17 Mean change in absorbance vs time in BPU indicating CaOx crystallisation kinetics in the presence of IP6.



Comparisons:

BPU vs BPU2: p > 0.05

BPUvs BPU3: p < 0.001*

BPU vs BPU4: p < 0.001*

BPUvs BPU5: p < 0.001*

BPU2 vs BPU3: p < 0.001*

BPU2 vs BPU4: p < 0.001*

BPU2 vs BPU5: p < 0.001*

BPU3 vs BPU4: p < 0.001*

BPU3 vs BPU5: p < 0.001*

BPU4 vs BPU5: p < 0.001*

Figure 5.18 Effect of IP6 on the rate of CaOx crystallisation in BPU. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.10 Rate of CaOx crystallisation in BPU and BPU dosed with IP6. Mean ± SE.

Sample	Rate of crystallisation ($x10^{-3}$ min^{-1})
BPU (1.00 µM IP6)	3.64 ± 0.01
BPU2 (2.27 µM IP6)	3.93 ± 0.04
BPU3 (4.45 µM IP6)	1.47 ± 0.04
BPU4 (15 µM IP6)	0.77 ± 0.08
BPU5 (30 µM IP6)	0.35 ± 0.02

The effect of IP6 on the mean change in absorbance with respect to time upon crystallisation in WPU is shown in Figure 5.19. Each experiment was performed in triplicate. All raw data are reported in Appendix 5.5.

The mean rate of crystallisation was determined from the slope between 0 and 45 mins (an increase in absorbance) and is shown in Figure 5.20. Actual values are reported in Table 5.11. These results indicate that IP6 increased the rate of crystallisation significantly in WPU3 relative to WPU and WPU2. The following trend in the rate of crystallisation was observed:

WPU = WPU2 < WPU3

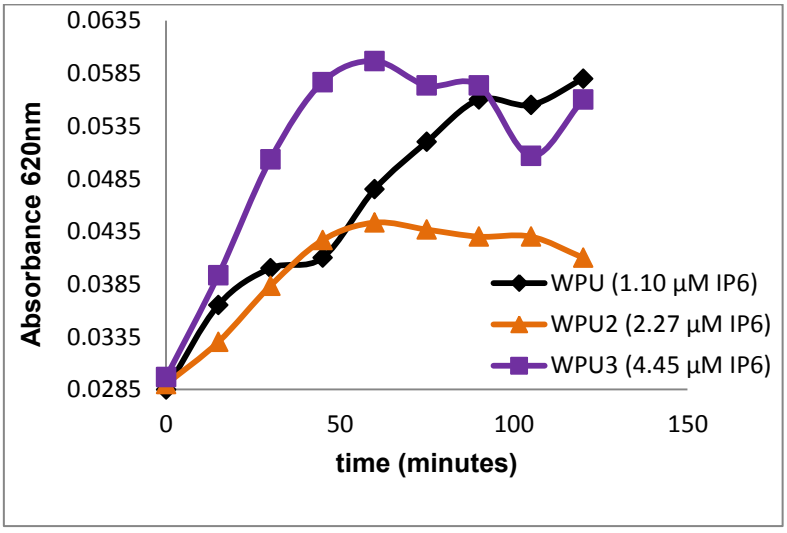
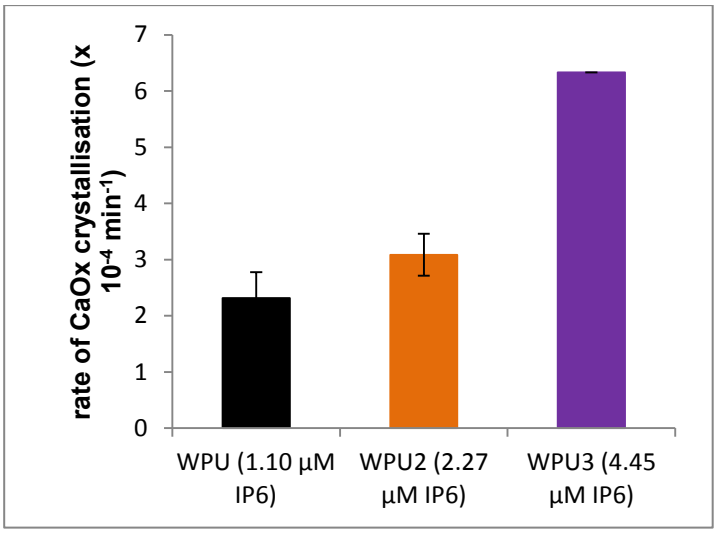


Figure 5.19 Mean change in absorbance vs time in WPU indicating CaOx crystallisation kinetics in the presence of IP6.



Comparisons:

WPU vs WPU2: $p > 0.05$

WPU vs WPU3: $p < 0.001$*

WPU2 vs WPU3: $p < 0.01$*

Figure 5.20 Effect of IP6 on the rate of CaOx crystallization in WPU. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.11 Rate of CaOx crystallisation in WPU and WPU dosed with IP6. Mean ± SE.

Sample	Rate of crystallisation (x 10^{-4} min^{-1})
WPU (1.10 µM IP6)	2.31 ± 0.46
WPU2 (2.27 µM IP6)	3.09 ± 0.37
WPU3 (4.45 µM IP6)	6.33 ± 0.01

Scanning Electron Microscopy

Electron micrographs of crystals deposited in BPU and BPU dosed with different concentrations of IP6 are shown in Figure 5.21.

Small COM crystals (approximate size 3 µm) together with a few large COD crystals (approximate size 8 µm) were observed in BPU (Figure 5.21 a). COM crystals remained predominant at the lower concentrations of IP6 (Figure 5.21 b and c). However, at the higher concentrations, the small COM crystals were replaced by large single COD crystals (approximate size 19 µm). There is little evidence of aggregation of COM or COD crystals at any concentration of IP6 including the control urine (BPU; Figure 5.21 a).

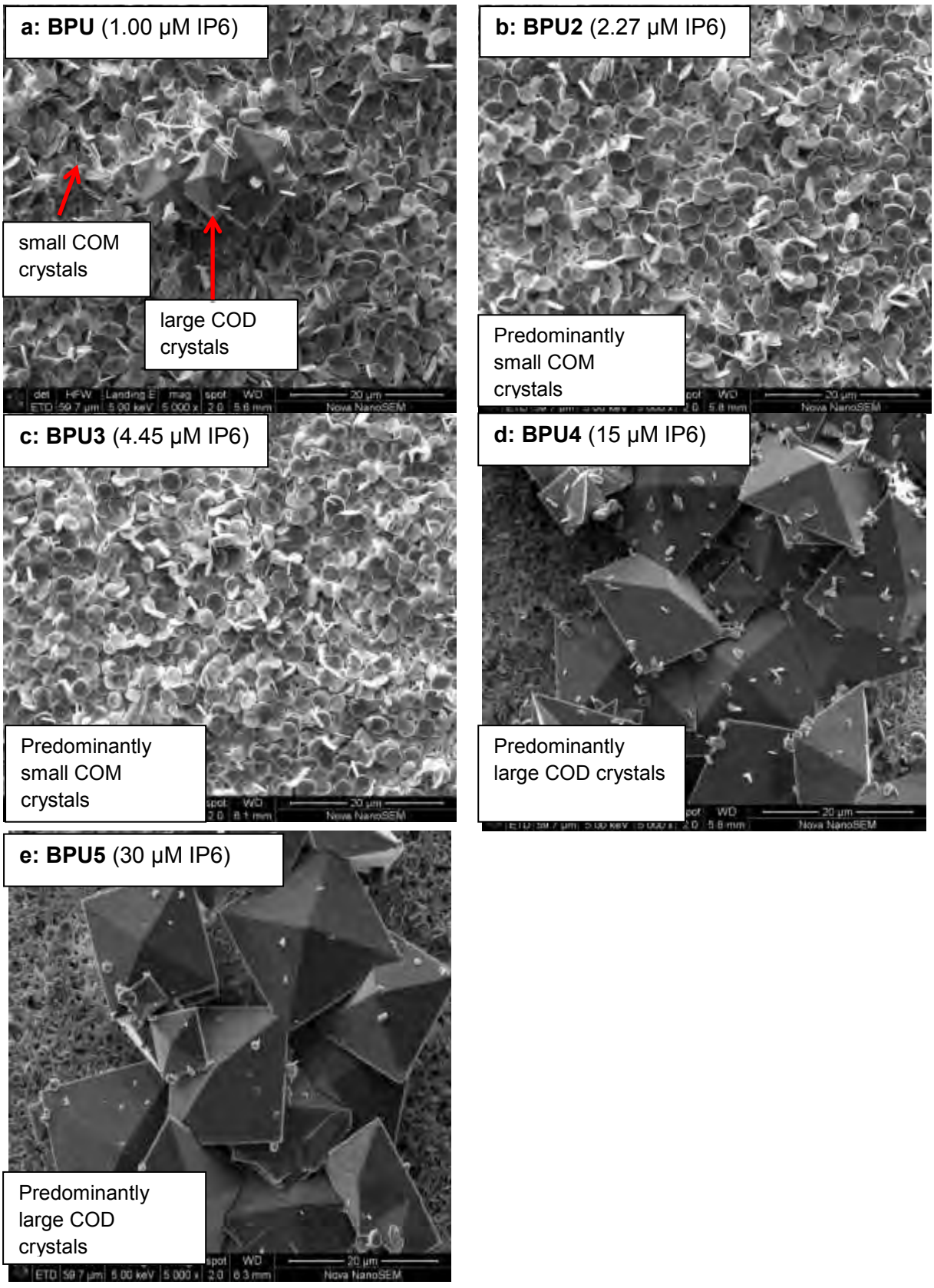


Figure 5.21 CaOx crystals deposited in BPU. All micrographs shown are at a magnification of x 5000.

Crystals deposited in WPU before and after the addition of IP6 are shown in Figure 5.22. COD crystals were observed at both of the concentrations of IP6 which were used. The approximate size of these crystals decreased with increasing concentration from 8 µm in the control urine (Figure 5.22 a) to 4 µm (Figure 5.22 c).

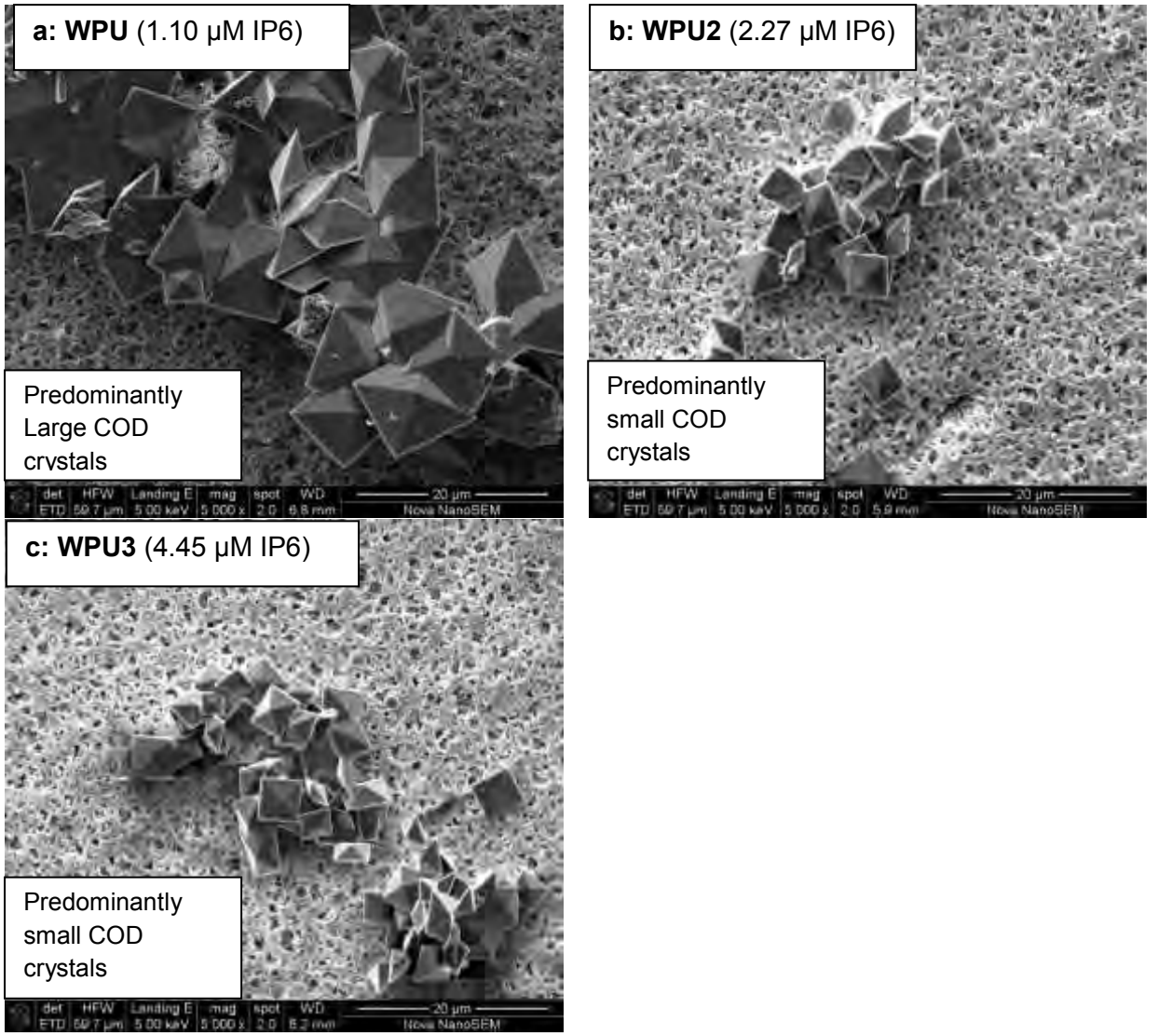


Figure 5.22 CaOx crystals deposited in WPU. All micrographs shown are at a magnification of x 5000

Inter-group comparisons

These comparisons are reported in Table 5.12. Attention is drawn to the following. The significantly higher concentration of Ca^{2+} in BPU relative to WPU, the substantially higher CaOx MSL in BPU relative to WPU and the significantly higher rate of CaOx crystallisation in BPU relative to WPU.

The presence of mostly COM crystals in BPU (Figure 5.21a) relative to the predominance of COD in WPU (Figure 5.22a) is also noted.

Table 5.12 Ca^{2+} concentrations and crystallisation propensity in pooled urine of black and white subjects: Inter-group comparisons

experiment	BPU vs WPU	BPU2 vs WPU2	BPU3 vs WPU3	BPU4 vs WPU4	BPU5 vs WPU5
Ionized calcium (Ca^{2+})	B > W p = 0.01*	B > W p= 0.02*	B > W p = 0.09	B > W p= 0.29	B > W p= 0.006*
CaOx MSL **% difference indicated**	B > W 54 %**	B > W 91 %**	B > W 91 %**	B < W 26 %	B = W 0 %
Rate of crystallisation	B > W p = 0.002*	B > W p < 0.0001*	B > W p = 0.002*	_	_

*Indicates statistical significant difference. **Indicates "substantial" difference as defined previously.

Specific crystallisation kinetic mechanisms

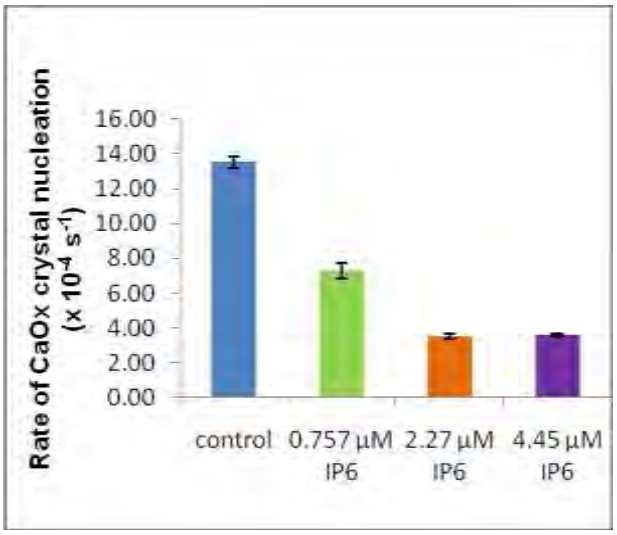
Nucleation and aggregation

Test substance: IP6

The mean rates of CaOx crystal nucleation and aggregation in the presence of physiological concentrations of IP6 is shown in Figure 5.23 and Figure 5.24; respectively. Actual values and the percentage inhibition of each mechanism are reported in Table 5.13. The metastable solution of CaOx without the addition of IP6 served as the control. Plots of all raw data are in Appendix 5.6.

The results indicate that IP6 significantly decreased the rate of both CaOx crystal nucleation and aggregation in a concentration dependent manner with the following trend:

control > 0.757 µM IP6 > 2.27 µM IP6 = 4.45 µM IP6.



Comparisons:

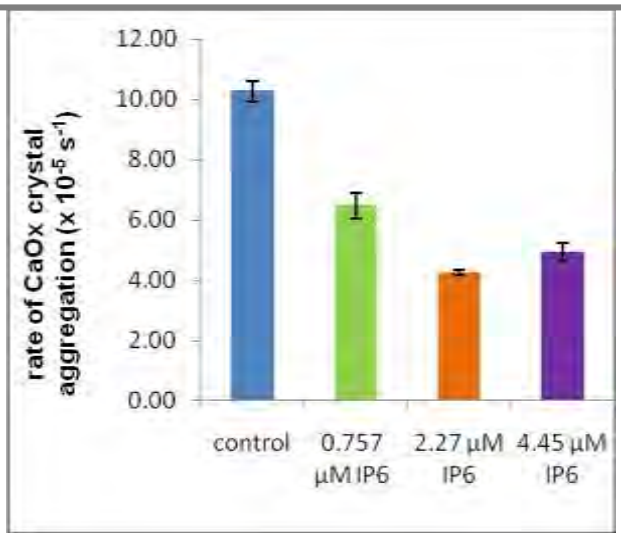
control vs 0.757 µM IP6 : p <0.0001*

0.757 µM IP6 vs 2.27 µM IP6 : p < 0.001*

0.757 µM IP6 vs 4.45 µM IP6 : p < 0.001*

2.27 µM IP6 vs 4.45 µM IP6 : p > 0.05

Figure 5.23 Effect of IP6 on the rate of CaOx crystal nucleation. Error bars indicate standard error of the mean. *Indicates statistical significance.



Comparisons:

control vs 0.757 µM IP6: p < 0.0001*

0.757 µM IP6 vs 2.27 µM IP6 : p < 0.05*

0.757 µM IP6 vs 4.45 µM IP6: p < 0.05*

2.27 µM IP6 vs 4.45 µM IP6: p > 0.05

Figure 5.24 Effect of IP6 on the rate of CaOx crystal aggregation. Error bars indicate standard error of the mean. *Indicates statistical significance

Table 5.13 Rates of CaOx crystal nucleation and aggregation and the inhibition of each mechanism in the presence of IP6. Mean ± SE.

[IP6] µM	Rate of nucleation (x 10^{-3} s^{-1})	% I_N	Rate of aggregation (x 10^{-4} s^{-1})	% I_A
control	13.50 ± 0.33	--	10.26 ± 0.34	--
0.757	7.28 ± 0.45	46 ± 3	6.48 ± 0.42	37 ± 4
2.27	3.52 ± 0.14	74 ± 1	4.25 ± 0.10	59 ± 1
4.45	3.58 ± 0.07	73 ± 1	4.93 ± 0.30	52 ± 3

Test substance: AU and AU dosed with IP6

The effect of these test substances on the mean rate of CaOx nucleation and aggregation is shown in Figure 5.25 and Figure 5.26. Actual values together with the percentage inhibition of each mechanism are reported in Table 5.14. The metastable solution without the addition of the AU test substances served as the control. It is noted that the control values are different in the experiments presented in Table 5.13 and Table 5.14. Fresh CaOx metastable solutions were prepared for these experiments on the days on which they were performed. Since the actual conditions under which the metastable solutions were prepared cannot be fully controlled it is likely that CaOx crystals of different sizes and degrees of aggregation would be present, giving rise to different rates in the control samples. Plots of all raw data are shown in Appendix 5.6.

The rate of CaOx crystal nucleation and aggregation decreased significantly in the presence of AU and AU dosed with IP6 and showed the following trend:

Control> AU0 = AU1 > AU2 = AU3

These results indicate that the addition of IP6 at concentrations of 2.27 µM and 4.45 µM increased the inhibitory activity of AU.

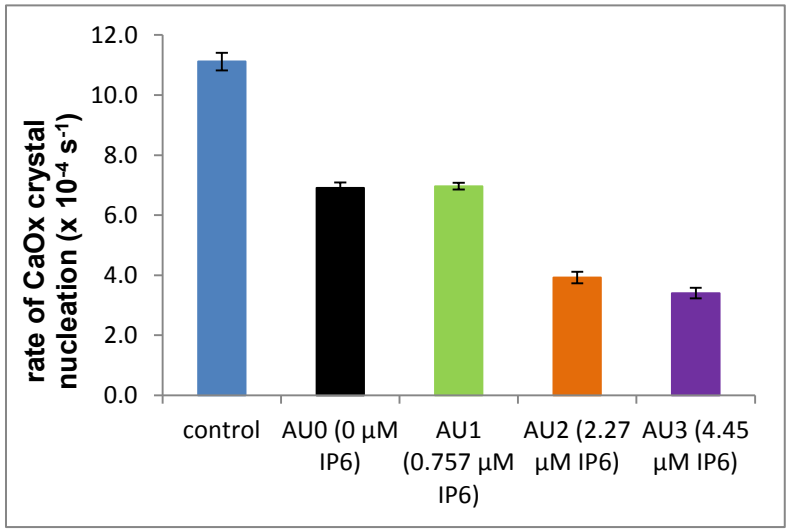
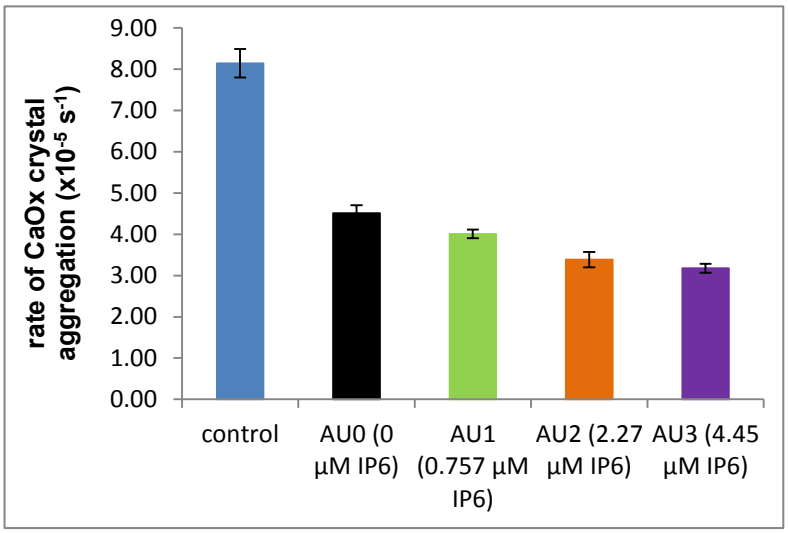


Figure 5.25 Effect of AU and AU dosed with IP6 on the rate of CaOx crystal nucleation. Error bars indicate standard error of the mean. *Indicates statistical significance.



Comparisons:

Control vs AU0: p <0.0001*

AU0 vs AU1: P= 0.073

AU0 vs AU2: P < 0.05*

AU0 vs AU3: P= 0.01*

AU1 vs AU2: P < 0.05*

AU1 vs AU3: P < 0.05*

AU2 vs AU3: P > 0.05

Figure 5.26 Effect of AU and AU dosed with IP6 on the rate of CaOx crystal aggregation. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.14 Rates of CaOx crystal nucleation and aggregation and the inhibition of each mechanism in the presence of AU and AU dosed with IP6. Mean ± SE.

	Rate of nucleation (x 10^{-4} s^{-1})	% I_N	Rate of aggregation (x 10^{-5} s^{-1})	% I_A
control	11.10 ± 0.29	--	8.14 ± 0.35	--
AU0 (0 µM IP6)	6.91 ± 0.18	38 ± 2	4.51 ± 0.20	45 ± 2
AU1 (0.757 µM IP6)	6.97 ± 0.12	37 ± 1	4.01 ± 0.11	51 ± 1
AU2 (2.27 µM IP6)	3.93 ± 0.19	65 ± 2	3.39 ± 0.19	58 ± 2
AU3 (4.45 µM IP6)	3.40 ± 0.17	69 ± 2	3.17 ± 0.11	61 ± 1

Test substance: PU and PU dosed with IP6

The effect of these test substances on the mean rate of CaOx crystal nucleation and aggregation is shown in Figure 5.27 and Figure 5.28; respectively. Actual values and the percentage inhibition of each mechanism are reported in Table 5.15. The metastable CaOx solution without the addition of the PU test substances served as the control. As noted above, the values of these controls differ to that of the controls presented in other experiments. The reason for this is stated above. Plots of all raw data are reported in Appendix 5.6.

These results indicate that the rate of CaOx crystal nucleation decreased significantly in the presence of PU and PU dosed with IP6 and showed the following trend:

Control > PU = PU2

The above trend indicates that 2.27 µM IP6 did not increase the urine's inhibitory activity with regard to nucleation.

The results for the rate of CaOx crystal aggregation on the other hand showed the following trend:

Control >> PU > PU2

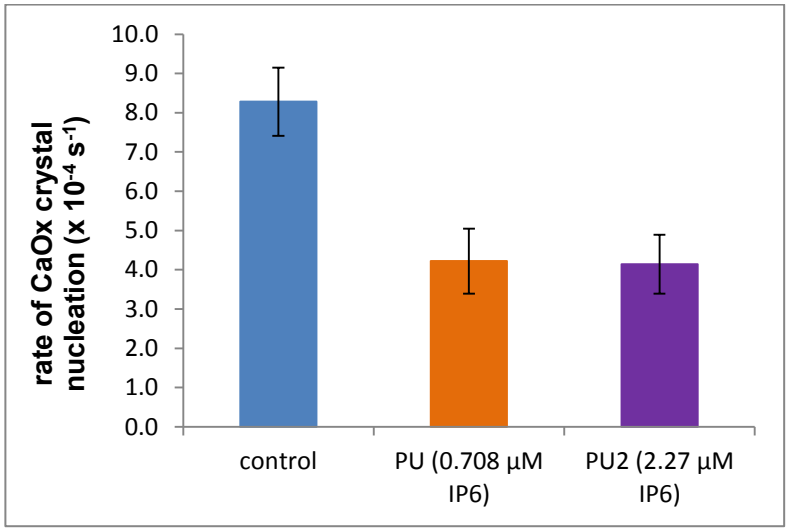


Figure 5.27 Effect of PU on the rate of CaOx crystal nucleation. Error bars indicate standard error of the mean. *Indicates statistical significance.

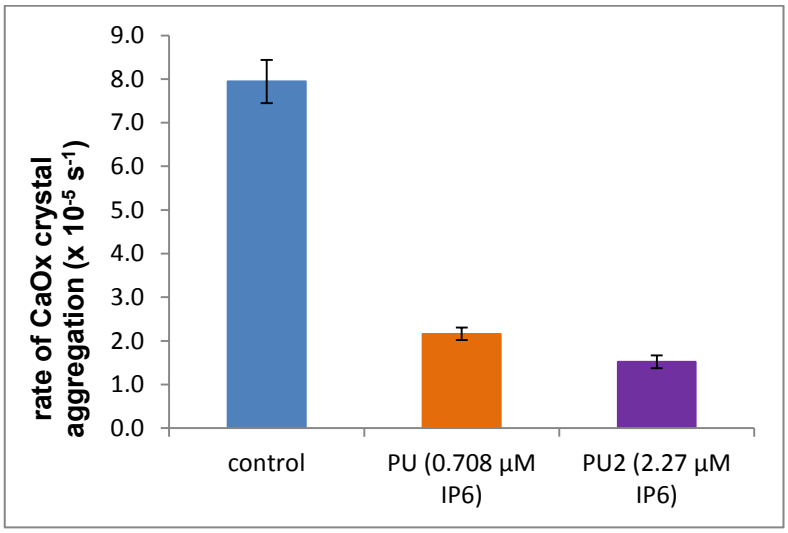


Figure 5.28 Effect of PU on the rate of CaOx crystal aggregation. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.15 Rates of CaOx crystal nucleation and aggregation and the percentage inhibition of each mechanism in the presence of PU and PU dosed with IP6. Mean ± SE.

	Rate of nucleation ($x\ 10^{-4}\ s^{-1}$)	% I_N	Rate of aggregation ($x\ 10^{-5}\ s^{-1}$)	% I_A
control	8.30 ± 0.87	--	7.90 ± 0.50	--
PU (0.708 µM IP6)	4.22 ± 0.83	49 ± 10	2.16 ± 0.14	73 ± 2
PU2 (2.27 µM IP6)	4.14 ± 0.75	50 ± 9	1.52 ± 0.15	81 ± 2

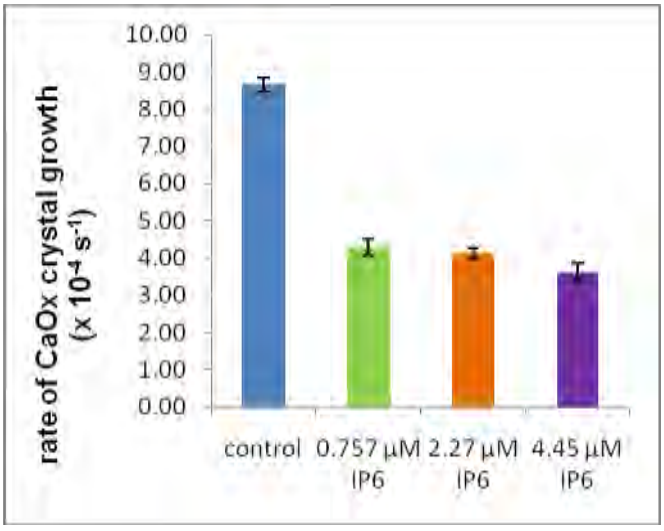
Growth

Test substance: IP6

Figure 5.29 shows the effect of IP6 on the mean rate of CaOx crystal growth. Actual values and the percentage inhibition of growth are reported in Table 5.16. The metastable solution of CaOx without the addition of IP6 served as the control. Plots of all raw data are in Appendix 5.7.

The results indicate that the rate of CaOx crystal growth decreased significantly in the presence of IP6 and is not concentration dependent:

Control > 0.757 µM IP6 = 2.27 µM IP6 = 4.45 µM IP6



Comparisons:

control vs 0.757 µM IP6: $p < 0.001$*

control vs 2.27 µM IP6 : $p < 0.001$*

control vs 4.45 µM IP6: $p < 0.001$*

0.757 µM IP6 vs 2.27 µM IP6: $p > 0.05$

0.757 µM IP6 vs 4.45 µM IP6: $p > 0.05$

2.27 µM IP6 vs 4.45 µM IP6: $p > 0.05$

Figure 5.29 Effect of IP6 on the rate of CaOx crystal growth. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.16 Rates of CaOx crystal growth and its percentage inhibition in the presence of IP6. Mean ± SE.

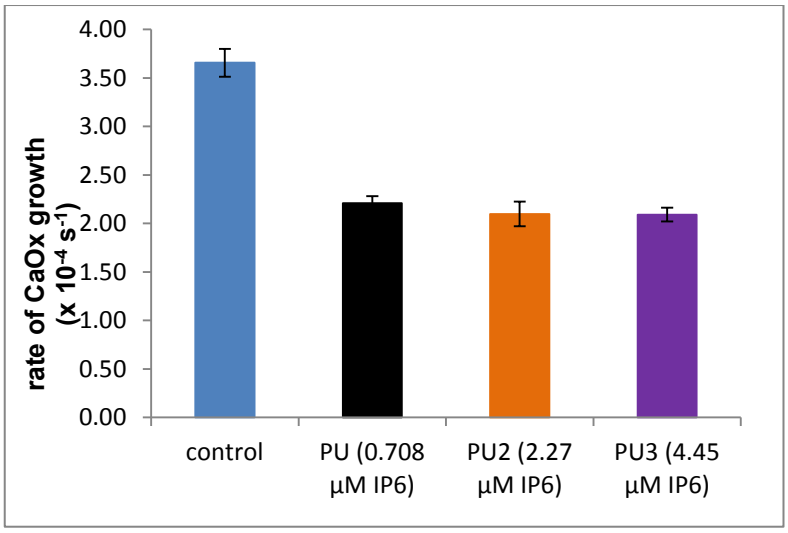
[IP6] µM	Rate of growth ($x10^{-3}$ s^{-1})	% I_G
control	8.64 ± 0.19	--
0.757	4.29 ± 0.22	50 ± 3
2.27	4.11 ± 0.15	52 ± 2
4.45	3.61 ± 0.25	58 ± 3

Test substance: PU and PU dosed with IP6

The effect of these test substances on the mean rate of CaOx crystal growth is shown in Figure 5.30. Actual values and the percentage inhibition of CaOx crystal growth are reported in Table 5.17. The metastable CaOx solution without the addition of the PU test substances served as the control. It is noted that the control value in Table 5.17 differs to the control presented in Table 5.16. The reason for this has already been stated. Plots of all raw data are reported in Appendix 5.7.

The rate of CaOx crystal growth decreased significantly in the presence of PU and PU dosed with IP6 relative to the control and showed the following trend:

Control > PU = PU2 = PU3



Comparisons:

Control vs PU: $p < 0.001$*

PU vs PU2: $p > 0.05$

PU vs PU3: $p > 0.05$

PU2 vs PU3: $p > 0.05$

Figure 5.30 Effect of PU on the rate of CaOx crystal aggregation. Error bars indicate standard error of the mean. *Indicates statistical significance.

Table 5.17 Rates of CaOx crystal growth and its percentage inhibition in the presence of PU and PU dosed with IP6. Mean ± SE.

	Rate of growth ($x\ 10^{-4}\ s^{-1}$)	**% I_G**
control	3.66 ± 0.14	--
PU (0.708 µM IP6)	2.21 ± 0.07	40 ± 32
PU2 (2.27 µM IP6)	2.10 ± 0.13	43 ± 3
PU3 (4.45 µM IP6)	2.09 ± 0.07	43 ± 2

Discussion

As mentioned in Chapter 1, crystallisation is governed by thermodynamic (supersaturation) and kinetic (nucleation, aggregation and growth) factors.[35] Inhibitors of CaOx crystallisation may therefore either decrease the urinary supersaturation of CaOx, by decreasing the concentration of Ca^{2+} or Ox^{2-}, and/ or modulate crystallisation kinetics by inhibiting the rates of CaOx crystal nucleation, aggregation and growth.[15] The studies described in the present chapter were undertaken to test experimentally the prediction of the theoretical modelling that the concentration of Ca^{2+} is unaffected by IP6 under normal physiological conditions, and to investigate the notion that its inhibitory activity towards CaOx crystallization processes were of a kinetic nature.

Physiological concentrations of IP6 had no effect on the empirically derived concentration of Ca^{2+} in both AU and pooled urine of black and white subjects in the present study. This is in agreement with the theoretical prediction of the modelling performed in the present thesis as well as with the results of a previous *in vitro* study[285].

Regarding crystallisation propensity, the CaOx MSL can be interpreted as the ability of a solution to resist spontaneous nucleation.[50] Thus, in the present study, the substantial increase in the CaOx MSL in both AU and pooled urine of black and white subjects in the presence of IP6 suggests that it retards crystal nucleation. However, the effective IP6 concentration at which this was achieved in human urine was non-physiological (15 μM). This finding is in agreement with that reported in a previous study in which the effective concentrations of IP6 were even higher than those in the present study (AU: 12 μM vs 4.45 μM; human urine: 120 μM vs 15 μM). The latter difference is due to the higher baseline concentration of Ca^{2+} in the media of the previous study (≈ 4mM and ≈ 7 mM, respectively) relative to the concentrations of Ca^{2+} in the present study (AU: 1.80 mM, BPU: 0.93 mM and WPU: 0.69 mM). The results of these *in vitro* investigations (present and previous studies) indicate that IP6 at physiological concentrations is unlikely to affect the *in vivo* urinary concentration of Ca^{2+} or the CaOx MSL. Since both of these properties are thermodynamically related to the supersaturation (SS) of CaOx, it appears that other mechanisms (which are kinetic in nature) are more likely to be involved in any inhibitory activity of IP6.

Regarding the effect of IP6 on crystallisation kinetics, a significant decrease in the rate of CaOx crystallisation may be regarded as favourable as it decreases the risk of stone formation.[374] Thus, the decrease in the rate of CaOx crystallisation in both AU and pooled urine of black subjects in the presence of physiological concentrations of IP6 could be regarded as being favourable, thereby supporting the notion of IP6 playing a kinetic role in

inhibiting CaOx crystallisation. On the other hand, an increase in the rate of CaOx crystallisation was observed in the pooled urine of white subjects. This inter-group difference will be discussed later.

The formation of COD crystals in both AU and pooled urine of black subjects in the presence of IP6 is in agreement with recent studies which were conducted in AU.[385, 386] .The formation of COD is favourable as it has been demonstrated that it adheres to a lesser extent to renal tubule cells than COM.[222, 387] Other LMW (low molecular weight) molecules such as citrate and pyrophosphate as well as HMW (high molecular weight) compounds such as proteins (e.g. osteopontin and albumin) have been reported to favour the formation of COD.[222, 388, 389] The mechanism by which this occurs is due to specific interactions between the respective molecules and the crystal surface of COM which inhibits the growth of COM (a process known as “step-pinning”) and favours the formation of COD, as cited in a review article by Ryall.[17] It is therefore speculated that IP6 may favour the formation of COD by the same mechanism. In principle, this would support the notion of it being a protective agent in CaOx urolithiasis. However, in the present study the effective concentration of IP6 in pooled urine of black subjects was non-physiological, suggesting that this process might not occur *in vivo*.

With regard to the experiments in which different test substances were added to a metastable solution of CaOx, the observation that IP6 alone has the capacity to inhibit CaOx crystal nucleation (Figure 5.23 and Table 5.13) at all the physiological concentrations investigated in the present study, is promising. However, when 0.757 µM and 2.27 µM IP6 in AU and in pooled human urine were tested in the same metastable solutions, negligible inhibitory effects were observed (Figure 5.25 and Figure 5.27). This suggests that other urinary components may affect the inhibitory capacity of IP6 *in vivo*.

Similarly, the inhibition of CaOx crystal aggregation at all concentrations of IP6 in the present study is promising (Figure 5.24 and Table 5.13). However, 0.757 µM IP6 had a negligible effect on the inhibitory capacity of AU (Figure 5.26 and Table 5.14) indicating that the urine composition influences the inhibitory capacity of IP6 as suggested above. Interestingly the presence of 2.27 µM IP6 increased the inhibitory capacity of pooled human urine with regard to aggregation (Figure 5.28 and Table 5.15) whereas no effect was observed for nucleation, as stated above. This is noteworthy as aggregation is regarded as the most important stone-forming mechanism as it occurs at a faster rate than nucleation and may lead to the development of a relatively large concretion.[6, 33] The decrease in the rate of CaOx crystal aggregation in urine is therefore encouraging and supports the notion that IP6 may influence the kinetics of CaOx crystallisation *in vivo*. These results are important as no study has been

previously reported on the effect of IP6 on crystal aggregation in *in vitro* or *in vivo* studies in the context of urolithiasis.

The effect of IP6 alone on CaOx crystal growth in the present study is intriguing. The results demonstrated that its inhibitory capacity is not concentration dependent (Figure 5.29 and Table 5.16). This suggests that the endogenous IP6 concentration of 0.708 µM in the pooled human urine may have contributed towards the inhibition of growth of this particular urine. The result of the present study regarding the concentration required for growth inhibition is in agreement with a previous potentiometric study in which 0.75 µM IP6 inhibited CaOx crystal growth in an aqueous medium containing calcium and oxalate.[390] Since the inhibition of CaOx crystal growth was not concentration dependent in the present study, it suggests that any deficiency of IP6 may increase the risk of kidney stone formation. Alternatively, an increase in the urinary concentration of IP6 may decrease the risk of kidney stone formation.

The observation that a lower effective concentration of IP6 was required to inhibit CaOx crystal growth relative to that required for the inhibition of CaOx crystal nucleation and aggregation is also noteworthy. This indicates that IP6 has a stronger inhibitory capacity for the inhibition of CaOx crystal growth and that, as such, it may play a crucial role *in vivo*.

Consideration of all the physicochemical properties (CaOx MSL, rate of CaOx crystal nucleation, CaOx hydration state, CaOx crystal nucleation and growth) shows that higher effective concentrations of IP6 were required in real urine than in AU in the present study to achieve some measure of inhibition. This indicates that caution should be taken when extrapolating results from *in vitro* experiments in aqueous media to *in vivo* conditions. Nevertheless, AU is a useful model for providing insight into protocols and conditions that might be applied in *in vivo* studies.[50, 374]

Significant inter-group differences as reported in Table 5.12 are not likely due to the effect of IP6 but due to differences in inter-racial urinary compositions. Inter-group comparisons at baseline have been extensively discussed in several studies conducted in the KSRL at the University of Cape Town.[302, 305, 306, 384] It is therefore not necessary to repeat these discussions here. Of importance in the present study is to consider different effects which occurred in the urines of the two groups in the presence of IP6. Only two such differences were observed. Firstly, the rate of CaOx crystallisation decreased significantly in pooled urine of black subjects in the presence of 4.45 µM IP6 whereas a significant increase was observed in pooled urine of white subjects, as stated earlier in this discussion. Secondly, a decrease in crystal size was observed in the white group only (SEM) in the presence of this concentration of IP6. Decreases and increases in the rate of crystallisation have been given different interpretations in the literature. On the one hand, a decrease has been regarded as

a favourable effect which reduces the risk of stone formation[374] while on the other hand, some researchers regard an increase in the rate of crystallisation as favourable as it results in the formation of smaller crystals which can be excreted harmlessly and decreases the supersaturation.[378, 388] Thus, the significant increase in the CaOx crystallisation rate with a concomitant decrease in crystal size in the white group may be regarded as favourable. Furthermore, since a decrease in crystal size was observed in pooled urine of white subjects (SEM) but not in the black group; the increase in the crystallisation rate in the former group might be the overriding factor in this anomaly. The two interpretations of crystallisation rates can be confusing unless some other independent physicochemical property supports one or the other, as has happened in this particular case. Irrespective of the interpretation of this kinetic factor, of interest is that IP6 had different effects in the urines of black and white subjects, raising the possibility of its physicochemical activity being dependent on its urine environment.

It is recognized that the present study had certain limitations. These are as follows. Observations and conclusions were drawn from experiments which were conducted in single pooled urine samples from each respective group. This can be improved by using more than one pooled urine sample. Furthermore, urine flow dynamics were not modelled in the present experimental model. This can be improved by using an experimental model such as MSMPR (mixed suspension mixed product removal).[50, 374] In addition, the measured rate of CaOx crystallisation in AU and real urine in the present study does not distinguish between the different crystallisation mechanisms which occur in urine. This aspect can be improved in future studies by employing a superior crystallisation experiment such as MSMPR[374] which can be used to distinguish between crystal nucleation and growth.

In conclusion, the results of the studies described in this chapter are important for several reasons. Firstly, while previous studies have focussed on investigating the effect of IP6 on individual crystallisation processes, none has undertaken the comprehensive approach adopted in the present project. Secondly, the influence of IP6 on CaOx crystal aggregation kinetics is described here for the first time. Thirdly, the results of the present study indicate that the inhibitory effects of urinary CaOx crystallisation are more likely to be of a kinetic nature rather than a thermodynamic one.

Finally, it is recognised that the results of the present study were derived in *in vitro* models. The possibility remains that the ingestion of IP6 may demonstrate *in vivo* effects due to physiological processes arising from metabolic effects of IP6 itself or from its degradation products (phosphate and lower phosphorylated inositols). The following chapter describes

the effect of IP6 ingestion on risk factors associated with CaOx urolithiasis in a human model.

Chapter 6 : The effect of dietary phytate ingestion on its urinary excretion and on the risk factors associated with calcium oxalate urolithiasis in South African stone free and stone prone population groups

Introduction

A previous dietary survey by Modlin showed that the mean dietary intake of phytate (IP6) in black South Africans was higher than that of their white compatriots (4290 mg/day vs 275 mg/day).[318] This nutrient is of interest in the present study as it occurs in the staple diet of the black population and as such may contribute to the rarity of kidney stone disease in this group. A recent study in the KSRL at the University of Cape Town investigated the effects of this nutrient on the urine chemistry in South African black and white males[319] and was discussed in Chapter 1 of the present thesis. Briefly, the results of that study indicated that despite the higher dietary intake of IP6 in the black group, the baseline urinary excretion was lower than in the white group. It was concluded that the renal handling of IP6 in the two ethnic groups may be different. However, as mentioned in Chapter 1, the method used in the aforementioned study was limited whereby the separation of inorganic phosphate and IP6 during sample preparation was inadequate thus rendering the results and conclusions questionable. As such a more rigorous investigation using a more reliable and robust method is warranted.

Numerous studies by Grases et al, investigating the pharmacokinetic profile of IP6 in animals as well as in humans showed that urinary IP6 is correlated to dietary IP6.[265, 268, 391, 392] Interestingly, studies in both humans and rats have shown that the excretion of IP6 reaches a plateau which is not exceeded by an excess intake of dietary phytate.[268, 392] As such, urinary IP6 can be used as an indicator to identify a deficiency in the intake of IP6 but not necessarily an excess.

The objectives of the present study are to include the determination of the respective baseline levels of dietary IP6 intake in black and white subjects, investigation of its influence on urine chemistry and its effect on urinary crystallisation of CaOx in both groups. Such studies are described in the present chapter.

Methods

Subjects

Healthy black and white South African males between the ages of 18 -36, average weight of 70 ± 2 kg and BMI of 23 ± 1 kg/m^2, without any history of kidney stone disease, were recruited from the student and staff cohort at the University of Cape Town. Vegetarians and individuals with nut allergies were excluded. Eligible participants were selected after completing a general questionnaire assessing their health (Appendix 6.1). Race was self-declared.

Two groups of males per race group were recruited from the same cohort. The first group of males consisted of black (n= 16) and white (n= 16) males. These males completed food frequency questionnaires (FFQ) for the assessment of their typical nutrient intake (objective 1). The second group of males consisted of black (n= 12) and white (n = 11) males. These males participated in a 10 day dietary intervention described below (objective 2).

It should be noted that an overlap between the groups exists in which some of the males who participated in the study for objective 1, also participated in the study for objective 2. These studies were carried out at different time periods of the current thesis. Thus when objective 2 was undertaken, the same group of males who participated in objective 1 were not available to participate. However, the estimated intakes of nutrients obtained from the FFQs in objective 1 is assumed to be representative of subjects used in the present study since subjects were recruited from the same cohort.

Study design

FFQ's were self-administered and checked upon completion by the present researcher in the presence of the subject.

A 10- day cross-over study consisting of two phases (Phase 1 and Phase 2) was conducted. Phase 1 consisted of an IP6-deficient diet while Phase 2 was IP6-rich. These diets were administered at breakfast (Table 6.1). Subjects consumed their own meals for other meal times (lunch, supper and snacks) in which dietary IP6 intake was restricted for the duration of the study as follows. IP6 rich foods (wholegrains) indicated in Table 6.2 were excluded from meals while fruits and vegetables which contain IP6 to a lesser extent than that of wholegrains[259] were permissible. In addition to the restriction of dietary IP6, the intake of oxalate-rich foods was also restricted as shown in Table 6.3.

Prior to Phase 1, a baseline urine sample was provided (day 0) while subjects were on their free unrestricted diets. During Phase 1, the IP6-deficient breakfast was ingested for 3 consecutive days (days 1 – 3). Other meals were consumed in accordance with the

restrictions described in Table 6.2 and Table 6.3. A 24 hr urine sample was collected on day 3. Participants continued to avoid IP6-rich foods on days 4-7 to maintain a low urinary excretion of IP6 prior to Phase 2. On days 8 -10 (Phase 2) an IP6- rich breakfast (Table 6.1) in the form of oats (Jungle Oats, Tiger brands, South Africa) was administered followed by the collection of a 24 hr urine sample on day 10. Other meals were consumed as per Phase 1.

Urine aliquots were used for the determination of IP6 and other biochemical parameters as well as for crystallisation experiments. All participants were required to keep a dietary record for the duration of the study to check for compliance. This study was approved by the Human Research Ethics Committee of the University of Cape Town, HREC ref: 174/2011 (Appendix 6.2).

Table 6.1 IP6 deficient and rich breakfasts administered in the present study.

IP6 deficient	IP6 rich
70 g Kellogs Corn Flakes	70 g (dry weight) Oats*
200 ml full cream milk	200 ml full cream milk
200 ml Coffee	200 ml Coffee
2 slices brown bread	2 slices brown bread
8 g butter	8 g butter
15 g strawberry jam	15 g strawberry jam

*IP6 analysis is described on page 131.

Table 6.2 List of IP6 foods excluded and included in meals for the duration of the present study.[259]

Excluded from meals	Included in meals
Barley	All fruits and vegetables
Brown rice & wild rice	--
Legumes	--
Maize	--
Nuts (including peanut butter and nut derived products)	--
Oats (except that which is provided)	--
Soybeans/Tofu	--
Whole-wheat bread	--

Table 6.3 List of Oxalate foods excluded and included in meals for the duration of the present study.[83, 102]

Excluded from meals	Included in meals
Alcohol	≥ 200 ml black tea per day
Beetroot	≥ 100 g chocolate per day
Green beans	≥ 20 g cocoa powder per day
Rhubarb	--
Spinach	--
Strawberries	--
Waterblommetjies	--
Wheat germ	--

Nutrient intake

The typical nutrient intake in each respective group was assessed using a FFQ.[393] Briefly, the questionnaire contained different categories of foods as follows: breads, breakfast cereals, nuts & seeds, vegetables, legumes & legume products, cereals & wholegrains and pastas, eggs & dairy products, meat & meat products, beverages, fish & seafood, fruit as well as snacks, desserts & sugar. Each subject was required to indicate the relative frequency and usual portion size of a particular food type that they would typically consume in 30 days.

Questionnaires were analysed using a software program (FoodFinder 2) developed by the South African Medical Research Council (MRC).[394] This program contains a data-base of nutrient contents for foods that are typically consumed by South Africans. It allows for the calculation of macronutrients, minerals, vitamins, fatty acids and cholesterol, amino acids and other organic components. Lithogenic and antilithogenic components were analysed including total protein, calcium, oxalic acid, magnesium and in particular IP6. The concentration of the latter nutrient in food is variable and influenced by environmental factors including growing location, irrigation, soil type, plant variety and processing as well as the method used for IP6 determination.[259] Unfortunately, FoodFinder 2 does not have a complete data set for the content of organic acids in food. As such IP6 content obtained from FoodFinder2 only includes that derived from fruit and vegetables. Accordingly, IP6 content in cereals, legumes, oilseeds and nuts was obtained from various publications. Mean values are given in Table 6.4. These values were then used to estimate the IP6 content of each item in the FFQ.

The daily intake of each of the aforementioned nutrients was estimated by dividing by a factor of 30.

24 hr dietary records on day 3 and day 10 were also assessed using FoodFinder 2. The nutrient intake on these days was compared to the estimated daily intake obtained from the 30 day FFQs.

Table 6.4 Estimated IP6 content of foods obtained from literature.

Food type	IP6 content (mg/ 100g) edible
Cereals & bread	
Barley[395-397]	652 ± 206
Brown/wild rice[395, 396, 398]	743 ± 164
Maize[259, 399, 400]	1044 ± 414
Oats[391, 396, 397, 400-402]	785 ± 203
whole-wheat bread[391, 398, 401]	406 ± 116
Legumes	
Beans[391, 395, 396, 398, 401, 403]	845 ± 167
Chick peas[401, 403]	1030 ± 510
Lentils[369, 391, 401, 403, 404]	626 ± 135
Nuts & oil seeds	
Almonds[398, 405]	1527 ± 585
Brazil nuts[259]	290 ± 0
Cashews[398, 405]	864 ± 365
Macadamia[398, 405]	605 ± 342
Peacans[398, 405]	1180 ± 728
Peanut butter[398]	443 ± 0
Peanuts[391, 395, 398, 405]	1097 ± 328
Soybeans[259, 395, 401]	1421 ± 282
Walnuts[391, 405]	1135 ± 466

IP6 content in oats

The concentration of IP6 in oats (*Jungle Oats, Tiger Brands, South Africa*) was determined using anion exchange chromatography as described in previous studies[406-409], followed by a colorimetric assay[369]. **Extraction[406-409]:** Briefly, 2 g of oats was weighed and shaken with 20 ml of 0.8 M HCl for 2 h at room temperature. The mixture was then centrifuged at 3000 rpm for 10 mins and the supernatant was decanted and filtered through filter paper (*MN 615, Machery-Nagel, Germany*). 10 ml of this extract was mixed with an equal volume of 0.11 M EDTA and set to a pH of 6 by the addition of NaOH (1 M). 1ml of this mixture was diluted with 30 ml milli-Q water and added to a 150 ml beaker containing 0.5 g of Dowex 1x8 anion exchange resin (*Sigma Aldrich*). The mixture was then shaken at 150 rpm for 20 mins to facilitate the adsorption of IP6 to the resin. The mixture containing the resin was then poured into an empty 20 ml SPE tube to separate the liquid from the resin. The resin was then washed twice with 5 ml of H_2O followed by washing it twice with 5 ml portions of 0.3 M NaCl.

IP6 was then finally eluted with 4 ml of 0.8 M NaCl in a stepwise manner by employing 1 ml of the NaCl each time. Recovery was tested by the addition of 600 µM dipotassium phytic acid (*Sigma Aldrich*). **Quantification[369]:** The eluate was homogenised and a 1:20 dilution was prepared using milli-Q water. IP6 concentration was determined colorimetrically, using Wade's reagent as described by Latta and Eskin.[369] This assay is based on the reaction between iron (Fe^{3+}) and sulfosalicylic acid which results in a pink colour with maximum absorbance at 500 nm. In the presence of IP6, Fe^{3+} becomes bound to the phosphate ester resulting in a decrease in absorbance. A calibration curve was prepared using a series of standards of concentration 5 – 40 µg/ml IP6. 1 ml of Wade's reagent (0.03 % $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid in milli-Q water) was added to 3 ml of the standard and eluate and vortex mixed for 5 s followed by centrifugation at 3000rpm for 10 mins. The absorbance was then measured at 500 nm using water to blank the spectrophotometer (*Heλ10s, Spectronic Unicam*). The concentration of IP6 in each eluate was determined from the standard curve.

Urine collection

24 hr urine samples were collected in 2.5 L plastic containers without any preservative. Details of the procedure for a 24 hr collection were provided to each participant (Appendix 6.3). The collection of a complete 24 hr sample was monitored by measuring urinary creatinine; urine samples with an abnormal concentration (normal range: 9.0-17.7 mmol / 24 hr)[410] were excluded. Urine samples were tested for blood and bacteria upon receipt (*Medi-Test Combi 10, Machery-Nagel, Germany*) and were discarded if tested positive.

Urine composition

Urinary IP6 was measured in each sample as described in Chapter 4. Each measurement was performed in duplicate per sample. Measurements were performed on the same day that samples were received. Urinary pH was measured using a pH meter (Hanna instruments, pH 211 microprocessor instruments); chloride was measured by using an ion-selective electrode; sodium, potassium, calcium and magnesium were measured using flame atomic absorption spectrophotometry;[411-413] oxalate was measured by using oxalate decarboxylase;[414] citrate was measured by using citrate lyase;[415] creatinine was determined by using picric acid;[416] urate was determined from uricase and phosphate from ammonium molybdate.[417, 418]

Physicochemical properties

The RS of urinary stone-forming salts were determined by using JESS.[321, 322] Concentrations (mol/L) of all of the aforementioned urinary parameters were used (except urinary creatinine) as input data (primary species) for the calculations. The SS of CaOx and CaP salts were determined.

The Tiselius risk index (TRI) of each sample was also determined using the following mathematical equation:[419]

$$\frac{(Ca/Cr)^{0.71} \times (Ox/Cr)}{(Mg/Cr)^{0.14} \times (Cit/Cr)^{0.10}}$$

This ratio is used to calculate the relative biochemical risk of CaOx crystallisation where Ca, Ox, Mg and Cit are the urinary concentrations of calcium, oxalate, magnesium and citrate in mmol/ 24 hr and Cr, the urinary creatinine in mol/ 24 hr.

Crystallisation experiments

The CaOx MSL was determined as described in Chapter 5. Examination of crystals deposited in urine on day 0, day 3 and day 10 was performed by SEM as described in Chapter 5. The effect of urine on the rate of CaOx crystal nucleation and aggregation on day 0, day 3 and day 10 was determined using the crystallisation assay described in Chapter 5. In this crystallisation assay a metastable solution containing calcium and oxalate only, served as the control. These kinetic experiments were conducted on different days and as such the control values varied from day to day. Therefore, experimental values were compared to the control measured on each particular day. All crystallisation experiments were performed a day after the sample was received.

Statistics

Statistical analysis was performed using the software program GraphPad InStat 3. Repeated measures ANOVA were performed for multiple intra-group comparisons followed by a post-hoc Tukey- Kramer test. Unpaired t-tests were performed for inter-group comparisons. A p value < 0.05 was considered statistically significant.

Results

Phytate content in oats

The concentration of IP6 in oats is presented in Table 6.5. Raw data are presented in Appendix 6.4. The mean concentration of IP6 in each 20 ml extract was 2.38 ± 0.02 mM (shown in row 1). Standard additions of 0.6 mM IP6 (row 2) allowed for the determination of percentage recovery which was calculated as 88 %. The mean amount of IP6 per 100 g of oats was 1.57 g. Thus, the administered amount of Oats (70 g) in the present study contained 1.10 g of IP6. It should be noted that this concentration was determined in uncooked oats. However, previous studies have shown that IP6 is heat stable up to 100 °C.[420, 421] Thus conventional cooking without pre-soaking of oats has minimal effect on IP6 concentration.[421]

Table 6.5 Concentration of IP6 in oats. Mean and SE are reported.

sample	IP6 in extract mM/ 20 ml	% recovery	IP6 g / 100g	IP6 g / 70g
2g Oats (n=5)	2.38 ± 0.02	88	1.57 ± 0.01	1.10 ± 0.01
2g Oats + 0.6 mM IP6 (n=5)	2.91 ± 0.07			

Nutrient intake

The mean nutrient intakes at baseline (FFQ) and on days 3 and 10 (24 hr food records) for the black subjects are given in Table 6.6.

Attention is drawn to the significant decrease in dietary oxalate and the total IP6 intake on day 3 relative to baseline. This indicates that participants were compliant with regard to decreasing their intake of both oxalate and IP6.

It is noted that several significant differences were observed on day 10 relative to baseline. Among these, is a significant decrease in oxalate hence confirming the subjects' compliance. No significant difference in the total IP6 intake is observed indicating that the administered amount of IP6 in the present study is similar to their estimated baseline intake. All other significant differences will be taken into account when urinary composition is interrogated.

Significant differences on day 3 relative to day 10 will also be taken into account when interrogating urinary composition.

Table 6.6 Nutrient intake in black subjects at baseline, day 3 and day 10. Mean ± SE.

	Baseline[a] (A)	Day 3[b] (B)	Day 10[b] (C)	p value (A vs B)	p value (A vs C)	p value (B vs C)
Total protein (g/day)	114.5 ± 14.8	104.0 ± 20.9	58.6 ± 9.2	> 0.05	< 0.05*	>0.05
Total fat (g/day)	100.8 ± 16.9	74.1 ± 12.3	49.1 ±6.1	> 0.05	< 0.05*	>0.05
Total carbohydrate (g/day)	418 ± 57	281 ± 10	227 ± 19	> 0.05	< 0.01*	< 0.05*
Fibre (g/day)	39.3 ± 6.0	16.6 ± 1.0	19.2 ± 2.2	<0.01*	< 0.01*	>0.05
Added sugar (g/day)	60.6 ± 15.4	43.8 ± 5.0	42.4 ± 6.4	> 0.05	> 0.05	>0.05
Oxalate (mg/day)	68.9 ± 18.2	23.8 ± 11.8	24.1 ± 17.6	< 0.05*	< 0.05*	>0.05
Calcium (mg/day)	992 ± 253	591 ± 63	548 ± 83	> 0.05	> 0.05	>0.05
Magnesium (mg/day)	534 ± 82	277 ± 20	284 ± 20	< 0.05*	< 0.05*	>0.05
Phosphorous (mg/day)	1955 ± 298	1302 ± 170	1081 ± 105	> 0.05	< 0.05*	>0.05
Potassium (mg/day)	4059 ± 663	2576 ± 284.7	1403 ± 229	> 0.05	< 0.05*	>0.05
Sodium (mg/day)	2836 ± 442	2540 ± 252	1403 ± 177	> 0.05	< 0.05*	< 0.05*
Vitamin A (RE/day)	1727 ± 445	1597 ± 1235	772 ± 434	> 0.05	> 0.05	>0.05
Vitamin B6	47.7 ± 44.2	3.14 ± 0.35	0.92 ± 0.20	> 0.05	< 0.01*	<0.001*
Vitamin C (mg/day)	151.5 ± 29.3	72.9 ± 16.9	50.3 ± 19.8	> 0.05	< 0.05*	>0.05
Vitamin D (ug/day)	8.6 ± 0.1	5.82 ± 1.22	0.81 ± 0.43	> 0.05	< 0.001*	<0.01*
Vitamin E (mg/day)	15.0 ± 3.2	7.70 ± 1.33	4.56 ± 0.89	> 0.05	< 0.05*	>0.05
Citric acid (mg/24h)	1566 ± 481	1101 ± 339	787 ± 138	> 0.05	> 0.05	>0.05
IP6 (fruit & veg) (mg/day)	290 ± 70	193 ± 47	120 ± 46	> 0.05	> 0.05	<0.0001*
IP6 (wholegrains and legumes) (mg/day)	1360 ± 178	0	1100 ± 0	< 0.0001*	> 0.05	<0.0001*
Total IP6 (mg/day)	1650 ± 202	193 ± 47	1220 ± 46	< 0.0001*	> 0.05	<0.0001*

[a] Baseline intake assessed using FFQ, [b]including nutrient content of administered breakfasts in the present study.

*indicates statistical significance.

The mean nutrient intakes at baseline, day 3 and day 10 are reported in Table 6.7 for the white group. The food diaries of 3 subjects were excluded as one subject deviated from the prescribed diet and two subjects collected incomplete urine samples. The results obtained from these subjects were therefore excluded from all analyses.

Both oxalate and the total IP6 intake decreased significantly on day 3 relative to baseline indicating the subjects' compliance with regard to the protocol of the present study. Oxalate was also significantly lower on day 10 relative to baseline. Dietary IP6 was significantly higher on day 10 relative to baseline indicating that the administered amount of IP6 in the present study was significantly higher than their usual intake.

The total dietary IP6 intake was significantly higher on day 10 relative to day 3 in accordance with the protocol of the present study.

Table 6.7 Nutrient intake in white subjects at baseline, day 3 and day 10. Mean ± SE.

	Baseline[a] (A)	Day 3[b] (B)	Day 10[b] (C)	p value (A vs B)	p value (A vs C)	p value (B vs C)
Total protein (g/day)	84.6 ± 11.4	82.2 ± 11.1	306.9 ± 234.1	> 0.05	> 0.05	> 0.05
Total fat (g/day)	91.7 ± 16.8	66.8 ± 15.6	68.7 ± 16.2	> 0.05	> 0.05	> 0.05
Total carbohydrate (g/day)	240 ± 30	249 ± 38	233 ± 30	> 0.05	> 0.05	> 0.05
Fibre (g/day)	26.3 ± 4.6	20.1 ± 3.8	15.8 ± 1.7	> 0.05	> 0.05	> 0.05
Added sugar (g/day)	46.2 ± 11.8	40.4 ± 7.8	61.7 ± 15.5	> 0.05	> 0.05	> 0.05
Oxalate (mg/day)	72.2 ± 16.6	17.5 ± 8.8	6.5 ± 3.0	< 0.05*	< 0.05*	> 0.05
Calcium (mg/day)	766 ± 88	870 ± 181	581 ± 67	> 0.05	> 0.05	> 0.05
Magnesium (mg/day)	331 ± 67	260 ± 36	278 ± 35	> 0.05	> 0.05	> 0.05
Phosphorous (mg/day)	1343 ± 161	1325 ± 213	1304 ± 234	> 0.05	> 0.05	> 0.05
Potassium (mg/day)	2823 ± 426	2285 ± 390	2541 ± 446	> 0.05	> 0.05	> 0.05
Sodium (mg/day)	1986 ± 298	2719 ± 440	1753 ± 537	> 0.05	> 0.05	> 0.05
Vitamin A (RE/day)	1023 ± 246	579 ± 175	562 ± 175	> 0.05	> 0.05	> 0.05
Vitamin B6	103 ± 101	2.4 ± 0.2	2.1 ± 0.4	> 0.05	> 0.05	> 0.05
Vitamin C (mg/day)	125.3 ± 31.4	42.1 ± 11.8	50.5 ± 12.2	> 0.05	> 0.05	> 0.05
Vitamin D (ug/day)	4.5 ± 0.9	4.81 ± 2.6	1.9 ± 0.2	> 0.05	> 0.05	> 0.05
Vitamin E (mg/day)	10.9 ± 2.0	6.7 ± 1.3	6.5 ± 1.0	> 0.05	> 0.05	> 0.05
Citric acid (mg/24h)	1079 ± 188	1106 ± 281	706 ± 60	> 0.05	> 0.05	> 0.05
IP6 (fruit & veg) (mg/day)	206 ± 42	167 ± 53	172 ± 62	> 0.05	> 0.05	> 0.05
IP6 (wholegrains and legumes) (mg/day)	434 ± 125	0	1100 ± 0	< 0.001*	< 0.001*	<0.0001*
Total IP6 (mg/day)	640 ± 134	167 ± 53	1272 ± 62	< 0.05*	< 0.01*	<0.001*

[a] Baseline intake assessed using FFQ, [b]including nutrient content of administered breakfasts in the present study.

*Indicates statistical significance.

Inter-group comparisons of the nutrient intakes are shown in Table 6.8. Attention is drawn to the following significant differences: total dietary carbohydrate and IP6 were significantly higher in the black group than in the white group at baseline.

Table 6.8 Inter-group comparison of nutrient intake

	p value		
	Baseline[a]	**Day 3**	**Day 10**
Total protein (g/day)	0.15	0.62	0.27
Total fat (g/day)	0.78	0.73	0.40
Total carbohydrate (g/day)	0.029* (B > W)	0.62	0.87
Fibre (g/day)	0.056	0.68	0.27
Added sugar (g/day)	0.47	0.70	0.49
Oxalate (mg/day)	0.84	>0.99	0.64
Calcium (mg/day)	0.98	0.34	0.49
Magnesium (mg/day)	0.021* (B > W)	0.67	0.86
Phosphorous (mg/day)	0.14	0.93	0.35
Potassium (mg/day)	0.11	0.55	0.17
Sodium (mg/day)	0.22	0.71	0.84
Vitamin A (RE/day)	0.32	0.52	0.54
Vitamin B6	0.13	0.21	0.0085* (B < W)
Vitamin C (mg/day)	0.36	0.20	0.42
Vitamin D (ug/day)	0.11	0.012* (B > W)	0.005* (B < W)
Vitamin E (mg/day)	0.23	0.64	0.17
Citric acid (mg/24h)	0.70	0.34	0.72
IP6 fruit & veg (mg/day)	0.42	0.72	0.50
IP6 (wholegrains and legumes) (mg/day)	0.0002* (B > W)	> 0.99	> 0.99
Total IP6 (mg/day)	0.0002* (B > W)	0.72	0.50

[a] Baseline intake assessed using FFQ, * Indicates statistical significance, "B"= black subjects, "W'= white subjects.

The type of wholegrains and legumes consumed by each group at baseline, the estimated portion size as well as the percentage of subjects who consume each type of IP6 containing food is reported in Table 6.9. Portion sizes were variable amongst subjects and are therefore reported as medians together with ranges. A notable difference in the consumption of beans, whole-wheat bread and maize meal was observed between the respective groups. A higher percentage of the black subjects consumed these foods relative to their white compatriots.

Table 6.9 Type of wholegrains consumed by each group in the present study. The median and the range, indicated in parentheses, are reported for the portion size.

Source of IP6	Black males (n =16)		White males (n =16)	
	% subjects	portion size (g)	% of subjects	portion size (g)
Beans	75	142.5 (30-410)	31	100 (50-400)
Whole wheat bread	94	120 (50 - 300)	50	105 (55-210)
Oats	31	250 (100 - 375)	19	70 (50 - 250)
Maize meal/samp	88	225 (75 - 1125)	19	160 (125 - 250)
Pecan nuts	6	100	0	0
Peanut butter	38	16 (10 -50)	56	15 (10 - 50)
Peanuts	44	125 (30 - 250)	6	60
Brown/wild rice	6	70	12	100 (50 -150)
Cashew nuts	0	0	31	50 (30 - 200)
Lentils	0	0	12	90 (80 - 100)

Urine analysis

The urinary excretions of IP6 on day 0, day 3 and day 10 are reported in Table 6.10 and Table 6.11 for the black and white group, respectively. As mentioned above the results of 3 white males were excluded thus n= 8. The mean, standard deviation (SD), standard error (SE), minimum & maximum, median and p values are reported for each intra-group comparison.

In the black group, no significant difference in the urinary excretion of IP6 was observed for the respective inter-day comparisons. In the white group, urinary IP6 was significantly higher on day 10 relative to both day 0 and day 3 ($p < 0.05$). All raw data are reported in Appendix 6.5.

Table 6.10 Urinary IP6 excretion in black subjects (n= 12).

Parameter	Urinary IP6 (μM)					
	Black subjects (n= 12)			p value		
	Day 0 (A)	Day 3 (B)	Day 10 (C)	A vs B	A vs C	B vs C
mean	0.89	0.82	0.95	> 0.05	> 0.05	> 0.05
SD	0.50	0.51	0.34			
SE	0.14	0.15	0.10			
min	0.31	0.44	0.46			
max	1.69	1.98	1.53			
median	0.76	0.61	0.96			

Table 6.11 Urinary IP6 excretion in white subjects (n= 8).

Parameter	Urinary IP6 (μM)					
	white males (n= 8)			p value		
	Day 0 (A)	Day 3 (B)	Day 10 (C)	A vs B	A vs C	B vs C
mean	0.39	0.39	0.67	> 0.05	< 0.05*	< 0.05*
SD	0.16	0.24	0.2			
SE	0.06	0.08	0.07			
min	0.2	0.16	0.47			
max	0.68	0.75	1.04			
median	0.38	0.32	0.65			

*Indicates statistical significance.

Inter-group comparisons on day 0, day 3 and day 10 are shown in Figure 6.1. On day 0 urinary IP6 was significantly higher in the black group (0.89 ± 0.14 μM) than the white group (0.39 ± 0.06 μM)) (p = 0.02). On day 3, urinary IP6 remained significantly higher in the black group (0.82 ± 0.15 μM) than the white group (0.39 ± 0.16 μM) (p = 0.04). Urinary IP6 was also higher in the black group (0.95 ± 0.10 μM) than the white group (0.67 ± 0.07 μM) on day 10 and approached statistical significance (p = 0.057).

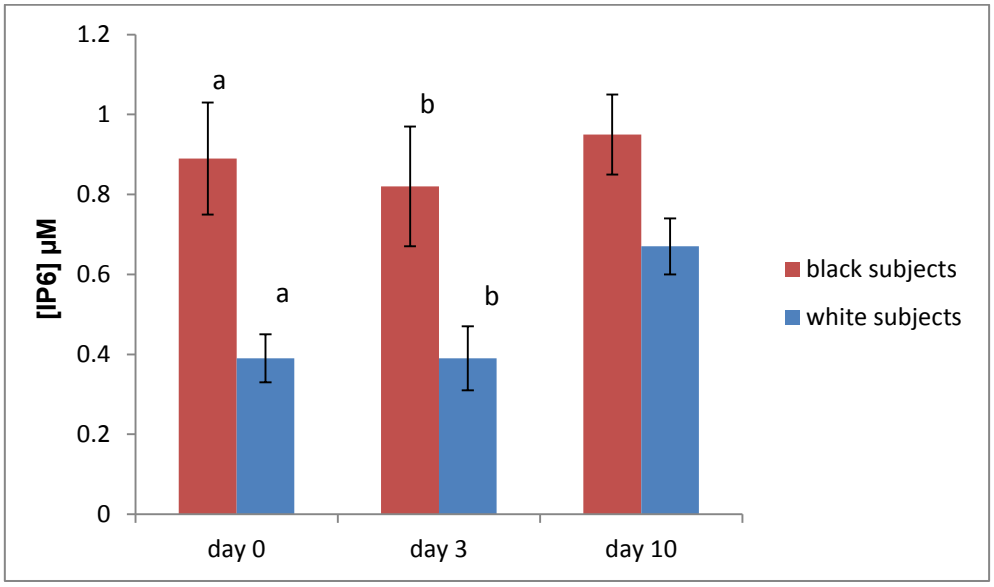


Figure 6.1 Inter-group comparison of urinary IP6 excretion on day 0, day 3 and day 10. The statistical difference between bars labelled with the same letter is significant. [a]p= 0.02. [b]p= 0.04.

The mean urine composition and physicochemical properties on day 0, day 3 and day 10 are summarised in Table 6.12 and Table 6.13 for the black and white group, respectively. All raw data are in Appendix 6.6.

In the black group, attention is drawn to the significant increase in urinary oxalate, magnesium and sodium on day 3 relative to day 0 as well as the significant increase in urinary citrate on day 10 relative to day 3. Since dietary IP6 consumption was similar on day 0 and day 10, the inter-day significant changes in urinary parameters are not of interest and will not be discussed.

In the white group the significant increase in urinary citrate on day 10 relative to day 0 as well as its increase on day 10 relative day 3 (tending toward statistical significance; $p = 0.07$) is of interest.

Table 6.12 Urinary composition and physicochemical properties of black subjects (n=12).

	Day 0 (A)	Day 3 (B)	Day 10 (C)	p value		
	mean ± SE	mean ± SE	mean ± SE	A vs B	Avs C	B vs C
pH	6.70 ± 0.26	6.32 ± 0.15	6.11 ± 0.09	> 0.05	< 0.05*	> 0.05
Volume (ml)	1453 ± 167	1479 ± 196	1686 ± 207	>0.05	> 0.05	> 0.05
Citrate (mmol/24hr)	1.55 ± 0.21	1.61 ± 0.20	2.66 ± 0.33	> 0.05	< 0.01*	< 0.01*
Oxalate (mmol/24hr)	0.22 ± 0.02	0.30 ± 0.03	0.29 ± 0.04	< 0.05*	> 0.05	> 0.05
Calcium (mmol/24hr)	1.97 ± 0.12	2.16 ± 0.17	2.36 ± 0.19	> 0.05	< 0.01*	> 0.05
Magnesium (mmol/24hr)	1.86 ± 0.46	2.35 ± 0.15	2.47 ± 0.20	< 0.01*	< 0.01*	> 0.05
Sodium (mmol/24hr)	102 ± 6.9	130.0 ± 6.7	120.2 ± 8.3	0.002*	> 0.05	> 0.05
Potassium (mmol/24hr)	37.1 ± 2.1	41.0 ± 2.3	36.8 ± 2.8	> 0.05	> 0.05	> 0.05
Urate (mmol/24hr)	3.1 ± 0.8	2.8 ± 0.3	2.8 ± 0.2	> 0.05	> 0.05	> 0.05
Creatinine (mmol/24hr)	14.6 ± 0.93	15.0 ± 0.6	14.5 ± 0.8	> 0.05	> 0.05	> 0.05
Phosphate (mmol/24hr)	22.2 ± 2.9	22.2 ± 1.8	19.1 ± 1.8	> 0.05	> 0.05	> 0.05
Chloride (mmol/24hr)	112 ± 10	146 ± 14	128 ± 10	> 0.05	> 0.05	> 0.05
Phytate (µM)	0.89 ± 0.14	0.82 ± 0.15	0.95 ± 0.10	> 0.05	> 0.05	> 0.05
TRI	165 ± 20	224 ± 37	208 ± 20	> 0.05	> 0.05	> 0.05
RS CaOx	3.30 ± 0.51	4.60 ± 0.54	4.95 ± 0.91	> 0.05	> 0.05	> 0.05
RS Brush	1.29 ± 0.16	1.34 ± 0.19	0.96 ± 0.13	> 0.05	> 0.05	> 0.05
RS Uric	1.02 ± 0.40	1.03 ± 0.34	0.98 ± 0.22	> 0.05	> 0.05	> 0.05
CaOx MSL (mM) (n=8)	1.39 ± 0.20	1.35 ± 0.16	0.94 ± 0.19	> 0.05	> 0.05	> 0.05

*Indicates statistical significance.

Table 6.13 Urinary composition and physicochemical properties of white subjects (n=8).

	Day 0 (A)	Day3 (B)	Day 10 (C)	p value		
	mean ± SE	mean ± SE	mean ± SE	A vs B	Avs C	B vs C
pH	6.01 ± 0.07	5.94 ± 0.12	6.00 ± 0.15	> 0.05	> 0.05	> 0.05
Volume (ml)	1339 ± 131	1428 ± 210	1598 ± 195	> 0.05	> 0.05	> 0.05
Citrate (mmol/24hr)	1.88 ± 0.24	2.48 ± 0.45	2.94 ± 0.41	> 0.05	< 0.05*	> 0.05
Oxalate (mmol/24hr)	0.20 ± 0.03	0.24 ± 0.05	0.31 ± 0.04	> 0.05	> 0.05	> 0.05
Calcium (mmol/24hr)	1.13 ± 0.42	2.53 ± 0.65	2.61 ± 0.64	> 0.05	> 0.05	> 0.05
Magnesium (mmol/24hr)	1.80 ± 0.33	2.18 ± 0.44	1.96 ± 0.28	> 0.05	> 0.05	> 0.05
Sodium (mmol/24hr)	92.8 ± 4.2	105.3 ± 14.1	95.4 ± 12.2	> 0.05	> 0.05	> 0.05
Potassium (mmol/24hr)	26.0 ± 2.7	30.7 ± 2.9	29.0 ± 3.6	> 0.05	> 0.05	> 0.05
Urate (mmol/24hr)	3.7 ± 0.5	3.4 ± 0.5	4.0 ± 0.3	> 0.05	> 0.05	> 0.05
Creatinine (mmol/24hr)	14.8 ± 1.6	15.7 ± 2.1	16.8 ± 1.5	> 0.05	> 0.05	> 0.05
Phosphate (mmol/24hr)	29.6 ± 3.2	26.9 ± 5.0	34.1 ± 2.9	> 0.05	> 0.05	> 0.05
Chloride (mmol/24hr)	117 ± 9.7	136.8 ± 17.4	124.4 ± 13.9	> 0.05	> 0.05	> 0.05
Phytate (µM)	0.39 ± 0.06	0.39 ± 0.08	0.67 ± 0.07	> 0.05	< 0.05*	< 0.05*
TRI	161 ± 32	163 ± 33	231 ± 64	> 0.05	> 0.05	> 0.05
RS CaOx	4.86 ± 0.91	4.45 ± 0.93	4.96 ± 1.04	> 0.05	> 0.05	> 0.05
RS Brushite	1.77 ± 0.27	1.23 ± 0.38	1.13 ± 0.24	> 0.05	> 0.05	> 0.05
RS Urate	2.38 ± 1.21	1.69 ± 0.42	1.90 ± 0.53	> 0.05	> 0.05	> 0.05
CaOx MSL (mM)	1.05 ± 0.16	0.96 ± 0.06	1.31 ± 0.16	> 0.05	> 0.05	> 0.05

*Indicates statistical significance.

Inter-group comparisons of urinary composition and physicochemical properties are shown in Table 6.14. Attention is drawn to the significantly lower concentration of urinary phosphate in the black group than the white group on day 10.

Table 6.14 Inter-group comparisons of urinary composition and physicochemical properties.

	p value		
	Day 0	**Day 3**	**Day 10**
pH	0.14	0.080	0.52
Volume (ml)	0.19	0.86	0.77
Citrate (mmol/24hr)	0.32	0.063	0.59
Oxalate (mmol/24hr)	0.66	0.35	0.75
Calcium (mmol/24hr)	0.91	0.52	0.43
Magnesium (mmol/24hr)	0.59	0.67	0.14
Sodium (mmol/24hr)	0.79	0.097	0.098
Potassium (mmol/24hr)	0.002* (B > W)	0.012* (B > W)	0.11
Urate (mmol/24hr)	0.32 (B < W)	0.23 (B < W)	0.005* (B < W)
Creatinine (mmol/24hr)	0.94	0.70	0.18
Phosphate (mmol/24hr)	0.12	0.32	0.001* (B < W)
Chloride (mmol/24hr)	0.73	0.68	0.83
Phytate (μM)	0.020* (B > W)	0.039* (B > W)	0.057 (B> W)
TRI	0.92	0.26	0.73
RS CaOx	0.12	0.88	0.99
RS Brushite	0.12	0.77	0.49
RS Urate	0.20	0.23	0.09
CaOx MSL (mM)	0.21	0.058	0.16

* Indicates statistical significance. “B”= black subjects. “W”= white subjects.

Crystallisation experiments

CaOx MSL

In the black group, baseline urinary samples of 4 subjects tested positive for bacteria on the day of which crystallisation experiments were conducted. These samples were therefore excluded from crystallisation experiments. Comparisons of the CaOx MSL of these subjects on day 0 relative to day 3 and day 10 could therefore not be made. Thus, statistical comparisons of the CaOx MSL of n= 8 black subjects were calculated.

The mean CaOx MSL for each group on the respective days is reported in Table 6.12 and Table 6.13. Raw data are reported in Appendix 6.7. No statistically significant differences were observed in either group for the respective inter-day comparisons.

Inter-group comparisons of the mean CaOx MSL are reported in Table 6.14. No statistically significant differences were observed on each respective day.

CaOx nucleation and aggregation

The mean percentage inhibition of CaOx crystal nucleation and aggregation rates relative to the control was calculated and is reported in Table 6.15 and Table 6.16, respectively. A negative percentage indicates promotion of CaOx crystal nucleation and aggregation, respectively.

Intra-group comparisons showed no statistically significant differences. Attention is drawn to the significantly higher percentage inhibition of CaOx crystal nucleation in the black group relative to their white counterparts on day 3 and day 10. (It is also noted that the percentage inhibition of CaOx crystal nucleation was higher in the black group on day 0 albeit not statistically significant). Plots of the raw data are shown in Appendix 6.8.

Table 6.15 Intra- and inter-group comparisons of the percentage inhibition of CaOx crystal nucleation. Mean ± SE.

	% inhibition of the rate of CaOx crystal nucleation			p value (intra-group)		
	Day 0 (A)	Day 3 (B)	Day 10 (C)	A vs B	A vs C	B vs C
Black subjects (n=8)	22 ± 5	43 ± 10	43 ± 6	> 0.05	> 0.05	> 0.05
White subjects (n=8)	13 ± 13	11 ± 8	-10 ± 15	> 0.05	> 0.05	> 0.05
p value (inter-group)	0.15	0.031*	0.0003*			

Negative percentage indicates promotion of CaOx crystal nucleation. *Indicates significant difference.

Table 6.16 Intra- and inter-group comparison of the percentage inhibition of CaOx crystal aggregation. Mean ± SE.

	% inhibition of the rate of CaOx crystal aggregation			p value (intra-group)		
	Day 0 (A)	Day 3 (B)	Day 10 (C)	A vs B	A vs C	B vs C
Black subjects (n =8)	23 ± 11	4.7 ± 16	29 ± 13	> 0.05	> 0.05	> 0.05
White subjects (n=8)	33 ± 6	41 ± 5	33 ± 8	> 0.05	> 0.05	> 0.05
p value (inter-group)	0.46	0.23	0.51			

Scanning electron microscopy

Crystals deposited in the urines of 3 randomly chosen black and 3 randomly chosen white subjects were viewed on day 0, day 3 and day 10. The total surface-area of each stub was examined and micrographs of typical deposits with respect to morphology, size and aggregation were recorded as described in Chapter 5.

Figure 6.2 shows electron micrographs of crystals deposited in urine of 3 black subjects on day 0 (BM1a - BM3a), day 3 (BM1b - BM3b) and day 10 (BM1c - BM3c). At baseline (day 0) the presence of single, bipyramidal COD crystals (mean cross section of 6 μm) was observed for all subjects.

On day 3 the presence of single, oval-shaped COM crystals (mean cross section of 4 μm) was found in 2 out of 3 subjects (BM1b & BM2b) whereas single COD crystals (mean cross section of 8 μm) were present in all.

On day 10, different observations were recorded for the three subjects. Single oval-shaped COM crystals (mean cross section 6 μm) occurred in one subject (BM1c) while single COD crystals (mean cross section 8 μm) occurred in another (BM3c). Mixed COM and COD occurred in the final subject (mean cross sections of 4 μm and 6 μm; respectively). In addition, a few small aggregated COM crystals were observed in two of the subjects (BM1c and BM2c).

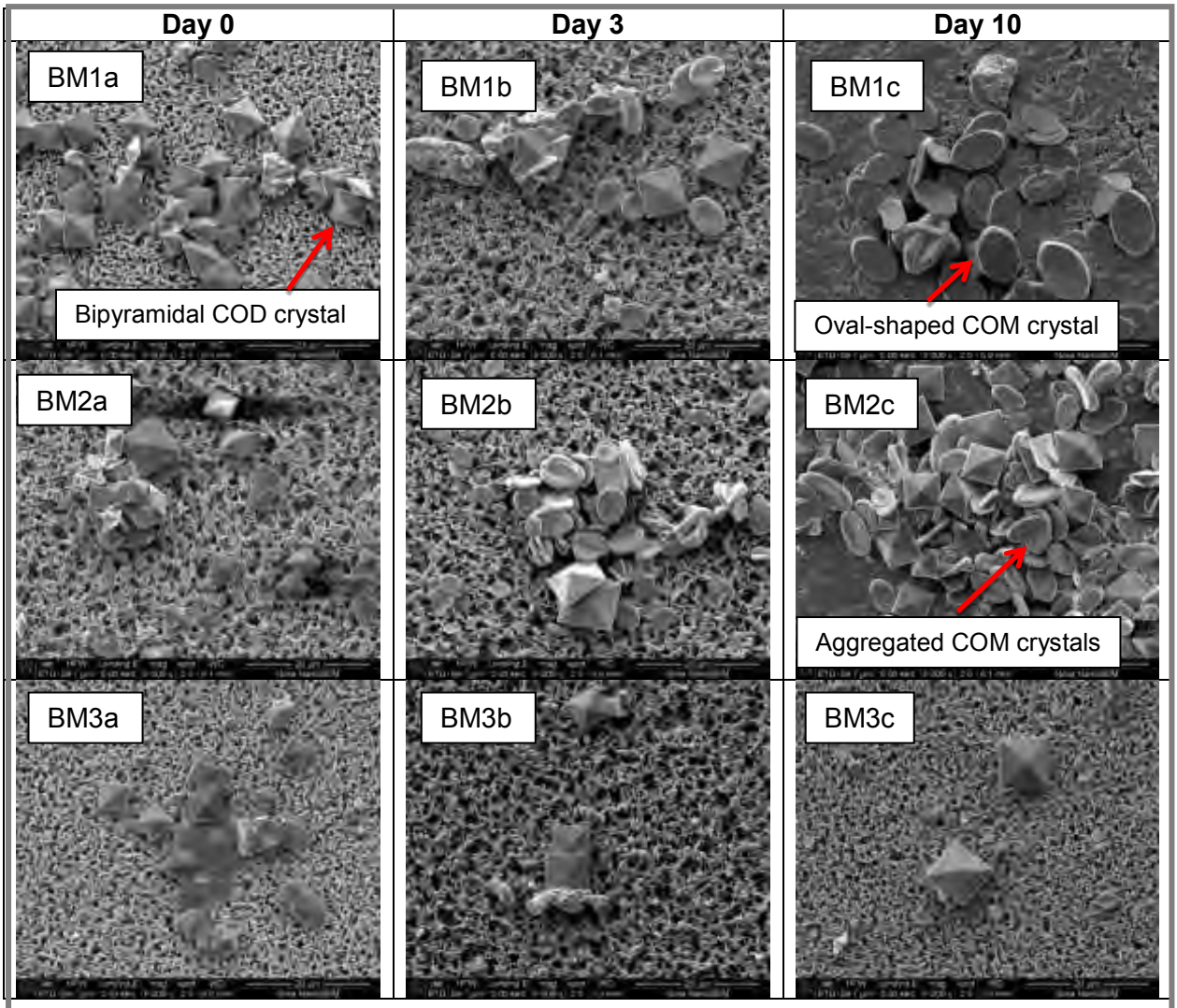


Figure 6.2 Electron micrographs of CaOx crystals deposited in urine of black subjects on day 0 (BM1a – BM3a), day 3 (BM1b – BM3b) and day 10 (BM1c – BM3c). Micrographs are at a magnification of x 5000.

Figure 6.3 shows electron micrographs of crystals deposited in urine of 3 white subjects on day 0 (WM1a – WM3a), day 3 (WM1b – WM3b) and day 10 (WM1c – WM3c). At baseline (day 0) a mixture of both COM and COD crystals (mean cross sections of 6 µm and 9 µm; respectively) were observed in 2 out of 3 subjects (WM2a & WM3a) whereas single COD crystals alone (mean cross section of 9 um) were observed in the other (WM1a). A few large COD crystals with an altered morphology were observed in one sample (WM2a).

On day 3, COM crystals (mean cross section of 5 µm) were predominant in all samples. On day 10 mostly single bipyramidal COD crystals were observed in all samples (mean cross section of 9 µm).

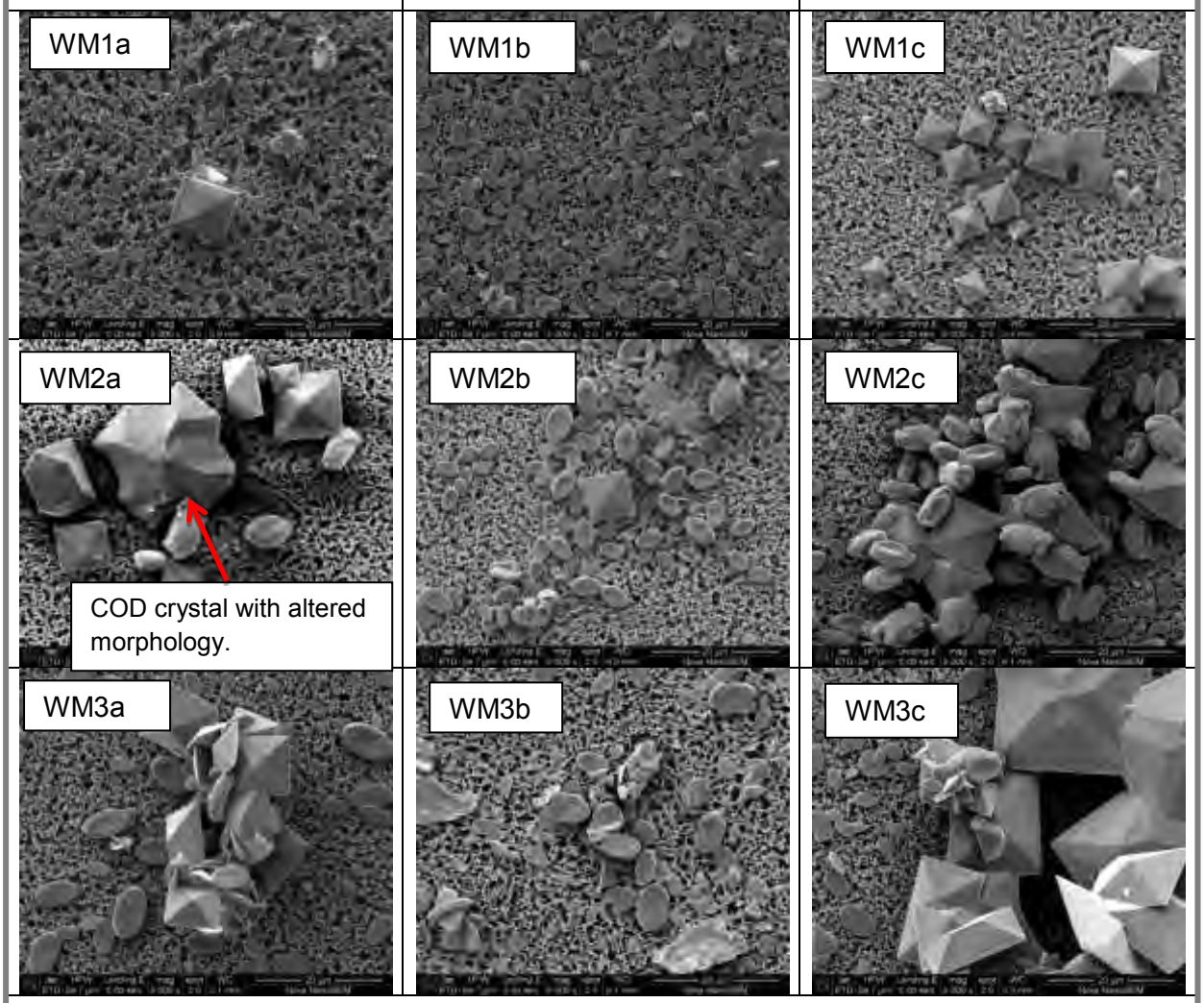


Figure 6.3 Electron micrographs of CaOx crystals deposited in urine of white subjects on day 0 (WM1a – WM3a), day 3 (WM1b – WM3b) and day 10 (WM1c – WM3c). Micrographs are at a magnification of x 5000.

Discussion

Based on a previous study by Modlin[318], the hypothesis under investigation in the present study was that a significantly higher intake of dietary IP6 in South African black subjects relative to their white compatriots leads to a higher urinary excretion level of this substance which, in turn, contributes to the relative rarity of kidney stone disease in this group. Since the study by Modlin was conducted 35 years ago, the first objective of the study described here was to assess whether black subjects still have a relatively high intake of this nutrient compared to their white compatriots. Results of the present study showed that dietary IP6 was indeed significantly higher in black subjects compared to white subjects.

The estimated total dietary intake of IP6 in both groups of the present study differed to those of Modlin's[318] study as follows. The intake of this nutrient in the black group was lower in the present study relative to Modlin's study (1650 ± 202 mg/ day vs 4290 mg/ day); and higher in the white group of the present study relative to Modlin's study (640 ± 134 mg/ day vs 275 mg/ day).While the former might be indicative of a change in lifestyle associated with a transition from rural to urban areas[422, 423], the reason for the latter is not obvious. Irrespective of these differences, the major contributors of the total IP6 intake in the black group which were identified in the present study, namely maize, beans and wholewheat bread are in agreement with those reported by Modlin[318]. Furthermore, a study in a large cohort of 96 245 females demonstrated a correlation between a high dietary intake of IP6 and the decreased risk of kidney stone formation.[424] Thus, the higher dietary intake of IP6 in the black subjects of the present study relative to their white compatriots might contribute to the relative rarity of kidney stone disease in this group.

Of importance to the present hypothesis is to establish whether the higher intake of dietary IP6 in black subjects manifests itself in a higher urinary excretion of this substance. The results of the present study showed that the mean urinary IP6 was indeed significantly higher in the black group than in the white group while following their free unrestricted diets (Figure 6.1). These results are however not in agreement with the only other study in which IP6 was measured in the urine of the respective groups. This latter study was conducted in the KSRL at the University of Cape Town, as stated earlier in the introduction of the present chapter.[319] Not only was the concentrations of IP6 in both groups higher than those of the present study, the urinary excretion of this substance in the black group was lower than in the white group in the previous study. This discrepancy can be attributed to a difference in the analytical methods used in the two studies, possibly linked to the insufficient separation of inorganic phosphate in the previous study during sample preparation (as mentioned in Chapter 1) which yields a false positive for the quantification of IP6. This explains the higher IP6 values in the previous study relative to the present study. Furthermore, previous studies

showed that urinary phosphate is higher in the white group relative to the black group.[4, 5, 294, 296, 302] Thus, it can be speculated that this might have resulted in a higher false positive detection of IP6 in the urine of white subjects in the previous study which, in turn, explains the reported lower urinary IP6 in black subjects relative to white subjects. The significantly higher urinary excretion of IP6 in South African black subjects relative to white subjects in the present study has therefore been reliably demonstrated for the first time.

A previous study reported that the urinary excretion of IP6 was significantly lower in CaOx stone formers than in healthy European (Spanish) individuals.[265] Furthermore, the urinary excretions of IP6 in the black subjects of the present study are within the normal urinary range reported for healthy European (Spanish) individuals (0.757- 4.54 μM)[259]. Therefore, the significantly higher urinary excretion of IP6 in the black subjects of the present study relative to their white compatriots may be consistent with the relative rarity of urolithiasis in this group.

With regard to the urinary excretion of IP6 in relation to its ingestion, the results of the present study showed that no significant decrease was observed after 3 days of IP6 restriction in either group. This suggests that 3 days of restriction is insufficient to observe a significant decrease. This is inconsistent with a previous study by Grases et al in which urinary IP6 decreased by 50 % after only 36 hrs of IP6 restriction.[391] Nonetheless, other studies by the same researchers demonstrated that urinary IP6 reached minute levels when its ingestion was restricted for a period of 15 days.[268, 392] When IP6 was administered in the present study no significant increase was observed in the black group on day 10 relative to day 0 (Table 6.10). This might be explained by the fact that the administered amount in the present study (1100 ± 10 mg) is similar to their mean estimated intake at baseline (1650 ± 202 mg). Accordingly, no significant increase in urinary IP6 was observed on day 10 relative to day 3 in this group. On the other hand, urinary IP6 increased significantly in the white group on day 10 relative to day 0 and day 3. This is consistent with the significant increase in the ingestion of IP6 in accordance with the protocol of the present study thereby demonstrating that an increase in urinary IP6 is correlated to its dietary intake.

Inter-group comparisons of urinary IP6 on day 10 demonstrated that when the amount of IP6 ingestion was the same between the two groups, the urinary excretion of IP6 in the black group was higher than in their white compatriots approaching statistical significance ($p= 0.057$). It is therefore tempting to speculate that the absorption of IP6 may be higher in the black group than the white group. However, studies have indicated that the rate of absorption of IP6 is low, requiring more than 10 days to reach normal levels when humans become deficient in this substance.[268, 269, 392] Thus, since urinary IP6 was significantly lower in

the white group relative to the black group on day 0 and day 3 (and possibly immediately prior to Phase 2), it is possible that the urinary excretion of this substance may approach that of the black group after a more prolonged ingestion of a diet rich in IP6.

With regard to possible metabolic effects of IP6 ingestion on the urine chemistry, the following should be borne in mind. As previously stated in Chapter 1, an excessive intake of IP6 in conjunction with a low intake of minerals such as calcium and magnesium may lead to a decrease in the bioavailability of these minerals and hence its urinary excretion.[270-272] Thus, the converse may be true, namely that a significant decrease in IP6 consumption may lead to an increase in these urinary parameters. Furthermore, studies have demonstrated that the ingestion of 1- 2 g of IP6 with a balanced diet had a negligible effect on the bioavailability of calcium and magnesium.[425, 426] Thus, in the present study, changes in the excretion of urinary calcium and magnesium were not anticipated since both the estimated baseline intakes of IP6 in the respective groups and the administered amount of IP6 were not in excess of the above mentioned range. However, it is speculated by the present researcher that changes in other biochemical urinary parameters associated with possible unknown metabolic effects of IP6 itself or its degradation products, namely phosphate and lower phosphorylated inositols, may occur.

In light of the above, the significant increase in both urinary calcium and magnesium in the black group on day 3 relative to day 0 is unlikely to be attributed to the significant decrease in IP6 ingestion in the present study. This might have occurred due to fluctuations in the subjects' dietary regime since meals were not standardized in the present study. This will not be discussed here as it digresses from the objectives of the present study.

Regarding significant changes in other biochemical urinary parameters, the significant increase in urinary citrate in both groups on day 10 (after IP6 ingestion) relative to day 3 (Table 6.12 and Table 6.13), is of interest. Citrate is well known for its inhibitory properties in CaOx urolithiasis as mentioned in Chapter 1. An increase in urinary citrate may be caused by an increase in plasma citrate (caused by an increase in citrate ingestion) or by metabolic alkalosis.[427, 428] Both of the aforementioned processes result in an increase in urinary pH. However, in the present study no increase in urinary pH was observed in either group thereby ruling out the aforementioned mechanisms as a possible explanation for the increase in urinary citrate on day 10 relative to day 3. Other possible causes of increased urinary citrate include that of an increase in vitamin E intake[298], dietary fat and dietary carbohydrate[429]. None of these increased in either group for the respective inter-day comparisons. Furthermore, no correlation between dietary IP6 ingestion and the excretion of urinary citrate has been reported. However, a previous study demonstrated that the ingestion

of alkaline orthophosphate (K_2HPO_4) increased the urinary excretion of citrate.[430] Another study also demonstrated that the ingestion of a potassium- phosphate preparation (Urophos-K) increased citraturia.[228] Thus, since the hydrolysis of IP6 yields phosphate,[431-433] it is speculated by the present researcher that the latter might have influenced the urinary excretion of citrate in the present study. The mechanism by which orthophosphate caused an increase in urinary citrate was however not explained in the previous studies. Nonetheless, this observation is encouraging. No significant difference in the excretion of citrate was observed between the two groups, suggesting that the renal handling of IP6 in this regard is similar between the two groups. However, a significantly lower excretion of phosphate was observed in the black group relative to their white compatriots on day 10 (Table 6.14) indicating that the white subjects are more sensitive to the absorption of phosphate. This is consistent with previous studies as stated earlier in this discussion.

The final objective of the present study was to determine whether a significant increase/ decrease in the urinary excretion of IP6 results in a significant effect on urinary CaOx crystallisation. This objective is based on the results presented in Chapter 5 of the present thesis is which certain crystallisation mechanisms were inhibited by IP6 in a concentration dependent manner *in vitro*. Furthermore, a previous *in vivo* study in CaOx stone formers demonstrated that an increase in urinary IP6 reduced the crystallisation propensity of the urine of these patients.[292] However, in the present *in vivo* study, since no significant difference in urinary IP6 was observed in the black group for the respective inter-day comparisons, a conclusion cannot be made on phytate's capacity to inhibit crystallisation in the urine of this group.

On the other hand, a significant increase in urinary IP6 was observed in the white group on day 10 relative to both day 3 and day 0 (Table 6.11), but it was not sufficient to increase the CaOx MSL (Table 6.13). This is consistent with the observation reported in the preceding chapter in which physiological concentrations of IP6 did not increase the CaOx MSL in pooled urine of white subjects. Furthermore, the significant increase in urinary IP6 in the present study was not sufficient to increase the urine's inhibitory capacity with regard to CaOx crystal nucleation and aggregation, nor was it sufficient to cause favourable changes in CaOx crystal size and/ hydration state (SEM). These results cast doubt on the purported inhibitory capacity of IP6 in CaOx urolithiasis. However, a recent *in vitro* study demonstrated that the effective concentration of IP6 required for the inhibition of artificially grown stones had no effect on the crystallisation of CaOx in suspension around the growing stone.[285] Therefore, although the significant increase in urinary IP6 failed to increase the CaOx MSL and inhibit CaOx crystal nucleation and aggregation in the present in vivo study, it may be crucial in the prevention of kidney stone growth at a macroscopic level.

The only noteworthy inter-group difference with regard to CaOx crystallisation was the higher percentage inhibition of CaOx crystal nucleation in the urine of black subjects relative to white subjects on each respective day (Table 6.15). This may not necessarily be due to the higher urinary excretion of IP6 in the black group *per se* as other urinary parameters such as the significantly higher urinary excretion of potassium and the lower urinary excretion of urate may have contributed towards this effect in this group (Table 6.14), although the mechanisms by which this may have been achieved are not obvious.

It is recognized that the outcomes of the present study were influenced by its duration which may have been too short with respect to the period of IP6 restriction. This would account for no significant decrease in urinary IP6 being observed in either group when dietary IP6 was restricted. A second limitation in the study design was the absence of urinary IP6 determinations immediately prior to initiating Phase 2 i.e. on day 7. As such it was not possible to demonstrate whether urinary IP6 was at a reduced level prior to the administration of the IP6 rich diet. At first glance it may appear that there are two further limitations to this study namely, that the dosage of IP6 might have been too small and that the period in which it was administered was too short. However, these limitations must be considered individually for the respective groups. Since urinary IP6 increased significantly in the white group after the ingestion of the administered amount of IP6 in the present study, it can be concluded that the dosage was satisfactory and that the duration during which it was administered was adequate. On the other hand, these variables (dosage and duration) might need further exploration in black subjects in future studies.

Irrespective of the above, the results of the present study are important since the significantly higher urinary excretion of IP6 in South African black subjects was demonstrated for the first time in the present study. Furthermore, results of the present study suggest that IP6 ingestion may increase the urinary excretion of citrate. This was also demonstrated for the first time.

In order to address the limitation relating to the suspected short duration of IP6 restriction, a further study was undertaken. This is described in the next chapter.

Chapter 7 : Investigation of the effect of an extended period of dietary restriction of IP6 followed by ingestion of an IP6 supplement on the urinary risk factors for calcium oxalate urolithiasis in South African black and white subjects.

Introduction

The study described in this chapter is a follow up to that described in the previous chapter in which a decrease in the urinary excretion of IP6 was not observed after 3 days of dietary IP6 restriction. The present study was conducted to address this limitation.

Methods

Study design

A similar design as described in Chapter 6, was used in the present study. However, instead of a 10- day cross-over study, an 18- day cross-over study (again consisting of two phases) was conducted.

Prior to the first phase of the study, a 24 hr baseline urine sample was provided by subjects while following their free unrestricted diets. Phase 1 (IP6 restricted) had a duration of 15 days (compared to 7 days in the previous study), during which urinary IP6 excretion was determined in 24 hr urine samples on days 3, 7 and 15. This duration period was used as previous studies by Grases et al demonstrated that urinary IP6 approached values close to zero after 15 days of dietary IP6 restriction.[268, 392]

During Phase 2 of the present study, an IP6 supplement (*IP6 Gold, immune support formula, USA*) containing 800 mg of IP6 was administered on days 16 -18. The switch from an IP6 rich diet (preceding chapter) to an IP6 supplement was implemented due to its ease of administration and to reduce participant burden with respect to the dietary protocol. Although no studies have been previously reported on the bioavailability of IP6 from the diet relative to its supplemental form, it can be reasonably speculated that its bioavailability from the latter may be greater as it is not part of a complex food matrix.

Although the dosage of IP6 delivered by the supplement is lower than in the study presented in the previous chapter (1100 ± 10 mg), it is within the range reported in a previous study (400- 3200 mg).[268] The latter study demonstrated that the ingestion of IP6 supplements at different dosages within this range resulted in the same urinary excretion of IP6. In the present study, subjects were required to ingest the IP6 supplement on an empty stomach, as directed on the supplement bottle, 1 hr before breakfast. Urinary IP6 was measured on day 18.

Urine aliquots were kept for the determination of biochemical urine parameters as well as for crystallisation experiments.

As in the preceding study (Chapter 6), dietary IP6 and oxalate were restricted for the duration of the study (Table 6.2 and Table 6.3). Each subject was required to keep a dietary record on days of urine collection. In an attempt to minimize confounding dietary factors, subjects were asked not to vary their dietary intakes on all days of urine collection (excluding day 0). This also ensured that each subject served as their own control.

This study was approved by the Human Research Ethics Committee of the University of Cape Town, HREC ref: 072/2014 (Appendix 7.1).

Subjects

Healthy black (n =7) and white (n =7) South African males between the ages of 18 -25, without any history of kidney stone disease were recruited from the student and staff cohort at the University of Cape Town. Vegetarians and individuals with nut allergies were excluded. Eligible participants were selected after completing a general questionnaire assessing their health. Race was self-declared.

Dietary analysis

24 hr dietary records were analysed using FoodFinder 2 as described in Chapter 6.

Urine collection, treatment and composition

24 hr urine samples were collected and treated as described in Chapter 6. Urinary composition, including IP6 analysis was determined as described in Chapter 6. Ionized calcium was determined as described in Chapter 5.

Physicochemical properties

The RS of CaOx, brushite and uric acid and the TRI was determined as described in Chapter 6.

Crystallisation experiments

The CaOx MSL was determined; this was then followed by determining the rate of CaOx crystallisation. Both methods are fully described in Chapter 5. These experiments were performed in urines collected on days 0, day 15 and day 18. The effect of urine samples on the rate of CaOx crystal nucleation and aggregation was not determined in the present study (as had been done in the study described in the previous chapter), as the required instrument malfunctioned at the time at which this study was undertaken.

Statistics

Statistical analyses of data on days 0, 15 and 18 were determined using GraphPad Instat 3. Repeated measures ANOVA were performed for multiple intra-group comparisons followed by a post-hoc Tukey- Kramer test. Unpaired t-tests for inter-group comparisons were performed. A p value < 0.05 was considered statistically significant.

Results

Dietary analysis

Inter-day comparisons of the nutrient intake on days 0, 15 and 18 were determined and are shown in Table 7.1 and Table 7.2 for the black and white group, respectively.

In the black group, the only significant difference was in the intake of vitamin B6 which was significantly higher on day 18 relative to day 0 (Table 7.1). Since vitamin B6 may influence oxaluria.[434-436], its higher intake in the black group may be a confounding factor which needs to be taken into consideration when interrogating the urine chemistry (page 161). Attention is drawn to the decrease in the ingestion of total dietary IP6 on days 15 and 18 relative to day 0 (albeit not statistically significant) in accordance with the protocol of the present study in which subjects were required to restrict their dietary intake of IP6.

In the white group, no significant differences in the nutrient intake were observed for the respective inter-day comparisons thus eliminating the possibility of confounding dietary factors. (Table 7.2). A decrease in the ingestion of total dietary IP6 was observed on day 15 and day 18 relative to day 0 (albeit not statistically significant) in accordance with the protocol of the present study in which subjects were required to restrict their dietary intake of IP6.

Inter-group comparisons showed no significant differences in the nutrient intake as shown in Table 7.3.

It should be noted that the dietary intake on day 0 (baseline) corresponds to the subjects' nutrient intake on that particular day and is not necessarily representative of their typical dietary intake (unlike that obtained in Chapter 6 for the FFQs). The nutrient analyses of these single 24 hr dietary records were therefore not used as a means to compare the usual/ typical intakes in black versus white subjects but rather as a means to account for any intra- or inter-group differences in urine parameters which may have occurred due to differences in their nutrient intakes on days of urine collection.

Table 7.1 Nutrient intake in black subjects (n= 7) on day 0, day 15 and day 18. Mean ± SE.

	Day 0 (A)	Day 15 (B)	Day 18 (C)	p value (A vs B)	p value (A vs C)	p value (B vs C)
Total protein (g/ day)	75.4 ± 18.3	55.6 ± 14.3	67.1 ± 15.9	> 0.05	> 0.05	> 0.05
Total fat (g/ day)	52.2 ± 6.8	39.2 ± 9.9	56.2 ± 9.6	> 0.05	> 0.05	> 0.05
Total carbohydrate (g/ day)	197.7 ± 20.0	211.6 ± 41.7	241.8 ± 36.0	> 0.05	> 0.05	> 0.05
Fibre (g/ day)	15.0 ± 3.1	17.8 ± 7.8	25.0 ± 7.0	> 0.05	> 0.05	> 0.05
Added sugar (g/ day)	13.1 ± 4.8	4.4 ± 2.3	6.2 ± 2.0	> 0.05	> 0.05	> 0.05
Oxalate (mg/ day)	8.85 ± 5.9	52.7 ± 44.8	56.8 ± 44.4	> 0.05	> 0.05	> 0.05
Calcium (mg/ day)	407.4 ± 92.7	427.7 ± 139.2	536.6 ± 120.7	> 0.05	> 0.05	> 0.05
Magnesium (mg/ day)	225.7 ± 35.2	223.6 ± 93.4	306.6 ± 84.3	> 0.05	> 0.05	> 0.05
Phosphorous (mg/ day)	947.3 ± 155.1	818.7 ± 257.1	1071.7 ± 247.7	> 0.05	> 0.05	> 0.05
Potassium (mg/ day)	1591.1 ± 115.2	1776.3 ± 538.7	2677.1 ± 115.2	> 0.05	> 0.05	> 0.05
Sodium (mg/ day)	2043.1 ± 405.1	2278.6 ± 615.6	2533.1 ± 564.6	> 0.05	> 0.05	> 0.05
Vitamin A (RE/ day)	228.0 ± 65.1	249.3 ± 78.8	816.0 ± 564.4	> 0.05	> 0.05	> 0.05
Vitamin B6	1.29 ± 0.16	1.36 ± 0.36	2.03 ± 0.18	> 0.05	< 0.01*	> 0.05
Vitamin C (mg/ day)	67.1 ± 27.8	80.7 ± 32.2	103.4 ± 36.6	> 0.05	> 0.05	> 0.05
Vitamin D (ug/ day)	5.31 ± 1.54	2.20 ± 1.23	2.61 ± 1.30	> 0.05	> 0.05	> 0.05
Vitamin E (mg/ day)	11.3 ± 3.1	9.3 ± 3.8	9.7 ± 3.7	> 0.05	> 0.05	> 0.05
Citric acid (mg/ day)	464.9 ± 166.9	671.1 ± 451.6	750.4 ± 443.6	> 0.05	> 0.05	> 0.05
IP6 (fruit & veg) (mg/ day)	270.3 ± 100.6	304.6 ± 60.8	255.9 ± 57.0	> 0.05	> 0.05	> 0.05
IP6(wholegrains and legumes) (mg/ day)	232.0 ± 149.8	0	0	> 0.05	> 0.05	> 0.05
Total IP6 (mg/day)	502.3 ± 122.8	304.6 ± 60.8	255.9 ± 57.0	> 0.05	> 0.05	> 0.05

*Indicates statistical significance.

Table 7.2 Nutrient intake in white subjects (n= 7) on day 0, day 15 and day 18. Mean ± SE.

	Day 0 (A)	Day 15 (B)	Day 18 (C)	p value (A vs B)	p value (A vs C)	p value (B vs C)
Total protein (g/ day)	84.0 ± 17.7	87.0 ± 14.1	81.7 ± 17.5	> 0.05	> 0.05	> 0.05
Total fat (g/ day)	74.0 ± 14.1	78.0 ± 14.2	101.7 ± 23.3	> 0.05	> 0.05	> 0.05
Total carbohydrate (g/ day)	207.4 ± 58.6	159.7 ± 81.6	157.9 ± 24.6	> 0.05	> 0.05	> 0.05
Fibre (g/ day)	21.1 ± 7.5	11.9 ± 2.8	11.2 ± 3.0	> 0.05	> 0.05	> 0.05
Added sugar (g/ day)	21.8 ± 10.9	17.0 ± 9.9	21.2 ± 8.9	> 0.05	> 0.05	> 0.05
Oxalate (mg/ day)	13.1 ± 7.5	41.4 ± 28.5	24.0 ± 14.2	> 0.05	> 0.05	> 0.05
Calcium (mg/ day)	756.3 ± 239.0	461.3 ± 75.9	430.9 ± 55.7	> 0.05	> 0.05	> 0.05
Magnesium (mg/ day)	670.4 ± 542.7	190.9 ± 28.3	186.3 ± 23.4	> 0.05	> 0.05	> 0.05
Phosphorous (mg/ day)	1131.1 ± 237.4	1003.6 ± 95.6	1031.7 ± 168.2	> 0.05	> 0.05	> 0.05
Potassium (mg/ day)	7545.7 ± 5907.7	2086.7 ± 346.9	2235.6 ± 427.6	> 0.05	> 0.05	> 0.05
Sodium (mg/ day)	2837.7 ± 412.2	1730.1 ± 266.6	2465.3 ± 946.2	> 0.05	> 0.05	> 0.05
Vitamin A (RE/ day)	540.2 ± 306.4	327.1 ± 49.1	205.4 ± 40.4	> 0.05	> 0.05	> 0.05
Vitamin B6	1.64 ± 0.68	1.58 ± 0.40	1.42 ± 0.34	> 0.05	> 0.05	> 0.05
Vitamin C (mg/ day)	125.9 ± 77.4	102.1 ± 58.3	49.6 ± 18.8	> 0.05	> 0.05	> 0.05
Vitamin D (ug/ day)	7.6 ± 3.5	7.0 ± 2.2	6.4 ± 2.4	> 0.05	> 0.05	> 0.05
Vitamin E (mg/ day)	65.6 ± 51.3	12.6 ± 2.8	10.8 ± 2.4	> 0.05	> 0.05	> 0.05
Citric acid (mg/ day)	990.4 ± 499.3	512.3 ± 207.9	991.9 ± 364.4	> 0.05	> 0.05	> 0.05
IP6 (fruit & veg) (mg/ day)	112.7 ± 34.1	180.4 ± 67.8	204.8 ± 40.4	> 0.05	> 0.05	> 0.05
IP6(wholegrains and legumes) (mg/ day)	650.6 ± 357.4	0	0	> 0.05	> 0.05	> 0.05
Total IP6 (mg/ day)	763.4 ± 354.8	180.4 ± 67.8	204.9 ± 40.4	> 0.05	> 0.05	> 0.05

Table 7.3 Inter-group comparisons (p values) of nutrient intake on day 0, day 15 and day 18.

	p value		
	Day 0	**Day 15**	**Day 18**
Total protein (g/ day)	0.74	0.14	0.55
Total fat (g/ day)	0.19	0.04	0.1
Total carbohydrate (g/ day)	0.88	0.34	0.08
Fibre (g/ day)	0.47	0.48	0.09
Added sugar (g/ day)	0.48	0.24	0.06
Oxalate (mg/ day)	0.67	0.83	0.49
Calcium (mg/ day)	0.2	0.84	0.44
Magnesium (mg/ day)	0.43	0.74	0.19
Phosphorous (mg/ day)	0.53	0.51	0.89
Potassium (mg/ day)	0.33	0.64	0.51
Sodium (mg/ day)	0.19	0.43	0.95
Vitamin A (RE/ day)	0.34	0.41	0.3
Vitamin B6	0.62	0.69	0.14
Vitamin C (mg/ day)	0.49	0.75	0.22
Vitamin D (ug/ day)	0.56	0.07	0.2
Vitamin E (mg/ day)	0.31	0.5	0.66
Citric acid (mg/ day)	0.34	0.75	0.68
IP6 (fruit & veg) (mg/ day)	0.16	0.2	0.48
IP6(whole grains) (mg/ day)	0.31	>0.99	>0.99
Total IP6 (mg/ day)	0.51	0.2	0.47

Urine analysis

The mean urinary excretion of IP6 for the duration of the study is shown in Figure 7.1 while actual values are reported in Table 7.4. All raw data are reported in Appendix 7.2.

In the black group, the mean urinary excretion of IP6 decreased within 3 days of the commencement of the study and remained constant up to day 15. Urinary excretion of IP6 was significantly lower on day 15 relative to day 0 ($p < 0.05$). After 3 days of IP6 supplementation (day 18), urinary IP6 increased (relative to day 15) albeit not statistically significant ($p > 0.05$).

In the white group, urinary IP6 remained constant during Phase 1 of the study but increased significantly on day 18 relative to day 0 and day 15 ($p < 0.05$).

Inter-group comparisons showed that urinary IP6 was higher in the black group than the white group on day 0, approaching statistical significance ($p = 0.06$). No significant difference in the urinary excretion of IP6 was observed between the respective groups after 15 days of IP6 restriction ($p = 0.59$) and on day 18 after IP6 supplementation ($p = 0.89$).

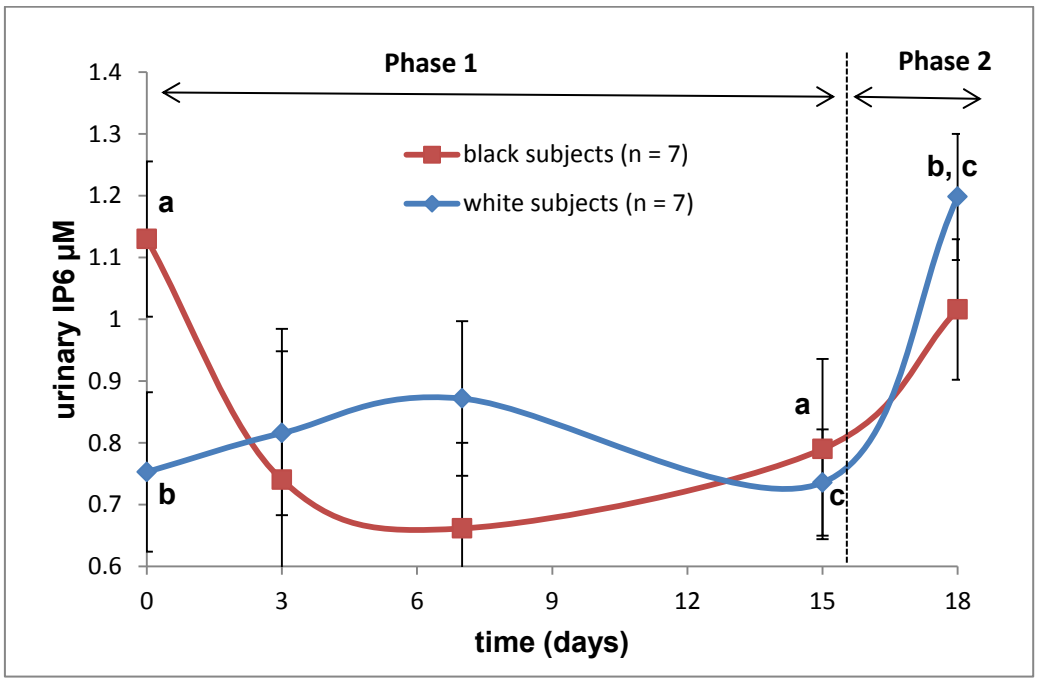


Figure 7.1 Mean urinary IP6 in black (red curve) and white (blue curve) subjects. Error bars indicate standard error of the mean. Phase 1: IP6 deficient diet. Phase 2: IP6 deficient diet + 800 mg IP6 supplement. Pairwise comparisons of data points labelled with the same letter are significantly different; $p < 0.05$.

Table 7.4 Urinary IP6 excretion in black and white subjects for the duration of the study. Mean ± SE

	Day 0 (A)	Day 3 (B)	Day 7 (C)	Day 15 (D)	Day 18 (E)	p values intra-group				
						A vs B	A vs C	A vs D	A vs E	D vs E
Black subjects (n=7)	1.13 ± 0.13	0.74 ± 0.24	0.66 ± 0.14	0.79 ± 0.15	1.02 ± 0.11	> 0.05	< 0.01*	< 0.05*	> 0.05	> 0.05
White subjects (n= 7)	0.75 ± 0.13	0.82 ± 0.13	0.87 ± 0.13	0.74 ± 0.09	1.20 ± 0.10	> 0.05	> 0.05	> 0.05	< 0.05*	< 0.05*
p value inter-group	0.06	0.79	0.29	0.74	0.26					

*Indicates statistical significance.

The mean urine composition of other biochemical and physicochemical risk factors on days 0, 15 and 18 are shown in Table 7.5 and Table 7.6 for the black and white group, respectively. All raw data are shown in Appendix 7.3.

In the black group no significant changes in urinary composition was observed for the respective inter-day comparisons. As stated above, the observation vitamin B6 being significantly higher on day 18 relative to day 0 in this group could potentially lead to a lower excretion of oxalate. This did not occur (Table 7.5) thereby discounting any confounding dietary factors.

Attention is drawn to the significant increase in urinary citrate on day 18 relative to day 0 in the white group.

Inter-group comparisons are shown in Table 7.7. Attention is drawn to the significantly lower urinary excretion of citrate and phosphate in the black group relative to the white group on day 18. This is in agreement with the results presented in Chapter 6.

Table 7.5 Urinary composition and physicochemical properties of black subjects (n= 7).

	Day 0 (A)	Day 15 (B)	Day 18 (C)	p value		
	mean ± SE	mean ± SE	mean ± SE	A vs B	A vs C	B vs C
pH	6.44 ± 0.17	6.55 ± 0.12	6.54 ± 0.20	> 0.05	> 0.05	> 0.05
Volume (ml)	864 ± 97	898 ± 137	1049 ± 129	> 0.05	> 0.05	> 0.05
Citrate (mmol/ 24 hr)	1.78 ± 0.22	1.58 ± 0.27	1.81 ± 0.22	> 0.05	> 0.05	> 0.05
Oxalate (mmol/ 24 hr)	0.20 ± 0.02	0.22 ± 0.03	0.26 ± 0.02	> 0.05	> 0.05	> 0.05
Calcium (mmol/ 24 hr)	2.04 ± 0.19	1.84 ± 0.20	2.41 ± 0.22	> 0.05	> 0.05	> 0.05
Magnesium (mmol/ 24 hr)	1.29 ± 0.16	1.66 ± 0.16	1.77 ± 0.24	> 0.05	> 0.05	> 0.05
Sodium (mmol/ 24 hr)	114 ± 12	96 ± 6.5	119 ± 8.82	> 0.05	> 0.05	> 0.05
Potassium (mmol/ 24 hr)	30.2 ± 4.7	26.1 ± 4.0	33.8 ± 3.2	> 0.05	> 0.05	> 0.05
Urate (mmol/ 24 hr)	2.9 ± 0.4	2.6 ± 0.3	2.9 ± 0.2	> 0.05	> 0.05	> 0.05
Creatinine (mmol/ 24 hr)	13.7 ± 1.1	12.4 ± 0.6	13.5 ± 0.4	> 0.05	> 0.05	> 0.05
Phosphate (mmol/ 24 hr)	22.8 ± 3.9	25.0 ± 3.2	23.91 ± 1.8	> 0.05	> 0.05	> 0.05
Chloride (mmol/ 24 hr)	129 ± 19	120 ± 16	163 ± 13	> 0.05	> 0.05	> 0.05
Phytate (µM)	1.13 ± 0.12	0.79 ± 0.15	1.02 ± 0.11	<0.05*	> 0.05	> 0.05
TRI	179 ± 26	193 ± 26	256 ± 39	> 0.05	> 0.05	> 0.05
RS CaOx	5.75 ± 1.9	4.95 ± 0.96	2.75 ± 0.48	> 0.05	> 0.05	> 0.05
RS Brush	1.94 ± 0.43	3.19 ± 0.88	1.85 ± 0.46	> 0.05	> 0.05	> 0.05
RS UA	1.06 ± 0.37	0.64 ± 0.16	0.86 ± 0.35	> 0.05	> 0.05	> 0.05
CaOx MSL (mM)	0.72 ± 0.14	1.30 ± 0.21	0.88 ± 0.15	> 0.05	> 0.05	> 0.05
Ionized calcium (mmol/ 24 hr)	0.56 ± 0.11	0.57 ± 0.19	0.76 ± 0.19	> 0.05	> 0.05	> 0.05

*Indicates statistical significance.

Table 7.6 Urinary composition and physicochemical properties of white subjects (n= 7).

	Day 0 (A)	Day 15 (B)	Day 18 (C)	p value		
	Mean ± SE	Mean ± SE	Mean ± SE	A vs B	A vs C	B vs C
pH	6.53 ± 0.20	6.34 ± 0.28	6.33 ± 0.11	0.28	> 0.05	> 0.05
Volume (ml)	1414 ± 300	1594 ± 318	1583 ± 252	0.13	> 0.05	> 0.05
Citrate (mmol/ 24 hr)	2.30 ± 0.34	2.70 ± 0.47	3.00 ± 0.38	0.13	< 0.05*	> 0.05
Oxalate (mmol/ 24 hr)	0.19 ± 0.02	0.19 ± 0.03	0.27 ± 0.06	0.94	> 0.05	> 0.05
Calcium (mmol/ 24 hr)	1.60 ± 0.32	1.99 ± 0.42	2.11 ± 0.24	0.44	> 0.05	> 0.05
Magnesium (mmol/ 24 hr)	1.32 ± 0.16	1.78 ± 0.28	1.78 ± 0.13	0.18	> 0.05	> 0.05
Sodium (mmol/ 24 hr)	173.6 ± 30.0	162.7 ± 25.0	198.1 ± 26.4	0.7	> 0.05	> 0.05
Potassium (mmol/ 24 hr)	24.5 ± 4.1	27.1 ± 5.6	31.5 ± 2.8	0.53	> 0.05	> 0.05
Urate (mmol/ 24 hr)	3.6 ± 0.4	3.6 ± 0.5	4.6 ± 0.4	0.8	> 0.05	> 0.05
Creatinine (mmol/ 24 hr)	14.8 ± 1.6	15.8 ± 1.9	17.8	0.25	> 0.05	> 0.05
Phosphate (mmol/ 24 hr)	28.7 ± 4.6	27.8 ± 4.2	35.9 ± 2.3	0.8	> 0.05	> 0.05
Chloride (mmol/ 24 hr)	163 ± 21	164 ± 24	185 ± 18	0.95	> 0.05	> 0.05
Phytate (µM)	0.75 ± 0.13	0.73 ± 0.08	1.20 ± 0.10	0.91	< 0.05*	< 0.05*
TRI	123 ± 18	133 ± 35	155 ± 42	0.81	> 0.05	> 0.05
RS CaOx	1.84 ± 0.31	2.68 ± 0.83	2.34 ± 0.32	0.21	> 0.05	> 0.05
RS Brush	1.05 ± 0.71	1.08 ± 0.31	1.38 ± 0.28	0.92	> 0.05	> 0.05
RS UA	0.73 ± 0.22	1.05 ± 0.41	1.11 ± 0.38	0.32	> 0.05	> 0.05
CaOx MSL (mM)	1.14 ± 0.17	0.81 ± 0.24	1.20 ± 0.15	0.22	> 0.05	> 0.05
Ionized calcium (mmol/ 24 hr)	0.51 ± 0.10	0.79 ± 0.11	0.62 ± 0.10	0.08	> 0.05	> 0.05

*Indicates statistical significance.

Table 7.7 Inter-group comparisons of urinary composition and physicochemical risk factors.

	p value		
	Day 0	**Day 15**	**Day 18**
pH	0.8	0.38	0.46
Volume (ml)	0.11	0.12	0.05
Citrate (mmol/ 24 hr)	0.22 (B < W)	0.06 (B < W)	0.01* (B < W)
Oxalate (mmol/ 24 hr)	0.48	0.28	0.71
Calcium (mmol/ 24 hr)	0.38	> 0.99	0.38
Magnesium (mmol/ 24 hr)	0.9	0.72	> 0.99
Sodium (mmol/ 24 hr)	0.16 (B < W)	0.04* (B < W)	0.03* (B < W)
Potassium (mmol/ 24 hr)	0.37	0.89	0.59
Urate (mmol/ 24 hr)	0.16 (B < W)	0.11 (B < W)	0.004* (B < W)
Creatinine (mmol/ 24 hr)	0.58	0.1	0.006* (B < W)
Phosphate (mmol/ 24 hr)	0.34	0.6	0.002* (B < W)
Chloride (mmol/ 24 hr)	0.26	0.15	0.34
Phytate (μM)	0.06 (B > W)	0.89	0.59
TRI	0.13	0.31	0.15
RS CaOx	0.07	0.10	0.42
RS Brush	0.31	0.04* (B > W)	0.40
RS UA	0.40	0.37	0.64
CaOx MSL (mM)	0.09	0.21	0.15
Ionized calcium (mmol/ 24 hr)	0.74	0.33	0.52

Crystallisation experiments

CaOx MSL

The mean CaOx MSL of both groups is indicated in the aforementioned tables (Table 7.5 and Table 7.6). Raw data are reported in Appendix 7.4. In both the black and the white groups, no significant difference in the CaOx MSL was observed for inter-day comparisons. Inter-group comparisons also showed no statistical difference as shown in Table 7.7.

Rate of crystallisation

Crystallisation was induced in urine samples by the addition of Na_2Ox at a concentration of 0.15 mM greater than the CaOx MSL. The urine of 1 black subject and 2 white subjects were supersaturated and had CaOx MSLs below 0.15 mM (the lowest concentration of Na_2Ox used in the present study). The rate of crystallisation was therefore not determined in these samples. Accordingly, the mean rate of crystallisation in urine of 6 black and 5 white subjects on days 0, 15 and 18 are reported in Table 7.8. Both intra- and inter-group comparisons were made; no statistical differences were observed. All raw data are reported in Appendix 7.5.

Table 7.8 Mean rate of CaOx crystallisation in urine of black (n= 6) and white (n= 5) subjects.

	Rate (change in OD_{620nm} x 10^{-3} min^{-1}) mean ± SE			**p value (intra-group)**		
	Day 0 (A)	**Day 15 (B)**	**Day 18 (C)**	**A vs B**	**A vs C**	**B vs C**
Black subjects	1.74 ± 0.78	0.82 ± 45	1.58 ± 0.36	> 0.05	> 0.05	> 0.05
White subjects	0.50 ± 0.20	0.54 ± 0.33	1.53 ± 1.10	> 0.05	> 0.05	> 0.05
p value Inter-group	0.18	0.64	0.97			

Discussion

The objective of the present study was to establish whether a period of more than 3 days of dietary IP6 restriction would initiate a decrease in its urinary excretion. Surprisingly, urinary IP6 decreased in the black group after 3 days of IP6 restriction (Figure 7.1 and Table 7.4). This is inconsistent with the results presented in Chapter 6 in which no such decrease was observed in either group after IP6 restriction for this period of time. These conflicting results might not occur in bigger sample sizes drawing attention to a limitation of the present study. Nevertheless, an extended period of restriction as described in this chapter is of interest in terms of examining whether a further decrease in urinary IP6 is observed (as discussed in the next paragraph). On the other hand, a similar decrease was not observed in the white group of the present study which is consistent with the preceding chapter.

After day 3, IP6 excretion remained constant up to day 15 in both groups despite the restriction of IP6 from whole-grains and legumes. This indicates that dietary IP6 derived from fruits and vegetables (which were not restricted in the present study and which contain IP6, although to a lesser extent than that of whole-grains[259]) must have contributed toward the total urinary excretion of IP6. Furthermore, foods containing refined wheat (such as pasta, white bread, pizza, biscuits, and cake), and refined white rice, all of which may contain IP6[259], were also not excluded from meals. These factors should therefore be taken into consideration when planning future studies.

In the present study, a period of 3 days of IP6 supplementation was sufficient to increase its urinary excretion (in both groups) to levels within the normal range. However, it must be noted that longer periods of IP6 ingestion may be required when urinary IP6 is extremely low.[268, 392]

Regarding inter-group comparisons of the urinary excretion of IP6, the results of the present study demonstrated that the excretion levels of this substance are similar when their IP6 intake is the same (Table 7.4, day 15 and day 18). This suggests that differences in the physiological handling of IP6 with respect to its absorption and subsequent urinary excretion may not exist between the respective groups.

Based on the results presented in the previous chapter for both groups, it was speculated that a significant increase in IP6 consumption may influence the excretion of urinary citrate. However, no such effect was observed in the black group of the present study on day 18 relative to day 15 (Table 7.5). This suggests that the conditions under which the supplement was ingested in the present study namely, on an empty stomach, might have affected the metabolism of this substance. Since it is speculated that the increase in urinary citrate might be due to an increase in phosphate (derived from IP6 hydrolysis) it might be possible that the

hydrolysis of IP6 has not occurred on an empty stomach. Indeed, studies in both ileostomy patients and in pigs demonstrated that the hydrolysis of IP6 in the stomach and the small intestine is dependent on phytases (an enzyme for IP6 hydrolysis) obtained from food such as wheat, barley and oats.[431-433] On the other hand, a significant increase in urinary citrate was observed in the present study in the white group on day 18 relative to day 0 ($p < 0.05$; Table 7.6) with a concomitant increase in urinary phosphate ($p > 0.05$). This is puzzling since it suggests that IP6 hydrolysis has occurred in this group. This suggests that the transit time of the IP6 supplement in the stomach or the small intestine relative to the time at which a meal was ingested (which possibly contained wheat) might have influenced the hydrolysis of IP6 in this group. Nonetheless, the observation that urinary citrate increased in the white group only, in response to IP6 supplementation, demonstrates that the respective groups may have a different renal response to IP6 supplementation. This is not dissimilar to results of previous studies which were conducted in the KSRL at the University of Cape Town in which the renal response to five different supplemental challenges (calcium, vitamin B6, L-glutamine and L-cystine supplement) were different in the respective groups.[299] In these studies, no effect was observed in the black group whereas significant effects were observed in the white group.[299]

Crystallisation properties (CaOx MSL and rate of CaOx crystallization) were not affected in either of the groups when urinary IP6 increased significantly (following ingestion of the IP6 supplement). These results are consistent with the findings reported in the previous chapter thereby supporting the notion that if urinary IP6 inhibits the formation of kidney stones it does so by inhibiting stone growth at a macroscopic level as demonstrated in a previous *in vitro* study by Saw et al[285].

Direct comparison of the results of the present study to those presented in the preceding chapter cannot be made since the relative bioavailability of IP6 in the two forms (dietary and supplemental) is not known, nor was the net delivered dosage of IP6 standardized in the respective studies. Furthermore, identical crystallisation assays in the two studies were not possible due to various circumstances as stated in the methods of the present chapter (page 156), as such inter-study comparisons cannot be made.

In conclusion, results of the present study demonstrated that increasing the period during which IP6 ingestion is restricted from 3 to 15 days may not be necessary to detect a significant decrease in urinary IP6 and conversely, that urinary IP6 can be increased with IP6 supplementation. These findings are important because urinary IP6 is hypothesized to play a role in the inhibition of CaOx urolithiasis. The finding of an increase in citraturia following ingestion of the IP6 supplement is consistent with the finding reported for ingestion of dietary

IP6 and shows that this effect is independent of the form in which IP6 is administered. Results (with regard to citrate excretion) also showed evidence of different renal handling of the IP6 supplement in the two race groups. Since this phenomenon has been previously reported for different supplements, researchers should consider focusing on these physiological handling processes in their efforts to identify the cause of the different stone incidence rates in the two groups.

Chapter 8 : Concluding comments

Studies on the ingestion of IP6 in the context of urolithiasis have hypothesized that this nutrient may provide protection against CaOx kidney stone formation via two mechanisms. Firstly, the administration of large quantities of IP6 can be undertaken with a view to increasing the formation of insoluble calcium-phytate complexes in the gastrointestinal tract thereby culminating in a decrease in the concentration of total urinary calcium. While this mechanism may be useful in treating patients with hypercalciuria it may not be helpful to treat patients with idiopathic CaOx urolithiasis. Secondly, the administration of moderate amounts of IP6 can be undertaken with a view to increasing urinary IP6 in order to inhibit CaOx crystallisation. This mechanism may be useful in treating patients with idiopathic CaOx urolithiasis. The work conducted in the present PhD project was undertaken on the basis of these mechanisms.

Another aspect of the work undertaken in this project was based on a hypothesis published 35 years ago that the relative rarity of CaOx renal lithiasis in the South African black population compared to the white population may be due to their higher intake of IP6. Testing this hypothesis in relation to whether the former group has a higher urinary excretion of IP6 had been a challenge in a previous study which was conducted in the KSRL at the University of Cape Town since the methods which were available for urinary IP6 quantification required sophisticated instrumentation and in some cases relied upon the adequate separation of inorganic phosphate. This difficulty rendered the conclusions of that study questionable. Thus, the present project sought to test the aforementioned hypothesis after identifying and testing a more robust method for the quantification of urinary IP6.

Regarding the first hypothesis of the present PhD project, the results have demonstrated that although soluble calcium-phytate complexes do form in urine, they do not influence the crystallisation of CaOx. Thus, the first hypothesis is rebutted. This led the researcher to speculate that the inhibitory effect of IP6 might be more of a kinetic nature than a thermodynamic one. This was confirmed in *in vitro* experiments in AU and pooled urine of South African black and white subjects. These *in vitro* experiments demonstrated two important kinetic properties of IP6, namely that physiological concentrations of IP6 have the capacity to inhibit CaOx crystal aggregation and that its effect on the inhibition of CaOx crystal growth may not be concentration dependent. These findings may be important for the inhibition of CaOx stone formation *in vivo*.

Regarding the second hypothesis under investigation in the present project, the results have demonstrated that a higher ingestion of IP6 in black subjects compared to white subjects manifests itself in a higher urinary excretion. This was demonstrated for the first time. Thus, the employment of a more robust method for the quantification of urinary IP6 was successful. Furthermore, since urinary IP6 may play an important role in the inhibition of CaOx crystal aggregation and growth *in vivo* as well as the notion of its inhibitory capacity at a macroscopic level, its higher concentration in the black group might be a contributory factor to the relative rarity of CaOx urolithiasis in this group. Therefore, the second hypothesis of the present PhD project has been validated.

Regarding future studies, the present project demonstrated that an increase in IP6 ingestion resulted in an increase in urinary citrate. Although, it was speculated that an increase in phosphate (derived from IP6 hydrolysis) might have resulted in this effect, future studies can be undertaken to validate this.

The work presented in this thesis contributes to the body of knowledge about kidney stone disease with regard to the role of urinary phytate. More importantly, the hypothesis that the relative rarity of renal stone formation in South Africa's black population compared to the white population may be attributed to the much higher dietary intake of IP6 in this group has been validated.

Chapter 9 : References

1. Eknoyan G. History of urolithiasis. *Clin Rev Bone Min Metab* 2004; **2:** 177.

2. Shah J, Whitfield HN. Urolithiasis through the ages. *Br J Urol* 2002; **89:** 801.

3. Wise RO, Kark AE. Urinary calculi and serum calcium levels in Africans and Indians. *S Afr Med J* 1961; **35:** 47.

4. Modlin M. The aetiology of renal stones: A new concept arising from studies on a stone-free population. *Ann R Coll Surg Engl* 1967; **40**(3)**:** 155.

5. Whalley N, Moreas M, Shar T, Pretorius S, Meyer A. Lithogenic risk factors in the urine of black and white subjects. *Br J Urol* 1998; **82:** 785.

6. Hess B, Kok DJ. Kidney Stones: Medical and Surgical Management. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger G (eds). Lippincott-Raven Publishers: Philadelphia, 1996.

7. Boskey AL. Mineralization of bones and teeth. *Elements* 2007; **3:** 387.

8. Bonucci E. Bone mineralization. *Front Biosci* 2012; **17:** 100.

9. Kok DJ, Schelle-Feith EA. Risk factors for crystallization in the nephron: the role of renal development. *J Am Soc Nephrol* 1999; **10**(14)**:** S364.

10. Tiselius HG, Ackermann D, Hess B, Boeve E. Stone disease: diagnosis and medical management. *European Urology* 2002; **41**(5)**:** A1.

11. Finlayson B. Calcium stones: some physical and clinical aspects. In: David DS (ed) *Calcium metabolism in renal failure and nephrolithiasis*. Wiley: New York, 1977, pp 337-382.

12. Khan SR, Hackett RL. Identification of urinary stone and sediment crystals by scanning and X-ray microanalysis. *J Urol* 1987; **135:** 818.

13. Pak CYC. Etiology & treatment of urolithiasis. *Am J Kidney Dis* 1991; **18**(6)**:** 624.

14. Park S, Pearle MS. Pathophysiology and management of calcium stones. *Urol Clin N Am* 2007; **34**(3)**:** 323.

15. Khan SR, Kok DJ. Modulators of urinary stone formation. *Front Biosci* 2004; **9:** 1450.

16. Lewandowski S, Rodgers AL. Idiopathic calcium oxalate urolithiasis: risk factors and conservative treatment. *Clin Chim Acta* 2004; **345:** 17.

17. Ryall RL. The possible roles of inhibitors, promoters, and macromolecules in formation of calcium kidney stones. In: Rao PN, Preminger GM, Kavanagh JP (eds). *Urinary Tract Stone Disease*. Springer: London, 2011.

18. Bithelis G, Bouropoulos N, Liatsikos EN, Perimenis MD, Koutsoukos PG, Barbalias GA. Assessment of encrustations on polyurethane ureteral stents. *J Endourol* 2004; **18:** 550.

19. Tiselius HG. A hypothesis of calcium stone formation: an interpretation of stone research in the past decades. *Urol Res* 2011; **39**(4)**:** 231.

20. Paliouras C, Tsampikaki E, Polichronis A, Aperis G. Pathophysiology of nephrolithiasis. *Nephrology Reviews* 2012; **4:** e14.

21. Gault MH, Chafe L. Relationship of frequency, age, sex, stone weight and composition in 15,624 stones: comparison results for 1980 to 1983 and 1995 to 1998. *J Urol* 2000; **164**(2)**:** 302.

22. Evan AP, Coe FL, Lingeman JE, Worcester E. Insights on the pathology of kidney stone formation. *Urol res* 2005; **33:** 383.

23. Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest* 2005; **115**(10)**:** 2598.

24. Worcester EM, Coe FL. Nephrolithiasis. *Prim Care Clin Office Pract* 2008; **35:** 370.

25. Worcester EM, Coe FL. Calcium kidney stones. *N Engl J Med* 2010; **363**(10)**:** 954.

26. Parks JH, Worcester EM, Coe FL, Evan AP, Lingeman JE. Clinical implications of abundant calcium phosphate in routinely analysed kidney stones. *Kidney Int* 2004; **66:** 777.

27. Ramello A, Vitale C, Marangella M. Epidemiology of nephrolithiasis *J Nephrol* 2000; **13**(3)**:** S65.

28. Bruyeni F, Traxer O, Sausinne C, Lechevallier E. Infection and urinary lithiasis. *Prog Urol* 2008; **18**(12)**:** 1015.

29. Gómez-Núnez JG, Fernández F, Aceves JG, Loske AM. Interaction of intracorporeal lithotripters with Proteus mirabilis inoculated inside artificial calcium and struvite stones. *J Endourol* 2009; **23:** 519.

30. Robertson WG, Peacock M. Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone formers. *Clin Sci* 1972; **43:** 499.

31. Hallson PC, Rose GA. Crystalluria in normal subjects and stone formers with and without thiazide and cellulose phosphate treatment. *Br J Urol* 1976; **48:** 515.

32. Huang ZJ, Li JJ, He JY, Ouyang JM. Study on nano- and microcrystallites in the urines of calcium oxalate stone formers. *Spectroscopy and spectral analysis* 2010; **30**(7)**:** 1913.

33. Finlayson B. Physicochemical aspects of uroliths. *Kidney Int* 1978; **13:** 344.

34. Mandel N. Mechanism of stone formation. *Semin Nephrol* 1996; **16:** 364.

35. Grases F, Costa-Bauza A, Kónigsberger E, Kónigsberger LC. Kinetic versus thermodynamic factors in calcium renal lithiasis. *Int Urol and Nephrol* 2000; **32:** 19.

36. Coe FL, Evan AP, Worcester EM, Lingeman JE. Three pathways of kidney stone formation. *Urol Res* 2010; **38:** 147.

37. Evan AP. Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatr Nephrol* 2010; **25:** 831.

38. Evan AP, Lingeman JE, Worcester EM, Sommer AJ, Phillips CL, Williams JC *et al.* Contrasting histopathology and crystal deposits in kidneys of idiopathic stone formers who produce hydroxy apatite, brushite, or calcium oxalate stones. *Anat Rec* 2014; **297:** 731.

39. Randall A. The origin and growth of renal calculi. *Ann Surg* 1937; **105:** 1009.

40. Khan S, Canales B, Monga M. Formation and growth of Randall's plaques: an ultrastructure of renal papillae from idiopathic stone formers. *J Urol* 2011; **185:** S861.

41. Evan A, Lingeman J, Coe FL, Worcester E. Randall's plaque: pathogenesis and role in calcium oxalate nephrolithiasis. *Kidney Int* 2006; **69:** 1313.

42. Evan AP, Coe FL, Lingeman JE, Shao Y, Sommer AJ, Bledsoe SB *et al.* Mechanism of formation of human calcium oxalate renal stones on Randall's plaque. *Anat Rec (Hoboken)* 2007; **290**(10)**:** 1315.

43. Kavanagh JP. Supersaturation and renal precipitation: the key to kidney stone formation? *Urol Res* 2006; **34:** 81.

44. Marangella M, Vitale C, Bagnis C, Bruno M, Ramello A. Idiopathic calcium nephrolithiasis. *Nephron* 1999; **81**(1)**:** 38.

45. Ryall RL. The scientific basis of calcium oxalate urolithiasis. *World J Urol* 1993; **11:** 59.

46. Rodgers AL. Urinary saturation: casual or causal risk factor in urolithiasis. *BJU Int* 2014; **114**(1)**:** 104.

47. Bazin D, Daudon M, Combes C, Rey C. Characterization and some physicochemical aspects of pathological microcalcifications. *Chem Rev* 2012; **112:** 5092.

48. Kavanagh JP. Physicochemical aspects of uro-crystallization and stone formation. In: Rao NP, Preminger GM, Kavanagh JP (eds). *Urinary tract stone disease*, vol. 34. Springer: London, 2011, p 81.

49. Messa P, marangella M, Paganin L. Different dietary calcium intake and relative supersaturation of calcium oxalate in the urine of patients forming renal stones. *Clin Sci* 1997; **93:** 257.

50. Kavanagh JP. In vitro calcium oxalate crystallisation methods. *Urol Res* 2006; **34:** 139.

51. Low RK, Stoller ML. Endoscopic mapping of renal papillae for Randall's plaques in patients with urinary stone disease. *J of Urol* 1999; **158:** 2062.

52. Low RK, Stoller ML, Schreiber CK. Metabolic and urinary risk factors associated with Randall's papillary plaques. *J Endourol* 2000; **14:** 507.

53. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y *et al.* Randall's plaque of patients with nephrolithiasis begins in basement membranes of this loops of Henle. *J Clin Invest* 2003; **111:** 607.

54. Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB *et al.* Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney Int* 2003; **64**(6)**:** 2150.

55. Kim SC, Coe FL, Tinmouth WW, Kuo RL, Paterson RF, Parks JH *et al.* Stone formation is proportional to papillary surface coverage by Randall's plaque. *J Urol* 2005; **173**(1)**:** 117.

56. Miller NL, Gillen DL, Williams JC, Evan AP, Bledsoe SB, Coe FL *et al.* A formal test for the hypothesis that idiopathic calcium oxalate stones grow on Randall's plaque. *BJU Int* 2009; **103**(7)**:** 966.

57. Miller N, Williams JCJ, Evans AP, Bledsoe SB, Coe FL, Worcester EM *et al.* In idiopathic calcium oxalate stone formers, unattached stones shown evidence of having originated as attached stones on Randall's plaque. *BJU Int* 2010; **105:** 242.

58. Matlaga BR, Williams JC, Kim SC, Kuo RL, Evan AP, Bledsoe SB *et al.* Endoscopic evidence of calculus attachment to Randall's plaque. *J Urol* 2006; **175**(5)**:** 1720.

59. Worcester EM, Coe FL. New insights into the pathogenesis of idiopathic hypercalciuria. *Semin Nephrol* 2008; **28**(2)**:** 120.

60. Hodkinson A, Pyrah LN. The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. *Br J Surg* 1958; **48:** 10.

61. Pak CY, Ohata M, Lawrence EC, Snyder W. The hypercalciurias: causes, parathyroid functions, and diagnostic criteria. *J Clin Invest* 1974; **54**(2)**:** 387.

62. Flocks RH. Calcium and phosphorus excretion in the urine of patients with renal or ureteral calculi. *JAMA* 1939; **113:** 1466.

63. Khan SR. Renal cellular dysfunction/ damage and the formation of kidney stones. In: Rao NP, Preminger GM, Kavanagh JP (eds). *Urinary tract stone disease*. Springer: London, 2011, p 61.

64. Sakhaee K, Maalouf NM, Sinnott B. Kidney stones 2012: pathogenesis, diagnosis, and management. *J Clin Endocrinol Metab* 2012; **97**(6)**:** 1847.

65. Zerwekh JE, Reed-Gitomer BY, Pak CYC. Pathogenesis of hypercalciuric nephrolithiasis. *Endocrinol Metab Clin N Am* 2002; **31:** 869.

66. Levy FL, Adams-Huet B, Pak CYC. Ambulatory evaluation of nephrolithiasis: An update of a 1980 protocol. *American Journal of Medicine* 1995; **98:** 50.

67. Jaeger P. Pathophysiology of idiopathic hypercalciuria: the current concept. *Current Op in Urol* 1998; **8:** 321.

68. Broadus AE, Insogna KL, Lang R, Ellison AF, Dreyer BE. Evidence of disordered control of 1,25-dihydroxyvitamin D production in absorptive hypercalciuria. *N Engl J Med* 1984; **311:** 73.

69. Prié D, Ravery V, Boccon-Gibod L, Friedlander G. Frequency of renal phosphate leak among patients with calcium nephrolithiasis. *Kidney Int* 2001; **60:** 272.

70. Mollerup CL, Vestergaard P, Frokjaer VG, Mosekilde L, Christiansen P, Blichert-Toft M. Risk of renal stone events in primary hyperthyroidism before and after parathyroid surgery: controlled retrospective follow up study. *BMJ* 2002; **13:** 2186.

71. Weisinger JR. New insights in the pathogenesis of idiopathic hypercalciuria; the role of bone. *Kidney Int* 1996; **49:** 1507.

72. Sakhaee K, Harvey JA, Padalino PK. The role of salt abuse on the risk of kidney stone formation. *J Urol* 1993; **150:** 310.

73. Blackwood AM, Cappuccio FP, Sagnella GA. Epidemiology of blood pressure and urinary calcium excretion: importance of ethnic origin and diet. *J Hum Hypertens* 1999; **13:** 892.

74. Haleblian GE, Preminger GM. Calcium metabolism and hypercalciuria. In: Rao NP, Preminger GM, Kavanagh JP (eds). *Urinary tract stone disease*. Springer: London, 2011, p 159.

75. Martini LA, Cuppari L, Cunha MA, Schhor N, Heilberg IP. Potassium and sodium intake and excretion in calcium stone forming patients. *J Renal Nutr* 1998; **8**(3)**:** 127.

76. Rao PN, Prendiville V, Buxton A. Dietary management of urinary risk factors in renal stone formers. *Br J Urol* 1982; **54:** 578.

77. Marshall RW, Cochran M, Hodgkinson A. Relationships between calcium and oxalic acid intake in the diet and their excretion in the urine of normal and renal-stone forming subjects. *Clin Sci* 1972; **43:** 91.

78. Vahlensieck W. The importance of diet in urinary stones. *Urol Res* 1986; **14:** 283.

79. Robertson WG, Peacock M. The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria? *Nephron* 1980; **26:** 105.

80. Ito H, Suzuki F, Yamaguchi K, Nishikawa Y, Kotake T. Reduction of urinary oxalate by combined calcium and citrate administration without increase in urinary calcium oxalate stone formers. *Clin Nephrol* 1992; **37:** 14.

81. Wilson DM, Smith LH, Erickson SB. *Renal oxalate handling in normal subjects and patients with ideopathic renal lithiasis: primary and secondary hyperoxaluria*, Plenum: New York, 1989.

82. Schwille PO, Manoharan M, Rumenapf G. Oxalate measurement in the picamole range by ion chromatography: values in fasting plasma urine of controls and patients with ideopathic calcium urolithiasis. *J Clin Chem Clin Biochem* 1989; **27:** 87.

83. Holmes RP, Goodman HO, Assimos DG. Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int* 2001; **59:** 270.

84. Robertson WG. Mild hyperoxaluria: a critical review and future outlook. In: Borghi L, Meschi T, Briganti A, Schianchi T, Novarini A (eds). *Kidney stones. 8th European symposium on urolithiasis*, vol. 33. Editoriale Bios: Cosenza, 1999.

85. Allison MJ, Cook HM, Milne DB. Oxalate degradation by gastrointestinal bacteria from humans. *J Nutr* 1986; **116:** 455.

86. Ogawa Y, Miyazato T, Hatono T. Oxalate and urinary stones. *World J Surg* 2000; **24:** 1154.

87. Kaufman DW, Kelly JP, Curhan GC, Anderson TE, Dretler SP, Preminger GM *et al.* Oxalobacter Formigenes may reduce the risk of calcium oxalate kidney stones. *J Am Soc Nephrol* 2008; **19**(6)**:** 1197.

88. Robertson WG, Peacock M, Heyburn PJ, Marshall DH, Clark PB. Risk factors in calcium stone disease on the urinary tract. *Br J Urol* 1978; **50:** 449.

89. Griffith HM, O'Shea B, Maguire M, Keogh B, Kevany JP. A case-control study of dietary intake of renal stone patients. *Urol Res* 1986; **14:** 75.

90. Robertson WG, Peacock M, Heyburn PJ, Marshall DH. The role of affluence and diet in the genesis of calcium containing stones. *Fortschr Urol Nephrol* 1978; **11:** 15.

91. Coe FL. Hyperuricosuric calcium oxalate nephrolithiasis. *Kidney Int* 1978; **13:** 418.

92. Ryall RL, HIbberd CM, Marshall VR. The effect of crystalline monosodium urate on crystallisation of calcium oxalate in whole human urine. *Urol Res* 1986; **14:** 63.

93. Ettinger B. Allopurinol for the treatment of uric acid and calcium calculi. In: Pak CYC, (ed) *Pharmacological treatment of endocrinopathies*. Basel: Karger, 1991.

94. Grases F, Rodriguez A, Costa-Bauza A. Theobromine inhibits uric acid crystallization. A potential application in the treatment of uric acid nephrolithiasis. *PloS ONE* 2014; **9**(10)**:** e111184.

95. Bilobrov VM, Chugaj AV, Bessarabov VI. Urine pH variation dynamics in healthy individuals and stone formers. *Urol Int* 1990; **45:** 326.

96. Pak CYC. Citrate and renal calculi: an update. *Miner Electrolyte Metab* 1994; **20:** 371.

97. Wagner CA, Mohebbi N. Urinary pH and stone formation. *J Nephrol* 2010; **23:** S165.

98. Tiselius HG. A simplified estimate of the iron-activity product of calcium phosphate in urine. *Eur Urol* 1984; **10:** 191.

99. Ngo TC, Assimos DG. Uric acid nephrolithiasis: recent progress and future directions. *Rev Urol* 2007; **9:** 17.

100. Tiselius HG. Epidemiology and medical management of stone disease. *BJU Int* 2003; **91:** 758.

101. Brinkley LJ, Gregory J, Pak CYC. A further study of oxalate bioavailability in foods. *J Urol* 1990; **144:** 94.

102. Holmes RP, Kennedy M. Estimation of the oxalate content of foods and daily oxalate intake. *Kidney Int* 2000; **57:** 1662.

103. Mendonca OGCd, Martini LA, Baxmann AC. Effects of an oxalate load on urinary excretion in calcium stone formers. *J Renal Nutr* 2003; **13:** 39.

104. Hesse A, Siener R, Heynek H, Jahnen A. The influence of dietary factors on the risk of urinary stone formation. *Scanning Microsc* 1993; **7:** 1119.

105. Finch AM, Kasidas GP, Rose GA. Urine composition in normal subjects after oral ingestion of oxalate-rich foods. *Clin Sci* 1981; **60:** 411.

106. Massey LK. Dietary influences on urinary oxalate and risk of kidney stones. *Front Biosci* 2003; **8:** 584.

107. Hess B. Low calcium diet in hypercalciuric calcium nephrolithiasis: first do no harm. *Scanning Microsc* 1996; **10:** 547.

108. Breslau NA, Brinkley L, Hill KD. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endocrinol Metab* 1988; **66:** 140.

109. Heilberg IP. Update on dietary recommendations and medical treatment of renal stone disease. *Nephrol Dial Transplant* 2000; **15:** 117.

110. Krieg C. The role if diet in the prevention of common kidney stones. *Urol Nursing* 2005; **25:** 451.

111. Massey LK, Roman-Smith H, Sutton RL. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. *J Am Diet Assoc* 1993; **93:** 901.

112. Bleich HL, Moore MJ, Lemann JJ, Adams ND, Gary RW. Urinary calcium excretion in human beings. *New Engl J Med* 1979; **301:** 535-541.

113. Robertson WG. Diet and calcium stones. *Miner Electrolyte Metab* 1987; **13:** 228.

114. Galosy R, Clarke L, Ward DL, Pak CY. Renal oxalate excretion in calcium urolithiasis. *J Urol* 1980; **123:** 320.

115. Curhan GC, Willet WC, Rimm EB, Stampfer MJ. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 1993; **328:** 833.

116. Bataille P, Charransol G, Gregoire I. Effect of calcium restriction on renal excretion of oxalate and the probability of stones in the carious pathophysiological groups with calcium stones. *J Urol* 1983; **130:** 218.

117. Sorensen MD, Khan AJ, Reiner AP, Tseng TY, Shikany JM, Wallace RB *et al.* Impact of nutritional factors on incident kidney stone formation: A report from the WHI OS. *J Urology* 2012; **187:** 1645.

118. Wahl C, Hess B. Kidney calculi- is nutrition a trigger or treatment. *Rev Ther* 2000; **57:** 138.

119. Robertson WG, Peacock M, Hodgskinson A. Dietary changes and the incidence of urinary calculi in the UK between 1958 and 1976. *J Chronic Dis* 1979; **32:** 469.

120. Hesse A, Brandle E, Wilbert D, Kohrmann KU, Alken P. Study in the prevalence and incidence of urolithiasis in Germany comparing years 1979 vs 2000. *Eur Urol* 2003; **44**(6)**:** 709.

121. Siener R. Impact of dietary habits on stone incidence. *Urol Res* 2006; **34:** 131.

122. Basiri A, Shakhssalim N, Khoshdel AR, Radfar MH, Pakmanesh H. Influential nutrient in urolithiasis incidence: nutrient or protein. *J Renal Nutr* 2009; **19**(5)**:** 396-400.

123. Robertson WG, Heyburn PJ, Peacock M. The effect of high protein intake on the risk of calcium stone formation in the urinary tract. *Clin Sci* 1979; **57:** 285-288.

124. Brockis JG, Levitt J, Cruthers SM. The effects of vegetable and animal protein diets on calcium, urate and oxalate excretion. *Br J Urol* 1982; **54:** 590-593.

125. Siener R, Hesse A. The effect of different diets on urine compositions and the risk of calcium oxalate crystallisation in healthy subjects. *Eur Urol* 2002; **42:** 289-296.

126. Nguyen QV, Kalin A, Drouve S. Sensitivity to mean protein intake and hyperoxaluria in idiopathic calcium stone former. *Kidney Int* 2001; **59:** 2273.

127. Sterrett SP, Nakada SY. Medical management of idiopathic calcium stone disease. In: Rao NP, Preminger GM, Kavanagh JP (eds). *Urinary tract stone disease*. Springer: London 2011, p 667.

128. Schuette SA, Hegsted M, Zemel MB, Linkswiler HM. Renal acid, urinary cyclic AMP, and hydroxyproline excretion as affected by level of protein, sulfur amino acid, and phosphorus intake. *J Nutr* 1981; **111:** 2106.

129. Barzel US, Massey LK. Excess dietary protein can adversely affect bone. *J Nutr* 1998; **128:** 1051.

130. Ferrari P, Piazza R, Ghidini N, Bisi M, Galizia G, Ferrari G. Lithiasis and risk factors. *Urol Int* 2007; **79**(1)**:** 8.

131. Kerstetter JE, O'Brien KO, Isonga KL. Low protein intake: the impact on calcium and bone homeostasis in humans. *J Nutr* 2003; **133:** 855S.

132. Lemann JJ, Pleuss JA, Rwet JAG. Dietary NaCl-restriction prevents the calciuria of KCl-deprivation and blunts the calciuria of KHCO3 deprivation in healthy adults. *Kidney Int* 1995; **47:** 899.

133. Kok DJ, Iestra JA, Doorenbos CJ. The effect of dietary excesses in animal protein and in sodium on the composition and the crystallisation kinetics of calcium oxalate monohydrate in urines of healthy men. *J Clin Endocrinol Metab* 1990; **4:** 861.

134. Nouvenne A, Meschi T, Prati B, Guerra A, Allegri F, Vezzoli G *et al.* Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial. *Am J Clin Nutr* 2010; **91**(3)**:** 565.

135. Brutis WJ, Gay L, Insogna KL. Dietary hypercalciuria in patients with calcium oxalate kidney stones. *Am J Clin Nutr* 1994; **60:** 424.

136. Baggio B, Plebani M, Gambaro G. Pathogenesis of idiopathic calcium nephrolithiasis: update 1997. *Crit Rev Clin Lab Sci* 1998; **35:** 153.

137. Kurien TB, Selvan R. Induction of lipid peroxidation in calcium oxalate stone formation. *Indian J Exp Biol* 1989; **27:** 450.

138. Naya Y, Ito H, Masai M, Yamaguchi K. Association of dietary fatty acids with urinary oxalate excretion in calcium oxalate stone formers in their fourth decade. *BJU Int* 2002; **89:** 842.

139. Roche CH-L, Rodriguez-Iturbe B, Parra G. Increased urinary excretion of prostoglandin E2 in patients with idiopathic hypercalciuria is a primary phenomenon. *Clin Sci* 1992; **83:** 75.

140. Baggio B, Budakovic A, Priante G, Gambaro G, Manzato E, Khan S. Dietary fatty supplementation modulates the urinary excretion of calcium and oxalate in the rat. Insight into calcium lithogenesis. *Nephron* 2002; **91:** 486.

141. Hirayama H, Ikegami K, Shimomura T. The possible role of prostaglandins E2 in urinary stone formation. *J Urol* 1988; **139:** 451.

142. Buck AC, Davies RL, Harrison T. The protective role of eicosapentaenoic acid (EPA) in the pathogenesis of urolithiasis. *J Urol* 1991; **146:** 188.

143. Ortiz-Alvarado O, Miyaoka R, Kriedberg C, Leavitt DA, Moeding A, Stressman M *et al.* Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid in the management of hypercalciuric stone formers. *Urology* 2012; **79:** 282.

144. Seltzer MA, Low RK, McDonald M, Shami GS, Stroller ML. Dietary manipulation with lemonade to treat hypocitraturia calcium nephrolithiasis. *J Urol* 1996; **156:** 907.

145. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Stampfer MJ. Prospective study of beverage use and the risk of kidney stones. *Am J Epidemiol* 1996; **143:** 240.

146. Lda CB, Baxmann AC, Moreira SR, Holmes RP, Heilberg IP. Noncitrus alkaline fruit: a dietary alternative for the treatment of hypocitraturic stone formers. *J Endourol* 2012; **26**(9)**:** 1221.

147. Tosukhowong P, Yachantha C, Sasivongsbhakdi T, Ratchanon S, Chaisawasdi S, Boonla C *et al.* Citraturic, alkalinizing and antioxidative effects of limeade-based regimen in nephrolithiasis patients. *Urol Res* 2008; **36**(3-4)**:** 149.

148. McHarg T, Rodgers A, Charlton K. Influence of cranberry juice on the urinary risk factors for calcium oxalate kidney stone formation. *BJU Int* 2003; **92:** 765.

149. Rodgers AL. Effect of cola consumption on urinary biochemical and physicochemical risk factors associated with calcium oxalate urolithiasis. *Urol Res* 1999; **27:** 77.

150. Borghi L, Meschi T, Amato F. Urinary volume, water, and the recurrence in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol* 1996; **155:** 839.

151. Ackermann D, Baumann JM, Futterlieb A, Zingg EJ. Influence of calcium content in mineral water on chemistry and crystallization conditions in urine of calcium stone formers. *Eur Urol* 1988; **14:** 305.

152. Rodgers AL. Effect of mineral water containing calcium and magnesium on calcium oxalate urolithiasis risk factors. *Urol Int* 1997; **58:** 93.

153. Rodgers AL. The influence of South African mineral water on reduction of risk of calcium kidney stone formation. *SA Med J* 1998; **88:** 448.

154. Bellizzi V, Nicola LD, Minutolo R. Effects of water hardness on urinary risk factors for kidney stones in patients with idiopathic nephrolithiasis. *Nephron* 1999; **81:** 66.

155. Kessler T, Hesse A. A cross-over study of the influence of bicarbonate-rich mineral water on urinary composition in comparison with sodium potassium citrate in healthy male subjects. *Br J Nutr* 2000; **84:** 865.

156. Goldfarb S. Dietary factors in the pathogenisis and prophylaxis of calcium nephrolithiasis. *Kidney Int* 1988; **34:** 544.

157. Goldfarb S. The role of diet in the pathogenesis and therapy of nephrolithiasis *Endocrinol Metab Clin N Ann* 1990; **19:** 805.

158. Pak CYC, Lynwood HS, Resnick MI, Weinerth JL. Dietary management of idiopathic calcium urolithiasis. *J Urol* 1984; **131:** 850.

159. Robertson WG. Stone formation in the Middle Eastern Gulf States: A review. *Arab Journal of Urology* 2012; **10**(3)**:** 265.

160. Schwille PO, Herrmann U. Environmental factors in the pathophysiology of recurrent idiopathic calcium urolithiaisis (RCU), with emphasis on nutrition. *Urol Res* 1992; **20:** 72.

161. Johri N, Jaeger P, Robertson W, Choong S, Unwin R. Renal stone disease. *Medicine* 2011; **39:** 7.

162. Masterson JH, Jourdain VJ, Collard DA, Choe CH, Christman MS, L'Esperance JO *et al.* Changes in urine parameters after desert exposure: assessment of stone risk in United States marines transiently exposed to a desert environment. *J Urol* 2013; **189:** 165.

163. Hadramy MSA. Seasonal variations of urinary stone colic in Arabia. *J Pak Med Assoc* 1997; **47:** 184.

164. Brikowski TH, Lotan Y, Pearle MS. Climate-related increase in the prevalence of urolithiasis in the United States. *PNAS* 2008; **105**(28)**:** 9841.

165. Sirohi M, Katz BF, Moreira DM, Dinlenc C. Monthly variations in urolitihiasis presentations and their association with meteorologic factors in New York City. *J Endourol* 2014; **28**(5)**:** 599.

166. Ferrie BG, Scott R. Occupation and urinary tract stone disease. *Urology* 1984; **24:** 443.

167. Borghi L, Meschi T, Amato F, Novarini A, Romanelli A, Cigala F. Hot occupation and nephrolithiasis. *J Urol* 1993; **150:** 1757.

168. Zheng W, Beiko DT, Segura JW, Preminger GM, Albala DM, Denstedt JD. Urinary calculi in aviation pilots: what is the best therapeutic approach? *J Urol* 2002; **168:** 1341.

169. Hyams ES, Nelms D, Silberman WS, Feng Z, Matlaga BR. The incidence of urolithiasis among commercial aviation pilots. *J Urol* 2011; **186:** 914.

170. Ekane S, Wildschutz T, Simon J, Schulman CC. Urinary lithiasis: epidemiology and pysiology. *Acta Urol Belg* 1997; **65**(3)**:** 1.

171. Atan L, Anderoni C, Ortiz V, Silva EK, Pitta R, Atan F *et al.* High kidney stone risk in men working in steel industry at hot temperatures *Urology* 2005; **65:** 858.

172. Parmar MS. Kidney stones. *BMJ* 2004; **328:** 1420.

173. Heller HJ, Sakhaee K, Moe OW, Pak CY. Etiological role of estrogen status in renal stone formation. *J Urol* 2002; **168**(5)**:** 1923.

174. Curhan GC. Epidemiologic evidence for the role of oxalate in idiopathic nephrolithiasis. *J Endourol* 1999; **13:** 629.

175. Ferrari P, Bonny O. Diagnosis and prevention of uric acid stones. *Ther Umsch* 2004; **61**(9)**:** 571.

176. Lee YH, Huang WC, Huang JK, Chang LS. Testosterone enhances estrogen inhibits calcium oxalate stone formation in ethylene glycol treated rats. *J Urol* 1996; **156:** 502.

177. Yoshihara H, Yamaguchi S, Yachiku S. Effect of sex hormones on oxalate-synthesizing enzymes in male and female rat livers. *J Urol* 1999; **161**(2)**:** 668.

178. Cary MK. The racial incidence of urolithiasis. *J Urol* 1937; **37:** 651.

179. Akoudad S, Szklo M, McAdams MA, Fulop T, Anderson CA, Coresh J *et al.* Correlates of kidney stone disease differ by race in a multi-ethnic middle-aged population: the ARIC study. *Prev Med* 2010; **51**(5)**:** 416.

180. Rodgers AL. Race, ethnicity and urolithiasis: a critical review. *Urolithiasis* 2013; **41:** 99.

181. Sarmina I, Spirnak JP, Resnick MI. Urinary lithiasis in the black population: an epidemiological study and review of the literature. *J Urol* 1987; **138:** 14.

182. Taylor EN, Curhan GC. Difference in 24 h urine composition between black and white women. *J Am Soc Nephrol* 2007; **18:** 654.

183. López M, Hoppe B. History, epidemiology and regional diversities of urolithiasis. *Pediatr Nephrol* 2010; **25:** 49.

184. Whalley NA, Martins MC, Dyk RCV, Meyers AM. Lithogenic risk factors in normal black and white recurrent stone formers. *BJU Int* 1999; **84:** 243.

185. Goad EHA, Bereczky ZB. Metabolic risk factors in patients with renal stones in KwaZulu Natal: an inter-racial study (Asian and Whites). *BJU Int* 2003; **93:** 120.

186. Robertson WG, Peacock M. calcium oxalate crystalluria and inhibitors of crystallisation in recurrent renal stone-formers. *Clin Sci* 1972; **43:** 499-506.

187. Khan SR, Kok DJ. Modulators of urinary stone formation. *Frontiers in Bioscience* 2004; **9:** 1450-1482.

188. Nishio S, Abe Y, Wakatsuki A, Iwata H, Ochi K, Takeuchi M *et al.* Matrix glycosaminoglycans in urinary stones. *J Urol* 1985; **134:** 503.

189. Roberts D, Resnick MI. Glycosaminoglycans content of stone matrix. *J Urol* 1986; **135:** 1078.

190. Goldberg JM, Cotlier E. Specific isolation and analysis of mucopolysaccharides (glycosaminoglycans) from human urine. *Clin Chim Acta* 1972; **41:** 19.

191. Wessler E. Nature of non-ultrafilterable glycosaminoglycans of normal human urine. *Biochem J* 1971; **122:** 373.

192. Fellstrom B, Monica L, Danielson BG, Karlsson FA, Ljunghall S. Binding of glycosaminoglycan inhibitors to calcium-oxalate crystals in relation to ionic-stregth. *Clin Chim Acta* 1989; **180:** 213.

193. Ryall RL, Harnett RM, Marshall VR. The effect of urine, pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals in vitro. *Clin Chim Acta* 1981; **112:** 349.

194. Scurr DS, Robertson WG. Modifiers of calcium oxalate crystallization found in urine. II. Studies on their mode of action in artificial urine. *J Urol* 1986; **136:** 128.

195. Gjaldbaek JC. Inhibition of chondroitin sulphate and heparin on the growth and agglomeration of calcium oxalate monohydrate crystals *in vitro*. *Clin Chim Acta* 1982; **120:** 363.

196. Yamaguchi S, Yoshioka T, Utsunomya M, Koide T, Osafune M, Okuyama A. Heparan sulfate in the stone matrix and its inhibitory effect on calcium oxalate crystallization. *Urol Res* 1993; **21:** 187.

197. Michelacci YM, Boim MA, Rovigatti CT, Schor N. Possible role of chondroitin sulphate in urolithiasis: *in vivo* studies in an experimental model. *Clin Chim Acta* 1992; **208:** 1.

198. Ryall RL, Harnett RM, Hibberd CM, Edyvane KA, Marshall VR. Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. *Urol Res* 1991; **19:** 181-188.

199. Michelacci Y, Glashan R, Schor N. Urinary-excretion of glycosaminoglycans in normal and stone forming subjects. *Kidney International* 1989; **36:** 1022.

200. Nesse A, Garbossa G, Romero MC, Bogado CE, Zanchetta JR. Glycosaminoglycans in urolithiasis. *Nephron* 1992; **62:** 36.

201. Hwang IS, Preminger GM, Pondexter J, Pak CYC. Urinary glycosaminoglycans in normal subjects and patients with stones. *J Urol* 1988; **139:** 995.

202. Samuell CT. A study of glycosaminoglycans excretion in normal and stone-forming subjects using a modified cetylpyridinium chloride technique. *Clin Chim Acta* 1981; **117:** 63.

203. Akinici N, Essen T, Kocack T, Ozsoy C, Tellaloglu S. The role if inhibitor deficiency in urolithiasis. I. Rationale of urinary magnesium, citrate, pyrophosphate and glycosaminoglycan determinations. *Eur Urol* 1992; **19:** 240.

204. Nikkila MT. Urinary glycosaminoglycans excretion in normal and stone-forming subjects: significant disturbance in recurrent stone formers. *Urol Int* 1989; **44:** 157.

205. Adachi J, Kumar C, Zhang Y, Olsen JV, Mann M. The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins. *Genome Biology* 2006; **7:** R80.

206. Ryall RL. Urinary inhibitors of calcium oxalate crystallisation and their potential role in stone formation. *World J Urol* 1997; **15:** 155.

207. Nakagawa Y, Abram V, Kezdy FJ, Kaiser ET, Coe FL. Purification and characterization of the principal inhibitor of calcium oxalate monohydrate crystal growth in human urine. *J Biol Chem* 1983; **258:** 12594-12600.

208. Asplin J, DeGanello S, Nakagawa Y, Coe FL. Evidence that nephrocalcin and urine inhibit nucleation of calcium oxalate monohydrate crystals. *Am J Physiol* 1991; **261:** F824-F830.

209. Hess B. The role of Tamm-Horsfall Glycoprotein and nephrocalcin in calcium oxalate monohydrate crystallization processes. *Scanning Microsc* 1991; **5:** 689-696.

210. Hess B, Nakagawa Y, Coe FL. Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. *Am J Physiol* 1989; **257:** F99.

211. Worcester EM, Snyder C, Beshensky AM. Osteopontin inhibits heterogeneous nucleation of calcium oxalate. *J Am Soc Nephrol* 1995; **6:** 956.

212. Asplin JR, Hoyer J, Gillespie C, Coe FL. Uropontin (UP) inhibits aggregation of calcium oxalate monohydrate (COM) crystals. *J Am Soc Nephrol* 1995; **6:** 941.

213. Doyle IR, Ryall RL, Marshall VR. Inclusion of proteins into calcium oxalate crystals precipitated from human urine: highly selective phenomenon. *Clin Chem* 1991; **37:** 1589.

214. Fleisch H, Bisaz S. Isolation from urine of pyrophosphate, a calcification inhibitor. *Am J Physiol* 1962; **203:** 671.

215. Doremus RH, Teich S, Silvis PX. Crystallization of calcium oxalate from synthetic urine. *Invest Urol* 1978; **15:** 469.

216. Meyer JL, Smith LH. Growth of calcium oxalate crystals. II. Inhibition by natural crystal growth inhibitors. *Invest Urol* 1975; **13:** 36.

217. Sutor DJ. Growth studies of calcium oxalate in the presence of various compounds. *Br J Urol* 1969; **41:** 171.

218. Welshman SG, McGeown MG. A quantitative investigation of the effects on the growth of calcium oxalate crystals of potential inhibitors. *Br J Urol* 1972; **44:** 677.

219. Robertson WG. Factors affecting the precipitation of calcium phosphate *in vitro*. *Calcif Tissu Res* 1973; **11:** 311.

220. Grases F, Ramis M, Costa-Bauza A. Effects of phytate and pyrophosphate on brushite and hydroxyapatite crystallisation- Comparison with the action of other polyphosphates *Urol Res* 2000; **28:** 136.

221. Yuzawa M, Tozuka K, Tokue A. Effect of citrate and pyrophosphate on the stability of calcium oxalate dihydrate. *Urol Res* 1998; **26:** 83-88.

222. Wesson JA, Worcester EM, Weissner JH, Mandel NS, Kleinman JG. Control of calcium oxalate crystal structure and cell adherence by urinary macromolecules. *Kidney Int* 1998; **53:** 952.

223. Fleisch H, Bisaz S. Mechanism of calcification: inhibitory role of pyrophosphate. *Nature* 1962; **195:** 911.

224. Sharma S, Vaidyanathan S, Thind SK, Nath R. Urinary-excretion of inorganic pyrophosphate by normal subjects and patients with renal calculi in north-western India and the effect of Diclodefac sodium upon urinary-excretion of pyrophosphate in stone formers. *Urol Int* 1992; **48:** 404-408.

225. O'Brien MM, Uhlemann I, McIntosh HW. Urinary pyrophosphate in normal subjects and in stone formers. *Can Med Assoc J* 1967; **96:** 100-103.

226. Russell RGG, Hodgkinson A. The urinary excretion of inorganic pyrophosphate by normal subjects and patients with renal calculus. *Clin Sci* 1966; **31:** 51-62.

227. Russell RGG, Bisaz S. The influence of orthophosphate on the renal handling of inorganic pyrophosphate in man and dog. *Clin Sci Mol Med* 1976; **51:** 435-443.

228. Breslau NA, Padalino P, Kok DJ, Kim YG, Pak CYC. Physicochemical effects of a new slow-release potassium phosphate preparation (Urophos-K) in absorptive hypercalciuria. *J Bone Min Res* 1995; **10:** 394-400.

229. Doremus RH, Teich S, Silvis PX. Crystallisation of calcium oxalate from synthetic urine. *Invest Urol* 1978; **15:** 469-472.

230. Schwille PO, Schmiedl A, Herrmann U, Fan J, Gottlieb D, Manoharan M. Magnesium, citrate, magnesium citrate and magnesium-alkali citrate as modulators of calcium oxalate crystallization in urine: observations in patients with recurrent idiopathic calcium urolithiasis. *Urol Res* 1999; **27:** 117.

231. Hess B, Jordi S, Zipperle L, Ettinger E, Giovanoli R. Citrate determines calcium oxalate crystallization kinetics and crystal morphology- studies in the presence of Tamm-Horsfall protein of a healthy subject and a severely recurrent stone former. *Nephrol Dial Transplant* 2000; **15:** 366-374.

232. Tiselius HG, Fornander AM, Nilsson MA. The effects of citrate and urine on calcium oxalate crystal aggregation. *Urol Res* 1993; **21:** 363.

233. Ryall RL, Hibberd CM, Marshall VR. A method for studying inhibitory activity in whole urine. *Urol Res* 1985; **13:** 285.

234. Hallson PC, Rose GA, Sulaiman S. Raising urinary citrate lowers calcium oxalate and calcium phosphate crystal formation in whole urine. *Urol Int* 1983; **38:** 179-181.

235. Elliot JS, Ribiero ME. The urinary excretion of citrate, hippuric acid and lactic acid in normal adults and in patients with calcium oxalate urinary calculus disease. *Invest Urol* 1972; **10:** 102-106.

236. Hodgkinson A. Citric acid excretion in normal adults and in patients with renal calculus. *Clin Sci* 1962; **23:** 203-212.

237. Menon M, Mahle CJ. Urinary citrate excretion in patients with renal calculi. *J Urol* 1983; **129:** 1158.

238. Nicar MJ, Skula C, Sakhaee K, Pak CYC. Low citrate excretion in nephrolithiasis. *Urology* 1983; **21:** 8-14.

239. Laminski NA, Meyers AM, Sonnekus MI, Smyth AE. Prevalence of hypocitraturia and hypopyrophosphaturia in recurrent calcium stone formers: as isolated defects or association with other metabolic abnormalities. *Nephron* 1990; **56:** 379.

240. Cupisti A, Morelli E, Lupetti S, Meola M, Barsotti G. Low urine citrate excretion as main risk factor for recurrent calcium oxalate nephrolithiasis in males. *Nephron* 1992; **61:** 73.

241. Kok DJ, Papapoulos SE, Bijvoet OLM. Excessive crystal agglomeration with low citrate excretion in recurrent stone formers. *The Lancet* 1986**:** 1056.

242. Kok DJ, Papapoulos SE, Bijvoet OLM. Crystal agglomeration is a major element in calcium oxalate urinary stone formation. *Kidney Int* 1990; **37:** 51.

243. Erwin DT, Kok DJ, Alam J, Vaughn J, Coker O, Carriere BT *et al.* Predicting recurrent renal stone formation and therapy using crystal agglomeration inhibition. *Am J Kidney Dis* 1994; **24:** 893.

244. Berg C, Larsson L, Tiselius HG. Effects of different doses of alkaline citrate on urine composition and crystallization of calcium oxalate. *Urol Res* 1990; **18:** 13.

245. Hofbauer J, Hobarth K, Szabo N, Marberger M. Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis- a prospective randomized study. *Br J Urol* 1994; **73:** 362.

246. Ogawa Y. Impact of sodium-potassium citrate on the diurnal variations in urinary calcium oxalate and calcium phosphate saturation levels in normal individuals. *Br J Urol* 1994; **73:** 136.

247. Sakhaee K, Baker S, Zerwekh J, Poindexter J, Garcia-Hernandez PA. Limited risk of kidney stone formation during long-term calcium citrate supplementation in nonstone forming subjects. *J Urol* 1994; **152:** 324.

248. Levine BS, Rodman JS, Wienerman S, Bockman RS, Lane JM. Effect of calcium citrate supplementation on urinary calcium oxalate saturation in female stone formers: implications for prevention of osteoporosis. *Am J Clin Nutr* 1994; **60:** 592.

249. Allie-Hamdulay S, Rodgers AL. Prophylactic and therapeutic properties of a sodium citrate preparation in the management of calcium oxalate urolithiasis: randomized, placebo-controlled trial. *Urol Res* 2005; **33:** 116.

250. Li MK, Blacklock NJ, Garside J. Effects of magnesium on calcium oxalate crystallisation. *J Urol* 1985; **133:** 123.

251. Azoury R, Garside J, Robertson WG. Calcium oxalate precipitation in a flow system: an attempt to simulate the early stages of stone formation in the renal tubules. *J Urol* 1986; **136:** 150.

252. Bertoni E, Bigi A, Cojazzi G, Gandolfi M, Panzavolta S, Roveri N. Nanocrystals of magnesium and flouride substituted hydroxyapatite *J Inorganic Biochem* 1998; **72:** 29.

253. Robertson WG, Peacock M, Nordin BEC. Inhibitors of growth and aggregation of calcium oxalate crystals in vitro. *Clin Chim Acta* 1981; **112:** 349.

254. Ryall RL, Grover PK, Harnett RM, Hibberd CM, Marshall VR. Small molecular weight inhibitors. In: Walker VR, Sutton RAL, Cameron EC, Pak CYC, Robertson WG (eds). *Urolithiasis*. Plenum: New York, 1989, pp 91-96.

255. Hallson PC, Rose GA, Sulaiman S. Magnesium reduces calcium oxalate crystal formation in human whole urine. *Clin Sci* 1982; **62:** 17.

256. Emsley J, Niazi S. The structure of myoinositol hexaphosphate in solution- ^{31}P NMR investigation. *Phosphorous Sulfur ands Silicon and Related Elements* 1981; **10:** 159.

257. Jonson LF, Tate ME. Structure of phytic acids. *Can J Chem* 1969; **47:** 63.

258. Barrientos LG, Murthy PPN. Conformational studies of myo-inositol phosphates. *Carbohydr Res* 1996; **296:** 39.

259. Schlemmer U, Frolich W, Prieto RM, Grases F. Phytate in foods and significance in humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Mol Nutr Food Res* 2009; **53:** s330.

260. Bohn L, Meyer AS, Rasmussen SK. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Univ Sci B* 2008; **9**(3)**:** 165.

261. Johnson LF, Tate ME. Structure of phytic acids. *Can J Chem* 1969; **47:** 63.

262. Nahapetian A, Young VR. Metabolism of ^{14}C-phytate in rats: effect of low and high dietary calcium intakes. *J Nutr* 1980; **110:** 1458.

263. Sakumoto K, Vucenik I, Shamsuddin AM. [^{3}H] phytic acid (inositol hexaphosphate) is absorbed and distributed to various tissues in rats. *J Nutr* 1993; **123:** 713.

264. Grases F, Simonet B, Prieto RM, March JG. Variation of InsP4, InsP5 and InsP6 levels in tissues and biological fluids depending on dietary phytate. *J Nutr Biochem* 2001; **12:** 595.

265. Grases F, March JG, Prieto RM, Simonet BM. Urinary phytate in calcium oxalate stone formers and healthy people- dietary effects on phytate excretion. *Scand J Urol Nephrol* 2000; **34:** 162.

266. Grases F, Simonet BM, Prieto RM, March JG. Phytate levels in diverse rat tissues: influence of dietary phytate. *Br J Nutr* 2001; **86**(225)**:** 225.

267. F. Grases BMS, I. Vucenik, J. Perelló, R. M. Prieto, A. M. Shamsuddin. Effects of exogenous inositol hexakisphosphate (InsP6) on the levels of InsP6 and of inositol triphosphate (InsP3) in malignant cells, tissues and biological fluids. *Life Sciences* 2002; **71:** 1535.

268. Grases F, Simonet BM, Vucenik I, Prieto RM, Costa-Bauza A, March JG *et al.* Absorption and excretion of orally administered inositol hexaphosphate (IP6 or phytate) in humans. *BioFactors* 2001; **15:** 53.

269. Grases F, Costa-Bauza A, Perello J, Isern B, Vucenik I, Valiente M *et al.* Influence of concomitant food intake on the excretion of orally administered myo-inositol hexaphosphate in humans. *J Med Food* 2006; **9**(1)**:** 72.

270. McCance RA, Widdowson GM. Mineral metabolism of healthy adults on white and brown bread dietaries. *J Physiol* 1942; **101:** 44.

271. McCance RA, Walsham CM. The digestibility and absorption of the calories, proteins, purines, fat and calcium in wholemeal wheaten bread. *Br J Nutr* 1948; **2:** 26.

272. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am J Med* 1960; **31:** 532.

273. Graf E, Empson KL, Eaton JW. Phytic acid- A natural antioxidant *J Biol Chem* 1987; **262:** 11647.

274. Shamsuddin AM. Inositol phosphates have novel anticancer function. *J Nutr* 1995; **125:** 7255.

275. Jariwalla RJ, Sabin R, Lawson S, Herman ZS. Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. *J Appl Nutr* 1990; **42:** 18.

276. Lee SH, Park HJ, Chun HK, Cho SY, Jung HJ, Cho SM *et al.* Dietary phytic acid improves serum hepatic lipid levels in aged ICR mice fed a high cholesterol diet. *Nutr Res* 2007; **27:** 505.

277. Grases F, Costa-Bauza A. Phytate (IP6) is a powerful agent for preventing calcifications in biological fluids: usefulness in renal lithiasis treatment. *Anticancer Res* 1999; **19:** 3717.

278. Henneman PH, Benedict PH, Forbes AP, Dudley HR. Idiopathic hypercalciuria. *N Engl J Med* 1958; **259:** 802.

279. Ohkawa T, Ebisuno S, Kitagawa M, Morimoto S, Miyazaki Y, Yasukawa S. Rice bran treatment for patients with hypercalciuric stones: experimental and clinical studies. *J Urol* 1984; **132:** 1140.

280. Shah PJ, Green NA, Williams G. Unprocessed bran and its effect on urinary calcium excretion in idiopathic hypercalciuria. *Br Med J* 1980; **281:** 426.

281. Grases F, Garcia-Ferragut L, Costa-Bauza A, March JG. Study of the effects of different substances on the early stages of papillary stone formation. *Nephron* 1996; **73:** 561.

282. Grases F, Garcia-Ferragut L, Costa-Bauza A. A study of early ages of renal stone formation: experimental model using urothelium of pig bladder. *Urol Res* 1996; **24:** 305.

283. Grases F, Garcia-Ferragut L, Costa-Bauza A. Development of calcium oxalate crystals on urothelium: effect of free radicals. *Nephron* 1998; **78:** 296.

284. Grases F, Isern B, Sanchis P, Perello J, Torres JJ, Costa-Bauza A. Phytate acts as an inhibitor in formation of renal calculi. *Front Biosci* 2007; **12:** 2580.

285. Saw NK, Chow K, Rao PN, Kavanagh JP. Effects of inositol hexaphosphate (phytate) on calcium binding, calcium oxalate crystallization and in vitro stone growth. *J Urol* 2007; **177:** 2366.

286. Costa-Bauza A, Isern B, Perello J, Sanchis P, Grases F. Factors affecting the regrowth of renal stones in vitro: a contribution to the understanding of renal stone development. *Scand J Urol Nephrol* 2005; **39:** 194.

287. Costa-Bauza A, Perello J, Isern B, Sanchis P, Grases F. Factors affecting calcium oxalate dihydrate fragmented regrowth. *BMC Urol* 2006; **5:** 6.

288. Grases F, Garcia-Gonzalez R, Torress JJ, Llobera A. Effects of phytic acid on renal stone formation in rats. *Scand J Urol Nephrol* 1998; **32:** 261.

289. F. Grases RMP, B.M. Simonet and J.G. March. Phytate prevents tissue calcifications in female rats. *BioFactors* 2000; **11:** 171.

290. Wu N, Thon WF, Krah H, Schlick R, Jonas U. Effects of magnesium citrate and phytin on reducing urinary calcium excretion in rats. *World J Urol* 1994; **12:** 323.

291. Grases F, Perello J, Simonet BM, Prieto RM, Garcia-Raja A. Study of potassium phytate effects on decreasing urinary calcium in rats. *Urol Int* 2004; **72:** 237.

292. Conte A, Piza P, Garcia-Raja A, Grases F, Costa-Bauza A, Prieto RM. Urinary lithogen risk test: usefulness in the evaluation of renal lithiasis treatment using crystallization inhibitors (citrate and phytate). *Arch Esp Urol* 1999; **52**(3)**:** 305.

293. Grases F, García-Ferragut L, Costa-Bauzá A, Conte A, García-Raja A. Simple test to evaluate the risk of urinary calcium stone formation. *Clin Chim Acta* 1997; **263:** 43.

294. Meyers AM. Chemical composition of the urine in the normal black and white population. In: Ryall RL (ed) *Urolithiasis 2*, vol. 422. Plenum: New York, 1994.

295. Lewandowski S, Rodgers AL, Schloss I. The influence of a high-oxalate/low-calcium diet on calcium oxalate renal stone risk factors in non-stone-forming black and white South African subjects. *BJU Int* 2001; **87:** 307.

296. Rodgers AL, Lewandowski S. Effects of 5 different diets on urinary risk factors for calcium oxalate kidney stone formation: evidence of different renal handling mechanisms in different race groups. *J Urol* 2002; **168:** 931.

297. Rodgers AL. The riddle of kidney stone disease: lessons from Africa. *Urol Res* 2006; **34:** 92.

298. Theka T, Rodgers A, Lewandwoski S, Webber D, Allie-Hamdulay S. Effects of vitamin E ingestion on plasma and urinary risk factors for calcium oxalate urolithiasis in two population groups having different stone-risk profiles: evidence of physiological handling mechanisms. *Urol Res* 2012; **40**(2)**:** 113.

299. Lewandowski S, Rodgers AL. Renal response to lithogenic and anti-lithogenic supplement challenges in a stone-free population group. *J Renal Nutr* 2004; **14**(3)**:** 170.

300. Rodgers AL, Bungane N, Allie-Hamdulay S, Lewandowski S, Webber D. Calciuria, oxaluria and phosphaturia after ingestion of glucose, xylitol and sorbitol in two population groups with different stone-risk profiles. *Urol Res* 2009; **37:** 121.

301. Rodgers AL, Lewandowski S, Allie-Hamdulay S, Pinnock D, Baretta G, Gambaro G. Evening primrose oil supplementation increases citraturia and decreases other urinary risk factors for calcium oxalate urolithiasis. *J Urol* 2009; **182:** 2957.

302. Lewandowski S, Rodgers A, Laube N, Unruh Gv, Zimmerman D, Hesse A. Oxalate and its handling in a low stone risk vs a stone-prone population group. *World J Urol* 2005; **23:** 330.

303. Durrbaum D, Rodgers A, Sturrock E. A study of crystal matrix extract and urinary prothrombin fragment 1 from a stone-prone and stone-free population. *Urol Res* 2001; **29:** 83.

304. Webber D, Rodgers AL, Sturrock ED. Synergism between urinary prothrombin fragment 1 and urine: a comparison of inhibitory activities in stone-prone and stone-free population groups. *Clin Chem Lab Med* 2002; **40**(9)**:** 930.

305. Webber D, Rodgers AL, Sturrock E. Selective inclusion of proteins into urinary calcium oxalate crystals: Comparison between stone-prone and stone-free population groups. *J Cryst Growth* 2003; **259:** 179.

306. Rodgers A, Mensah P, Schwager S, Sturrock E. Inhibition of calcium oxalate crsytallisation by commercial human serum albumin and human urinary albumin

isolated from two different race groups: evidence for molecular differences. *Urol Res* 2006; **34:** 373.

307. Mensah PDNA. Investigation of the crystallization inhibitory properties of albumin isolated from the urine of black and white South Africans. MSc, University of Cape Town, South Africa, 2004.

308. Mabizela NF, Rodgers AL, Webber D, Schwager SLU. Isolation of bikunin from urine and inhibitory effect of protein precipitation in the urine of black and white subjects. *Urol Res* 2005; **32**(2)**:** 161.

309. Mabizela NF, Rodgers AL, Webber D. Analysis of urinary bikunin in calcium oxalate crystals from the black and white population groups in South Africa. *Urol Res* 2004; **32**(2)**:** 140.

310. Rodgers AL, HIbbert BE, Hess B, Khan SR, Preminger GM (eds). Inhibitory activity of Tamm-Horsfall mucoprotein isolated from two different population groups. *Urolithiasis 2000*; Cape Town. University of Cape Town, 2000.

311. Rodgers AL, HIbbert BE, Hess B, Khan SR, Preminger GM (eds). Comparison of Tamm-Horsfall Mucoprotein (THM) in normal and stone-forming caucasian and african males in South Africa. *Urolithiasis 2000*; Cape Town. University of Cape Town, 2000.

312. Jappie-Mohamed D. Urinary glycosaminoglycans and their possible protective role in calcium oxalate kidney stone disease, with particular reference to the rarity of this condition in black South Africans: in vitro and in vivo investigations. Ph.D., University of Cape Town, South Africa, 2013.

313. Tiselius HG. The effect of pH on the urinary inhibition of calcium oxalate crystal growth. *Br J Urol* 1981; **53:** 470.

314. Pierratos A, Dharamsi N, Carr LK, Ibanez D, Jewett MAS, Honey RJ. Higher urinary potassium is associated with decreased stone growth after shock wave lithotripsy. *J Urol* 2000; **164:** 1486.

315. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. *J Physiol Biochem* 2001; **57**(1)**:** 43.

316. Zara V, Giudetti AM, Siculella L, Palmieri F, Gnoni GV. Covariance of tricarboxylate carrier activity and lipogenesis in liver of polyunsaturated fatty acid (n-6) fed rats. *Eur J Biochem* 2001; **268**(22)**:** 5734.

317. Siculella L, Sabetta S, Damiano F, Giudetta AM, Gnoni GV. Different dietary fatty acids have dissimilar effects on activity and gene expression of mitochondrial tricarboxylate carrier in rat liver. *FEBS lett* 2004; **578**(3)**:** 280.

318. Modlin M. Urinary phosphorylated inositols and renal stone *The Lancet* 1980; **2:** 1113.

319. Lesotho N. Investigation of the role of dietary myo-inositol hexakisphosphate (phytate) on the relative risk of calcium oxalate kidney stone formation in black and white South African subjects. MSc Dissertation, University of Cape Town, South Africa, 2006.

320. Grases F, Kroupa M, Costa-Bauza A. Studies on calcium oxalate monohydrate crystallization: influence of inhibitors. *Urol Res* 1994; **22:** 39.

321. May PM, Murray K. JESS, a joint expert speciation system-I. *Talanta* 1991; **38:** 1409.

322. May PM, Murray K. JESS, a joint expert speciation system- II. The thermodynamic database. *Talanta* 1991; **38:** 1419.

323. Rodgers A, Allie-Hamdulay S, Jackson G. Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the determinant factor. *Nephrol Dial Transplant* 2006; **21**(2)**:** 361.

324. Rodgers AL, Webber D, Charmoy Rd, Jackson GE, Ravenscroft N. Malic acid supplementation increases urinary citrate excretion and urinary pH: implications for the potential treatment of calcium oxalate stone disease. *J Endourol* 2014; **28**(2)**:** 229.

325. Rodgers AL, Allie-Hamdulay S, Jackson GE, Sutton RAL. Enteric hyperoxaluria secondary to small bowel resection: use of computer simulation to characterize urinary risk factors for stone formation and assess potential treatment protocols. *J Endourol* 2014; **28**(8)**:** 985.

326. Rodgers A, Gauvin D, Edeh S, Allie-Hamdulay S, Jackson GE, Lieske JC. Sulfate but not thiosulfate reduces calculated and measured urinary ionized calcium and supersaturation: implications for the treatment of calcium renal stones. *Plos One* 2014; **9**(7)**:** e103602.

327. Rodgers AL, Allie-Hamdulay S, Jackson GE, Durbach I. Theoretical modelling of the urinary supersaturation of calcium salts in healthy individuals and kidney stone patients: precursors, speciation and therapeutic protocols for decreasing its value. *J Cryst Growth* 2013; **382:** 67.

328. Torres J, Dominguez S, Cerda MF, Obal G, Mederos A, Irvine RF *et al.* Solution behaviour of myo-inositol hexakisphosphate in the presence of multivalent cations. Prediction of a neutral pentamagnesium species under cytosolic/nuclear conditions. *J Inorganic Biochem* 2005; **99:** 828.

329. Crea P, Robertis Ad, Stefano Cd, Sammartano S. Speciation of phytate ion in aqueous solution. Sequestration of magnesium and calcium by phytate at different temperatures and ionic strengths, in NaCl. *Biophys Chem* 2006; **124:** 18.

330. Graf E. Calcium binding to phytic acid. *J Agric Food Chem* 1983; **31:** 851.

331. Housecroft CE, Sharpe AG. Acids, bases and ions in aqueous solution. In: *Inorganic chemistry*, 3 edn. Pearson Education Limited: England, 2008, pp 181 - 211.

332. Crea F, Stefano CD, Milea D, Sammartano S. Formation and stability of phytate complexes in solution. *Coord Chem Rev* 2008; **252:** 1108.

333. Evans WJ, McCourtney EJ, Shrager RI. Titrations of phytic acid. *JAOCS* 1982; **59:** 189.

334. Li N, Wahlberg O. Equilibrium constants of phytate ions. 2. Equilibrium between phytate ions, sodium ions and protons in sodium perchlorate medium. *Acta Chem Scand* 1989; **43:** 401.

335. Stefano CD, Milea D, Sammartano S. Speciation of phytate ion in aqueous solution. Protonation constants in tetraethylammonium iodide and sodium chloride. *J Chem Eng Data* 2003; **48:** 114.

336. Duff MR, Grubbs J, Howell EE. Isothermal titration calorimetry for measuring macromolecule-ligand affinity. *J. Vis. Exp.* 2011; (55)**:** 2796.

337. Jelesarov I, Bosshard HR. Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. *J. Mol. Recognit.* 1999; **12:** 3.

338. Velazquez-Campoy A, Leavitt SA, Freire E. Characterization of protein-protein interactions by isothermal titration calorimetry. *Methods Mol. Biol.* 2004; **261:** 35.

339. Brown A. Analysis of cooperativity by isothermal titration calorimetry. *Int J Mol Sci* 2009; **10**(8)**:** 3457.

340. Pierce MM, Raman CS, Nall BT. Isothermal titration calorimetry of protein-protein interactions. *Methods* 1999; **19:** 213.

341. Templeton DM, Ariese F, Cornelis R, Danielsson LG, Muntau H, Leeuwen HPV *et al.* Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC recommendations 2000). *Pure Appl Chem* 2000; **72**(8)**:** 1453.

342. Skoog DA, Leary JJ. *Principles of instrumental analysis*, Saunders College Publishing: Philadelphia, 1992.

343. Gans P, O'Sullivan B. GLEE, a new computer program for glass electrode calibration. *Talanta* 2000; **51:** 33.

344. Lee YH. Slope of grans plot- useful function in the examination of precipitation, the water-soluble part of airborne particles, and lake water. *Water Air Soil Poll* 1978; **10**(4)**:** 457.

345. Nielsen SS. Complexometric determination of calcium. In: Nielsen SS (ed) *Food analysis laboratory manual*. Springer, 2010, pp 61 - 67.

346. Vlasak M, Luxemnurkova Z, Sychra V, Suchanek M. Complexometry with EDTA as a quality control tool for certified single-element aqueous standard solutions. *Accredit Qual Assur* 2013; **18**(6)**:** 491.

347. Schwartz WB, Bank N, Cutler RWP. The influence of urinary ionic strength on phosphate pK2 and the determination of titratable acid. *J Clin Invest* 1959; **38**(2)**:** 347.

348. Gillenwater JY, Grayhack JT, Howards SS, Mitchell ME (eds). *Adult and pediatric urology*. Lippincott Williams and Wilkins: Philadelphia, 2002.

349. Gans P, Sabatim A, Vacca A. Investigation of equilibrium constants with the HYPERQUAD suite of programs. *Talanta* 1996; **43:** 1739.

350. Gans P. SUPERQUAD: An improved general program for computation of formation constants from potentiometric data. *J Chem Soc Dalton Trans* 1985**:** 1195.

351. Alderighi L, Gans P, Ienco A, Peters D, Sabatini A, Vacca A. Hyperquad simulation and speciation (HySS): a utility program for the investigation equilibria involving soluble and partially soluble species. *Coord Chem Rev* 1999; **184:** 311.

352. Stefano CD, Milea D, Pettignano A, Sammartano S. Speciation of phytate ion in aqueous solution. Alkali metal complex formation in different ionic media. *Anal Bioanal Chem* 2003; **376:** 1030.

353. Crea P, Stefano CD, Milea D, Porcino N, Sammartano S. Speciation of phytate ion in aqueous solution. Protonation constants and copper(II) interactions in $NaNO_{3aq}$ at different ionic strengths. *Biophys Chem* 2007; **128:** 176.

354. Crea F, Crea P, Stefano CD, Milea D, Sammartano S. Speciation of phytate ion in aqueous solution. Protonation in CSCl(aq) at different ionic strengths and mixing effects in LiCl(aq)+CsCl(aq). *J Mol Liq* 2008; **138:** 76.

355. Bieth H, Jost P, Spiess B, Wehrer C. Effect of the alkali-metal cations on the protonation constants of myo-Inositol Hexakis(phosphate). *Anal Lett* 1989; **22:** 703.

356. Katsumi H. Chemical speciation of trace metals in seawater: a Review. *Analyt Sci* 2006; **22:** 1055.

357. Bernhard M, Brinckman FE, Sadler PJ. The importance of chemical speciation in envirmonmental processes. *Springer Verlag, Berlin* 1986.

358. Atkins PW, Paula Jd. *Physical chemistry*, 8 edn Oxford University Press: New York, 2006.

359. Deosarkar SD, Narwade ML, Hiwase VV, Khedkar KM. Influence of ionic strength of medium on stability constants of Cu(II) complex of 2-amino-5-chloro benzene sulphonic acid at 301K. *Orient J Chem* 2009; **25**(1)**:** 233.

360. Ramteke AA, Chavan SP, Patil SD, Narwade ML. Effect of ionic strength on the stability constants of complexes of 3-(4-chlorophenyl)-4-(3-pyridine)-5-(2-hydroxyphenyl)-pyrazole with Cu (II), TB (III), and ND (III) metal ions. *International Journal of Chemical Studies* 2014; **2**(4)**:** 1.

361. Wiberg E, Wiberg N. Molecular transformations. In: *Inorganic chemistry*. Academic Press, 2001, p 196.

362. Grases F, March P. A study about some phosphate derivatives as inhibitors of calcium oxalate growth. *J Cryst Growth* 1989; **96:** 993.

363. Costa-Bauza A, Grases F, Gomila I, Rodriguez A, Prieto RM, Tur F. A simple and rapid colorimetric method for determination of phytate in urine. *Urol Res* 2012; **40**(6)**:** 663.

364. Perello J, Isern B, Munoz JA, Valiente M, Grases F. Determination of phytate in urine by high-performance liquid chromatography-mass spectrometry. *Chromatographia* 2004; **60:** 265.

365. Grases F, Perello J, Isern B, Prieto RM. Determination of myo-inositol hexakisphosphate (phytate) in urine by inductively coupled plasma atomic emission spectrometry. *Anal Chim Acta* 2004; **510:** 41.

366. Munoz JA, Valiente M. Determination of phytic acid in urine by inductively coupled plasma mass spectrometry. *Anal Chem* 2003; **75:** 6374.

367. March JG, Simonet BM, Grases F. Determination of phytic acid by gas chromatrography-mass spectroscopy: application to biological samples. *J Chromatogr B Biomed Sci Appl* 2001; **757:** 247.

368. Chen Y, Chen J, Ma K, Cao S, Chen X. Fluorimetric determination of phytic acid in urine based on replacement reaction. *Anal Chim Acta* 2007; **605:** 185.

369. Latta M, Eskin M. A simple and rapid colorimetric method for phytate determination. *J Agric Food Chem* 1980; **28:** 1313.

370. Costa-Bauza A, Grases F, Fakier S, Rodriguez A. A novel metal-dye system for urinary phytate detection at micro-molar levels in rats. *Anal Methods* 2013; **5:** 3016.

371. Grases F, Torres CS, Rodriguez A, Costa-Bauza A, Rodrigo D, Frontera G *et al.* Urinary phytate (myo-inositol hexaphosphate) in healthy school children and risk of nephrolithiasis. *J Renal Nutr* 2014; **24:** 219.

372. Walton RC, Kavanagh JP, Heywood BR, Rao PN. The association of different urinary proteins with calcium oxalate hydromorphs. Evidence for non-specific interactions. *Biochim Biophys Acta* 2005; **1723:** 175.

373. Chumanov SR, Burgess RR. Artifact-inducing enrichment of ethylenediaminetetraacetic acid and ethyleneglycoltetraacetic acid on anion exchange resins. *Anal Biochem* 2011; **412:** 34.

374. Hess B, Ryall RL, Kavanagh JP, Khan SR, Kok D, Rodgers AL *et al.* Methods for measuring crystallisation in urolithiasis research: why, how and when. *Eur Urol* 2001; **40:** 220.

375. Ryall RL. Glycosamnioglycans, proteins and stone formation: adult themes and child's play. *Pediatr Nephrol* 1996; **10:** 656.

376. Walton RC, Kavanagh JP, Heywood BR, Rao PN. Calcium oxalates grown in human urine under different batch conditions. *J Cryst growth* 2005; **284:** 517.

377. Grover PK, Moritz RL, Simpson RJ, Ryall RL. Inhibition of growth and aggregation of calcium oxalate crystals in vitro- a comparison of four proteins. *Eur J Biochem* 1998; **253:** 637.

378. Kavanagh JP. Methods for the study of calcium oxalate crystallisation and their application to urolithiasis research. *Scan Microsc* 1992; **6:** 685.

379. Rodgers AL. Aspects of calcium oxalate crystallisation: theory, in vitro studies, and in vivo implementation. *J Am Soc Nephrol* 1999; **10:** s351.

380. Rodger AL, Hibbert BE, Hess B, Khan SR, Preminger GM (eds). Determination of the optimum number of subjects required for pooling urines: Statistical approach. *Urolithiasis 2000*; University of Cape Town, Cape Town, South Africa, 2000.

381. Hess B, Meinhardt U, Zipperle L, Giovanoli R, Jaeger P. Simultaneous measurement of calcium oxalate crystal nucleation and aggregation: impact of various modifiers. *Urol Res* 1995; **23:** 231.

382. Webber D, Rodgers AL, Sturrock ED. Glycosylation of prothrombin fragment 1 governs calcium oxalate crystal nucleation and aggregation, but not crystal growth. *Urol res* 2007; **35:** 277.

383. Pak CYC, Ohata M, Holt K. Effect of disphosphonate on crystallization of calcium oxalate in vitro. *Kidney Int* 1975; **7:** 154.

384. Ramsout R. Investigation of the in vitro and in vivo effects of some herbal preparations on risk factors for calcium oxalate kidney stone disease. Ph.D., University of Cape Town, South Africa, 2012.

385. Grases F, Rodriguez A, Costa-Bauza A. Efficacy of mixtures of magnesium, citrate and phytate as calcium oxalate crystallization inhibitors in urine. *J Urol* 2015; **194:** 812.

386. Munoz JA, López-Mesas M, Valiente M. Inhibitors of oxalocalcic lithiasis: effects of their interactions on calcium oxalate crystallization. *Urology* 2012; **80**(5)**:** 1163.e13.

387. Mandel N. Crystal-membrane interaction in kidney stone disease. *J Am Soc Nephrol* 1994; **5**(1)**:** S37.

388. Cerini C, Geider S, Dussol B, Henniquin C, Daudon M, Vessler S *et al.* Nucleation of calcium oxalate crystals by albumin: involvement in the prevention of stone formation. *Kidney Int* 1999; **55:** 1776.

389. Yuzawa MK, Tozuka A, Tokue A. Effect of citrate and pyrophosphate on the stability of calcium oxalate dihydrate. *Urol Res* 1998; **26:** 83.

390. Grases F, March P. Determination of phytic acid based on inhibition of crystalline growth of calcium oxalate monohydrate. *Anal Chim Act* 1989; **219:** 89.

391. Prieto RM, Fiol M, Perello J, Estruch R, Ros E, Sanchis P *et al.* Effects of Mediterranean diets with low and high proportions of phytate-rich foods on the urinary phytate excretion. *Eur J Nutr* 2010; **49:** 321.

392. Grases F, Simonet BM, March JG, Prieto RM. Inositol Hexakisphosphate in urine: the relationship between oral intake and urinary excretion. *Br J Urol Int* 2000; **85:** 138.

393. Sharma S, Yacavone M, Cao X, Pardilla M, Qi M, Gittelsohn J. Dietary intake and development of a quantitative FFQ for a nutritional intervention to reduce the risk of chronic disease in the Navajo Nation. *Public Health Nutr* 2009; **13**(3)**:** 350.

394. Wolmarans P, Humphreys J, Sayed N. FoodfinderTM 2. In. Cape Town: South African Medical Research Council, 2001.

395. Joung H, Nam G, Yoon S, Lee J, Shim JE, Paik HY. Bioavailable zinc intake of Korean adults in relation to the phytate content of Korean foods. *J Food Compos Anal* 2004; **17:** 713.

396. Lehrfeld J. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problems and solutions. *J Agric Food Chem* 1994; **42:** 2726.

397. Lolas GM, Palamidis N, Markakis P. The phytic acid-total phosphorus relationship in barley, oats, soybeans and wheat. *Cereal Chem* 1976; **53**(6)**:** 867.

398. Harland BF, Smikle-Williams S, Oberleas D. High performance liquid chromatography analysis of phytate (IP6) in selected foods. *J Food Compos Anal* 2004; **17:** 227.

399. Kasim AB, Edwards HMJ. The analysis of inositol phosphate forms in feed ingredients. *J Sci Food Agric* 1998; **76:** 1.

400. Shen YR, Yin YL, Chavez ER, Fan M. Methodological aspects of measuring phytate phosphorous content in selected cereal grains and digesta and feces of pigs. *J Agric Food Chem* 2005; **32:** 31.

401. Yoon JH, Thompson LU, Jenkins DJA. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *Am J Clin Nutr* 1983; **38:** 835.

402. Morris ER, Ellis R. Phytate-zinc molar ratio of breakfast cereals and bioavailability of zinc to rats. *Cereal Chem* 1981; **58:** 363.

403. Manez G, Alegria A, Farre R, Frigola A. Effect of traditional, microwave and industrial cooking on inositol phosphate content in beans, chickpeas and lentils. *Int J Food Sci Nutr* 2002; **53:** 503.

404. Davis KR. Effect of processing on composition and tetrahymena relative nutritive value of green and yellow peas, lentils and white pea beans. *Cereal Chem* 1981; **58**(5)**:** 454.

405. Chen QC. Determination of phytic acid and inositol pentakisphosphates in foods by HPLC. *Agric Food Chem* 2004; **52:** 4604.

406. Harland BF, Oberleas D. A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. *Cereal Chem* 1977; **54:** 827.

407. Ellis R, Morris ER. Improved ion-exchange phytate method. *Cereal Chem* 1983; **60:** 121.

408. Harland BF, Oberleas D. Anion-exchange method for determination of phytate in foods: collaborative study. *J Assoc Off Anal Chem* 1986; **69:** 667.

409. Klaas DB, Verbeek C, Eeden CHPv, Slump P, Wolters MGE. Improved determination of phytate by ion-exchange chromotography. *J Agric Food Chem* 1991; **39:** 1770.

410. McPherson RA, Ben-Ezra J. Basic examination of urine. In: McPherson RA, Pincus MR (eds). *Henry's clinical diagnosis and management by laboratory methods*, 22 edn. Elsevier Saunders: Philadelphia, 2011.

411. Fernandez FJ, Khan HL. Clinical methods for atomic absorption spectroscopy. *Clin Chem Newslett* 1971; **3:** 24.

412. Trudeau DL, Freier EF. Determination of calcium in urine and serum by atomic absorption spectrophotometry (AAS). *Clin Chem* 1967; **13:** 101.

413. Willis JB. Determination of calcium and magnesium in urine by atomic absorption spectroscopy. *Anal Chem* 1961; **33:** 556.

414. Chiriboga J. Some properties of an oxalic oxidase purified from barley seedlings. *Biochem Biophys Res Commun* 1963; **11:** 277.

415. Gruber W, Mollering H. Citrat-Lyase und Bestimmung von Citrat. *Biochem Z* 1966; **346:** 85.

416. Rock RC, Walker WG, Jennings CD. Nitrogen metabolites and renal function. In: Tietz NW (ed) *Textbook of clinical chemistry*, vol. 2. W. B. Saunders: Philadelphia, 1986, p 1278.

417. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925; **66:** 375.

418. Dryer RL, Routh JI. Determination of serum inorganic phosphorus. *Clin Chem* 1963; **4:** 191.

419. Tiselius HG. *Clin Chim Acta* 1982; **122:** 409.

420. Bullock JI, Duffin PA, Nolan KB. In vitro hydrolysis of phytate at 95 C and the influence of metal ion on the rate. *J Sci Agric* 1993; **63:** 261.

421. Schlemmer U, Muller H, Jany KD. The degradation of phytic acid in legumes prepared by different methods. *Eur J Clin Nutr* 1995; **49:** 207.

422. MacIntyre UE, Kruger HS, Venter CS, Vorster HH. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutr Res* 2002; **22:** 239.

423. Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutr* 2005; **8**(5)**:** 480.

424. Curhan GC, Willet WC, Knight EL, Stampfer MJ. Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. *Arch Intern Med* 2004; **164:** 885.

425. Cullumbine H, Basnayake V, Lemotte J. Mineral metabolism on rice diets. *Brit J Nutr* 1950; **4:** 101.

426. Walwer ARP, Fox FW, Irving JT. Studies in human mineral metabolism. I. The effect of bread rich in phytate phosphorous on the metabolism of certain mineral salts with special reference to calcium. *Biochem J* 1948; **42:** 452.

427. Bashir S, Khan NA, Gilani AH. Physiology of renal handling of citrate. In: Talati J, Tiselius HG, Albala DL, YE Z (eds). *Urolithiasis: Basic Science and Clinical Practice*. Springer-Verlag: London, 2012.

428. Jack M, Zuckerman BS, Dean G, Assimos MD. Hypocitraturia: pathophysiology and medical management. *Reviews in Urology* 2009; **11**(3)**:** 134.

429. Smith AH, Meyer CE. The influence of diet on the endogenous production of citric acid. *J Biol Chem* 1939; **131:** 45.

430. William C, Thomas MD. Effectiveness and mode of action of orthophosphates in patients with calcareous renal calculi. *Trans Am Clin Climatol Assoc* 1972; **83:** 113.

431. Sandberg AS, Anderrson H, Carlsson NG, Sandstrom B. Degradation products of bran phytate formed during digestion in the human small intestine: Effect of extrusion cooling on digestibility. *J Nutr* 1987; **117:** 2061.

432. Sandberg AS, Anderrson H. Effect of dietary phytases on the digestion of phytate in the stomach and small intestine of humans. *J Nutr* 1988; **118:** 469.

433. Schlemmer U, Jany KD, Berk A, Schulz E. Degradation of phytate in the gut of pigs and enzymes involved. *Arch Anim Nutr* 2001; **55:** 255.

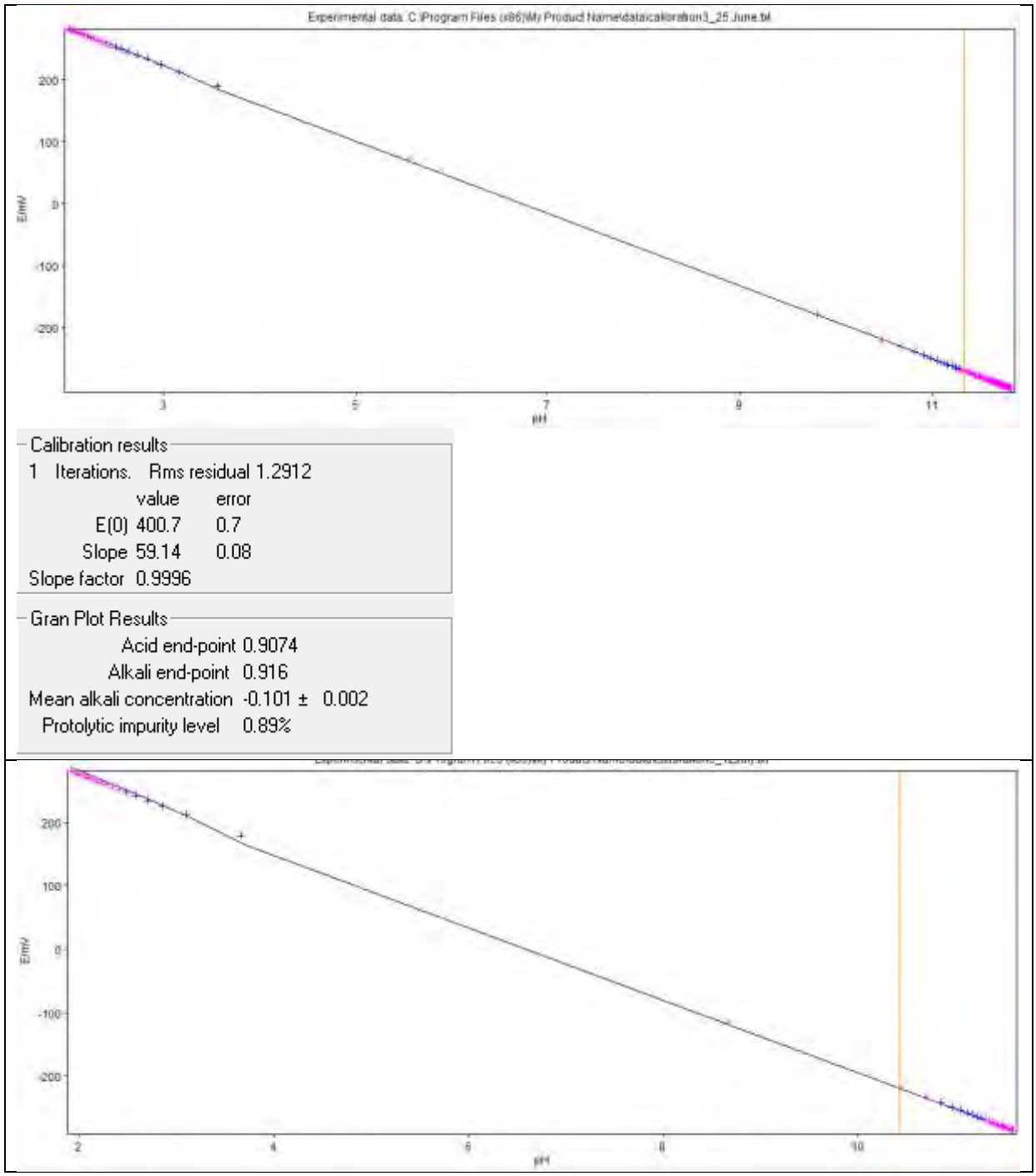
434. Siener R, Ebert D, Nicolay C, Hesse A. Dietary risk factors for hyperoxaluria in calcium oxalate stone formers. *Kidney Int* 2003; **63:** 1037.

435. Rao TVRK, Choudhary VK. Effect of pyridoxine (Vitamin-B(6)) supplementation on calciuria and oxaluria levels of some normal healthy persons and urinary stone patients. *Indian J Clin Biochem* 2005; **20**(2)**:** 166.

436. Hoyer-Kuhn H, Kohbrok S, Volland R, Franklin J, Hero B, Beck BB *et al.* Vitamin B6 in Primary Hyperoxaluria I: First prospective trial after 40 years of practice. *Clin J Am Soc Nephrol* 2014; **9**(3)**:** 468.

Appendix 2

Glass electrode calibration



Calibration results

1 Iterations. Rms residual 1.2912

	value	error
E(0)	400.7	0.7
Slope	59.14	0.08
Slope factor	0.9996	

Gran Plot Results

Acid end-point	0.9074
Alkali end-point	0.916
Mean alkali concentration	-0.101 ± 0.002
Protolytic impurity level	0.89%

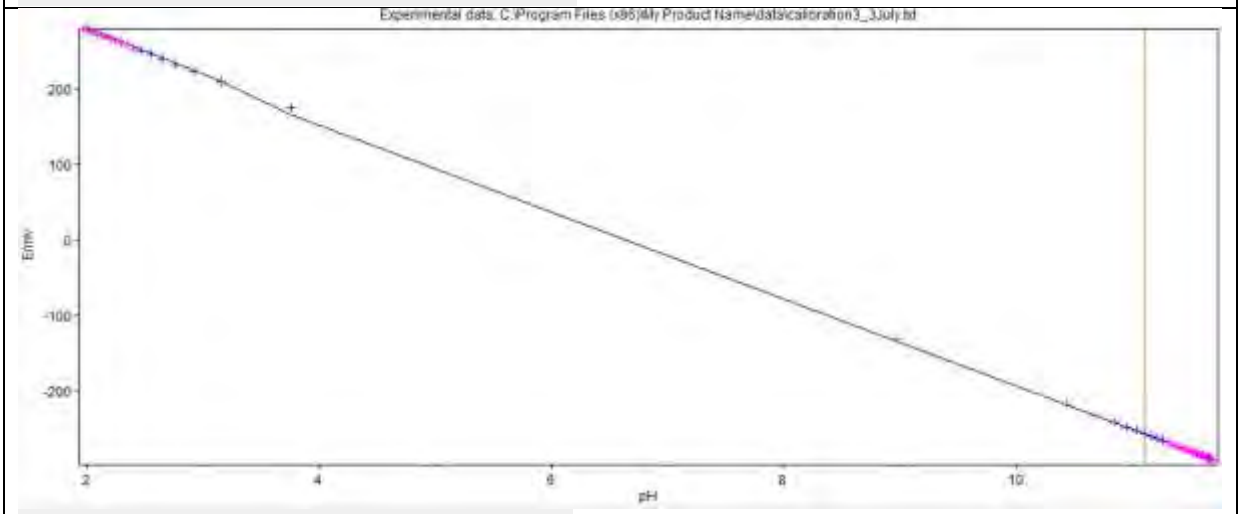
Calibration results

1 Iterations. Rms residual 3.6286

	value	error
E(0)	393.5	2.0
Slope	58.6	0.3
Slope factor	0.9910	

Gran Plot Results

Acid end-point	0.8667
Alkali end-point	0.870
Mean alkali concentration	-0.101 ± 0.006
Protolytic impurity level	0.37%



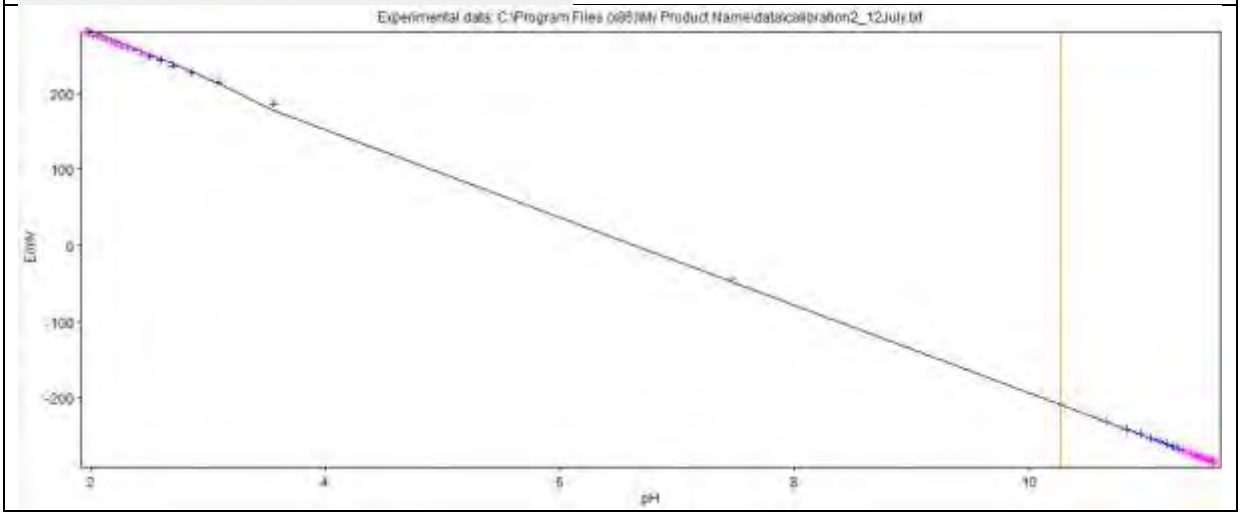
Calibration results

1 Iterations. Rms residual 2.608

	value	error
E(0)	395.9	1.0
Slope	58.9	0.2
Slope factor	0.9949	

Gran Plot Results

Acid end-point	0.9162
Alkali end-point	0.920
Mean alkali concentration	-0.101 ± 0.004
Protolytic impurity level	0.38%



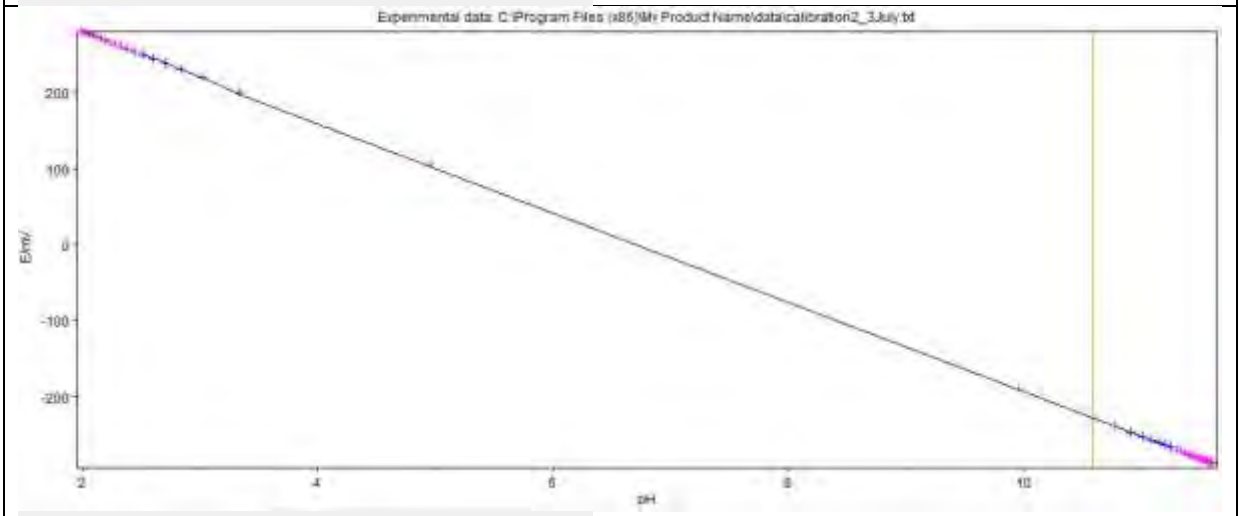
Calibration results

1 Iterations. Rms residual 2.629

	value	error
E(0)	395.5	2.0
Slope	58.8	0.2
Slope factor	0.9940	

Gran Plot Results

Acid end-point	0.8737
Alkali end-point	0.881
Mean alkali concentration	-0.101 ± 0.003
Protolytic impurity level	0.78%



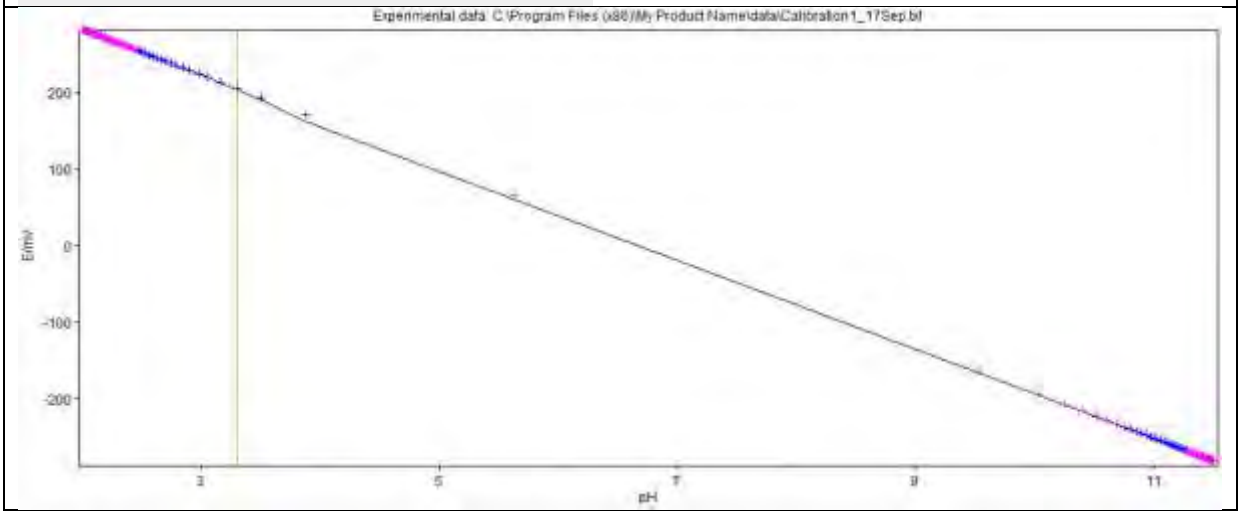
Calibration results

1 Iterations. Rms residual 0.9841

	value	error
E(0)	397.6	0.6
Slope	58.98	0.08
Slope factor	0.9970	

Gran Plot Results

Acid end-point	0.8947
Alkali end-point	0.897
Mean alkali concentration	-0.101 ± 0.003
Protolytic impurity level	0.22%



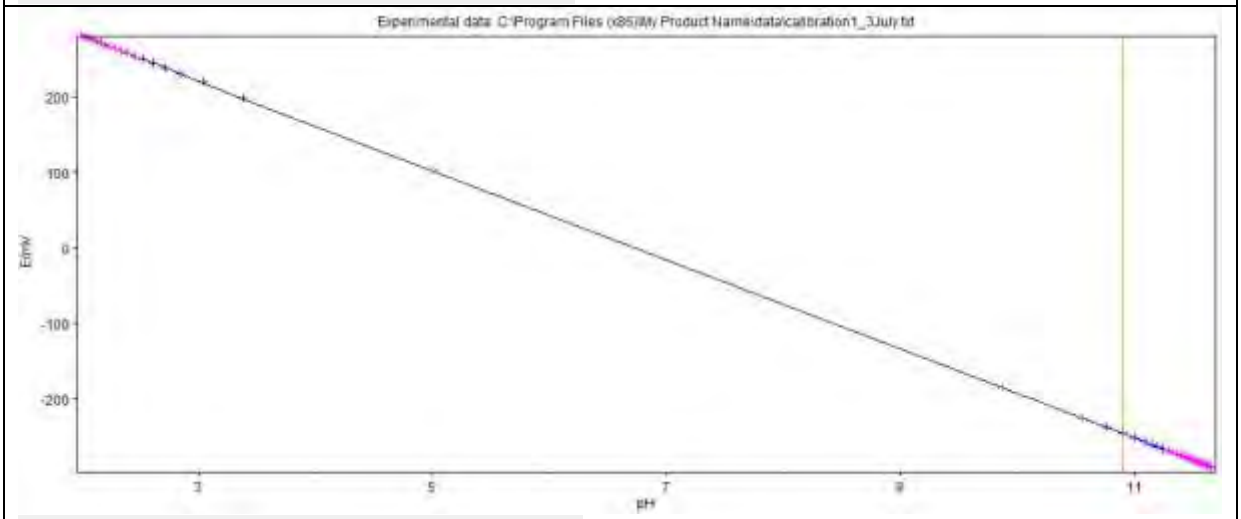
Calibration results

1 Iterations. Rms residual 1.7619

	value	error
E(0)	401.1	0.6
Slope	59.40	0.07
Slope factor	1.0042	

Gran Plot Results

Acid end-point	0.9947
Alkali end-point	1.0069
Mean alkali concentration	-0.0994 ± 0.0007
Protolytic impurity level	1.21%



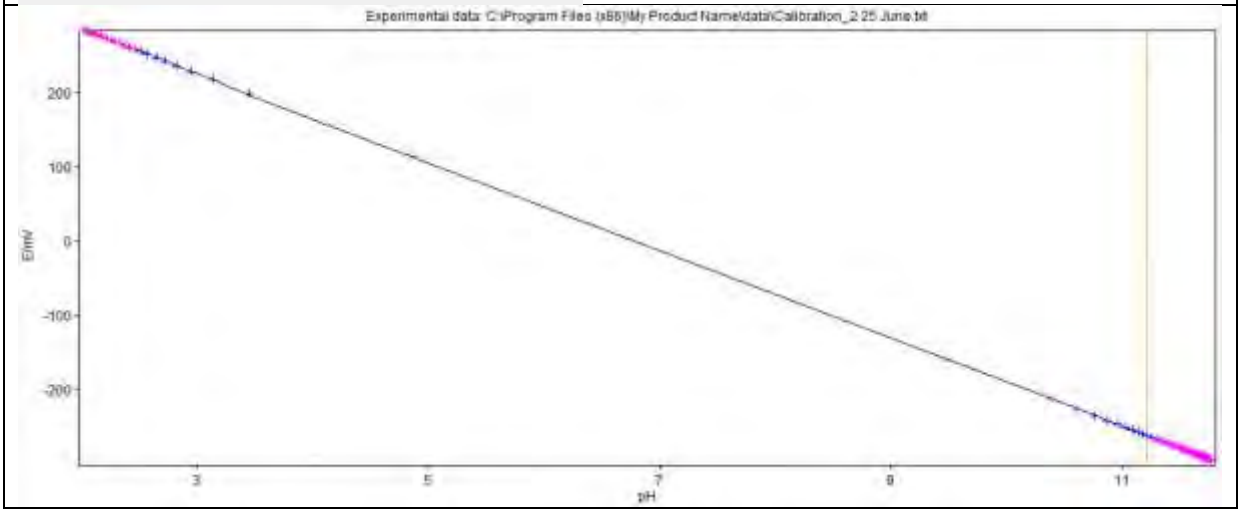
Calibration results

1 Iterations. Rms residual 0.8133

	value	error
E(0)	399.1	0.5
Slope	59.16	0.06
Slope factor	1.0000	

Gran Plot Results

Acid end-point	0.8917
Alkali end-point	0.905
Mean alkali concentration	-0.100 ± 0.004
Protolytic impurity level	1.44%



Calibration results

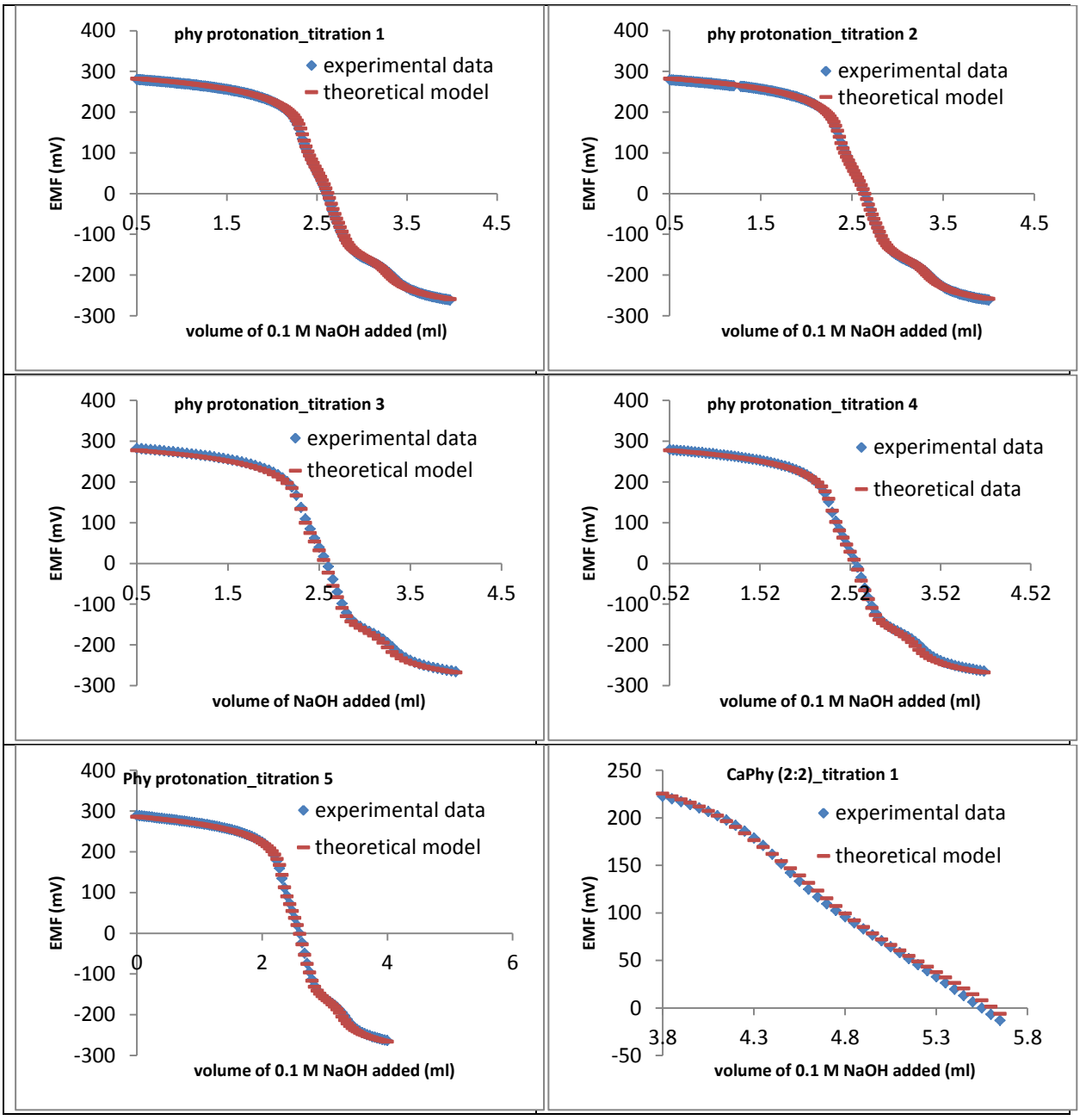
1 Iterations. Rms residual 0.7782

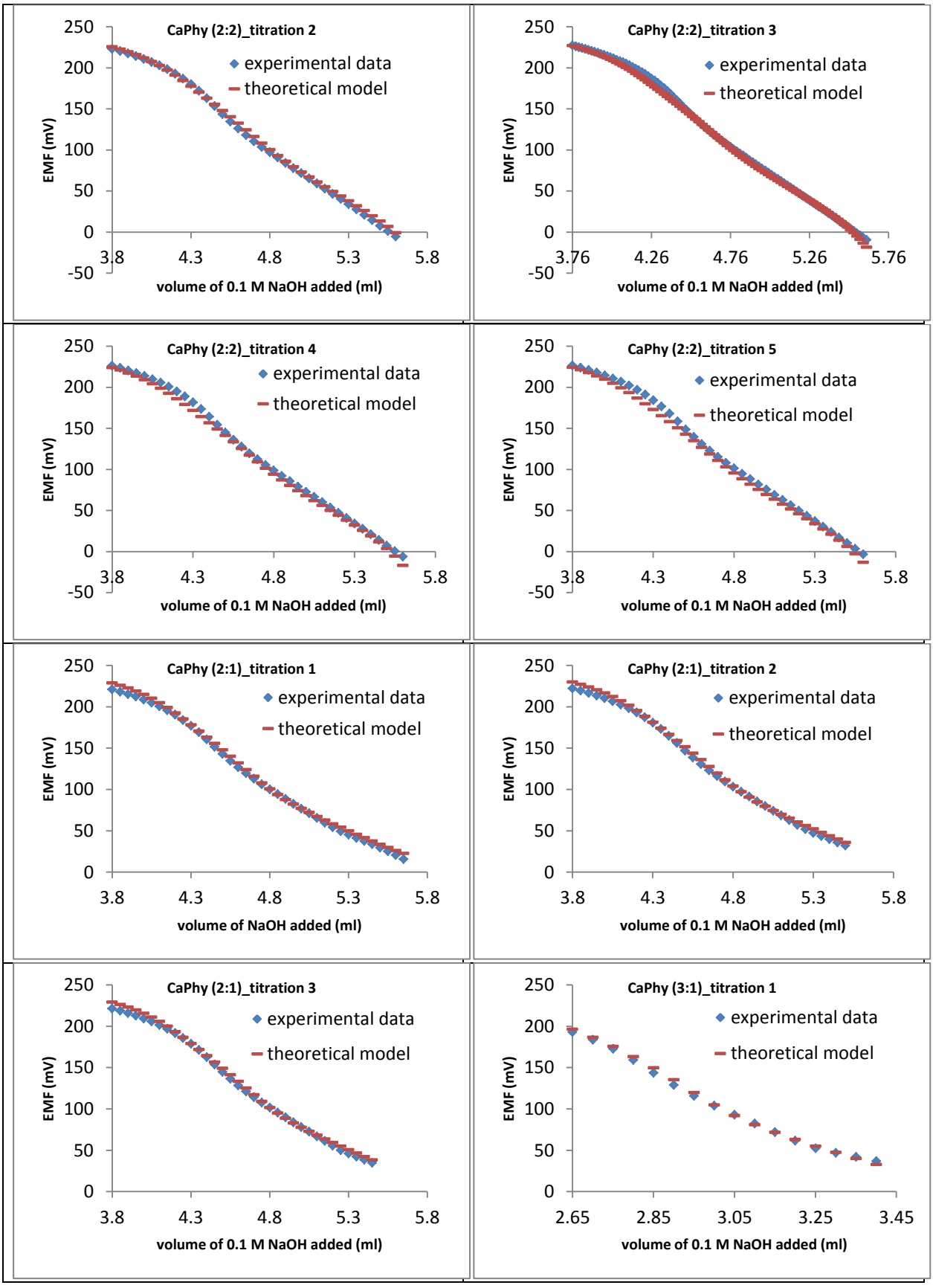
	value	error
E(0)	403.2	0.4
Slope	59.38	0.05
Slope factor	1.0037	

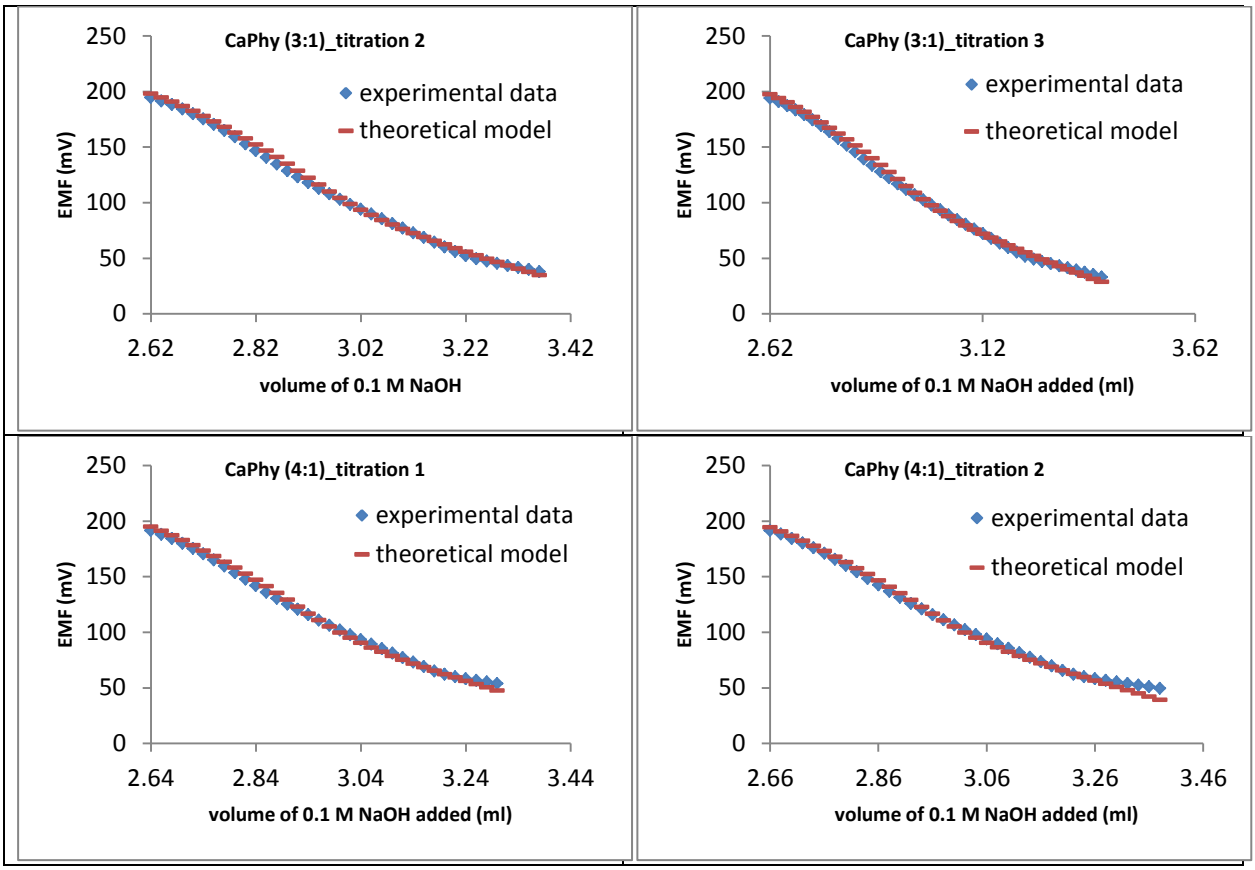
Gran Plot Results

Acid end-point	0.9164	
Alkali end-point	0.930	
Mean alkali concentration	-0.100 ±	0.004
Protolytic impurity level	1.45%	

Phytate protonation and calcium-phytate complexation_titration curves




CaPhy (2:2)_titration 2
experimental data
theoretical model
EMF (mV)
volume of 0.1 M NaOH added (ml)
CaPhy (2:2)_titration 3
CaPhy (2:2)_titration 4
CaPhy (2:2)_titration 5
CaPhy (2:1)_titration 1
volume of NaOH added (ml)
CaPhy (2:1)_titration 2
CaPhy (2:1)_titration 3
CaPhy (3:1)_titration 1


CaPhy (3:1)_titration 2
experimental data
theoretical model
EMF (mV)
250
200
150
100
50
0
2.62
2.82
3.02
3.22
3.42
volume of 0.1 M NaOH
CaPhy (3:1)_titration 3
experimental data
theoretical model
EMF (mV)
250
200
150
100
50
0
2.62
3.12
3.62
volume of 0.1 M NaOH added (ml)
CaPhy (4:1)_titration 1
experimental data
theoretical model
EMF (mV)
250
200
150
100
50
0
2.64
2.84
3.04
3.24
3.44
volume of 0.1 M NaOH added (ml)
CaPhy (4:1)_titration 2
experimental data
theoretical model
EMF (mV)
250
200
150
100
50
0
2.66
2.86
3.06
3.26
3.46
volume of 0.1 M NaOH added (ml)

Appendix 3

Appendix 3.1

Urine data of black subjects used for theoretical modelling. All units in mol/L

Lab no	pH	Cit	Ox	Ca	Mg	Na	K	Urate	Phos	Cl
BU1a	5.58	1.69E-03	1.80E-04	3.44E-03	2.89E-03	1.69E-01	5.26E-02	4.43E-03	3.52E-02	1.57E-01
BU2a	7.89	4.38E-04	1.23E-04	9.95E-04	1.00E-03	4.26E-02	2.14E-02	1.37E-03	9.09E-03	3.88E-02
BU3a	6.05	1.08E-03	1.41E-04	1.63E-03	1.39E-03	5.74E-02	1.94E-02	1.76E-03	1.07E-02	5.35E-02
BU4a	6.20	1.65E-03	2.29E-04	1.68E-03	1.41E-03	6.09E-02	2.85E-02	3.06E-03	1.44E-02	7.16E-02
BU5a	6.70	5.79E-04	1.02E-04	7.62E-04	5.75E-04	4.90E-02	1.85E-02	1.28E-03	8.81E-03	5.28E-02
BU6a	6.67	1.28E-03	2.22E-04	1.72E-03	2.36E-03	9.35E-02	3.17E-02	2.78E-03	1.65E-02	7.92E-02
BU7a	8.36	6.49E-04	1.10E-04	1.02E-03	1.12E-03	6.13E-02	2.00E-02	8.77E-04	3.90E-03	6.49E-02
BU8a	7.71	1.01E-03	2.60E-04	1.46E-03	1.21E-03	8.13E-02	3.72E-02	3.10E-03	1.55E-02	7.40E-02
BU9a	7.06	1.84E-03	1.34E-04	1.20E-03	1.39E-03	8.35E-02	1.92E-02	1.95E-03	1.66E-02	1.01E-01
BU10a	5.87	1.11E-03	1.68E-04	1.21E-03	1.00E-03	8.39E-02	3.94E-02	3.27E-03	2.36E-02	1.03E-01
BU11a	6.68	1.89E-03	1.41E-04	1.54E-03	1.42E-03	9.26E-02	2.80E-02	3.45E-03	3.29E-02	1.10E-01
BU12a	5.62	8.16E-04	1.46E-04	1.85E-03	1.69E-03	7.13E-02	2.73E-02	2.54E-03	2.51E-02	1.12E-01
b1a	6.5	2.39E-03	1.25E-04	2.13E-03	1.11E-03	1.72E-01	2.33E-02	4.43E-03	3.52E-02	1.55E-01
B2a	5.74	2.02E-03	4.04E-04	3.10E-03	2.27E-03	1.16E-01	3.04E-02	3.65E-03	2.96E-02	1.02E-01
B3a	6.32	2.05E-03	1.95E-04	1.54E-03	1.14E-03	1.13E-01	4.00E-02	2.93E-03	2.35E-02	1.66E-01
B4a	6.29	1.88E-03	3.54E-04	2.97E-03	2.32E-03	1.59E-01	4.74E-02	5.69E-03	3.94E-02	1.77E-01
B5a	6.81	1.73E-03	2.04E-04	2.14E-03	1.17E-03	8.13E-02	2.55E-02	1.48E-03	1.01E-02	1.00E-01
B7a	6.21	2.39E-03	2.60E-04	1.67E-03	7.10E-04	1.38E-01	2.36E-02	3.60E-03	3.68E-02	1.03E-01
average	6.57	1.47E-03	1.94E-04	1.78E-03	1.45E-03	9.59E-02	2.96E-02	2.87E-03	2.15E-02	1.01E-01
SE	0.18	1.45E-04	1.96E-05	1.75E-04	1.46E-04	9.55E-03	2.37E-03	2.99E-04	2.65E-03	9.57E-03

Urine data of white subjects used for theoretical modelling. All units in mol/L

Lab no	pH	Cit	Ox	Ca	Mg	Na	K	Urate	Phos	Cl
W1a	6.05	9.83E-04	1.66E-04	1.54E-03	1.33E-03	1.05E-01	2.91E-02	4.86E-03	4.12E-02	1.15E-01
W3a	5.64	1.67E-03	1.37E-04	2.20E-03	1.84E-03	1.51E-01	4.43E-02	1.08E-02	4.52E-02	1.11E-01
W4a	6.78	1.71E-03	1.79E-04	1.34E-03	9.11E-04	5.39E-02	1.74E-02	4.64E-03	1.54E-02	2.89E-01
W5a	6.08	1.90E-03	1.19E-04	8.97E-04	8.10E-04	5.28E-02	1.73E-02	2.06E-03	1.21E-02	1.17E-01
W7a	6.17	1.81E-03	2.16E-04	1.19E-03	9.61E-04	8.76E-02	2.71E-02	3.14E-03	3.81E-02	1.43E-01
W6A	6.29	1.32E-03	1.94E-04	2.21E-03	1.85E-03	6.08E-02	2.02E-02	2.06E-03	2.08E-02	7.00E-02
W9A	5.89	1.26E-03	1.64E-04	2.92E-03	2.50E-03	8.15E-02	1.66E-02	2.13E-03	1.76E-02	9.75E-02
W11A	6.75	2.39E-03	1.22E-04	1.03E-03	2.10E-03	1.03E-01	1.83E-02	2.10E-03	1.28E-02	1.07E-01
W10A	5.96	2.90E-03	2.41E-04	3.57E-03	1.70E-03	1.18E-01	3.65E-02	2.65E-03	2.55E-02	1.70E-01
W8A	6.07	1.68E-03	1.90E-04	1.52E-03	1.55E-03	6.21E-02	1.28E-02	2.66E-03	2.51E-02	5.89E-02
SP1a	6.12	1.39E-03	7.02E-05	1.13E-03	7.48E-04	3.49E-02	1.05E-02	1.57E-03	1.61E-02	5.17E-02
SP2a	6.19	1.97E-03	1.61E-04	8.14E-04	1.31E-03	1.96E-01	2.73E-02	3.90E-03	2.80E-02	1.85E-01
SP3a	7.49	1.35E-03	1.09E-04	8.94E-04	4.64E-04	1.04E-01	1.34E-02	1.70E-03	1.05E-02	9.28E-02
SP4a	6.07	2.14E-03	2.54E-04	9.72E-04	1.18E-03	1.09E-01	1.89E-02	3.24E-03	3.38E-02	1.55E-01
SP5a	6.25	2.06E-03	1.85E-04	2.07E-03	1.49E-03	2.30E-01	3.13E-02	4.35E-03	3.98E-02	1.80E-01
SP6a	6.8	1.72E-03	1.72E-04	1.29E-03	1.34E-03	1.27E-01	2.11E-02	3.19E-03	2.50E-02	9.05E-02
average	6.29	1.77E-03	1.67E-04	1.60E-03	1.38E-03	1.05E-01	2.26E-02	3.44E-03	2.54E-02	1.27E-01
SEM	0.11	1.18E-04	1.21E-05	1.99E-04	1.35E-04	1.31E-02	2.31E-03	5.54E-04	2.83E-03	1.49E-02

Intergroup comparison of urine data (comparison of means using Unpaired t-test)

Urine parameter	pH	Cit	Ox	Ca	Mg	Na	K	Urate	Phos	Cl
P-value	0.21	0.13	0.29	0.62	0.72	0.68	0.045*	0.33	0.37	0.22

Appendix 3.2
Supersaturation of salts in urine of black subjects

	0.76 µM (black subjects)									
SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
BU1a	6.85E+04	0.44	1.90	9.89	6.75	2.98	5.81	2.18	4.51	9.29
BU2a	2.54E+12	2360.48	1.73	42953.64	2.14	0.94	1.85	0.69	0.01	1.28
BU3a	2.01E+05	0.53	1.09	6.15	5.14	2.27	4.43	1.66	0.88	1.87
BU4a	9.04E+05	1.27	1.35	18.45	6.35	2.81	5.47	2.06	1.10	3.46
BU5a	9.66E+06	3.52	1.00	37.33	2.00	0.88	1.72	0.65	0.16	1.32
BU6a	4.02E+08	36.39	2.42	966.05	4.82	2.13	4.16	1.56	0.35	4.98
BU7a	3.95E+12	1614.36	0.51	8729.71	1.46	0.64	1.26	0.47	0.00	1.17
BU8a	4.08E+11	972.75	1.70	18155.16	1.36	3.09	2.67	1.00	0.04	4.95
BU9a	6.07E+07	8.81	0.93	90.57	0.99	0.44	0.86	0.32	0.10	3.12
BU10a	9.33E+03	0.11	0.90	1.07	3.57	1.58	3.08	1.16	2.14	4.31
BU11a	3.22E+07	9.20	1.86	192.31	1.41	0.62	1.21	0.45	0.41	5.64
BU12a	5.37E+03	0.09	1.08	1.05	5.06	2.23	4.37	1.64	2.56	2.47
B1a	5.30E+07	13.93	2.43	395.37	1.85	0.82	1.59	0.60	0.76	12.71
B2a	2.11E+05	0.77	2.02	17.46	15.85	7.00	13.68	5.13	2.97	6.10
B3a	1.56E+06	1.78	1.40	28.51	2.97	1.31	2.56	0.96	0.75	5.60
B4a	1.25E+08	27.54	3.93	1279.38	8.55	3.78	7.36	2.77	1.51	14.52
B5a	9.20E+08	49.32	1.94	1047.13	5.70	2.52	4.92	1.85	0.14	2.36
B7a	9.86E+05	1.57	1.74	30.76	4.06	1.79	3.49	1.31	1.18	8.24

	2.27 µM Phy (black subjects)									
SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
BU1a	6.82E+04	0.44	1.90	9.84	6.73	2.98	5.81	2.18	4.51	9.29
BU2a	2.48E+12	2328.09	1.72	42169.65	2.13	0.94	1.84	0.69	0.01	1.28
BU3a	1.99E+05	0.53	1.09	6.10	5.13	2.26	4.42	1.66	0.88	1.87
BU4a	8.93E+05	1.26	1.35	18.32	6.34	2.80	5.46	2.05	1.10	3.46
BU5a	9.42E+06	3.48	1.00	36.64	1.99	0.88	1.71	0.64	0.16	1.32
BU6a	3.97E+08	36.22	2.42	959.40	4.81	2.12	4.15	1.56	0.35	4.98
BU7a	3.85E+12	1592.21	0.50	8550.67	1.45	0.64	1.25	0.47	0.00	1.17
BU8a	4.02E+11	963.83	1.70	17947.34	3.08	1.36	2.66	1.00	0.04	4.95
BU9a	6.08E+07	8.83	0.93	90.78	1.00	0.44	0.86	0.32	0.10	3.12
BU10a	9.20E+03	0.11	0.90	1.06	3.56	1.57	3.07	1.15	2.14	4.31
BU11a	3.20E+07	9.18	1.86	191.43	1.40	0.62	1.21	0.45	0.41	5.64
BU12a	5.32E+03	0.09	1.08	1.04	5.05	2.23	4.36	1.63	2.56	2.47
B1a	5.27E+07	13.90	2.43	394.46	1.85	0.81	1.59	0.60	0.76	12.71
B2a	2.10E+05	0.77	2.02	17.42	15.85	7.00	13.65	5.13	2.97	6.10
B3a	1.55E+06	1.78	1.39	28.38	2.96	1.31	2.56	0.96	0.75	5.60
B4a	1.25E+08	27.48	3.93	1276.44	8.53	3.77	7.36	2.76	1.51	14.52
B5a	9.10E+08	48.98	1.94	1039.92	5.69	2.51	4.91	1.84	0.14	2.36
B7a	9.79E+05	1.56	1.73	30.55	4.05	1.79	3.49	1.31	1.18	8.24

	4.45 µM Phy (black subjects)									
SS	**HAP**	**tCaP**	**brushite**	**OCP**	**CaOx**	**COT**	**COM**	**COD**	**Uric**	**NaUric**
BU1a	6.78E+04	0.44	1.89	9.82	6.73	2.90	5.81	2.18	4.51	9.29
BU2a	2.39E+12	2285.60	1.71	41020.41	2.11	0.93	1.82	0.68	0.01	1.28
BU3a	1.96E+05	0.52	1.08	6.03	5.12	2.26	4.41	1.66	0.88	1.87
BU4a	8.79E+05	1.25	1.34	18.07	6.31	2.79	5.45	2.04	1.10	3.46
BU5a	9.12E+06	3.40	0.99	35.65	1.97	0.87	1.70	0.64	0.16	1.32
BU6a	3.92E+08	35.89	2.41	948.42	4.80	2.12	4.14	1.55	0.35	4.98
BU7a	3.71E+12	1555.97	0.50	8298.51	1.44	0.64	1.24	0.47	0.00	1.17
BU8a	3.92E+11	950.60	1.69	17579.24	3.07	1.36	2.64	0.99	0.04	4.95
BU9a	6.08E+07	8.83	0.93	90.78	1.00	0.44	0.86	0.32	0.10	3.12
BU10a	9.06E+03	0.11	0.89	1.04	3.55	1.57	3.06	1.15	2.14	4.31
BU11a	3.17E+07	9.12	1.86	190.11	1.40	0.62	1.21	0.45	0.41	5.64
BU12a	5.26E+03	0.09	1.07	1.04	5.04	2.23	4.35	1.63	2.56	2.47
B1a	5.25E+07	13.87	2.43	393.55	1.85	0.81	1.59	0.60	0.76	12.71
B2a	2.08E+05	0.77	2.01	17.30	15.81	6.98	13.65	5.12	2.97	6.10
B3a	1.53E+06	1.77	1.39	28.18	2.96	1.31	2.55	0.96	0.75	5.60
B4a	1.24E+08	27.42	3.92	1270.57	8.53	3.77	7.36	2.76	1.51	14.52
B5a	8.97E+08	48.53	1.93	1028.02	5.68	2.51	4.90	1.84	0.14	2.36
B7a	9.71E+05	1.55	1.73	30.34	4.04	1.78	3.48	1.31	1.18	8.24

	30 µM Phy (black subjects)									
SS	**HAP**	**tCaP**	**brushite**	**OCP**	**CaOx**	**COT**	**COM**	**COD**	**Uric**	**NaUric**
BU1a	6.35E+04	0.42	1.87	9.31	6.65	2.94	5.74	2.15	4.51	9.29
BU2a	1.60E+12	1790.61	1.57	29716.66	1.96	0.86	1.69	0.63	0.01	1.28
BU3a	1.63E+05	0.47	1.04	5.19	4.95	2.19	4.27	1.60	0.87	1.87
BU4a	7.29E+05	1.12	1.29	15.60	6.11	2.70	5.26	1.98	1.10	3.45
BU5a	6.03E+06	2.65	0.91	25.59	1.82	0.81	1.57	0.59	0.16	1.32
BU6a	3.29E+08	32.28	2.32	824.14	4.65	2.05	4.00	1.50	0.35	4.97
BU7a	2.33E+12	1177.61	0.46	5741.16	1.32	0.58	1.13	0.42	0.00	1.17
BU8a	2.97E+11	803.53	1.60	14092.89	2.90	1.28	2.51	0.94	0.04	4.95
BU9a	4.98E+07	7.83	0.90	77.27	0.95	0.42	0.82	0.31	0.10	3.11
BU10a	7.36E+03	0.09	0.86	0.89	3.41	1.51	2.94	1.10	2.14	4.31
BU11a	2.86E+07	8.57	1.82	174.98	1.37	0.61	1.18	0.44	0.41	5.62
BU12a	4.55E+03	0.08	1.04	0.92	4.91	2.17	4.24	1.59	2.56	2.47
B1a	4.97E+07	13.43	2.40	376.70	1.82	0.81	1.57	0.59	0.76	12.71
B2a	1.91E+05	0.73	1.98	16.11	15.60	6.89	13.43	5.05	2.96	6.10
B3a	1.40E+06	1.67	1.36	26.24	2.90	1.28	2.51	0.94	0.75	5.60
B4a	1.17E+08	26.55	3.87	1216.19	8.43	3.72	7.28	2.73	1.51	14.49
B5a	7.60E+08	44.06	1.87	901.57	5.51	2.43	4.75	1.78	0.14	2.36
B7a	8.71E+05	1.46	1.69	27.86	3.95	1.75	3.40	1.28	1.18	8.24
	1.50 mM Phy (black subjects)									

SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
BU1a	1.04E+04	0.15	1.27	2.32	4.58	2.02	3.95	1.48	4.39	9.04
BU2a	9.79E+09	82.79	0.49	459.20	0.66	0.29	0.57	0.21	0.01	1.14
BU3a	1.64E+02	0.01	0.25	0.02	1.23	0.54	1.06	0.40	0.81	1.72
BU4a	9.98E+03	0.09	0.52	0.51	2.42	1.07	2.08	0.78	1.01	3.20
BU5a	4.70E+04	0.14	0.31	0.51	0.60	0.27	0.52	0.19	0.15	1.19
BU6a	1.44E+07	5.00	1.16	67.45	2.39	1.06	2.07	0.77	0.33	4.63
BU7a	5.81E+10	127.06	0.19	277.97	0.60	0.26	0.51	0.19	0.00	1.05
BU8a	3.98E+10	240.44	0.96	2703.96	1.82	0.80	1.57	0.59	0.03	4.59
BU9a	1.05E+07	3.09	0.59	21.63	0.64	0.28	0.55	0.21	0.09	2.89
BU10a	6.93E+02	0.02	0.51	0.14	1.89	0.84	1.63	0.61	2.02	4.06
BU11a	9.89E+06	4.61	1.36	75.34	1.00	0.44	0.86	0.32	0.39	5.27
BU12a	8.07E+01	0.01	0.45	0.04	2.13	0.94	1.84	0.69	2.44	2.36
B1a	2.16E+07	8.34	1.91	198.61	1.42	0.63	1.22	0.46	0.72	12.13
B2a	7.60E+03	0.11	1.01	1.28	8.24	3.64	7.10	2.67	2.85	5.85
B3a	5.43E+05	0.97	1.07	12.71	2.17	0.96	1.87	0.70	0.71	5.32
B4a	4.76E+07	15.85	3.09	616.60	6.62	2.92	5.70	2.14	1.45	13.93
B5a	2.33E+07	5.48	0.86	54.95	2.64	1.16	2.28	0.85	0.13	2.19
B7a	3.05E+05	0.80	1.30	12.50	2.82	1.25	2.44	0.91	1.11	7.78

Supersaturation in urine of white subjects

	0.76 µM (white subjects)									
SS	**HAP**	**tCaP**	**brushite**	**OCP**	**CaOx**	**COT**	**COM**	**COD**	**Uric**	**NaUric**
W1a	5.96E+05	1.31	2.01	29.51	3.32	1.47	2.86	1.07	2.21	8.17
w3a	2.11E+04	0.22	1.58	4.02	3.71	1.64	3.20	1.20	10.14	21.23
W4a	7.94E+06	3.29	0.90	34.67	1.64	0.72	1.41	0.53	0.43	4.31
W5a	1.43E+03	0.03	0.40	0.12	1.65	0.73	1.42	0.53	0.93	1.91
W7a	1.19E+05	0.46	1.25	6.53	2.54	1.12	2.18	0.82	1.12	4.49
W6a	3.85E+07	12.25	2.94	387.26	6.67	2.94	5.74	2.15	0.61	2.34
W9a	1.08E+06	1.73	2.06	38.90	7.50	3.31	6.47	2.43	1.36	2.82
W11a	2.92E+06	1.71	0.72	13.68	1.06	0.47	0.92	0.34	0.22	4.11
W10a	2.59E+06	3.04	2.43	84.72	8.93	3.94	7.71	2.89	1.41	4.82
W8a	2.28E+05	0.67	1.48	10.57	4.53	2.00	3.90	1.47	1.25	2.93
SP1a	3.96E+04	0.21	0.89	1.98	1.64	0.72	1.41	0.53	0.70	1.04
SP2a	2.84E+04	0.18	0.73	1.55	1.29	0.57	1.11	0.42	1.28	12.22
SP3a	1.32E+08	8.77	0.43	41.30	0.58	0.25	0.50	0.19	0.03	3.51
SP4a	1.20E+04	0.12	0.84	1.14	2.56	1.13	2.21	0.83	1.40	5.61
Sp5a	1.10E+07	6.50	2.44	190.99	3.11	1.37	2.69	1.01	1.24	15.78
Sp6a	5.46E+07	10.76	1.51	181.97	1.67	0.74	1.44	0.54	0.29	7.41

	2.27uM Phy (white subjects)

SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
W1a	5.89E+05	1.297179	2.01	29.24	3.31	1.46	2.85	1.07	2.21	8.17
W3a	2.10E+04	0.2208	1.58	4.00	3.71	1.64	3.20	1.20	10.14	21.23
W4a	7.93E+06	3.280953	0.90	34.59	1.63	0.72	1.41	0.53	0.43	4.31
W5a	1.40E+03	0.027542	0.40	0.12	1.64	0.72	1.42	0.53	0.93	1.91
W7a	1.19E+05	0.460257	1.25	6.50	2.53	1.12	2.18	0.82	1.12	4.49
W6a	3.81E+07	12.16186	2.93	353.18	6.65	2.94	5.73	2.15	0.61	2.34
W9a	1.07E+06	1.725838	2.06	38.73	7.50	3.31	6.46	2.42	1.36	2.82
W11a	2.89E+06	1.698244	0.72	13.55	1.06	0.47	0.91	0.34	0.22	4.11
W10a	2.58E+06	3.033891	2.43	84.53	8.93	3.94	7.69	2.89	1.41	4.82
W8a	2.25E+05	0.660693	1.48	10.47	4.52	2.00	3.89	1.46	1.25	2.93
SP1a	3.90E+04	0.209411	0.89	1.95	0.72	1.63	1.41	0.53	0.70	1.04
SP2a	2.83E+04	0.178649	0.73	1.54	1.29	0.57	1.11	0.42	1.28	12.22
SP3a	1.30E+08	8.709636	0.43	40.93	0.57	0.25	0.50	0.19	0.03	3.51
SP4a	1.19E+04	0.119674	0.84	1.14	2.56	1.13	2.21	0.83	1.40	5.61
Sp5a	1.09E+07	6.501297	2.44	190.55	3.11	1.37	2.69	1.01	1.24	15.78
Sp6a	5.42E+07	10.71519	1.51	180.72	1.67	0.74	1.44	0.54	0.29	7.41

	4.45uM Phy (white subjects)									
SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
W1a	5.82E+05	1.28825	2.00	28.97	3.30	1.46	2.84	1.07	2.21	8.17
W3a	2.08E+04	0.219786	1.58	3.98	3.71	1.63	3.19	1.20	10.14	21.23
W4a	7.89E+06	3.280953	0.90	34.51	1.63	0.72	1.41	0.53	0.43	4.31
W5a	1.37E+03	0.027102	0.40	0.12	1.63	0.72	1.41	0.53	0.93	1.91
W7a	1.17E+05	0.458142	1.25	6.46	2.52	1.11	2.18	0.82	1.12	4.49
W6a	3.77E+07	12.07814	2.92	380.19	6.64	2.93	5.71	2.15	0.61	2.34
W9a	1.06E+06	1.717908	2.05	38.46	7.48	3.30	6.46	2.42	1.36	2.82
W11a	2.84E+06	1.682674	0.72	13.40	1.06	0.47	0.91	0.34	0.22	4.11
W10a	2.56E+06	3.019952	2.42	83.95	8.91	3.94	7.69	2.88	1.41	4.82
W8a	2.21E+05	0.654636	1.48	10.33	4.50	1.99	3.88	1.46	1.25	2.93
SP1a	3.80E+04	0.206538	0.88	1.91	1.63	0.72	1.40	0.53	0.70	1.04
SP2a	2.82E+04	0.178238	0.73	1.54	1.29	0.57	1.11	0.42	1.28	12.22
SP3a	1.28E+08	8.629785	0.43	40.36	0.57	0.25	0.49	0.18	0.03	3.51
SP4a	1.18E+04	0.119124	0.84	1.13	2.56	1.13	2.20	0.83	1.40	5.61
Sp5a	1.09E+07	6.486344	2.44	190.11	3.11	1.37	2.68	1.01	1.24	15.78
Sp6a	5.36E+07	10.64143	1.51	179.06	1.66	0.74	1.44	0.54	0.29	7.41

	30 uM Phy (white subjects)									
SS	HAP	tCaP	brushite	OCP	CaOx	COT	COM	COD	Uric	NaUric
W1a	5.09E+05	1.19	1.95	26.06	3.21	1.42	2.77	1.04	2.21	8.17
W3a	1.89E+04	0.21	1.55	3.68	3.63	1.61	3.13	1.17	10.14	21.23
W4a	7.60E+06	3.21	0.89	33.50	1.62	0.71	1.40	0.52	0.43	4.31
W5a	1.03E+03	0.02	0.38	0.09	1.55	0.68	1.33	0.50	0.93	1.91

W7a	1.06E+05	0.43	1.22	5.96	2.47	1.09	2.13	0.80	1.12	4.48
W6a	3.27E+07	11.12	2.84	340.41	6.47	2.86	5.58	2.09	0.61	2.34
W9a	9.64E+05	1.62	2.01	35.65	7.36	3.25	6.35	2.38	1.36	2.82
W11a	2.38E+06	1.51	0.69	11.59	1.02	0.45	0.88	0.33	0.22	4.10
W10a	2.38E+06	2.89	2.39	79.25	8.81	3.89	7.59	2.84	1.41	4.82
W8a	1.82E+05	0.58	1.42	8.81	4.34	1.91	3.74	1.40	1.25	2.92
SP1a	2.84E+04	0.17	0.83	1.52	1.54	0.68	1.33	0.50	0.70	1.04
SP2a	2.72E+04	0.17	0.73	1.50	1.28	0.56	1.10	0.41	1.28	12.22
SP3a	1.07E+08	7.76	0.41	34.99	0.55	0.24	0.48	0.18	0.03	3.51
SP4a	1.08E+04	0.11	0.82	1.05	2.51	1.11	2.16	0.81	1.40	5.61
Sp5a	1.05E+07	6.34	2.42	184.08	3.08	1.36	2.66	1.00	1.24	15.78
Sp6a	4.67E+07	9.82	1.46	160.69	1.62	0.72	1.40	0.52	0.29	7.40

	1.50uM Phy (white subjects)									
SS	**HAP**	**tCaP**	**brushite**	**OCP**	**CaOx**	**COT**	**COM**	**COD**	**Uric**	**NaUric**
W1a	1.19E+05	0.509331	1.39	8.49	2.15	0.95	1.85	0.68	2.09	7.73
W3a	3.71E+03	0.080538	1.08	1.06	2.44	1.08	2.10	0.79	9.77	20.51
W4a	7.89E+06	3.280953	0.90	34.51	1.63	0.72	1.41	0.53	0.43	4.31
W5a	1.91E+02	0.008492	0.25	0.03	0.93	0.41	0.80	0.30	0.86	1.77
W7a	5.21E+04	0.289068	1.01	3.52	1.87	0.82	1.61	0.60	1.05	4.23
W6a	2.30E+05	0.576766	1.00	6.56	2.36	1.04	2.04	0.76	0.57	2.17
W9a	4.73E+03	0.068077	0.68	0.52	2.76	1.22	2.38	0.89	1.30	2.69
W11a	5.56E+05	0.638263	0.48	3.60	0.70	0.31	0.61	0.23	0.20	3.83
W10a	3.73E+05	0.977237	1.59	18.88	5.86	2.59	5.06	1.90	1.35	4.62
W8a	3.49E+03	0.055208	0.61	0.38	1.77	0.78	1.53	0.57	1.15	2.70
SP1a	2.00E+02	0.008974	0.29	0.03	0.48	0.21	0.41	0.16	0.63	0.94
SP2a	1.74E+04	0.137404	0.63	1.09	1.06	0.47	0.92	0.34	1.22	11.72
SP3a	1.90E+07	2.741574	0.26	8.36	0.35	0.16	0.30	0.11	32.21	3.24
SP4a	6.07E+03	0.082414	0.70	0.70	1.95	0.86	1.69	0.63	1.33	5.32
Sp5a	5.71E+06	4.528976	2.04	118.58	2.52	1.11	2.18	0.82	1.20	15.21
Sp6a	1.23E+07	4.456562	1.03	54.95	1.13	0.50	0.97	0.37	0.27	6.93

Chemical speciation in urine of black subjects

Calcium speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Ca] mol/L								
Ca+2	2.04E-03	3.29E-04	8.93E-04	6.98E-04	3.50E-04	7.16E-04	3.02E-04	3.22E-04	1.93E-04
Ca+2_Citric-3_PO4-3	3.28E-05	3.53E-04	2.39E-05	9.72E-05	8.69E-05	3.42E-04	5.17E-04	8.27E-04	7.19E-04
Ca+2_Citric-3	4.75E-04	2.66E-05	4.43E-04	5.67E-04	1.72E-04	2.63E-04	2.99E-05	3.76E-05	1.49E-04
Ca+2_H+1_PO4-3	1.54E-04	1.40E-04	8.87E-05	1.10E-04	8.18E-05	1.97E-04	4.13E-05	1.38E-04	7.59E-05
Ca+2_H+1(3)_PO4-3(2)	2.19E-04	4.57E-06	4.78E-05	6.95E-05	2.28E-05	7.73E-05	1.53E-07	7.75E-06	1.75E-05
Ca+2_Cl-1	3.03E-04	1.83E-05	6.55E-05	6.36E-05	2.61E-05	6.60E-05	2.61E-05	2.78E-05	2.26E-05
Ca+2_H+1(2)_PO4-3	1.34E-04	6.94E-07	3.00E-05	2.58E-05	6.24E-06	1.53E-05	6.81E-08	9.80E-07	2.39E-06
Ca+2_PO4-3	7.25E-07	1.07E-04	9.97E-07	1.82E-06	4.05E-06	1.01E-05	9.63E-05	7.77E-05	9.64E-06
Ca+2_Oxalic-2	2.76E-05	7.86E-06	1.90E-05	2.38E-05	7.37E-06	1.85E-05	5.43E-06	1.18E-05	3.83E-06
Ca+2_H+1(2)_PO4-3(2)	2.04E-06	4.34E-06	6.95E-07	1.61E-06	1.43E-06	6.06E-06	4.74E-07	6.62E-06	3.44E-06
Ca+2(2)_Oxalic-2	1.22E-05	5.75E-07	3.77E-06	3.69E-06	5.74E-07	2.91E-06	3.63E-07	8.38E-07	1.63E-07
Ca+2_H+1_Citric-3	1.08E-05	4.81E-09	5.33E-06	4.48E-06	4.75E-07	6.40E-07	1.71E-09	8.37E-09	1.46E-07
Ca+2_Citric-3(2)	2.79E-06	1.19E-08	1.43E-06	4.07E-06	5.00E-07	1.19E-06	2.16E-08	5.33E-08	1.48E-06
Ca+2_H+1(2)_Citric-3(2)	1.02E-05	4.55E-12	2.26E-06	2.44E-06	4.32E-08	5.98E-08	7.37E-13	2.26E-11	1.19E-08
Ca+2_H+1_Citric-3_PO4-3	1.49E-06	1.54E-07	6.70E-07	1.69E-06	5.70E-07	1.74E-06	6.75E-08	3.85E-07	1.46E-06
Ca+2(2)_H+1_Phy-12	1.29E-06	1.51E-06	1.52E-06	1.50E-06	1.51E-06	1.43E-06	1.47E-06	1.18E-06	7.48E-07

Calcium speciation: 0.76 µM (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Ca] mol/L								
Ca+2	6.08E-04	2.89E-04	1.11E-03	5.68E-04	1.57E-03	5.14E-04	1.11E-03	7.91E-04	4.88E-04
Ca+2_Citric-3_PO4-3	2.81E-05	7.10E-04	9.02E-06	7.66E-04	5.34E-05	3.20E-04	5.25E-04	5.63E-04	3.13E-04
Ca+2_Citric-3	2.83E-04	1.98E-04	2.67E-04	2.99E-04	6.87E-04	3.70E-04	3.57E-04	4.46E-04	4.23E-04
Ca+2_H+1_PO4-3	7.32E-05	1.51E-04	8.76E-05	1.97E-04	1.64E-04	1.13E-04	3.18E-04	1.58E-04	1.41E-04
Ca+2_H+1(3)_PO4-3(2)	8.03E-05	1.15E-04	1.11E-04	1.55E-04	2.15E-04	8.46E-05	3.37E-04	3.25E-05	1.76E-04
Ca+2_Cl-1	7.14E-05	3.44E-05	1.45E-04	8.45E-05	1.74E-04	8.65E-05	1.83E-04	9.30E-05	5.30E-05
Ca+2_H+1(2)_PO4-3	3.56E-05	1.11E-05	7.62E-05	2.08E-05	1.06E-04	1.86E-05	5.36E-05	8.90E-06	3.04E-05
Ca+2_PO4-3	6.03E-07	8.33E-06	4.02E-07	7.69E-06	1.03E-06	2.83E-06	7.80E-06	1.11E-05	2.67E-06
Ca+2_Oxalic-2	1.38E-05	5.51E-06	1.94E-05	7.53E-06	6.19E-05	1.19E-05	3.53E-05	2.18E-05	1.60E-05
Ca+2_H+1(2)_PO4-3(2)	1.03E-06	1.06E-05	7.80E-07	1.18E-05	2.22E-06	3.83E-06	1.69E-05	3.47E-06	5.71E-06
Ca+2(2)_Oxalic-2	1.84E-06	3.48E-07	4.75E-06	9.28E-07	2.13E-05	1.33E-06	8.48E-06	3.80E-06	1.71E-06
Ca+2_H+1_Citric-3	4.24E-06	4.26E-07	7.26E-06	8.29E-07	1.31E-05	1.68E-06	1.53E-06	7.93E-07	2.62E-06
Ca+2_Citric-3(2)	1.75E-06	2.22E-06	7.98E-07	3.88E-06	4.72E-06	5.48E-06	3.11E-06	3.00E-06	6.53E-06
Ca+2_H+1(2)_Citric-3(2)	3.26E-06	8.03E-08	5.01E-06	2.10E-07	1.36E-05	8.25E-07	3.97E-07	8.17E-08	1.90E-06
Ca+2_H+1_Citric-3_PO4-3	8.70E-07	3.10E-06	5.12E-07	4.24E-06	2.07E-06	2.89E-06	4.51E-06	2.10E-06	3.89E-06
Ca+2(2)_H+1_Phy-12	1.36E-06	6.40E-07	1.48E-06	4.92E-07	1.47E-06	7.03E-07	8.27E-07	1.46E-06	9.05E-07

Calcium speciation: 1.50 mM Phy (black subjects)									
	BU1a	**BU2a**	**BU3a**	**BU4a**	**BU5a**	**BU6a**	**BU7a**	**BU8a**	**BU9a**
	[Ca] mol/L								
Ca+2	1.43E-03	1.40E-04	2.42E-04	3.16E-04	1.47E-04	4.11E-04	1.62E-04	2.39E-04	1.58E-04
Ca+2_Citric-3_PO4-3	3.49E-05	3.42E-04	1.80E-05	9.57E-05	8.20E-05	3.40E-04	5.14E-04	8.40E-04	7.87E-04
Ca+2_Citric-3	3.36E-04	1.07E-05	1.42E-04	2.60E-04	6.37E-05	1.40E-04	1.33E-05	2.08E-05	8.81E-05
Ca+2_H+1_PO4-3	1.02E-04	4.01E-05	2.02E-05	4.20E-05	2.53E-05	9.37E-05	1.56E-05	7.76E-05	4.81E-05
Ca+2_H+1(3)_PO4-3(2)	1.41E-04	1.03E-06	1.04E-05	2.49E-05	6.08E-06	3.29E-05	4.63E-08	3.55E-06	9.21E-06
Ca+2_Cl-1	1.90E-04	5.93E-06	1.43E-05	2.36E-05	8.39E-06	3.17E-05	1.09E-05	1.70E-05	1.52E-05
Ca+2_H+1(2)_PO4-3	8.35E-05	1.82E-07	6.39E-06	9.16E-06	1.78E-06	6.72E-06	2.35E-08	5.03E-07	1.38E-06
Ca+2_PO4-3	5.20E-07	3.59E-05	2.59E-07	7.88E-07	1.47E-06	5.40E-06	4.23E-05	4.94E-05	6.91E-06
Ca+2_Oxalic-2	1.98E-05	2.58E-06	4.80E-06	9.61E-06	2.37E-06	9.74E-06	2.37E-06	7.43E-06	2.63E-06
Ca+2_H+1(2)_PO4-3(2)	1.70E-06	1.54E-06	2.19E-07	8.28E-07	5.96E-07	3.67E-06	2.23E-07	4.46E-06	2.67E-06
Ca+2(2)_Oxalic-2	6.03E-06	7.91E-08	2.55E-07	6.64E-07	7.60E-08	8.69E-07	8.37E-08	3.85E-07	8.98E-08
Ca+2_H+1_Citric-3	6.29E-06	1.41E-09	1.33E-06	1.59E-06	1.30E-07	2.64E-07	5.62E-10	3.51E-09	6.49E-08
Ca+2_Citric-3(2)	2.86E-06	1.34E-08	1.30E-06	4.14E-06	4.69E-07	1.15E-06	2.12E-08	4.59E-08	1.30E-06
Ca+2_H+1(2)_Citric-3(2)	6.84E-06	1.83E-12	8.98E-07	1.15E-06	1.50E-08	2.90E-08	2.82E-13	9.18E-12	4.93E-09
Ca+2_H+1_Citric-3_PO4-3	1.36E-06	9.18E-08	3.42E-07	1.17E-06	3.37E-07	1.28E-06	4.35E-08	2.83E-07	1.16E-06
Ca+2(2)_H+1_Phy-12	1.08E-03	4.15E-04	1.17E-03	8.88E-04	4.22E-04	6.41E-04	2.59E-04	2.00E-04	7.77E-05

Calcium speciation: 1.50 mM Phy (black subjects)									
	BU10a	**BU11a**	**BU12a**	**B1a**	**B2a**	**B3a**	**B4a**	**B5a**	**B7a**
	[Ca] mol/L								
Ca+2	3.88E-04	2.53E-04	5.09E-04	5.06E-04	8.42E-04	4.45E-04	9.54E-04	4.27E-04	4.14E-04
Ca+2_Citric-3_PO4-3	3.24E-05	8.10E-04	8.37E-06	8.72E-04	5.20E-05	3.85E-04	5.91E-04	5.59E-04	3.93E-04
Ca+2_Citric-3	1.66E-04	1.28E-04	1.30E-04	2.21E-04	4.00E-04	2.68E-04	2.69E-04	2.41E-04	3.01E-04
Ca+2_H+1_PO4-3	4.13E-05	1.10E-04	3.63E-05	1.54E-04	8.16E-05	8.61E-05	2.49E-04	6.99E-05	1.05E-04
Ca+2_H+1(3)_PO4-3(2)	4.29E-05	7.28E-05	4.46E-05	1.08E-04	1.04E-04	5.83E-05	2.43E-04	1.27E-05	1.20E-04
Ca+2_Cl-1	3.82E-05	2.55E-05	5.65E-05	6.61E-05	8.21E-05	6.47E-05	1.40E-04	4.21E-05	3.84E-05
Ca+2_H+1(2)_PO4-3	1.85E-05	7.40E-06	2.94E-05	1.49E-05	4.93E-05	1.30E-05	3.86E-05	3.65E-06	2.07E-05
Ca+2_PO4-3	3.82E-07	6.78E-06	1.85E-07	6.60E-06	5.58E-07	2.38E-06	6.65E-06	5.52E-06	2.21E-06
Ca+2_Oxalic-2	7.77E-06	4.19E-06	8.63E-06	6.18E-06	3.38E-05	9.28E-06	2.91E-05	1.07E-05	1.19E-05
Ca+2_H+1(2)_PO4-3(2)	7.89E-07	9.68E-06	4.31E-07	1.14E-05	1.41E-06	3.70E-06	1.64E-05	1.92E-06	5.53E-06
Ca+2(2)_Oxalic-2	6.52E-07	2.28E-07	9.55E-07	6.66E-07	6.16E-06	8.85E-07	5.91E-06	9.92E-07	1.06E-06
Ca+2_H+1_Citric-3	1.92E-06	2.10E-07	2.82E-06	4.78E-07	6.27E-06	9.44E-07	9.15E-07	3.35E-07	1.44E-06
Ca+2_Citric-3(2)	1.82E-06	1.96E-06	7.69E-07	3.70E-06	4.84E-06	5.54E-06	3.01E-06	3.18E-06	6.87E-06
Ca+2_H+1(2)_Citric-3(2)	1.71E-06	3.64E-08	2.58E-06	1.18E-07	8.35E-06	4.68E-07	2.40E-07	4.38E-08	1.07E-06
Ca+2_H+1_Citric-3_PO4-3	7.49E-07	2.70E-06	3.61E-07	3.97E-06	1.63E-06	2.79E-06	4.29E-06	1.55E-06	3.81E-06
Ca+2(2)_H+1_Phy-12	4.66E-04	1.08E-04	1.02E-03	1.54E-04	1.42E-03	1.93E-04	4.17E-04	7.60E-04	2.41E-04

Phytate speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Phy] mol/L								
Ca+2(2)_H+1_Phy-12	6.43E-07	7.54E-07	7.58E-07	7.49E-07	7.53E-07	7.16E-07	7.33E-07	5.88E-07	3.74E-07
H+1_Phy-12	1.17E-07	6.41E-09	1.82E-09	1.12E-08	7.54E-09	4.40E-08	2.55E-08	1.69E-07	3.84E-07
Phy-12	4.24E-11	6.32E-11	3.23E-13	4.20E-12	5.23E-12	7.55E-11	1.08E-09	3.12E-09	1.72E-09
H+1(2)_Phy-12	2.84E-13	4.84E-16	7.78E-15	2.34E-14	8.16E-15	2.09E-14	4.65E-16	7.42E-15	7.05E-14
Ca+2(2)_H+1(2)_Phy-12	8.96E-16	1.66E-17	1.02E-15	5.64E-16	2.45E-16	1.41E-16	4.42E-18	1.07E-17	2.91E-17
H+1(3)_Phy-12	2.22E-19	9.94E-24	9.19E-21	1.40E-20	2.42E-21	2.94E-21	2.38E-24	9.67E-23	3.85E-21
Ca+2(2)_H+1(3)_Phy-12	3.04E-24	7.57E-28	2.87E-24	9.20E-25	1.65E-25	6.29E-26	5.67E-29	4.38E-28	5.11E-27
Ca+2_H+1(3)_Phy-12	3.14E-24	4.63E-28	8.36E-25	5.47E-25	1.05E-25	6.08E-26	5.82E-29	9.23E-28	1.97E-26
H+1(4)_Phy-12	1.17E-26	1.16E-32	6.28E-28	5.02E-28	4.09E-29	2.57E-29	7.10E-34	7.81E-32	1.31E-29
Ca+2_H+1(4)_Phy-12	1.78E-31	4.16E-37	4.58E-32	1.68E-32	1.39E-33	4.89E-34	0	6.85E-37	6.22E-35
H+1(5)_Phy-12	4.31E-34	0	2.60E-35	1.13E-35	4.14E-37	1.45E-37	0	0	0
Ca+2_H+1(5)_Phy-12	0	0	0	0	0	0	0	0	0
Ca+2_H+1(6)_Phy-12	0	0	0	0	0	0	0	0	0
H+1(6)_Phy-12	0	0	0	0	0	0	0	0	0
H+1(8)_Phy-12	0	0	0	0	0	0	0	0	0

Phytate speciation: 0.76 µM Phy (black subjects)									
	BU10a	**BU11a**	**BU12a**	**B1a**	**B2a**	**B3a**	**B4a**	**B5a**	**B7a**
	[phy] mol/L								
Ca+2(2)_H+1_Phy-12	6.78E-07	3.20E-07	7.40E-07	2.46E-07	7.32E-07	3.51E-07	4.14E-07	7.28E-07	4.52E-07
H+1_Phy-12	8.20E-08	4.39E-07	1.99E-08	5.13E-07	2.77E-08	4.08E-07	3.46E-07	3.23E-08	3.07E-07
Phy-12	2.48E-11	1.14E-09	3.09E-12	1.49E-09	7.73E-12	6.20E-10	7.06E-10	7.35E-11	2.99E-10
H+1(2)_Phy-12	2.23E-13	1.43E-13	1.05E-13	1.55E-13	8.30E-14	2.32E-13	1.51E-13	1.15E-14	2.68E-13
Ca+2(2)_H+1(2)_Phy-12	7.95E-16	4.93E-17	1.63E-15	4.21E-17	1.02E-15	1.04E-16	1.06E-16	1.07E-16	1.93E-16
H+1(3)_Phy-12	1.82E-19	1.43E-20	1.63E-19	1.50E-20	7.58E-20	4.14E-20	2.13E-20	1.21E-21	7.23E-20
Ca+2(2)_H+1(3)_Phy-12	2.12E-24	1.76E-26	8.05E-24	1.75E-26	3.27E-24	7.38E-26	6.72E-26	3.50E-26	1.94E-25
Ca+2_H+1(3)_Phy-12	2.73E-24	6.65E-26	5.11E-24	6.22E-26	2.11E-24	2.20E-25	1.42E-25	2.93E-26	4.88E-25
H+1(4)_Phy-12	9.26E-27	9.10E-29	1.58E-26	9.76E-29	4.41E-27	4.84E-28	2.03E-28	7.88E-30	1.26E-27
Ca+2_H+1(4)_Phy-12	1.30E-31	4.17E-34	4.57E-31	4.33E-34	1.19E-31	2.67E-33	1.49E-33	1.74E-34	8.50E-33
H+1(5)_Phy-12	3.09E-34	3.89E-37	9.94E-34	4.44E-37	1.71E-34	3.90E-36	1.37E-36	0	1.48E-35
Ca+2_H+1(5)_Phy-12	0	0	2.07E-37	0	0	0	0	0	0
Ca+2_H+1(6)_Phy-12	0	0	0	0	0	0	0	0	0
H+1(6)_Phy-12	0	0	0	0	0	0	0	0	0
H+1(8)_Phy-12	0	0	0	0	0	0	0	0	0

Phytate speciation: 1.50 mM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Phy] mol/L								
Ca+2(2)_H+1_Phy-12	5.38E-04	2.07E-04	5.83E-04	4.44E-04	2.11E-04	3.21E-04	1.30E-04	1.00E-04	3.89E-05
H+1_Phy-12	9.62E-04	1.24E-03	9.16E-04	1.06E-03	1.29E-03	1.18E-03	1.19E-03	1.34E-03	1.44E-03
Phy-12	5.59E-07	5.24E-05	5.21E-07	1.13E-06	3.63E-06	4.93E-06	1.84E-04	6.54E-05	1.68E-05
H+1(2)_Phy-12	1.52E-09	2.47E-11	1.35E-09	8.48E-10	3.85E-10	2.46E-10	6.59E-12	2.41E-11	1.10E-10
Ca+2(2)_H+1(2)_Phy-12	5.70E-13	1.96E-15	3.98E-13	1.82E-13	3.03E-14	3.76E-14	3.67E-16	1.03E-15	1.73E-15
H+1(3)_Phy-12	8.05E-16	1.51E-19	6.06E-16	2.13E-16	3.54E-17	1.65E-17	1.14E-20	1.40E-19	2.71E-18
Ca+2(2)_H+1(3)_Phy-12	1.53E-21	4.31E-26	6.28E-22	1.76E-22	1.02E-23	1.07E-23	2.46E-27	2.60E-26	1.89E-25
Ca+2_H+1(3)_Phy-12	3.91E-21	3.38E-25	2.61E-21	7.82E-22	7.90E-23	5.11E-23	2.14E-26	2.30E-25	2.70E-24
H+1(4)_Phy-12	2.97E-23	5.91E-29	1.73E-23	3.48E-24	2.10E-25	7.35E-26	1.29E-30	5.43E-29	4.51E-27
Ca+2_H+1(4)_Phy-12	1.69E-28	1.30E-34	7.26E-29	1.30E-29	4.63E-31	2.44E-31	2.46E-36	9.65E-35	4.89E-33
H+1(5)_Phy-12	8.02E-31	0.00E+00	3.30E-31	3.88E-32	8.33E-34	2.30E-34	0.00E+00	0.00E+00	5.29E-36
Ca+2_H+1(5)_Phy-12	4.13E-35	0.00E+00	1.05E-35	1.15E-36	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Ca+2_H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(6)_Phy-12	1.05E-37	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(8)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Phytate speciation: 1.50 mM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Phy] mol/L								
Ca+2(2)_H+1_Phy-12	2.33E-04	5.39E-05	5.08E-04	7.72E-05	7.11E-04	9.63E-05	2.08E-04	3.80E-04	1.21E-04
H+1_Phy-12	1.27E-03	1.44E-03	9.91E-04	1.42E-03	7.89E-04	1.40E-03	1.29E-03	1.11E-03	1.38E-03
Phy-12	9.16E-07	8.38E-06	3.49E-07	7.39E-06	4.18E-07	4.16E-06	4.38E-06	6.18E-06	2.83E-06
H+1(2)_Phy-12	1.55E-09	2.23E-10	2.46E-09	2.51E-10	1.32E-09	4.30E-10	3.52E-10	1.76E-10	6.06E-10
Ca+2(2)_H+1(2)_Phy-12	1.64E-13	5.17E-15	6.93E-13	9.39E-15	6.80E-13	1.93E-14	3.99E-14	3.31E-14	3.33E-14
H+1(3)_Phy-12	6.10E-16	1.13E-17	1.94E-15	1.50E-17	7.05E-16	4.39E-17	3.26E-17	8.84E-18	8.77E-17
Ca+2(2)_H+1(3)_Phy-12	2.83E-22	1.23E-24	2.28E-21	2.92E-24	1.59E-21	9.78E-24	1.95E-23	6.98E-24	2.30E-23
Ca+2_H+1(3)_Phy-12	1.58E-21	1.37E-23	8.19E-21	2.30E-23	4.03E-21	7.39E-23	8.70E-23	3.05E-23	1.63E-22
H+1(4)_Phy-12	1.62E-23	3.92E-26	1.02E-22	6.27E-26	2.54E-23	3.11E-25	2.12E-25	2.96E-26	8.72E-25
Ca+2_H+1(4)_Phy-12	4.53E-29	5.34E-32	4.55E-28	1.14E-31	1.57E-28	6.04E-31	6.78E-31	1.08E-31	1.84E-30
H+1(5)_Phy-12	3.01E-31	9.77E-35	3.72E-30	1.93E-34	6.43E-31	1.60E-33	1.02E-33	6.89E-35	6.24E-33
Ca+2_H+1(5)_Phy-12	7.12E-36	0.00E+00	1.37E-34	0.00E+00	3.34E-35	0.00E+00	0.00E+00	0.00E+00	1.16E-37
Ca+2_H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(6)_Phy-12	0.00E+00	0.00E+00	6.24E-37	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(8)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Oxalate speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Ox] mol/L								
Oxalic-2	7.33E-05	7.40E-05	7.04E-05	1.27E-04	6.68E-05	1.09E-04	6.22E-05	1.55E-04	8.51E-05
Na+1_Oxalic-2	3.40E-05	1.13E-05	1.42E-05	2.54E-05	1.17E-05	3.13E-05	1.33E-05	3.83E-05	2.13E-05
Mg+2_Oxalic-2	2.73E-05	2.35E-05	3.00E-05	3.81E-05	1.15E-05	4.92E-05	2.46E-05	3.64E-05	1.78E-05
Ca+2_Oxalic-2	2.76E-05	7.86E-06	1.90E-05	2.38E-05	7.37E-06	1.85E-05	5.43E-06	1.18E-05	3.83E-06
K+1_Oxalic-2	9.01E-06	4.83E-06	4.08E-06	1.01E-05	3.75E-06	9.07E-06	3.65E-06	1.51E-05	4.21E-06
Ca+2(2)_Oxalic-2	6.11E-06	2.87E-07	1.89E-06	1.84E-06	2.87E-07	1.46E-06	1.82E-07	4.19E-07	8.13E-08
Mg+2_H+1_Oxalic-2_PO4-3	5.61E-07	9.62E-07	3.14E-07	7.61E-07	2.67E-07	2.13E-06	3.80E-07	2.44E-06	1.14E-06
H+1_Oxalic-2	1.05E-06	7.52E-09	4.76E-07	5.66E-07	1.04E-07	1.52E-07	2.01E-09	1.97E-08	4.78E-08
Mg+2_Oxalic-2_Citric-3	2.31E-07	1.92E-08	1.70E-07	4.49E-07	6.00E-08	3.43E-07	3.04E-08	8.00E-08	2.72E-07
Ca+2_H+1_Oxalic-2_PO4-3	1.36E-07	1.12E-07	6.79E-08	1.54E-07	5.92E-08	2.42E-07	2.81E-08	2.41E-07	7.30E-08
Mg+2(2)_Oxalic-2	2.81E-07	9.06E-08	1.68E-07	1.76E-07	2.49E-08	4.06E-07	1.36E-07	1.55E-07	7.01E-08
Mg+2_Oxalic-2(2)	9.40E-08	6.26E-08	7.73E-08	1.84E-07	2.79E-08	2.13E-07	5.68E-08	2.24E-07	6.09E-08
Ca+2_Oxalic-2(2)	5.38E-08	1.55E-08	3.56E-08	8.06E-08	1.31E-08	5.35E-08	8.99E-09	4.87E-08	8.66E-09
Ca+2_Oxalic-2_Citric-3	4.98E-08	2.00E-09	3.28E-08	8.11E-08	1.18E-08	3.47E-08	2.00E-09	7.03E-09	1.55E-08
Ca+2_H+1_Oxalic-2_Citric-3	1.13E-07	3.68E-11	3.96E-08	6.27E-08	3.31E-09	8.15E-09	1.14E-11	1.51E-10	1.46E-09

Oxalate speciation: 0.76 µM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Ox] mol/L								
Oxalic-2	9.85E-05	8.97E-05	7.42E-05	7.15E-05	1.82E-04	1.17E-04	1.76E-04	1.15E-04	1.58E-04
Na+1_Oxalic-2	2.51E-05	2.32E-05	1.63E-05	3.27E-05	6.23E-05	3.69E-05	7.38E-05	2.92E-05	6.12E-05
Mg+2_Oxalic-2	1.79E-05	1.43E-05	2.64E-05	7.78E-06	6.69E-05	1.53E-05	3.99E-05	2.63E-05	1.25E-05
Ca+2_Oxalic-2	1.38E-05	5.51E-06	1.94E-05	7.53E-06	6.19E-05	1.19E-05	3.53E-05	2.18E-05	1.60E-05
K+1_Oxalic-2	1.00E-05	6.09E-06	5.29E-06	3.83E-06	1.39E-05	1.12E-05	1.89E-05	7.78E-06	9.08E-06
Ca+2(2)_Oxalic-2	9.21E-07	1.74E-07	2.37E-06	4.64E-07	1.07E-05	6.65E-07	4.24E-06	1.90E-06	8.53E-07
Mg+2_H+1_Oxalic-2_PO4-3	3.58E-07	1.45E-06	3.30E-07	7.19E-07	1.31E-06	7.76E-07	3.32E-06	8.10E-07	7.42E-07
H+1_Oxalic-2	8.49E-07	1.14E-07	1.16E-06	1.24E-07	2.03E-06	3.21E-07	4.81E-07	1.17E-07	5.79E-07
Mg+2_Oxalic-2_Citric-3	1.70E-07	2.38E-07	1.22E-07	1.45E-07	6.86E-07	3.30E-07	4.99E-07	2.75E-07	2.84E-07
Ca+2_H+1_Oxalic-2_PO4-3	8.17E-08	1.57E-07	7.33E-08	1.69E-07	3.43E-07	1.56E-07	6.81E-07	2.05E-07	2.59E-07
Mg+2(2)_Oxalic-2	6.21E-08	4.86E-08	1.73E-07	2.30E-08	5.14E-07	4.89E-08	2.61E-07	1.08E-07	2.22E-08
Mg+2_Oxalic-2(2)	7.13E-08	5.38E-08	7.81E-08	2.59E-08	5.08E-07	7.97E-08	3.37E-07	1.20E-07	8.47E-08
Ca+2_Oxalic-2(2)	3.61E-08	1.32E-08	3.83E-08	1.43E-08	3.00E-07	3.70E-08	1.65E-07	6.69E-08	6.72E-08
Ca+2_Oxalic-2_Citric-3	3.45E-08	2.30E-08	2.41E-08	3.04E-08	1.60E-07	5.92E-08	9.12E-08	6.19E-08	8.83E-08
Ca+2_H+1_Oxalic-2_Citric-3	4.97E-08	4.78E-09	6.32E-08	8.37E-09	2.95E-07	2.62E-08	3.95E-08	1.06E-08	5.28E-08

Oxalate speciation: 1.50 mM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Ox] mol/L								
Oxalic-2	8.36E-05	8.67E-05	9.15E-05	1.51E-04	7.67E-05	1.27E-04	7.31E-05	1.69E-04	9.16E-05
Na+1_Oxalic-2	3.75E-05	1.10E-05	1.59E-05	2.67E-05	1.12E-05	3.31E-05	1.33E-05	3.79E-05	2.08E-05
Mg+2_Oxalic-2	2.35E-05	1.68E-05	2.27E-05	2.78E-05	7.64E-06	3.86E-05	1.70E-05	2.75E-05	1.33E-05
Ca+2_Oxalic-2	1.98E-05	2.58E-06	4.80E-06	9.61E-06	2.37E-06	9.74E-06	2.37E-06	7.43E-06	2.63E-06
K+1_Oxalic-2	9.94E-06	4.72E-06	4.58E-06	1.07E-05	3.60E-06	9.61E-06	3.67E-06	1.49E-05	4.11E-06
Ca+2(2)_Oxalic-2	3.01E-06	3.96E-08	1.28E-07	3.32E-07	3.80E-08	4.34E-07	4.19E-08	1.92E-07	4.49E-08
Mg+2_H+1_Oxalic-2_PO4-3	6.51E-07	9.36E-07	3.54E-07	8.12E-07	2.61E-07	2.31E-06	3.60E-07	2.44E-06	1.15E-06
H+1_Oxalic-2	1.08E-06	6.76E-09	5.00E-07	5.55E-07	9.20E-08	1.49E-07	1.85E-09	1.79E-08	4.27E-08
Mg+2_Oxalic-2_Citric-3	2.92E-07	3.14E-08	3.11E-07	6.47E-07	8.32E-08	4.55E-07	3.96E-08	8.70E-08	2.80E-07
Ca+2_H+1_Oxalic-2_PO4-3	1.09E-07	4.02E-08	2.12E-08	7.45E-08	2.25E-08	1.43E-07	1.33E-08	1.58E-07	5.34E-08
Mg+2(2)_Oxalic-2	2.30E-07	7.00E-08	1.18E-07	1.21E-07	1.66E-08	3.15E-07	9.34E-08	1.23E-07	5.44E-08
Mg+2_Oxalic-2(2)	1.05E-07	6.14E-08	8.65E-08	1.84E-07	2.48E-08	2.26E-07	5.43E-08	2.18E-07	5.77E-08
Ca+2_Oxalic-2(2)	4.40E-08	5.95E-09	1.17E-08	3.87E-08	4.83E-09	3.29E-08	4.61E-09	3.34E-08	6.40E-09
Ca+2_Oxalic-2_Citric-3	4.36E-08	1.20E-09	1.66E-08	5.28E-08	6.37E-09	2.51E-08	1.31E-09	5.03E-09	1.16E-08
Ca+2_H+1_Oxalic-2_Citric-3	8.81E-08	1.54E-11	1.50E-08	3.14E-08	1.25E-09	4.70E-09	5.37E-12	8.48E-11	8.64E-10

Oxalate speciation: 1.50 mM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Ox] mol/L								
Oxalic-2	1.10E-04	9.50E-05	8.94E-05	7.46E-05	2.18E-04	1.24E-04	1.88E-04	1.33E-04	1.67E-04
Na+1_Oxalic-2	2.54E-05	2.27E-05	1.79E-05	3.28E-05	6.98E-05	3.69E-05	7.63E-05	3.05E-05	6.04E-05
Mg+2_Oxalic-2	1.32E-05	1.08E-05	2.16E-05	5.98E-06	5.70E-05	1.15E-05	3.20E-05	1.98E-05	9.19E-06
Ca+2_Oxalic-2	7.77E-06	4.19E-06	8.63E-06	6.18E-06	3.38E-05	9.28E-06	2.91E-05	1.07E-05	1.19E-05
K+1_Oxalic-2	1.02E-05	5.99E-06	5.84E-06	3.85E-06	1.57E-05	1.12E-05	1.96E-05	8.14E-06	8.96E-06
Ca+2(2)_Oxalic-2	3.26E-07	1.14E-07	4.78E-07	3.33E-07	3.08E-06	4.42E-07	2.95E-06	4.96E-07	5.30E-07
Mg+2_H+1_Oxalic-2_PO4-3	3.89E-07	1.52E-06	3.89E-07	7.48E-07	1.52E-06	8.10E-07	3.60E-06	8.26E-07	7.79E-07
H+1_Oxalic-2	7.97E-07	1.03E-07	1.19E-06	1.14E-07	2.15E-06	2.96E-07	4.61E-07	1.14E-07	5.27E-07
Mg+2_Oxalic-2_Citric-3	2.08E-07	2.39E-07	1.84E-07	1.47E-07	9.90E-07	3.43E-07	5.31E-07	3.76E-07	3.02E-07
Ca+2_H+1_Oxalic-2_PO4-3	5.47E-08	1.30E-07	3.88E-08	1.48E-07	2.15E-07	1.35E-07	6.09E-07	1.11E-07	2.18E-07
Mg+2(2)_Oxalic-2	4.41E-08	3.82E-08	1.36E-07	1.74E-08	4.13E-07	3.56E-08	2.04E-07	7.75E-08	1.60E-08
Mg+2_Oxalic-2(2)	6.79E-08	5.13E-08	8.82E-08	2.45E-08	5.85E-07	7.43E-08	3.38E-07	1.20E-07	7.74E-08
Ca+2_Oxalic-2(2)	2.26E-08	1.06E-08	2.05E-08	1.23E-08	1.96E-07	3.06E-08	1.46E-07	3.78E-08	5.29E-08
Ca+2_Oxalic-2_Citric-3	2.60E-08	1.81E-08	1.63E-08	2.59E-08	1.25E-07	5.09E-08	8.01E-08	4.49E-08	7.54E-08
Ca+2_H+1_Oxalic-2_Citric-3	3.02E-08	3.07E-09	3.48E-08	6.18E-09	1.96E-07	1.91E-08	3.06E-08	6.17E-09	3.75E-08

Magnesium speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Mg] mol/L								
Mg+2	1.69E-03	5.62E-04	8.24E-04	6.88E-04	3.15E-04	1.26E-03	8.14E-04	6.50E-04	6.01E-04
Mg+2_H+1_PO4-3	1.67E-04	3.37E-04	1.15E-04	1.50E-04	1.03E-04	4.74E-04	1.56E-04	3.84E-04	3.23E-04
Mg+2_Citric-3	3.09E-04	2.75E-05	2.51E-04	3.55E-04	9.43E-05	3.09E-04	5.01E-05	5.08E-05	3.13E-04
Mg+2_H+1(2)_PO4-3	3.66E-04	3.98E-06	9.29E-05	8.50E-05	1.88E-05	8.94E-05	6.15E-07	6.61E-06	2.48E-05
Mg+2_Cl-1	2.50E-04	2.97E-05	5.76E-05	6.01E-05	2.23E-05	1.12E-04	6.74E-05	5.45E-05	6.81E-05
Mg+2_H+1(3)_PO4-3(2)	6.90E-05	2.23E-06	1.28E-05	2.07E-05	5.90E-06	4.33E-05	1.22E-07	4.99E-06	1.75E-05
Mg+2_Oxalic-2	2.73E-05	2.35E-05	3.00E-05	3.81E-05	1.15E-05	4.92E-05	2.46E-05	3.64E-05	1.78E-05
Mg+2_H+1(2)_PO4-3(2)	1.68E-06	7.42E-06	6.42E-07	1.58E-06	1.29E-06	1.06E-05	1.28E-06	1.34E-05	1.07E-05
Mg+2_Citric-3(2)	2.81E-06	1.91E-08	1.26E-06	3.95E-06	4.24E-07	2.16E-06	5.62E-08	1.11E-07	4.81E-06
Mg+2_H+1_Citric-3_PO4-3	1.38E-06	2.96E-07	6.94E-07	1.86E-06	5.76E-07	3.43E-06	2.04E-07	8.75E-07	5.09E-06
Mg+2_H+1_Citric-3	5.79E-06	4.52E-09	2.73E-06	2.50E-06	2.36E-07	6.58E-07	2.58E-09	9.90E-09	2.67E-07
Mg+2(2)_H+1(2)_PO4-3(2)	5.75E-07	2.16E-06	2.50E-07	4.33E-07	2.03E-07	4.39E-06	4.63E-07	2.88E-06	2.05E-06

Magnesium speciation: 0.76 µM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Ox] mol/L								
Mg+2	5.32E-04	5.32E-04	9.99E-04	4.82E-04	1.20E-03	5.09E-04	1.08E-03	6.25E-04	2.79E-04
Mg+2_H+1_PO4-3	8.74E-05	3.77E-04	1.08E-04	2.20E-04	1.69E-04	1.50E-04	4.04E-04	1.71E-04	1.09E-04
Mg+2_Citric-3	1.68E-04	2.58E-04	1.61E-04	1.99E-04	3.66E-04	2.73E-04	2.80E-04	2.35E-04	1.74E-04
Mg+2_H+1(2)_PO4-3	1.04E-04	6.83E-05	2.29E-04	5.84E-05	2.67E-04	6.10E-05	1.72E-04	2.35E-05	5.77E-05
Mg+2_Cl-1	6.07E-05	6.21E-05	1.26E-04	7.17E-05	1.30E-04	8.48E-05	1.79E-04	7.12E-05	2.98E-05
Mg+2_H+1(3)_PO4-3(2)	2.28E-05	7.19E-05	3.20E-05	5.00E-05	5.48E-05	3.01E-05	1.29E-04	8.16E-06	3.47E-05
Mg+2_Oxalic-2	1.79E-05	1.43E-05	2.64E-05	7.78E-06	6.69E-05	1.53E-05	3.99E-05	2.63E-05	1.25E-05
Mg+2_H+1(2)_PO4-3(2)	9.05E-07	1.95E-05	7.02E-07	1.01E-05	1.69E-06	3.80E-06	1.64E-05	2.74E-06	3.27E-06
Mg+2_Citric-3(2)	1.61E-06	4.49E-06	7.45E-07	3.99E-06	3.90E-06	6.26E-06	3.77E-06	2.45E-06	4.16E-06
Mg+2_H+1_Citric-3_PO4-3	8.54E-07	6.42E-06	5.16E-07	4.04E-06	1.77E-06	3.22E-06	4.92E-06	1.86E-06	2.50E-06
Mg+2_H+1_Citric-3	2.19E-06	4.76E-07	3.83E-06	4.55E-07	6.02E-06	1.04E-06	9.81E-07	3.66E-07	9.18E-07
Mg+2(2)_H+1(2)_PO4-3(2)	1.50E-07	2.84E-06	2.28E-07	1.00E-06	5.68E-07	4.55E-07	3.41E-06	5.73E-07	2.37E-07

Magnesium speciation: 1.50 mM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Mg] mol/L								
Mg+2	1.70E-03	6.51E-04	8.06E-04	6.88E-04	3.41E-04	1.33E-03	8.72E-04	7.34E-04	6.77E-04
Mg+2_H+1_PO4-3	1.56E-04	2.52E-04	9.12E-05	1.23E-04	7.94E-05	4.00E-04	1.13E-04	3.14E-04	2.70E-04
Mg+2_Citric-3	3.61E-04	3.51E-05	3.30E-04	4.15E-04	1.05E-04	3.51E-04	5.26E-05	5.05E-05	3.02E-04
Mg+2_H+1(2)_PO4-3	3.26E-04	2.82E-06	7.07E-05	6.62E-05	1.37E-05	7.18E-05	4.20E-07	5.11E-06	1.95E-05
Mg+2_Cl-1	2.33E-04	2.70E-05	4.65E-05	5.06E-05	1.91E-05	1.02E-04	5.82E-05	5.23E-05	6.55E-05
Mg+2_H+1(3)_PO4-3(2)	7.43E-05	1.62E-06	1.16E-05	1.91E-05	4.82E-06	4.00E-05	8.81E-08	4.17E-06	1.53E-05
Mg+2_Oxalic-2	2.35E-05	1.68E-05	2.27E-05	2.78E-05	7.64E-06	3.86E-05	1.70E-05	2.75E-05	1.33E-05
Mg+2_H+1(2)_PO4-3(2)	2.03E-06	7.17E-06	7.28E-07	1.80E-06	1.38E-06	1.18E-05	1.20E-06	1.37E-05	1.14E-05
Mg+2_Citric-3(2)	4.76E-06	6.84E-08	4.69E-06	1.02E-05	1.20E-06	4.46E-06	1.30E-07	1.72E-07	6.89E-06
Mg+2_H+1_Citric-3_PO4-3	1.81E-06	4.79E-07	1.28E-06	2.86E-06	8.79E-07	4.65E-06	2.63E-07	9.76E-07	5.58E-06
Mg+2_H+1_Citric-3	5.30E-06	3.99E-09	2.66E-06	2.15E-06	1.84E-07	5.49E-07	1.88E-09	7.02E-09	1.82E-07
Mg+2(2)_H+1(2)_PO4-3(2)	5.24E-07	1.27E-06	1.65E-07	3.04E-07	1.26E-07	3.30E-06	2.56E-07	2.04E-06	1.52E-06

Magnesium speciation: 1.50 mM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Ox] mol/L								
Mg+2	5.50E-04	5.94E-04	1.02E-03	5.10E-04	1.19E-03	5.33E-04	1.13E-03	6.35E-04	2.94E-04
Mg+2_H+1_PO4-3	7.71E-05	3.35E-04	9.65E-05	1.97E-04	1.51E-04	1.32E-04	3.70E-04	1.38E-04	9.67E-05
Mg+2_Citric-3	1.87E-04	2.52E-04	2.01E-04	2.06E-04	4.46E-04	2.83E-04	2.99E-04	2.76E-04	1.82E-04
Mg+2_H+1(2)_PO4-3	8.66E-05	5.72E-05	1.95E-04	4.91E-05	2.30E-04	5.10E-05	1.49E-04	1.80E-05	4.86E-05
Mg+2_Cl-1	5.43E-05	6.05E-05	1.13E-04	6.88E-05	1.16E-04	7.93E-05	1.71E-04	6.24E-05	2.77E-05
Mg+2_H+1(3)_PO4-3(2)	2.34E-05	7.00E-05	3.32E-05	4.96E-05	5.61E-05	3.01E-05	1.33E-04	7.05E-06	3.54E-05
Mg+2_Oxalic-2	1.32E-05	1.08E-05	2.16E-05	5.98E-06	5.70E-05	1.15E-05	3.20E-05	1.98E-05	9.19E-06
Mg+2_H+1(2)_PO4-3(2)	1.12E-06	2.27E-05	8.63E-07	1.15E-05	1.98E-06	4.43E-06	1.93E-05	2.85E-06	3.93E-06
Mg+2_Citric-3(2)	3.18E-06	5.98E-06	1.83E-06	5.34E-06	8.35E-06	9.04E-06	5.20E-06	5.64E-06	6.42E-06
Mg+2_H+1_Citric-3_PO4-3	1.19E-06	7.10E-06	8.13E-07	4.49E-06	2.58E-06	3.75E-06	5.68E-06	2.59E-06	3.04E-06
Mg+2_H+1_Citric-3	1.78E-06	3.34E-07	3.61E-06	3.47E-07	5.75E-06	7.88E-07	7.87E-07	3.19E-07	6.96E-07
Mg+2(2)_H+1(2)_PO4-3(2)	1.23E-07	2.37E-06	1.91E-07	8.44E-07	4.75E-07	3.77E-07	3.02E-06	3.90E-07	1.99E-07

Citrate speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Cit] mol/L								
Ca+2_Citric-3_PO4-3	3.28E-05	3.53E-04	2.39E-05	9.72E-05	8.69E-05	3.42E-04	5.17E-04	8.27E-04	7.19E-04
Ca+2_Citric-3	4.75E-04	2.66E-05	4.43E-04	5.67E-04	1.72E-04	2.63E-04	2.99E-05	3.76E-05	1.49E-04
Mg+2_Citric-3	3.09E-04	2.75E-05	2.51E-04	3.55E-04	9.43E-05	3.09E-04	5.01E-05	5.08E-05	3.13E-04
Citric-3	1.28E-04	1.61E-05	1.10E-04	2.20E-04	1.02E-04	1.24E-04	2.37E-05	3.92E-05	2.69E-04
Na+1_Citric-3	1.65E-04	8.85E-06	7.84E-05	1.49E-04	6.37E-05	1.14E-04	1.75E-05	3.11E-05	2.13E-04
Na+1(2)_Citric-3	1.56E-04	3.02E-06	3.53E-05	6.54E-05	2.49E-05	7.08E-05	8.26E-06	1.67E-05	1.15E-04
H+1_Na+1_Citric-3	2.38E-04	8.77E-08	5.19E-05	6.50E-05	9.65E-06	1.57E-05	5.52E-08	3.92E-07	1.19E-05
H+1_Citric-3	1.02E-04	1.21E-07	5.37E-05	6.75E-05	1.16E-05	1.12E-05	5.42E-08	3.25E-07	9.74E-06
K+1_Citric-3	2.04E-05	1.78E-06	1.05E-05	2.77E-05	9.58E-06	1.54E-05	2.26E-06	5.71E-06	1.97E-05
Ca+2_Citric-3(2)	5.58E-06	2.39E-08	2.87E-06	8.13E-06	1.00E-06	2.37E-06	4.32E-08	1.07E-07	2.96E-06
Mg+2_Citric-3(2)	5.62E-06	3.82E-08	2.52E-06	7.90E-06	8.48E-07	4.32E-06	1.12E-07	2.23E-07	9.61E-06
Ca+2_H+1(2)_Citric-3(2)	2.03E-05	9.10E-12	4.51E-06	4.87E-06	8.64E-08	1.20E-07	1.47E-12	4.53E-11	2.37E-08
Ca+2_H+1_Citric-3	1.08E-05	4.81E-09	5.33E-06	4.48E-06	4.75E-07	6.40E-07	1.71E-09	8.37E-09	1.46E-07
Mg+2_H+1_Citric-3_PO4-3	1.38E-06	2.96E-07	6.94E-07	1.86E-06	5.76E-07	3.43E-06	2.04E-07	8.75E-07	5.09E-06
Ca+2_H+1_Citric-3_PO4-3	1.49E-06	1.54E-07	6.70E-07	1.69E-06	5.70E-07	1.74E-06	6.75E-08	3.85E-07	1.46E-06

Citrate speciation: 0.76 µM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Cit] mol/L								
Ca+2_Citric-3_PO4-3	2.81E-05	7.10E-04	9.02E-06	7.66E-04	5.34E-05	3.20E-04	5.25E-04	5.63E-04	3.13E-04
Ca+2_Citric-3	2.83E-04	1.98E-04	2.67E-04	2.99E-04	6.87E-04	3.70E-04	3.57E-04	4.46E-04	4.23E-04
Mg+2_Citric-3	1.68E-04	2.58E-04	1.61E-04	1.99E-04	3.66E-04	2.73E-04	2.80E-04	2.35E-04	1.74E-04
Citric-3	1.66E-04	2.82E-04	8.20E-05	2.84E-04	1.75E-04	3.44E-04	1.85E-04	1.87E-04	3.76E-04
Na+1_Citric-3	1.33E-04	2.20E-04	5.73E-05	3.63E-04	1.82E-04	3.14E-04	2.12E-04	1.52E-04	4.35E-04
Na+1(2)_Citric-3	7.31E-05	1.21E-04	2.72E-05	3.40E-04	1.33E-04	2.05E-04	1.80E-04	8.35E-05	3.55E-04
H+1_Na+1_Citric-3	1.14E-04	2.78E-05	8.85E-05	6.35E-05	2.03E-04	8.64E-05	5.88E-05	1.53E-05	1.59E-04
H+1_Citric-3	9.17E-05	2.19E-05	8.28E-05	2.76E-05	1.21E-04	5.52E-05	2.77E-05	1.24E-05	8.32E-05
K+1_Citric-3	2.49E-05	2.71E-05	8.71E-06	1.99E-05	1.91E-05	4.44E-05	2.54E-05	1.90E-05	3.01E-05
Ca+2_Citric-3(2)	3.51E-06	4.45E-06	1.60E-06	7.75E-06	9.44E-06	1.10E-05	6.21E-06	6.01E-06	1.31E-05
Mg+2_Citric-3(2)	3.23E-06	8.98E-06	1.49E-06	7.97E-06	7.79E-06	1.25E-05	7.54E-06	4.90E-06	8.33E-06
Ca+2_H+1(2)_Citric-3(2)	6.53E-06	1.61E-07	1.00E-05	4.21E-07	2.71E-05	1.65E-06	7.94E-07	1.63E-07	3.80E-06
Ca+2_H+1_Citric-3	4.24E-06	4.26E-07	7.26E-06	8.29E-07	1.31E-05	1.68E-06	1.53E-06	7.93E-07	2.62E-06
Mg+2_H+1_Citric-3_PO4-3	8.54E-07	6.42E-06	5.16E-07	4.04E-06	1.77E-06	3.22E-06	4.92E-06	1.86E-06	2.50E-06
Ca+2_H+1_Citric-3_PO4-3	8.70E-07	3.10E-06	5.12E-07	4.24E-06	2.07E-06	2.89E-06	4.51E-06	2.10E-06	3.89E-06

Citrate speciation: 1.50 mM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[Cit] mol/L								
Ca+2_Citric-3_PO4-3	3.49E-05	3.42E-04	1.80E-05	9.57E-05	8.20E-05	3.40E-04	5.14E-04	8.40E-04	7.87E-04
Ca+2_Citric-3	3.36E-04	1.07E-05	1.42E-04	2.60E-04	6.37E-05	1.40E-04	1.33E-05	2.08E-05	8.81E-05
Mg+2_Citric-3	3.61E-04	3.51E-05	3.30E-04	4.15E-04	1.05E-04	3.51E-04	5.26E-05	5.05E-05	3.02E-04
Citric-3	1.65E-04	3.14E-05	2.33E-04	3.78E-04	1.83E-04	1.81E-04	3.78E-05	4.79E-05	3.16E-04
Na+1_Citric-3	1.88E-04	1.20E-05	1.24E-04	1.95E-04	8.03E-05	1.32E-04	2.02E-05	2.98E-05	1.97E-04
Na+1(2)_Citric-3	1.65E-04	3.23E-06	4.60E-05	7.22E-05	2.48E-05	7.08E-05	7.69E-06	1.36E-05	9.06E-05
H+1_Na+1_Citric-3	2.52E-04	9.37E-08	6.76E-05	7.19E-05	9.62E-06	1.57E-05	5.14E-08	3.19E-07	9.33E-06
H+1_Citric-3	1.08E-04	1.50E-07	7.90E-05	8.21E-05	1.34E-05	1.20E-05	5.68E-08	2.83E-07	8.14E-06
K+1_Citric-3	2.33E-05	2.42E-06	1.67E-05	3.65E-05	1.21E-05	1.80E-05	2.60E-06	5.47E-06	1.82E-05
Ca+2_Citric-3(2)	5.73E-06	2.68E-08	2.60E-06	8.27E-06	9.39E-07	2.29E-06	4.24E-08	9.19E-08	2.60E-06
Mg+2_Citric-3(2)	9.51E-06	1.37E-07	9.38E-06	2.05E-05	2.40E-06	8.92E-06	2.60E-07	3.45E-07	1.38E-05
Ca+2_H+1(2)_Citric-3(2)	1.37E-05	3.67E-12	1.80E-06	2.30E-06	3.00E-08	5.80E-08	5.64E-13	1.84E-11	9.85E-09
Ca+2_H+1_Citric-3	6.29E-06	1.41E-09	1.33E-06	1.59E-06	1.30E-07	2.64E-07	5.62E-10	3.51E-09	6.49E-08
Mg+2_H+1_Citric-3_PO4-3	1.81E-06	4.79E-07	1.28E-06	2.86E-06	8.79E-07	4.65E-06	2.63E-07	9.76E-07	5.58E-06
Ca+2_H+1_Citric-3_PO4-3	1.36E-06	9.18E-08	3.42E-07	1.17E-06	3.37E-07	1.28E-06	4.35E-08	2.83E-07	1.16E-06

Citrate speciation: 1.50 mM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[Cit] mol/L								
Ca+2_Citric-3_PO4-3	3.24E-05	8.10E-04	8.37E-06	8.72E-04	5.20E-05	3.85E-04	5.91E-04	5.59E-04	3.93E-04
Ca+2_Citric-3	1.66E-04	1.28E-04	1.30E-04	2.21E-04	4.00E-04	2.68E-04	2.69E-04	2.41E-04	3.01E-04
Mg+2_Citric-3	1.87E-04	2.52E-04	2.01E-04	2.06E-04	4.46E-04	2.83E-04	2.99E-04	2.76E-04	1.82E-04
Citric-3	2.38E-04	3.14E-04	1.32E-04	3.17E-04	2.62E-04	4.06E-04	2.10E-04	2.94E-04	4.62E-04
Na+1_Citric-3	1.53E-04	2.00E-04	7.51E-05	3.49E-04	2.33E-04	3.12E-04	2.11E-04	1.91E-04	4.41E-04
Na+1(2)_Citric-3	7.24E-05	9.54E-05	3.11E-05	2.96E-04	1.52E-04	1.82E-04	1.64E-04	9.03E-05	3.17E-04
H+1_Na+1_Citric-3	1.13E-04	2.20E-05	1.01E-04	5.54E-05	2.32E-04	7.69E-05	5.38E-05	1.66E-05	1.43E-04
H+1_Citric-3	9.63E-05	1.80E-05	1.01E-04	2.42E-05	1.44E-04	5.01E-05	2.53E-05	1.44E-05	7.70E-05
K+1_Citric-3	2.86E-05	2.46E-05	1.14E-05	1.92E-05	2.44E-05	4.42E-05	2.53E-05	2.38E-05	3.06E-05
Ca+2_Citric-3(2)	3.65E-06	3.92E-06	1.54E-06	7.39E-06	9.68E-06	1.11E-05	6.02E-06	6.36E-06	1.37E-05
Mg+2_Citric-3(2)	6.35E-06	1.20E-05	3.66E-06	1.07E-05	1.67E-05	1.81E-05	1.04E-05	1.13E-05	1.29E-05
Ca+2_H+1(2)_Citric-3(2)	3.43E-06	7.28E-08	5.15E-06	2.37E-07	1.67E-05	9.37E-07	4.80E-07	8.76E-08	2.14E-06
Ca+2_H+1_Citric-3	1.92E-06	2.10E-07	2.82E-06	4.78E-07	6.27E-06	9.44E-07	9.15E-07	3.35E-07	1.44E-06
Mg+2_H+1_Citric-3_PO4-3	1.19E-06	7.10E-06	8.13E-07	4.49E-06	2.58E-06	3.75E-06	5.68E-06	2.59E-06	3.04E-06
Ca+2_H+1_Citric-3_PO4-3	7.49E-07	2.70E-06	3.61E-07	3.97E-06	1.63E-06	2.79E-06	4.29E-06	1.55E-06	3.81E-06

Phosphate speciation: 0.76 µM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[PO4] mol/L								
H+1(2)_Na+1_PO4-3	4.66E-03	2.74E-05	6.17E-04	7.50E-04	2.72E-04	7.02E-04	4.54E-06	8.62E-05	3.61E-04
H+1(2)_PO4-3	1.91E-02	4.11E-04	6.83E-03	8.09E-03	3.53E-03	5.08E-03	4.71E-05	7.23E-04	2.98E-03
H+1_Na+1(2)_PO4-3	1.28E-04	4.03E-05	1.79E-05	3.21E-05	3.00E-05	1.34E-04	2.90E-05	1.55E-04	1.48E-04
H+1_Na+1_PO4-3	1.97E-03	1.73E-03	5.77E-04	1.05E-03	1.12E-03	3.08E-03	8.85E-04	4.14E-03	3.93E-03
H+1_K+1_PO4-3	3.80E-04	5.70E-04	1.27E-04	3.17E-04	2.75E-04	6.73E-04	1.86E-04	1.22E-03	5.84E-04
H+1(2)_K+1_PO4-3	1.00E-03	9.86E-06	1.48E-04	2.49E-04	7.31E-05	1.68E-04	1.04E-06	2.80E-05	5.90E-05
H+1(3)_PO4-3(2)	6.93E-05	3.12E-06	1.35E-05	3.08E-05	1.53E-05	4.20E-05	1.38E-07	9.29E-06	3.64E-05
H+1_K+1(2)_PO4-3	1.25E-05	1.04E-05	2.07E-06	7.17E-06	4.34E-06	1.58E-05	3.09E-06	3.36E-05	8.13E-06
H+1(4)_PO4-3(2)	4.58E-03	2.04E-06	5.68E-04	8.11E-04	1.51E-04	3.23E-04	1.92E-03	6.56E-06	1.12E-04
H+1_PO4-3	1.84E-03	5.29E-03	1.33E-03	2.40E-03	2.98E-03	4.86E-03	5.17E-04	7.56E-03	7.09E-03
Ca+2_Citric-3_PO4-3	3.28E-05	3.53E-04	2.39E-05	9.72E-05	8.69E-05	3.42E-04	9.63E-05	8.27E-04	7.19E-04
Ca+2_PO4-3	7.25E-07	1.07E-04	9.97E-07	1.82E-06	4.05E-06	1.01E-05	6.81E-08	7.77E-05	9.64E-06

Phosphate speciation: 0.76 µM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[PO4] mol/L								
H+1(2)_Na+1_PO4-3	1.76E-03	1.28E-03	1.74E-03	2.53E-03	2.90E-03	1.59E-03	3.17E-03	3.23E-04	3.19E-03
H+1(2)_PO4-3	1.42E-02	9.94E-03	1.64E-02	1.06E-02	1.71E-02	9.89E-03	1.43E-02	2.66E-03	1.64E-02
H+1_Na+1(2)_PO4-3	4.78E-05	2.34E-04	2.26E-05	5.65E-04	8.02E-05	1.59E-04	4.05E-04	7.48E-05	2.99E-04
H+1_Na+1_PO4-3	1.25E-03	6.12E-03	6.81E-04	8.83E-03	1.58E-03	3.51E-03	7.04E-03	1.95E-03	5.27E-03
H+1_K+1_PO4-3	3.74E-04	1.20E-03	1.66E-04	7.56E-04	2.63E-04	7.82E-04	1.31E-03	3.91E-04	5.79E-04
H+1(2)_K+1_PO4-3	5.82E-04	2.76E-04	4.66E-04	2.41E-04	5.34E-04	3.94E-04	6.59E-04	7.13E-05	3.88E-04
H+1(3)_PO4-3(2)	5.43E-05	1.90E-04	3.93E-05	1.74E-04	6.31E-05	9.19E-05	2.06E-04	1.57E-05	1.82E-04
H+1_K+1(2)_PO4-3	1.07E-05	2.27E-05	3.35E-06	1.09E-05	5.63E-06	2.04E-05	3.73E-05	7.46E-06	9.22E-06
H+1(4)_PO4-3(2)	2.54E-03	1.25E-03	3.37E-03	1.40E-03	3.69E-03	1.23E-03	2.55E-03	8.86E-05	3.38E-03
H+1_PO4-3	2.21E-03	1.06E-02	1.40E-03	8.37E-03	2.06E-03	4.90E-03	7.21E-03	3.48E-03	6.03E-03
Ca+2_Citric-3_PO4-3	2.81E-05	7.10E-04	9.02E-06	7.66E-04	5.34E-05	3.20E-04	5.25E-04	5.63E-04	3.13E-04
Ca+2_PO4-3	6.03E-07	8.33E-06	4.02E-07	7.69E-06	1.03E-06	2.83E-06	7.80E-06	1.11E-05	2.67E-06

Phosphate speciation: 1.50 mM Phy (black subjects)									
	BU1a	BU2a	BU3a	BU4a	BU5a	BU6a	BU7a	BU8a	BU9a
	[PO4] mol/L								
H+1(2)_Na+1_PO4-3	4.69E-03	2.12E-05	5.78E-04	7.01E-04	2.30E-04	6.38E-04	3.63E-06	7.20E-05	3.09E-04
H+1(2)_PO4-3	1.90E-02	3.35E-04	6.70E-03	7.80E-03	3.14E-03	4.69E-03	3.91E-05	6.12E-04	2.57E-03
H+1_Na+1(2)_PO4-3	1.27E-04	3.08E-05	1.66E-05	2.96E-05	2.50E-05	1.20E-04	2.28E-05	1.28E-04	1.25E-04
H+1_Na+1_PO4-3	2.22E-03	1.65E-03	6.41E-04	1.15E-03	1.16E-03	3.28E-03	8.64E-04	4.12E-03	4.00E-03
H+1_K+1_PO4-3	4.18E-04	5.26E-04	1.38E-04	3.41E-04	2.77E-04	6.96E-04	1.76E-04	1.18E-03	5.74E-04
H+1(2)_K+1_PO4-3	9.94E-04	7.48E-06	1.37E-04	2.29E-04	6.07E-05	1.51E-04	8.19E-07	2.30E-05	4.95E-05
H+1(3)_PO4-3(2)	8.31E-05	3.52E-06	1.98E-05	4.22E-05	2.02E-05	5.01E-05	1.54E-07	9.61E-06	3.90E-05
H+1_K+1(2)_PO4-3	1.25E-05	7.98E-06	1.93E-06	6.63E-06	3.63E-06	1.42E-05	2.44E-06	2.78E-05	6.87E-06
H+1(4)_PO4-3(2)	4.42E-03	1.42E-06	5.66E-04	7.69E-04	1.25E-04	2.76E-04	1.93E-08	4.70E-06	8.30E-05
H+1_PO4-3	2.06E-03	5.79E-03	1.65E-03	2.88E-03	3.53E-03	5.46E-03	2.09E-03	7.94E-03	7.59E-03
Ca+2_Citric-3_PO4-3	3.49E-05	3.42E-04	1.80E-05	9.57E-05	8.20E-05	3.40E-04	5.14E-04	8.40E-04	7.87E-04
Ca+2_PO4-3	5.20E-07	3.59E-05	2.59E-07	7.88E-07	1.47E-06	5.40E-06	4.23E-05	4.94E-05	6.91E-06

Phosphate speciation: 1.50 mM Phy (black subjects)									
	BU10a	BU11a	BU12a	B1a	B2a	B3a	B4a	B5a	B7a
	[PO4] mol/L								
H+1(2)_Na+1_PO4-3	1.70E-03	1.16E-03	1.70E-03	2.37E-03	2.87E-03	1.51E-03	3.06E-03	2.90E-04	3.03E-03
H+1(2)_PO4-3	1.39E-02	8.98E-03	1.63E-02	9.72E-03	1.71E-02	9.28E-03	1.36E-02	2.43E-03	1.55E-02
H+1_Na+1(2)_PO4-3	4.56E-05	2.08E-04	2.19E-05	5.20E-04	7.84E-05	1.48E-04	3.84E-04	6.62E-05	2.80E-04
H+1_Na+1_PO4-3	1.41E-03	6.51E-03	7.71E-04	9.54E-03	1.76E-03	3.86E-03	7.74E-03	2.04E-03	5.85E-03
H+1_K+1_PO4-3	4.12E-04	1.23E-03	1.84E-04	7.91E-04	2.88E-04	8.33E-04	1.40E-03	3.99E-04	6.23E-04
H+1(2)_K+1_PO4-3	5.54E-04	2.45E-04	4.52E-04	2.22E-04	5.23E-04	3.66E-04	6.27E-04	6.30E-05	3.63E-04
H+1(3)_PO4-3(2)	7.25E-05	2.13E-04	5.31E-05	1.87E-04	8.03E-05	1.06E-04	2.30E-04	1.82E-05	2.20E-04
H+1_K+1(2)_PO4-3	1.03E-05	2.02E-05	3.26E-06	1.01E-05	5.53E-06	1.91E-05	3.56E-05	6.62E-06	8.66E-06
H+1(4)_PO4-3(2)	2.43E-03	1.00E-03	3.36E-03	1.15E-03	3.65E-03	1.06E-03	2.23E-03	7.40E-05	3.00E-03
H+1_PO4-3	2.63E-03	1.16E-02	1.67E-03	8.97E-03	2.38E-03	5.43E-03	7.84E-03	3.86E-03	6.85E-03
Ca+2_Citric-3_PO4-3	3.24E-05	8.10E-04	8.37E-06	8.72E-04	5.20E-05	3.85E-04	5.91E-04	5.59E-04	3.93E-04
Ca+2_PO4-3	3.82E-07	6.78E-06	1.85E-07	6.60E-06	5.58E-07	2.38E-06	6.65E-06	5.52E-06	2.21E-06

Chemical speciation in urine of white subjects

Calcium speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ca] mol/L							
Ca+2	6.36E-04	1.14E-03	2.67E-04	2.97E-04	3.34E-04	9.40E-04	1.71E-03	2.59E-04
Ca+2_Citric-3	2.23E-04	3.84E-04	1.81E-04	4.23E-04	2.95E-04	4.83E-04	5.27E-04	2.62E-04
Ca+2_Citric-3_PO4-3	9.46E-05	3.64E-05	6.93E-04	4.55E-05	2.07E-04	1.66E-04	3.67E-05	3.82E-04
Ca+2_H+1(3)_PO4-3(2)	2.60E-04	2.36E-04	2.40E-05	1.98E-05	1.47E-04	2.00E-04	1.40E-04	1.61E-05
Ca+2_H+1_PO4-3	1.63E-04	1.28E-04	7.32E-05	3.30E-05	1.02E-04	2.39E-04	1.68E-04	5.88E-05
Ca+2_Cl-1	7.85E-05	1.26E-04	7.58E-05	4.29E-05	5.08E-05	8.37E-05	1.99E-04	3.15E-05
Ca+2_H+1(2)_PO4-3	5.12E-05	1.01E-04	4.06E-06	1.01E-05	2.41E-05	4.57E-05	7.89E-05	3.76E-06
Ca+2_Oxalic-2	1.30E-05	1.49E-05	6.67E-06	6.24E-06	9.99E-06	2.51E-05	2.86E-05	4.10E-06
Ca+2_Citric-3(2)	1.32E-06	2.72E-06	3.00E-06	6.09E-06	4.60E-06	2.21E-06	1.83E-06	3.61E-06
Ca+2_H+1_Citric-3	2.04E-06	8.28E-06	2.65E-07	4.24E-06	2.01E-06	3.09E-06	7.92E-06	5.14E-07
Ca+2_H+1(2)_PO4-3(2)	5.68E-06	2.26E-06	3.46E-06	3.67E-07	4.32E-06	5.69E-06	1.75E-06	1.59E-06
Ca+2_PO4-3	2.12E-06	6.72E-07	5.44E-06	4.23E-07	1.75E-06	4.88E-06	1.41E-06	3.69E-06
Ca+2_H+1(2)_Citric-3(2)	8.47E-07	9.21E-06	4.53E-08	5.59E-06	1.62E-06	8.68E-07	3.64E-06	1.14E-07
Ca+2(2)_Oxalic-2	1.81E-06	3.69E-06	3.86E-07	4.09E-07	7.30E-07	5.21E-06	1.08E-05	2.34E-07
Ca+2_H+1_Citric-3_PO4-3	1.75E-06	1.56E-06	2.02E-06	9.79E-07	2.84E-06	2.34E-06	1.17E-06	1.55E-06
Ca+2_H+1(2)_Citric-3_PO4-3	1.54E-06	3.29E-06	2.93E-07	9.54E-07	1.87E-06	1.46E-06	1.70E-06	2.92E-07
Ca+2_H+1(4)_PO4-3(2)	1.76E-06	3.92E-06	2.79E-08	1.37E-07	7.44E-07	8.68E-07	1.47E-06	2.26E-08
Ca+2(2)_H+1_Phy-12	1.16E-06	1.21E-06	1.50E-07	1.32E-06	6.27E-07	1.51E-06	1.51E-06	8.77E-07

Calcium speciation: 0.76 µM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ca] mol/L							
Ca+2	1.52E-03	5.53E-04	3.83E-04	3.30E-04	1.26E-04	3.06E-04	8.04E-04	2.96E-04
Ca+2_Citric-3	9.96E-04	5.03E-04	4.92E-04	1.69E-04	7.32E-05	2.85E-04	2.85E-04	1.65E-04
Ca+2_Citric-3_PO4-3	2.29E-04	7.86E-05	4.50E-05	1.22E-04	6.27E-04	1.24E-04	3.79E-04	5.87E-04
Ca+2_H+1(3)_PO4-3(2)	2.00E-04	1.41E-04	6.08E-05	4.99E-05	2.09E-06	9.04E-05	1.98E-04	5.58E-05
Ca+2_H+1_PO4-3	1.97E-04	1.21E-04	7.25E-05	5.93E-05	3.49E-05	6.80E-05	1.97E-04	1.23E-04
Ca+2_Cl-1	2.59E-04	4.27E-05	2.90E-05	5.70E-05	1.37E-05	4.96E-05	1.29E-04	2.92E-05
Ca+2_H+1(2)_PO4-3	7.38E-05	3.86E-05	2.12E-05	1.26E-05	4.10E-07	2.01E-05	3.57E-05	6.90E-06
Ca+2_Oxalic-2	3.58E-05	1.69E-05	6.02E-06	5.33E-06	2.21E-06	1.02E-05	1.31E-05	6.53E-06
Ca+2_Citric-3(2)	1.38E-05	3.64E-06	3.24E-06	2.31E-06	5.17E-07	4.94E-06	3.08E-06	1.45E-06
Ca+2_H+1_Citric-3	1.02E-05	5.50E-06	5.33E-06	9.17E-07	2.70E-08	2.38E-06	1.26E-06	2.75E-07
Ca+2_H+1(2)_PO4-3(2)	4.01E-06	2.32E-06	9.54E-07	1.96E-06	1.08E-06	2.18E-06	9.79E-06	6.61E-06
Ca+2_PO4-3	2.16E-06	1.46E-06	9.27E-07	1.15E-06	1.18E-05	9.39E-07	4.52E-06	8.85E-06
Ca+2_H+1(2)_Citric-3(2)	1.06E-05	4.36E-06	4.58E-06	4.75E-07	6.03E-10	2.60E-06	4.14E-07	3.16E-08
Ca+2(2)_Oxalic-2	1.18E-05	2.08E-06	5.14E-07	3.80E-07	6.12E-08	6.79E-07	2.26E-06	4.23E-07
Ca+2_H+1_Citric-3_PO4-3	4.69E-06	1.93E-06	1.19E-06	1.33E-06	4.84E-07	2.09E-06	3.40E-06	1.98E-06
Ca+2_H+1(2)_Citric-3_PO4-3	4.72E-06	2.08E-06	1.33E-06	7.27E-07	1.73E-08	1.70E-06	1.56E-06	3.18E-07
Ca+2_H+1(4)_PO4-3(2)	1.59E-06	1.04E-06	4.25E-07	2.21E-07	5.44E-10	5.72E-07	7.43E-07	6.79E-08
Ca+2(2)_H+1_Phy-12	1.32E-06	1.50E-06	1.51E-06	1.53E-07	5.18E-07	4.84E-07	5.25E-07	7.36E-07

Calcium speciation: 1.50 mM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ca] mol/L							
Ca+2	4.88E-04	8.27E-04	2.50E-04	2.21E-04	3.02E-04	3.72E-04	6.10E-04	2.11E-04
Ca+2_Citric-3	1.50E-04	2.68E-04	1.31E-04	2.54E-04	2.14E-04	2.15E-04	2.36E-04	1.65E-04
Ca+2_Citric-3_PO4-3	1.15E-04	4.21E-05	7.61E-04	6.03E-05	2.71E-04	1.51E-04	2.88E-05	4.47E-04
Ca+2_H+1(3)_PO4-3(2)	1.68E-04	1.54E-04	1.67E-05	1.14E-05	1.08E-04	6.43E-05	4.49E-05	9.05E-06
Ca+2_H+1_PO4-3	1.13E-04	8.73E-05	5.87E-05	2.06E-05	8.13E-05	8.15E-05	5.48E-05	3.85E-05
Ca+2_Cl-1	5.15E-05	8.06E-05	6.21E-05	2.59E-05	3.91E-05	2.75E-05	6.18E-05	2.14E-05
Ca+2_H+1(2)_PO4-3	3.24E-05	6.32E-05	2.98E-06	5.80E-06	1.76E-05	1.45E-05	2.44E-05	2.25E-06
Ca+2_Oxalic-2	9.00E-06	1.04E-05	5.65E-06	3.77E-06	7.87E-06	9.35E-06	1.10E-05	2.90E-06
Ca+2_Citric-3(2)	1.38E-06	2.88E-06	2.62E-06	6.62E-06	4.75E-06	2.29E-06	1.77E-06	3.50E-06
Ca+2_H+1_Citric-3	1.07E-06	4.60E-06	1.48E-07	1.92E-06	1.12E-06	1.09E-06	2.95E-06	2.45E-07
Ca+2_H+1(2)_PO4-3(2)	5.16E-06	1.99E-06	3.36E-06	3.16E-07	4.55E-06	2.56E-06	7.30E-07	1.31E-06
Ca+2_PO4-3	1.62E-06	5.00E-07	4.80E-06	3.01E-07	1.56E-06	1.86E-06	5.04E-07	2.73E-06
Ca+2_H+1(2)_Citric-3(2)	4.77E-07	5.80E-06	2.31E-08	2.71E-06	8.88E-07	4.44E-07	2.06E-06	5.37E-08
Ca+2(2)_Oxalic-2	9.47E-07	1.84E-06	3.01E-07	1.81E-07	5.11E-07	7.60E-07	1.46E-06	1.33E-07
Ca+2_H+1_Citric-3_PO4-3	1.65E-06	1.48E-06	1.82E-06	9.09E-07	2.88E-06	1.54E-06	7.20E-07	1.33E-06
Ca+2_H+1(2)_Citric-3_PO4-3	1.21E-06	2.68E-06	2.28E-07	6.77E-07	1.56E-06	7.57E-07	8.75E-07	2.00E-07
Ca+2_H+1(4)_PO4-3(2)	1.00E-06	2.30E-06	1.74E-08	6.79E-08	4.82E-07	2.46E-07	4.26E-07	1.10E-08
Ca+2(2)_H+1_Phy-12	3.98E-04	6.43E-04	3.88E-05	2.81E-04	1.31E-04	1.26E-03	1.84E-03	1.23E-04

Calcium speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ca] mol/L							
Ca+2	1.07E-03	2.69E-04	1.57E-04	3.13E-04	1.02E-04	2.83E-04	7.32E-04	2.47E-04
Ca+2_Citric-3	7.07E-04	2.29E-04	1.83E-04	1.39E-04	4.25E-05	2.16E-04	2.29E-04	1.06E-04
Ca+2_Citric-3_PO4-3	2.57E-04	8.26E-05	4.74E-05	1.54E-04	6.71E-04	1.68E-04	4.43E-04	6.60E-04
Ca+2_H+1(3)_PO4-3(2)	1.24E-04	5.41E-05	1.81E-05	3.89E-05	1.02E-06	6.93E-05	1.50E-04	3.25E-05
Ca+2_H+1_PO4-3	1.29E-04	4.92E-05	2.33E-05	5.08E-05	2.10E-05	5.62E-05	1.64E-04	8.33E-05
Ca+2_Cl-1	1.61E-04	1.67E-05	9.04E-06	4.77E-05	9.06E-06	3.93E-05	1.05E-04	2.05E-05
Ca+2_H+1(2)_PO4-3	4.49E-05	1.46E-05	6.28E-06	9.89E-06	2.26E-07	1.52E-05	2.72E-05	4.28E-06
Ca+2_Oxalic-2	2.48E-05	7.04E-06	1.88E-06	4.69E-06	1.45E-06	8.29E-06	1.13E-05	4.72E-06
Ca+2_Citric-3(2)	1.52E-05	3.65E-06	3.28E-06	2.48E-06	4.56E-07	5.38E-06	3.11E-06	1.33E-06
Ca+2_H+1_Citric-3	5.90E-06	1.92E-06	1.47E-06	5.88E-07	1.18E-08	1.39E-06	8.00E-07	1.34E-07
Ca+2_H+1(2)_PO4-3(2)	3.30E-06	1.31E-06	4.44E-07	2.11E-06	7.79E-07	2.38E-06	1.00E-05	5.56E-06
Ca+2_PO4-3	1.53E-06	6.81E-07	3.50E-07	1.08E-06	8.10E-06	8.63E-07	4.08E-06	6.73E-06
Ca+2_H+1(2)_Citric-3(2)	7.22E-06	1.92E-06	1.68E-06	3.07E-07	2.46E-10	1.53E-06	2.64E-07	1.48E-08
Ca+2(2)_Oxalic-2	5.69E-06	4.13E-07	6.45E-08	3.12E-07	3.18E-08	5.04E-07	1.76E-06	2.51E-07
Ca+2_H+1_Citric-3_PO4-3	4.37E-06	1.39E-06	7.74E-07	1.40E-06	3.72E-07	2.21E-06	3.40E-06	1.69E-06
Ca+2_H+1(2)_Citric-3_PO4-3	3.82E-06	1.13E-06	5.99E-07	6.68E-07	1.04E-08	1.49E-06	1.38E-06	2.20E-07
Ca+2_H+1(4)_PO4-3(2)	8.95E-07	3.42E-07	1.06E-07	1.55E-07	2.28E-10	3.86E-07	5.06E-07	3.46E-08
Ca+2(2)_H+1_Phy-12	1.01E-03	7.86E-04	6.76E-04	4.81E-05	3.60E-05	1.00E-04	1.83E-04	1.17E-04

Phytate speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Phy] mol/L							
H+1_Phy-12	1.80E-07	1.56E-07	6.81E-07	9.99E-08	4.36E-07	6.45E-09	5.59E-09	3.21E-07
Ca+2(2)_H+1_Phy-12	5.80E-07	6.04E-07	7.49E-08	6.60E-07	3.14E-07	7.54E-07	7.54E-07	4.38E-07
Phy-12	1.12E-10	5.07E-11	3.74E-09	3.40E-11	3.82E-10	3.01E-12	1.42E-12	7.56E-10
H+1(2)_Phy-12	2.44E-13	4.14E-13	1.09E-13	2.34E-13	4.22E-13	1.09E-14	1.77E-14	1.12E-13
Ca+2(2)_H+1(2)_Phy-12	3.75E-16	8.46E-16	6.77E-18	5.90E-16	1.48E-16	4.58E-16	9.59E-16	6.67E-17
H+1(3)_Phy-12	1.01E-19	3.47E-19	5.61E-21	1.59E-19	1.26E-19	5.25E-21	1.65E-20	1.18E-20
Ca+2(2)_H+1(3)_Phy-12	9.97E-25	3.94E-24	1.11E-26	2.01E-24	5.95E-25	2.71E-25	9.65E-25	7.20E-26
Ca+2_H+1(3)_Phy-12	5.64E-25	2.83E-24	1.48E-27	1.16E-24	1.64E-25	6.05E-25	2.72E-24	2.31E-26
H+1(4)_Phy-12	2.70E-27	1.91E-26	1.93E-29	6.55E-27	2.44E-27	1.52E-28	9.45E-28	7.73E-29
Ca+2_H+1(4)_Phy-12	2.63E-32	2.25E-31	4.08E-35	7.30E-32	1.15E-32	6.71E-33	5.00E-32	4.46E-34
H+1(5)_Phy-12	4.84E-35	7.23E-34	0	1.72E-34	3.18E-35	2.74E-36	3.48E-35	3.33E-37
Ca+2_H+1(5)_Phy-12	0	0	0	0	0	0	0	0
Ca+2_H+1(6)_Phy-12	0	0	0	0	0	0	0	0
H+1(6)_Phy-12	0	0	0	0	0	0	0	0
H+1(8)_Phy-12	0	0	0	0	0	0	0	0

Phytate speciation: 0.76 µM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Phy] mol/L							
H+1_Phy-12	9.86E-08	1.13E-08	3.39E-09	6.82E-07	4.95E-07	5.18E-07	6.96E-07	3.91E-07
Ca+2(2)_H+1_Phy-12	6.61E-07	7.49E-07	7.57E-07	7.65E-08	2.59E-07	2.42E-07	2.62E-07	3.68E-07
Phy-12	6.75E-11	2.75E-12	5.13E-13	1.09E-09	5.55E-09	3.89E-10	1.51E-09	1.25E-09
H+1(2)_Phy-12	1.25E-13	3.63E-14	1.65E-14	3.81E-13	3.60E-14	5.88E-13	2.90E-13	1.02E-13
Ca+2(2)_H+1(2)_Phy-12	4.41E-16	8.23E-16	1.04E-15	2.51E-17	7.80E-18	1.38E-16	6.77E-17	4.46E-17
H+1(3)_Phy-12	4.96E-20	3.29E-20	2.17E-20	6.88E-20	7.74E-22	2.08E-19	3.95E-20	8.14E-21
Ca+2(2)_H+1(3)_Phy-12	7.41E-25	1.24E-24	1.37E-24	1.40E-25	2.86E-27	7.98E-25	1.52E-25	4.27E-26
Ca+2_H+1(3)_Phy-12	7.04E-25	1.94E-24	2.94E-24	2.01E-26	5.28E-28	1.85E-25	4.35E-26	1.25E-26
H+1(4)_Phy-12	1.30E-27	1.76E-27	1.61E-27	8.39E-28	1.03E-30	4.76E-27	3.69E-28	4.13E-29
Ca+2_H+1(4)_Phy-12	2.02E-32	5.57E-32	7.71E-32	1.87E-33	3.51E-36	1.85E-32	1.60E-33	2.11E-34
H+1(5)_Phy-12	2.34E-35	5.82E-35	7.01E-35	7.24E-36	0	7.41E-35	2.47E-36	1.40E-37
Ca+2_H+1(5)_Phy-12	0	0	0	0	0	0	0	0
Ca+2_H+1(6)_Phy-12	0	0	0	0	0	0	0	0
H+1(6)_Phy-12	0	0	0	0	0	0	0	0
H+1(8)_Phy-12	0	0	0	0	0	0	0	0

Phytate speciation: 1.50 mM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Phy] mol/L							
H+1_Phy-12	1.30E-03	1.18E-03	1.47E-03	1.36E-03	1.43E-03	8.67E-04	5.81E-04	1.43E-03
Ca+2(2)_H+1_Phy-12	1.99E-04	3.22E-04	1.94E-05	1.40E-04	6.56E-05	6.32E-04	9.19E-04	6.15E-05
Phy-12	1.73E-06	7.03E-07	1.46E-05	1.35E-06	2.70E-06	1.06E-06	3.03E-07	8.43E-06
H+1(2)_Phy-12	8.80E-10	1.80E-09	1.36E-10	1.19E-09	6.87E-10	6.03E-10	9.57E-10	2.16E-10
Ca+2(2)_H+1(2)_Phy-12	8.28E-14	3.16E-13	1.24E-15	6.72E-14	1.98E-14	2.19E-13	7.69E-13	5.48E-15
H+1(3)_Phy-12	1.95E-16	9.09E-16	4.26E-18	3.32E-16	1.09E-16	1.30E-16	4.91E-16	1.06E-17
Ca+2(2)_H+1(3)_Phy-12	8.54E-23	7.82E-22	2.02E-25	7.75E-23	1.50E-23	1.78E-22	1.52E-21	1.20E-24
Ca+2_H+1(3)_Phy-12	4.75E-22	3.03E-21	3.23E-24	6.26E-22	1.47E-22	6.21E-22	3.50E-21	1.34E-23
H+1(4)_Phy-12	2.95E-24	3.18E-23	9.36E-27	6.14E-24	1.18E-24	1.82E-24	1.64E-23	3.49E-26
Ca+2_H+1(4)_Phy-12	8.04E-30	1.21E-28	8.39E-33	1.22E-29	1.81E-30	8.76E-30	1.19E-28	4.85E-32
H+1(5)_Phy-12	3.19E-32	8.04E-31	1.51E-35	7.87E-32	9.23E-33	1.72E-32	3.76E-31	8.19E-35
Ca+2_H+1(5)_Phy-12	7.57E-37	2.74E-35	0.00E+00	1.28E-36	1.24E-37	6.50E-37	2.16E-35	0.00E+00
Ca+2_H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(8)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Phytate speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Phy] mol/L							
H+1_Phy-12	9.95E-04	1.11E-03	1.16E-03	1.47E-03	1.44E-03	1.45E-03	1.40E-03	1.43E-03
Ca+2(2)_H+1_Phy-12	5.04E-04	3.93E-04	3.38E-04	2.41E-05	1.80E-05	5.00E-05	9.14E-05	5.83E-05
Phy-12	1.20E-06	8.35E-07	7.55E-07	4.05E-06	4.35E-05	2.27E-06	4.88E-06	1.06E-05
H+1(2)_Phy-12	7.50E-10	1.25E-09	1.49E-09	4.99E-10	4.21E-11	8.39E-10	3.79E-10	1.74E-10
Ca+2(2)_H+1(2)_Phy-12	2.42E-13	2.23E-13	1.99E-13	5.73E-15	3.04E-16	1.85E-14	1.79E-14	4.35E-15
H+1(3)_Phy-12	1.87E-16	4.41E-16	5.83E-16	5.72E-17	3.96E-19	1.61E-16	3.49E-17	6.94E-18
Ca+2(2)_H+1(3)_Phy-12	2.91E-22	2.98E-22	2.71E-22	3.50E-24	1.25E-26	1.72E-23	9.09E-24	8.03E-25
Ca+2_H+1(3)_Phy-12	8.42E-22	1.48E-21	1.69E-21	4.87E-23	2.67E-25	1.90E-22	6.03E-23	8.70E-24
H+1(4)_Phy-12	3.20E-24	1.01E-23	1.45E-23	4.63E-25	2.51E-28	2.12E-24	2.29E-25	1.88E-26
Ca+2_H+1(4)_Phy-12	1.65E-29	3.41E-29	4.07E-29	4.72E-31	1.84E-34	2.86E-30	4.82E-31	2.64E-32
H+1(5)_Phy-12	3.97E-32	1.57E-31	2.39E-31	2.77E-33	1.12E-37	2.02E-32	1.12E-33	3.66E-35
Ca+2_H+1(5)_Phy-12	1.81E-36	4.17E-36	5.04E-36	0.00E+00	0.00E+00	2.42E-37	0.00E+00	0.00E+00
Ca+2_H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(6)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
H+1(8)_Phy-12	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Oxalate speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ox] mol/L							
Oxalic-2	9.69E-05	6.69E-05	0.0001341	8.26E-05	0.0001441	9.95E-05	6.84E-05	6.94E-05
Na+1_Oxalic-2	2.89E-05	2.82E-05	1.93E-05	1.39E-05	3.52E-05	1.96E-05	1.76E-05	2.16E-05
Mg+2_Oxalic-2	1.74E-05	1.63E-05	1.19E-05	1.11E-05	1.48E-05	3.89E-05	3.88E-05	2.19E-05
Ca+2_Oxalic-2	1.30E-05	1.49E-05	6.67E-06	6.24E-06	9.99E-06	2.51E-05	2.86E-05	4.10E-06
K+1_Oxalic-2	6.89E-06	7.09E-06	5.27E-06	3.88E-06	9.38E-06	5.57E-06	3.04E-06	3.28E-06
Ca+2(2)_Oxalic-2	9.07E-07	1.84E-06	1.93E-07	2.05E-07	3.65E-07	2.61E-06	5.39E-06	1.17E-07
Mg+2_H+1_Oxalic-2_PO4-3	8.81E-07	4.29E-07	8.65E-07	1.71E-07	9.16E-07	1.26E-06	5.65E-07	8.40E-07
H+1_Oxalic-2	5.20E-07	8.72E-07	1.22E-07	4.69E-07	5.80E-07	3.60E-07	5.86E-07	7.84E-08
Mg+2_Oxalic-2_Citric-3	1.52E-07	1.67E-07	2.84E-07	2.56E-07	3.38E-07	2.92E-07	2.12E-07	4.59E-07
Ca+2_H+1_Oxalic-2_PO4-3	1.83E-07	1.01E-07	1.18E-07	3.03E-08	1.70E-07	2.63E-07	1.28E-07	4.64E-08
Mg+2(2)_Oxalic-2	6.81E-08	9.78E-08	2.86E-08	2.47E-08	3.37E-08	2.35E-07	3.85E-07	1.34E-07
Mg+2_Oxalic-2(2)	7.14E-08	4.85E-08	7.41E-08	3.55E-08	9.08E-08	1.47E-07	1.04E-07	6.16E-08
Ca+2_Oxalic-2_Citric-3	2.82E-08	3.52E-08	3.45E-08	4.05E-08	5.60E-08	5.42E-08	4.29E-08	2.26E-08
Ca+2_H+1_Oxalic-2_Citric-3	2.48E-08	7.41E-08	5.00E-09	3.94E-08	3.68E-08	3.39E-08	6.23E-08	4.26E-09
Ca+2_Oxalic-2(2)	3.36E-08	2.65E-08	2.38E-08	1.37E-08	3.83E-08	6.63E-08	5.20E-08	7.57E-09

Oxalate speciation: 0.76 µM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ox] mol/L							
Oxalic-2	1.21E-04	1.10E-04	4.71E-05	8.93E-05	7.37E-05	1.63E-04	9.34E-05	1.02E-04
Na+1_Oxalic-2	3.99E-05	2.24E-05	5.95E-06	4.70E-05	2.36E-05	4.95E-05	5.61E-05	3.72E-05
Mg+2_Oxalic-2	2.47E-05	3.36E-05	8.68E-06	1.21E-05	6.37E-06	2.07E-05	1.29E-05	1.86E-05
Ca+2_Oxalic-2	3.58E-05	1.69E-05	6.02E-06	5.33E-06	2.21E-06	1.02E-05	1.31E-05	6.53E-06
K+1_Oxalic-2	1.05E-05	3.96E-06	1.53E-06	5.61E-06	2.60E-06	7.38E-06	6.58E-06	5.35E-06
Ca+2(2)_Oxalic-2	5.91E-06	1.04E-06	2.57E-07	1.90E-07	3.06E-08	3.40E-07	1.13E-06	2.12E-07
Mg+2_H+1_Oxalic-2_PO4-3	7.54E-07	8.73E-07	1.50E-07	6.26E-07	2.76E-07	9.81E-07	1.02E-06	1.44E-06
H+1_Oxalic-2	7.55E-07	6.74E-07	2.87E-07	3.08E-07	1.56E-08	8.14E-07	2.71E-07	9.91E-08
Mg+2_Oxalic-2_Citric-3	4.97E-07	4.08E-07	1.06E-07	2.38E-07	6.98E-08	5.27E-07	2.00E-07	2.43E-07
Ca+2_H+1_Oxalic-2_PO4-3	2.82E-07	1.45E-07	3.68E-08	6.44E-08	2.90E-08	1.29E-07	2.28E-07	1.44E-07
Mg+2(2)_Oxalic-2	1.25E-07	1.50E-07	1.87E-08	4.70E-08	1.00E-08	6.07E-08	5.50E-08	7.06E-08
Mg+2_Oxalic-2(2)	1.33E-07	1.38E-07	1.46E-08	5.17E-08	1.87E-08	1.46E-07	5.99E-08	7.89E-08
Ca+2_Oxalic-2_Citric-3	1.65E-07	6.05E-08	2.31E-08	2.18E-08	6.53E-09	6.19E-08	3.97E-08	2.16E-08
Ca+2_H+1_Oxalic-2_Citric-3	1.66E-07	6.53E-08	2.57E-08	1.20E-08	2.33E-10	5.03E-08	1.81E-08	3.47E-09
Ca+2_Oxalic-2(2)	1.15E-07	4.93E-08	7.54E-09	1.27E-08	4.33E-09	4.41E-08	3.24E-08	1.77E-08

Oxalate speciation: 1.50 mM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ox] mol/L							
Oxalic-2	1.05E-04	7.39E-05	1.38E-04	8.95E-05	1.51E-04	1.23E-04	8.97E-05	7.63E-05
Na+1_Oxalic-2	2.92E-05	2.96E-05	1.91E-05	1.34E-05	3.44E-05	2.15E-05	2.12E-05	2.16E-05
Mg+2_Oxalic-2	1.32E-05	1.29E-05	9.11E-06	7.55E-06	1.08E-05	3.09E-05	3.53E-05	1.62E-05
Ca+2_Oxalic-2	9.00E-06	1.04E-05	5.65E-06	3.77E-06	7.87E-06	9.35E-06	1.10E-05	2.90E-06
K+1_Oxalic-2	6.97E-06	7.46E-06	5.23E-06	3.73E-06	9.20E-06	6.13E-06	3.67E-06	3.29E-06
Ca+2(2)_Oxalic-2	4.74E-07	9.21E-07	1.51E-07	9.04E-08	2.56E-07	3.80E-07	7.31E-07	6.63E-08
Mg+2_H+1_Oxalic-2_PO4-3	9.65E-07	4.81E-07	8.81E-07	1.75E-07	9.74E-07	1.43E-06	6.97E-07	8.59E-07
H+1_Oxalic-2	4.87E-07	8.53E-07	1.11E-07	4.15E-07	5.23E-07	3.71E-07	6.72E-07	7.22E-08
Mg+2_Oxalic-2_Citric-3	1.74E-07	2.01E-07	2.68E-07	2.84E-07	3.47E-07	4.82E-07	3.86E-07	4.92E-07
Ca+2_H+1_Oxalic-2_PO4-3	1.46E-07	8.10E-08	1.05E-07	2.20E-08	1.53E-07	1.17E-07	5.76E-08	3.58E-08
Mg+2(2)_Oxalic-2	5.07E-08	7.41E-08	2.18E-08	1.65E-08	2.45E-08	1.78E-07	3.28E-07	9.90E-08
Mg+2_Oxalic-2(2)	6.88E-08	4.92E-08	6.92E-08	3.07E-08	8.26E-08	1.64E-07	1.38E-07	5.92E-08
Ca+2_Oxalic-2_Citric-3	2.35E-08	3.01E-08	2.84E-08	3.17E-08	4.85E-08	3.51E-08	2.85E-08	1.83E-08
Ca+2_H+1_Oxalic-2_Citric-3	1.71E-08	5.45E-08	3.56E-09	2.36E-08	2.64E-08	1.73E-08	3.46E-08	2.75E-09
Ca+2_Oxalic-2(2)	2.52E-08	2.04E-08	2.08E-08	8.97E-09	3.17E-08	3.06E-08	2.62E-08	5.89E-09

Oxalate speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ox] mol/L							
Oxalic-2	1.36E-04	1.29E-04	5.49E-05	9.28E-05	7.77E-05	1.72E-04	9.71E-05	1.09E-04
Na+1_Oxalic-2	4.28E-05	2.29E-05	5.73E-06	4.73E-05	2.25E-05	4.88E-05	5.70E-05	3.68E-05
Mg+2_Oxalic-2	1.98E-05	2.43E-05	5.56E-06	9.12E-06	4.52E-06	1.51E-05	1.00E-05	1.40E-05
Ca+2_Oxalic-2	2.48E-05	7.04E-06	1.88E-06	4.69E-06	1.45E-06	8.29E-06	1.13E-05	4.72E-06
K+1_Oxalic-2	1.13E-05	4.07E-06	1.48E-06	5.64E-06	2.47E-06	7.29E-06	6.70E-06	5.29E-06
Ca+2(2)_Oxalic-2	2.84E-06	2.06E-07	3.22E-08	1.56E-07	1.59E-08	2.52E-07	8.78E-07	1.26E-07
Mg+2_H+1_Oxalic-2_PO4-3	8.24E-07	9.52E-07	1.52E-07	6.50E-07	2.59E-07	1.04E-06	1.08E-06	1.48E-06
H+1_Oxalic-2	7.59E-07	6.43E-07	2.57E-07	2.85E-07	1.36E-08	7.39E-07	2.54E-07	8.99E-08
Mg+2_Oxalic-2_Citric-3	6.14E-07	5.67E-07	1.49E-07	2.42E-07	6.97E-08	5.44E-07	2.05E-07	2.53E-07
Ca+2_H+1_Oxalic-2_PO4-3	2.19E-07	7.40E-08	1.47E-08	6.15E-08	1.98E-08	1.21E-07	2.11E-07	1.12E-07
Mg+2(2)_Oxalic-2	9.32E-08	1.06E-07	1.16E-08	3.38E-08	7.33E-09	4.34E-08	4.12E-08	5.45E-08
Mg+2_Oxalic-2(2)	1.38E-07	1.36E-07	1.27E-08	4.78E-08	1.66E-08	1.33E-07	5.72E-08	7.52E-08
Ca+2_Oxalic-2_Citric-3	1.46E-07	3.93E-08	1.28E-08	2.04E-08	4.73E-09	5.62E-08	3.60E-08	1.70E-08
Ca+2_H+1_Oxalic-2_Citric-3	1.27E-07	3.20E-08	9.91E-09	9.77E-09	1.31E-10	3.80E-08	1.46E-08	2.22E-09
Ca+2_Oxalic-2(2)	9.01E-08	2.41E-08	2.74E-09	1.16E-08	2.99E-09	3.78E-08	2.93E-08	1.37E-08

Magnesium speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ox] mol/L							
Mg+2	6.11E-04	9.61E-04	3.91E-04	3.34E-04	3.59E-04	9.01E-04	1.50E-03	9.36E-04
Mg+2_Citric-3	1.52E-04	2.43E-04	2.07E-04	3.09E-04	2.28E-04	2.95E-04	3.05E-04	6.45E-04
Mg+2_H+1_PO4-3	2.12E-04	1.45E-04	1.41E-04	5.12E-05	1.47E-04	3.18E-04	2.02E-04	2.90E-04
Mg+2_H+1(2)_PO4-3	1.64E-04	2.82E-04	1.97E-05	3.80E-05	8.59E-05	1.46E-04	2.31E-04	4.53E-05
Mg+2_Cl-1	7.40E-05	1.06E-04	1.11E-04	4.65E-05	5.37E-05	7.69E-05	1.69E-04	1.11E-04
Mg+2_H+1(3)_PO4-3(2)	8.53E-05	7.19E-05	1.33E-05	6.88E-06	5.43E-05	5.78E-05	3.86E-05	1.90E-05
Mg+2_Oxalic-2	1.74E-05	1.63E-05	1.19E-05	1.11E-05	1.48E-05	3.89E-05	3.88E-05	2.19E-05
Mg+2_Citric-3(2)	1.39E-06	2.67E-06	5.31E-06	6.89E-06	5.49E-06	2.09E-06	1.64E-06	1.38E-05
Mg+2_H+1(2)_PO4-3(2)	5.46E-06	1.91E-06	5.07E-06	4.13E-07	4.65E-06	5.45E-06	1.54E-06	5.75E-06
Mg+2_H+1_Citric-3_PO4-3	1.88E-06	1.48E-06	3.32E-06	1.24E-06	3.42E-06	2.51E-06	1.15E-06	6.27E-06
Mg+2_H+1_Citric-3	1.19E-06	4.40E-06	2.50E-07	2.74E-06	1.32E-06	1.68E-06	4.04E-06	1.10E-06
Mg+2(2)_H+1(2)_PO4-3(2)	9.00E-07	4.26E-07	4.13E-07	5.08E-08	4.36E-07	1.95E-06	7.98E-07	1.66E-06

Magnesium speciation: 0.76 µM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ox] mol/L							
Mg+2	8.09E-04	6.64E-04	3.13E-04	6.39E-04	2.40E-04	4.63E-04	7.19E-04	5.91E-04
Mg+2_Citric-3	3.99E-04	3.79E-04	2.41E-04	2.63E-04	9.30E-05	3.13E-04	2.14E-04	2.31E-04
Mg+2_H+1_PO4-3	1.40E-04	2.02E-04	8.33E-05	1.51E-04	9.09E-05	1.38E-04	2.29E-04	3.33E-04
Mg+2_H+1(2)_PO4-3	1.31E-04	1.55E-04	5.80E-05	8.08E-05	2.60E-06	1.01E-04	1.05E-04	4.59E-05
Mg+2_Cl-1	1.37E-04	4.90E-05	2.25E-05	1.11E-04	2.52E-05	7.40E-05	1.17E-04	5.71E-05
Mg+2_H+1(3)_PO4-3(2)	3.85E-05	5.04E-05	1.40E-05	3.77E-05	1.27E-06	4.79E-05	7.26E-05	3.74E-05
Mg+2_Oxalic-2	2.47E-05	3.36E-05	8.68E-06	1.21E-05	6.37E-06	2.07E-05	1.29E-05	1.86E-05
Mg+2_Citric-3(2)	8.56E-06	4.25E-06	2.45E-06	5.56E-06	1.02E-06	8.42E-06	3.58E-06	3.15E-06
Mg+2_H+1(2)_PO4-3(2)	2.14E-06	2.78E-06	7.78E-07	3.81E-06	2.05E-06	3.31E-06	8.76E-06	1.32E-05
Mg+2_H+1_Citric-3_PO4-3	2.81E-06	2.59E-06	1.09E-06	2.89E-06	1.03E-06	3.54E-06	3.41E-06	4.45E-06
Mg+2_H+1_Citric-3	3.44E-06	3.71E-06	2.37E-06	1.17E-06	3.01E-08	2.23E-06	7.64E-07	3.31E-07
Mg+2(2)_H+1(2)_PO4-3(2)	4.00E-07	7.80E-07	1.31E-07	4.74E-07	1.62E-07	3.85E-07	1.11E-06	2.20E-06

Magnesium speciation: 1.50 mM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Ox] mol/L							
Mg+2	6.44E-04	4.19E-04	3.91E-04	3.52E-04	3.83E-04	9.09E-04	1.47E-03	1.01E-03
Mg+2_Citric-3	1.65E-04	2.03E-04	2.07E-04	3.09E-04	2.31E-04	3.81E-04	4.18E-04	6.37E-04
Mg+2_H+1_PO4-3	1.93E-04	1.25E-04	1.41E-04	4.36E-05	1.34E-04	2.68E-04	1.77E-04	2.41E-04
Mg+2_H+1(2)_PO4-3	1.41E-04	1.64E-05	1.97E-05	3.06E-05	7.34E-05	1.18E-04	1.96E-04	3.56E-05
Mg+2_Cl-1	6.87E-05	1.08E-04	1.11E-04	4.10E-05	5.04E-05	6.61E-05	1.47E-04	1.03E-04
Mg+2_H+1(3)_PO4-3(2)	8.99E-05	1.28E-05	1.33E-05	6.74E-06	5.69E-05	5.48E-05	3.82E-05	1.70E-05
Mg+2_Oxalic-2	1.32E-05	9.11E-06	1.19E-05	7.55E-06	1.08E-05	3.09E-05	3.53E-05	1.62E-05
Mg+2_Citric-3(2)	2.34E-06	6.32E-06	5.31E-06	1.25E-05	7.95E-06	6.29E-06	4.85E-06	2.09E-05
Mg+2_H+1(2)_PO4-3(2)	6.80E-06	5.64E-06	5.07E-06	5.03E-07	5.77E-06	6.26E-06	1.76E-06	6.25E-06
Mg+2_H+1_Citric-3_PO4-3	2.44E-06	3.43E-06	3.32E-06	1.63E-06	4.10E-06	4.22E-06	1.95E-06	7.15E-06
Mg+2_H+1_Citric-3	9.47E-07	1.79E-07	2.50E-07	1.95E-06	9.66E-07	1.64E-06	4.42E-06	7.74E-07
Mg+2(2)_H+1(2)_PO4-3(2)	7.83E-07	3.41E-07	4.13E-07	3.89E-08	3.79E-07	1.44E-06	6.36E-07	1.22E-06

Magnesium speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Ox] mol/L							
Mg+2	7.98E-04	6.89E-04	3.25E-04	6.59E-04	2.67E-04	4.88E-04	7.43E-04	6.52E-04
Mg+2_Citric-3	4.56E-04	4.27E-04	2.64E-04	2.78E-04	8.83E-05	3.21E-04	2.30E-04	2.32E-04
Mg+2_H+1_PO4-3	1.24E-04	1.70E-04	6.55E-05	1.35E-04	7.24E-05	1.25E-04	2.07E-04	2.87E-04
Mg+2_H+1(2)_PO4-3	1.11E-04	1.24E-04	4.33E-05	6.82E-05	1.95E-06	8.59E-05	9.01E-05	3.73E-05
Mg+2_Cl-1	1.23E-04	4.21E-05	1.83E-05	1.04E-04	2.38E-05	6.89E-05	1.12E-04	5.48E-05
Mg+2_H+1(3)_PO4-3(2)	3.91E-05	4.85E-05	1.25E-05	3.85E-05	1.02E-06	5.02E-05	7.46E-05	3.47E-05
Mg+2_Oxalic-2	1.98E-05	2.43E-05	5.56E-06	9.12E-06	4.52E-06	1.51E-05	1.00E-05	1.40E-05
Mg+2_Citric-3(2)	1.51E-05	1.06E-05	7.35E-06	7.70E-06	1.47E-06	1.24E-05	4.84E-06	4.53E-06
Mg+2_H+1(2)_PO4-3(2)	2.47E-06	3.36E-06	9.21E-07	4.44E-06	2.04E-06	4.10E-06	1.02E-05	1.47E-05
Mg+2_H+1_Citric-3_PO4-3	3.67E-06	3.99E-06	1.80E-06	3.30E-06	1.10E-06	4.27E-06	3.87E-06	5.01E-06
Mg+2_H+1_Citric-3	3.03E-06	3.03E-06	1.83E-06	9.08E-07	2.01E-08	1.65E-06	6.10E-07	2.38E-07
Mg+2(2)_H+1(2)_PO4-3(2)	3.30E-07	5.79E-07	8.53E-08	4.01E-07	1.09E-07	3.32E-07	9.63E-07	1.73E-06

Citrate speciation: 0.76 µM Phy (white males)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Cit] mol/L							
Ca+2_Citric-3	9.96E-04	5.03E-04	4.92E-04	1.69E-04	7.32E-05	2.85E-04	2.85E-04	1.65E-04
Mg+2_Citric-3	3.99E-04	3.79E-04	2.41E-04	2.63E-04	9.29E-05	3.13E-04	2.14E-04	2.31E-04
Citric-3	3.20E-04	2.30E-04	2.42E-04	2.92E-04	1.95E-04	4.17E-04	2.21E-04	2.23E-04
Ca+2_Citric-3_PO4-3	2.29E-04	7.86E-05	4.50E-05	1.22E-04	6.27E-04	1.24E-04	3.79E-04	5.87E-04
Na+1_Citric-3	3.04E-04	1.61E-04	1.12E-04	4.21E-04	2.00E-04	3.74E-04	3.52E-04	2.49E-04
Na+1(2)_Citric-3	2.08E-04	7.29E-05	3.18E-05	4.50E-04	1.39E-04	2.39E-04	4.23E-04	1.93E-04
H+1_Na+1_Citric-3	1.91E-04	9.74E-05	6.64E-05	1.48E-04	4.19E-06	1.87E-04	1.05E-04	2.41E-05
H+1_Citric-3	1.16E-04	9.92E-05	1.11E-04	5.54E-05	2.68E-06	1.24E-04	3.41E-05	1.34E-05
K+1_Citric-3	3.75E-05	1.33E-05	1.35E-05	2.35E-05	1.03E-05	2.61E-05	1.93E-05	1.67E-05
Mg+2_Citric-3(2)	1.71E-05	8.49E-06	4.91E-06	1.11E-05	2.04E-06	1.69E-05	7.16E-06	6.29E-06
Ca+2_Citric-3(2)	2.76E-05	7.28E-06	6.48E-06	4.61E-06	1.04E-06	9.88E-06	6.15E-06	2.90E-06
Ca+2_H+1(2)_Citric-3(2)	2.13E-05	8.72E-06	9.15E-06	9.50E-07	1.21E-09	5.20E-06	8.28E-07	6.33E-08
Ca+2_H+1_Citric-3	1.02E-05	5.50E-06	5.33E-06	9.17E-07	2.70E-08	2.38E-06	1.26E-06	2.75E-07
Mg+2_H+1_Citric-3_PO4-3	2.81E-06	2.59E-06	1.09E-06	2.89E-06	1.03E-06	3.54E-06	3.41E-06	4.45E-06
Ca+2_H+1_Citric-3_PO4-3	4.69E-06	1.93E-06	1.19E-06	1.33E-06	4.84E-07	2.09E-06	3.40E-06	1.98E-06

Citrate speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Cit] mol/L							
Ca+2_Citric-3	2.23E-04	3.84E-04	1.81E-04	2.23E-04	2.95E-04	4.83E-04	5.27E-04	2.62E-04
Mg+2_Citric-3	1.52E-04	2.43E-04	2.07E-04	1.52E-04	2.28E-04	2.95E-04	3.05E-04	6.45E-04
Citric-3	1.47E-04	1.64E-04	3.64E-04	1.47E-04	3.81E-04	1.40E-04	9.85E-05	3.67E-04
Ca+2_Citric-3_PO4-3	9.46E-05	3.64E-05	6.93E-04	9.46E-05	2.07E-04	1.66E-04	3.67E-05	3.82E-04
Na+1_Citric-3	1.32E-04	1.99E-04	1.47E-04	1.32E-04	2.78E-04	9.29E-05	8.19E-05	3.57E-04
Na+1(2)_Citric-3	8.33E-05	1.75E-04	4.33E-05	8.33E-05	1.43E-04	4.03E-05	4.58E-05	2.38E-04
H+1_Na+1_Citric-3	7.07E-05	2.62E-04	1.35E-05	7.07E-05	1.12E-04	3.30E-05	6.94E-05	4.00E-05
H+1_Citric-3	4.81E-05	1.25E-04	1.86E-05	4.81E-05	9.28E-05	3.48E-05	5.56E-05	2.64E-05
K+1_Citric-3	1.47E-05	2.34E-05	1.87E-05	1.47E-05	3.47E-05	1.23E-05	6.62E-06	2.54E-05
Mg+2_Citric-3(2)	2.78E-06	5.33E-06	1.06E-05	2.78E-06	1.10E-05	4.17E-06	3.29E-06	2.75E-05
Ca+2_Citric-3(2)	2.63E-06	5.45E-06	6.01E-06	2.63E-06	9.19E-06	4.41E-06	3.66E-06	7.21E-06
Ca+2_H+1(2)_Citric-3(2)	1.70E-06	1.84E-05	9.06E-08	1.70E-06	3.24E-06	1.74E-06	7.27E-06	2.28E-07
Ca+2_H+1_Citric-3	2.04E-06	8.28E-06	2.65E-07	2.04E-06	2.01E-06	3.09E-06	7.92E-06	5.14E-07
Mg+2_H+1_Citric-3_PO4-3	1.88E-06	1.48E-06	3.32E-06	1.88E-06	3.42E-06	2.51E-06	1.15E-06	6.27E-06
Ca+2_H+1_Citric-3_PO4-3	1.75E-06	1.56E-06	2.02E-06	1.75E-06	2.84E-06	2.34E-06	1.17E-06	1.55E-06

Citrate speciation: 1.50 mM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[Cit] mol/L							
Ca+2_Citric-3	1.50E-04	2.68E-04	1.31E-04	2.54E-04	2.14E-04	2.15E-04	2.36E-04	1.65E-04
Mg+2_Citric-3	1.65E-04	2.76E-04	2.03E-04	3.09E-04	2.31E-04	3.81E-04	4.18E-04	6.37E-04
Citric-3	1.89E-04	2.14E-04	3.80E-04	5.87E-04	4.50E-04	2.57E-04	1.78E-04	4.50E-04
Ca+2_Citric-3_PO4-3	1.15E-04	4.21E-05	7.61E-04	6.03E-05	2.71E-04	1.51E-04	2.88E-05	4.47E-04
Na+1_Citric-3	1.40E-04	2.23E-04	1.32E-04	2.49E-04	2.70E-04	1.33E-04	1.23E-04	3.48E-04
Na+1(2)_Citric-3	7.76E-05	1.76E-04	3.52E-05	7.68E-05	1.22E-04	4.90E-05	6.10E-05	2.00E-04
H+1_Na+1_Citric-3	6.61E-05	2.65E-04	1.10E-05	1.17E-04	9.56E-05	4.03E-05	9.27E-05	3.35E-05
H+1_Citric-3	4.68E-05	1.28E-04	1.52E-05	1.56E-04	8.21E-05	4.65E-05	7.90E-05	2.34E-05
K+1_Citric-3	1.56E-05	2.63E-05	1.69E-05	3.25E-05	3.38E-05	1.77E-05	9.98E-06	2.48E-05
Mg+2_Citric-3(2)	4.68E-06	9.19E-06	1.26E-05	2.50E-05	1.59E-05	1.26E-05	9.70E-06	4.18E-05
Ca+2_Citric-3(2)	2.75E-06	5.77E-06	5.25E-06	1.33E-05	9.51E-06	4.58E-06	3.54E-06	7.00E-06
Ca+2_H+1(2)_Citric-3(2)	9.55E-07	1.16E-05	4.62E-08	5.43E-06	1.78E-06	8.87E-07	4.11E-06	1.08E-07
Ca+2_H+1_Citric-3	1.07E-06	4.60E-06	1.48E-07	1.92E-06	1.12E-06	1.09E-06	2.95E-06	2.45E-07
Mg+2_H+1_Citric-3_PO4-3	2.44E-06	1.97E-06	3.43E-06	1.63E-06	4.10E-06	4.22E-06	1.95E-06	7.15E-06
Ca+2_H+1_Citric-3_PO4-3	1.65E-06	1.48E-06	1.82E-06	9.09E-07	2.88E-06	1.54E-06	7.20E-07	1.33E-06

Citrate speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[Cit] mol/L							
Ca+2_Citric-3	7.07E-04	2.29E-04	1.83E-04	1.39E-04	4.25E-05	2.16E-04	2.29E-04	1.06E-04
Mg+2_Citric-3	4.56E-04	4.27E-04	2.64E-04	2.78E-04	8.83E-05	3.21E-04	2.30E-04	2.32E-04
Citric-3	4.30E-04	3.83E-04	4.59E-04	3.34E-04	2.32E-04	4.98E-04	2.47E-04	2.61E-04
Ca+2_Citric-3_PO4-3	2.57E-04	8.26E-05	4.74E-05	1.54E-04	6.71E-04	1.68E-04	4.43E-04	6.60E-04
Na+1_Citric-3	3.54E-04	2.01E-04	1.47E-04	4.19E-04	1.86E-04	3.70E-04	3.49E-04	2.36E-04
Na+1(2)_Citric-3	2.20E-04	7.53E-05	3.28E-05	4.08E-04	1.10E-04	2.09E-04	3.86E-04	1.59E-04
H+1_Na+1_Citric-3	2.02E-04	1.01E-04	6.85E-05	1.34E-04	3.29E-06	1.64E-04	9.59E-05	1.99E-05
H+1_Citric-3	1.25E-04	1.14E-04	1.34E-04	5.02E-05	2.25E-06	1.12E-04	3.09E-05	1.16E-05
K+1_Citric-3	4.37E-05	1.67E-05	1.77E-05	2.34E-05	9.56E-06	2.59E-05	1.92E-05	1.59E-05
Mg+2_Citric-3(2)	3.03E-05	2.11E-05	1.47E-05	1.54E-05	2.94E-06	2.47E-05	9.68E-06	9.06E-06
Ca+2_Citric-3(2)	3.03E-05	7.31E-06	6.57E-06	4.96E-06	9.13E-07	1.08E-05	6.21E-06	2.67E-06
Ca+2_H+1(2)_Citric-3(2)	1.44E-05	3.84E-06	3.35E-06	6.15E-07	4.92E-10	3.05E-06	5.28E-07	2.96E-08
Ca+2_H+1_Citric-3	5.90E-06	1.92E-06	1.47E-06	5.88E-07	1.18E-08	1.39E-06	8.00E-07	1.34E-07
Mg+2_H+1_Citric-3_PO4-3	3.67E-06	3.99E-06	1.80E-06	3.30E-06	1.10E-06	4.27E-06	3.87E-06	5.01E-06
Ca+2_H+1_Citric-3_PO4-3	4.37E-06	1.39E-06	7.74E-07	1.40E-06	3.72E-07	2.21E-06	3.40E-06	1.69E-06

Phosphate speciation: 0.76 µM Phy (white subjects)								
	W1a	W3a	W4a	W5a	W7a	W6a	W9a	W11a
	[PO4] mol/L							
H+1(2)_PO4-3	2.09E-02	2.43E-02	4.37E-03	7.72E-03	1.89E-02	1.07E-02	1.08E-02	3.55E-03
H+1_PO4-3	5.23E-03	2.54E-03	6.59E-03	1.80E-03	6.33E-03	3.90E-03	1.67E-03	4.20E-03
H+1_Na+1_PO4-3	3.50E-03	2.44E-03	2.19E-03	6.64E-04	3.48E-03	1.67E-03	9.43E-04	2.90E-03
H+1(4)_PO4-3(2)	5.49E-03	7.47E-03	2.40E-04	7.43E-04	4.51E-03	1.41E-03	1.45E-03	1.59E-04
H+1(2)_Na+1_PO4-3	3.11E-03	5.27E-03	3.30E-04	6.12E-04	2.32E-03	9.74E-04	1.32E-03	5.38E-04
H+1_K+1_PO4-3	6.21E-04	4.51E-04	4.37E-04	1.40E-04	6.89E-04	3.61E-04	1.23E-04	3.30E-04
H+1(2)_K+1_PO4-3	6.09E-04	1.08E-03	7.32E-05	1.42E-04	5.08E-04	2.30E-04	1.89E-04	6.75E-05
Ca+2_Citric-3_PO4-3	9.46E-05	3.64E-05	6.93E-04	4.55E-05	2.07E-04	1.66E-04	3.67E-05	3.82E-04
Ca+2_H+1(3)_PO4-3(2)	5.21E-04	4.72E-04	4.79E-05	3.96E-05	2.93E-04	3.99E-04	2.80E-04	3.22E-05
Mg+2_H+1_PO4-3	2.12E-04	1.45E-04	1.41E-04	5.12E-05	1.47E-04	3.18E-04	2.02E-04	2.90E-04
H+1_Na+1(2)_PO4-3	1.54E-04	1.48E-04	4.42E-05	1.71E-05	1.25E-04	5.06E-05	3.69E-05	1.36E-04
Ca+2_H+1_PO4-3	1.63E-04	1.28E-04	7.32E-05	3.30E-05	1.02E-04	2.39E-04	1.68E-04	5.88E-05
Mg+2_H+1(2)_PO4-3	1.64E-04	2.82E-04	1.97E-05	3.80E-05	8.59E-05	1.46E-04	2.31E-04	4.53E-05
Mg+2_H+1(3)_PO4-3(2)	1.71E-04	1.44E-04	2.65E-05	1.38E-05	1.09E-04	1.16E-04	7.72E-05	3.79E-05
Ca+2_H+1(2)_PO4-3	5.12E-05	1.01E-04	4.06E-06	1.01E-05	2.41E-05	4.57E-05	7.89E-05	3.76E-06

Phosphate speciation: 0.76 µM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[PO4] mol/L							
H+1(2)_PO4-3	1.34E-02	1.49E-02	1.06E-02	1.13E-02	7.73E-04	1.75E-02	1.36E-02	5.93E-03
H+1_PO4-3	2.93E-03	3.18E-03	2.27E-03	4.51E-03	4.88E-03	4.74E-03	6.52E-03	8.19E-03
H+1_Na+1_PO4-3	2.20E-03	1.41E-03	6.13E-04	5.52E-03	3.45E-03	3.26E-03	9.23E-03	6.69E-03
H+1(4)_PO4-3(2)	2.28E-03	2.73E-03	1.34E-03	1.60E-03	7.50E-06	3.86E-03	2.31E-03	4.43E-04
H+1(2)_Na+1_PO4-3	2.27E-03	1.39E-03	5.80E-04	3.15E-03	1.19E-04	2.69E-03	4.41E-03	1.07E-03
H+1_K+1_PO4-3	4.27E-04	1.91E-04	1.21E-04	4.78E-04	2.86E-04	3.59E-04	7.78E-04	7.18E-04
H+1(2)_K+1_PO4-3	4.89E-04	2.05E-04	1.25E-04	3.04E-04	1.09E-05	3.29E-04	4.17E-04	1.27E-04
Ca+2_Citric-3_PO4-3	2.29E-04	7.86E-05	4.50E-05	1.22E-04	6.27E-04	1.24E-04	3.79E-04	5.87E-04
Ca+2_H+1(3)_PO4-3(2)	3.99E-04	2.82E-04	1.22E-04	9.97E-05	4.18E-06	1.81E-04	3.97E-04	1.12E-04
Mg+2_H+1_PO4-3	1.40E-04	2.02E-04	8.33E-05	1.51E-04	9.09E-05	1.38E-04	2.29E-04	3.33E-04
H+1_Na+1(2)_PO4-3	1.04E-04	4.44E-05	1.19E-05	4.00E-04	1.68E-04	1.44E-04	7.40E-04	3.63E-04
Ca+2_H+1_PO4-3	1.97E-04	1.21E-04	7.25E-05	5.93E-05	3.49E-05	6.80E-05	1.97E-04	1.23E-04
Mg+2_H+1(2)_PO4-3	1.31E-04	1.55E-04	5.80E-05	8.08E-05	2.60E-06	1.01E-04	1.05E-04	4.59E-05
Mg+2_H+1(3)_PO4-3(2)	7.71E-05	1.01E-04	2.81E-05	7.54E-05	2.53E-06	9.57E-05	1.45E-04	7.48E-05
Ca+2_H+1(2)_PO4-3	7.38E-05	3.86E-05	2.12E-05	1.26E-05	4.10E-07	2.01E-05	3.57E-05	6.90E-06

Phosphate speciation: 1.50 mM Phy (white subjects)								
	W1a	**W3a**	**W4a**	**W5a**	**W7a**	**W6a**	**W9a**	**W11a**
	[PO4] mol/L							
H+1(2)_PO4-3	2.02E-02	2.41E-02	3.94E-03	7.39E-03	1.81E-02	1.04E-02	1.08E-02	3.16E-03
H+1_PO4-3	6.06E-03	2.92E-03	6.94E-03	2.17E-03	7.27E-03	4.65E-03	1.95E-03	4.60E-03
H+1_Na+1_PO4-3	3.95E-03	2.78E-03	2.32E-03	7.44E-04	3.91E-03	1.84E-03	1.05E-03	3.04E-03
H+1(4)_PO4-3(2)	5.10E-03	7.19E-03	1.89E-04	6.88E-04	4.05E-03	1.36E-03	1.47E-03	1.25E-04
H+1(2)_Na+1_PO4-3	3.01E-03	5.25E-03	3.02E-04	5.72E-04	2.22E-03	9.18E-04	1.30E-03	4.75E-04
H+1_K+1_PO4-3	6.80E-04	5.00E-04	4.48E-04	1.52E-04	7.49E-04	3.88E-04	1.34E-04	3.35E-04
H+1(2)_K+1_PO4-3	5.80E-04	1.06E-03	6.59E-05	1.30E-04	4.78E-04	2.15E-04	1.84E-04	5.85E-05
Ca+2_Citric-3_PO4-3	1.15E-04	4.21E-05	7.61E-04	6.03E-05	2.71E-04	1.51E-04	2.88E-05	4.47E-04
Ca+2_H+1(3)_PO4-3(2)	3.36E-04	3.08E-04	3.34E-05	2.29E-05	2.16E-04	1.29E-04	8.97E-05	1.81E-05
Mg+2_H+1_PO4-3	1.93E-04	1.33E-04	1.25E-04	4.36E-05	1.34E-04	2.68E-04	1.77E-04	2.41E-04
H+1_Na+1(2)_PO4-3	1.47E-04	1.45E-04	3.99E-05	1.58E-05	1.17E-04	4.71E-05	3.59E-05	1.18E-04
Ca+2_H+1_PO4-3	1.13E-04	8.73E-05	5.87E-05	2.06E-05	8.13E-05	8.15E-05	5.48E-05	3.85E-05
Mg+2_H+1(2)_PO4-3	1.41E-04	2.46E-04	1.64E-05	3.06E-05	7.34E-05	1.18E-04	1.96E-04	3.56E-05
Mg+2_H+1(3)_PO4-3(2)	1.80E-04	1.55E-04	2.56E-05	1.35E-05	1.14E-04	1.10E-04	7.64E-05	3.39E-05
Ca+2_H+1(2)_PO4-3	3.24E-05	6.32E-05	2.98E-06	5.80E-06	1.76E-05	1.45E-05	2.44E-05	2.25E-06

Phosphate speciation: 1.50 mM Phy (white subjects)								
	W10a	W8a	SP1a	SP2a	SP3a	SP4a	SP5a	SP6a
	[PO4] mol/L							
H+1(2)_PO4-3	1.32E-02	1.45E-02	1.01E-02	1.07E-02	6.48E-04	1.68E-02	1.28E-02	5.27E-03
H+1_PO4-3	3.30E-03	3.92E-03	2.91E-03	4.92E-03	5.10E-03	5.44E-03	7.00E-03	8.85E-03
H+1_Na+1_PO4-3	2.46E-03	1.58E-03	6.79E-04	6.12E-03	3.42E-03	3.68E-03	1.01E-02	7.01E-03
H+1(4)_PO4-3(2)	2.15E-03	2.64E-03	1.29E-03	1.37E-03	5.27E-06	3.50E-03	1.97E-03	3.46E-04
H+1(2)_Na+1_PO4-3	2.24E-03	1.30E-03	5.23E-04	3.03E-03	9.88E-05	2.60E-03	4.25E-03	9.52E-04
H+1_K+1_PO4-3	4.64E-04	2.07E-04	1.31E-04	5.12E-04	2.74E-04	3.93E-04	8.30E-04	7.27E-04
H+1(2)_K+1_PO4-3	4.76E-04	1.89E-04	1.11E-04	2.87E-04	8.82E-06	3.12E-04	3.95E-04	1.11E-04
Ca+2_Citric-3_PO4-3	2.57E-04	8.26E-05	4.74E-05	1.54E-04	6.71E-04	1.68E-04	4.43E-04	6.60E-04
Ca+2_H+1(3)_PO4-3(2)	2.49E-04	1.08E-04	3.62E-05	7.77E-05	2.03E-06	1.39E-04	2.99E-04	6.49E-05
Mg+2_H+1_PO4-3	1.24E-04	1.70E-04	6.55E-05	1.35E-04	7.24E-05	1.25E-04	2.07E-04	2.87E-04
H+1_Na+1(2)_PO4-3	1.01E-04	4.10E-05	1.06E-05	3.79E-04	1.37E-04	1.37E-04	7.01E-04	3.18E-04
Ca+2_H+1_PO4-3	1.29E-04	4.92E-05	2.33E-05	5.08E-05	2.10E-05	5.62E-05	1.64E-04	8.33E-05
Mg+2_H+1(2)_PO4-3	1.11E-04	1.24E-04	4.33E-05	6.82E-05	1.95E-06	8.59E-05	9.01E-05	3.73E-05
Mg+2_H+1(3)_PO4-3(2)	7.83E-05	9.70E-05	2.51E-05	7.70E-05	2.05E-06	1.01E-04	1.49E-04	6.94E-05
Ca+2_H+1(2)_PO4-3	4.49E-05	1.46E-05	6.28E-06	9.89E-06	2.26E-07	1.52E-05	2.72E-05	4.28E-06

Appendix 3.3
Intergroup comparison of urinary SS and chemical speciation (comparison of means using Unpaired t-test)

Intergroup comparisons of the SS of each stone-forming salt as well as the concentration of each species presented above were made and are reported below.

No statistically significant difference in the SS of salts was observed between the two groups at all concentrations of phytate modelled in the present study as shown below.

Comparison of urinary SS:

	P-value				
SS	**0.76 µM phytate**	**2.27 µM phytate**	**4.45 µM phytate**	**30 µM phyate**	**1.50 mM phytate**
COM	0.24	0.24	0.24	0.24	0.36
COD	0.24	0.24	0.24	0.24	0.36
COT	0.17	0.17	0.17	0.17	0.36
CaOx	0.28	0.28	0.28	0.28	0.36
Brushite	0.36	0.36	0.36	0.36	0.67
HAP	0.16	0.16	0.16	0.16	0.15
OCP	0.14	0.14	0.14	0.14	0.15
tCaP	0.11	0.11	0.11	0.11	0.11
Uric acid	0.48	0.48	0.48	0.48	0.21
Na-Urate	0.45	0.45	0.45	0.45	0.45

In addition to the above, the speciation of calcium, phytate oxalate and phosphate species was not significantly different between the two groups (except for that of KOx-1 due to a significant difference in urinary potassium between the two groups) at a physiological concentration of 0.76 µM phytate or at the non-physiological concentration of phytate (1.50 mM) as shown in Tables below.

Comparison of calcium speciation:

Species	P Values	
	0.76 µM phytate	**1.50mM phytate**
Ca^{+2}	0.40	0.71
$CaCitPO_4^{-4}$	0.54	0.57
$CaCit^{-1}$	0.56	0.66
$CaHPO_4$	0.33	0.87
$CaH_3(PO_4)_2^{-1}$	0.52	0.38
$CaCl^{+1}$	0.93	0.67

$CaH_2PO_4^{+1}$	0.62	0.55
$CaPO_4^{-1}$	0.10	0.19
CaOx	0.23	0.57
$CaH_2(PO_4)_2^{-2}$	0.66	0.96
Ca_2Ox^{+2}	0.30	0.59
CaHCit	0.38	0.34
$CaCit_2^{-4}$	0.18	0.16
$CaH_2Cit_2^{-2}$	0.21	0.23
$CaHCitPO_4^{-3}$	0.50	0.22
Ca_2HPhy^{-7}	0.16	0.25

Comparison of phytate speciation:

Species	P values	
	0.76 µM phytate	1.50 mM phytate
Ca_2HPhy^{-7}	0.12	0.65
$HPhy^{-11}$	0.36	0.53
Phy^{-12}	0.75	0.22

Comparison of oxalate speciation:

Species	P Value	
	0.76 µM phytate	1.50 mM phytate
Ox^{-2}	0.50	0.36
$NaOx^{-1}$	0.71	0.65
MgOx	0.09	0.12
CaOx	0.24	0.36
KOx^{-1}	0.03*	0.03*
Ca_2Ox^{+2}	0.30	0.59
$MgHOxPO_4^{-2}$	0.21	0.22
HOx^{-1}	0.84	0.76
$MgOxCit^{-3}$	0.54	0.76
$CaHOxPO_4^{-2}$	0.23	0.42
Mg_2Ox^{+2}	0.17	0.20
$MgOx_2^{-2}$	0.12	0.29
$CaOx_2^{-2}$	0.20	0.25
$CaOxCit^{-3}$	0.98	0.92
$CaHOxCit^{-2}$	0.82	0.87

Comparison of phosphate speciation:

Species	P value	
	0.76 µM phytate	1.50 mM phytate
$H_2PO_4^{-1}$	0.17	0.19
HPO_4^{-2}	0.90	0.87
$H_4(PO_4)_2^{-2}$	0.27	0.22
$HNaPO_4^{-1}$	0.91	0.85
H_2NaPO_4	0.38	0.38
$HKPO_4^{-1}$	0.14	0.18
$CaCitPO_4^{-4}$	0.23	0.30
$CaH_2PO_4^{+1}$	0.93	0.89
H_2KPO_4	0.92	0.90
$CaH_3(PO_4)_2^{-1}$	0.59	0.67
$MgH_2PO_4^{+1}$	0.75	0.86
HNa_2PO_4	0.68	0.71
$H_3(PO_4)_2^{-3}$	0.35	0.27
$CaHPO_4^{+1}$	0.33	0.87
$MgHPO_4$	0.25	0.32

Comparison of magnesium speciation:

Species	P value	
	0.76 uM	1.50 mM
Mg^{+2}	0.35	0.18
$MgHPO_4$	0.23	0.32
$MgCitric^{-1}$	0.10	0.17
$MgH_2PO_4^{+1}$	0.93	0.61
$MgCl^{+1}$	0.92	0.97
$MgH_3(PO_4)_2^{-1}$	0.52	0.78
MgOx	0.09	0.12
$MgH_2(PO_4)_2^{-2}$	0.29	0.32
$MgCit_2^{-4}$	0.03*	0.02*
$MgHCitPO_4^{-3}$	0.45	0.28
MgHCit	0.68	0.83
$Mg_2H_2(PO_4)_2$	0.18	0.22

Comparison of citrate speciation:

The concentration of ionized citrate (Cit^{-3}) and $MgCit_2^{-4}$ was significantly lower in the black group than the white group in the presence of 1.50 mM phytate as shown below (due to a difference in urinary citrate concentration):

Species	P value	
	0.76 µM phytate	1.50 mM phytate
$CaCitPO_4^{-4}$	0.24	0.30
$CaCit^{-1}$	0.56	0.37
$MgCit^{-1}$	0.16	0.14
Cit^{-3}	0.05 (B<W)	0.01* (B<W)
$NaCit^{-2}$	0.12	0.07
Na_2Cit^{-1}	0.21	0.22
$HNaCit^{-1}$	0.28	0.32
$HCit^{-2}$	0.17	0.14
$KCit^{-2}$	0.77	0.68
$CaCit_2^{-4}$	0.27	0.15
$MgCit_2^{-4}$	0.06 (B<W)	0.02* (B<W)
$CaH_2Cit_2^{-2}$	0.82	0.73
CaHCit	0.38	0.34
$MgHCitPO_4^{-3}$	0.40	0.35
$CaHCitPO_4^{-3}$	0.50	0.22

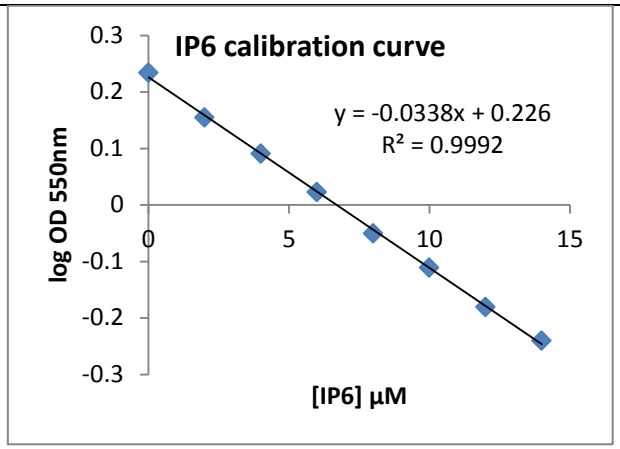
Appendix 4

Appendix 4.1

Raw data for the practical application of Method 2 (rat study)

18-Sep-12

[phytate]uM	OD 550nm	log OD 550nm
0	1.712	0.234
2	1.427	0.154
4	1.231	0.090
6	1.053	0.022
8	0.89	-0.051
10	0.774	-0.111
12	0.659	-0.181
14	0.575	-0.240

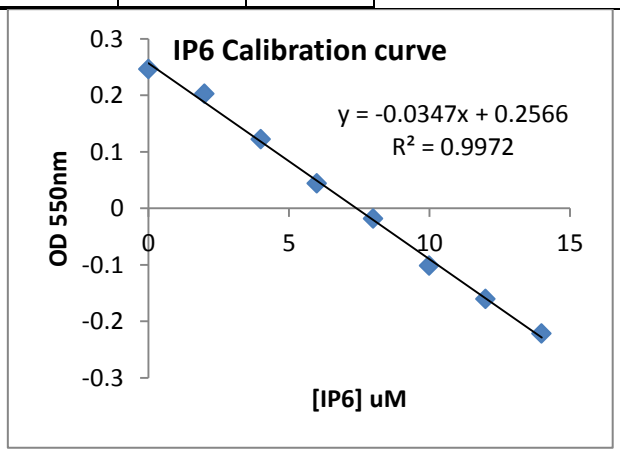


Day 0

rat	OD 550nm	log OD 550nm	conc in eluate	conc in urine	% recov
1	1.354	0.132	2.79	0.70	
2	1.403	0.147	2.34	0.58	
4	1.314	0.119	3.18	1.13	
5+6	1.371	0.137	2.63	1.74	
1+2 +IP6 (1.08uM)	1.109	0.045	5.36	1.46	76

24-Sep-12

[phytate] uM	OD 550 nm	log OD 550nm
0	1.762	0.246
2	1.594	0.202
4	1.324	0.122
6	1.107	0.044
8	0.958	-0.019
10	0.791	-0.102
12	0.691	-0.161
14	0.6	-0.222

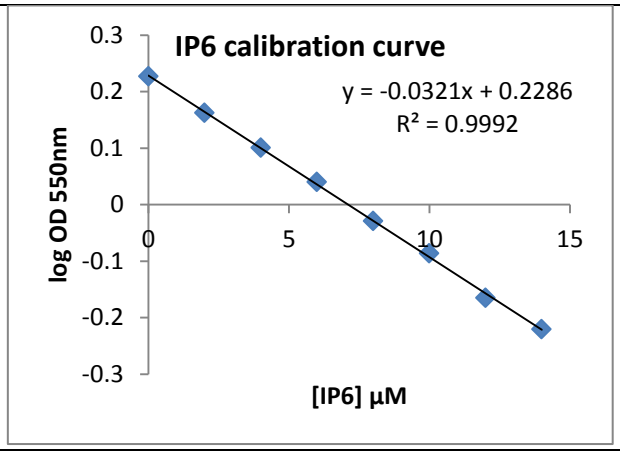


DAY 6 (IP6 restricted diet)

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine	% recov
1	1.623	0.210	1.334	0.333	
2	1.579	0.198	1.678	0.419	
2+1uM	1.191	0.0759	5.207	1.302	88.2
4	1.596	0.203	1.544	0.772	
5+6	1.656	0.219	1.082	0.832	

28-Sep-12

[phytate] uM	OD	logOD
0	1.686	0.227
2	1.454	0.163
4	1.26	0.100
6	1.097	0.040
8	0.935	-0.029
10	0.82	-0.086
12	0.684	-0.165
14	0.602	-0.220

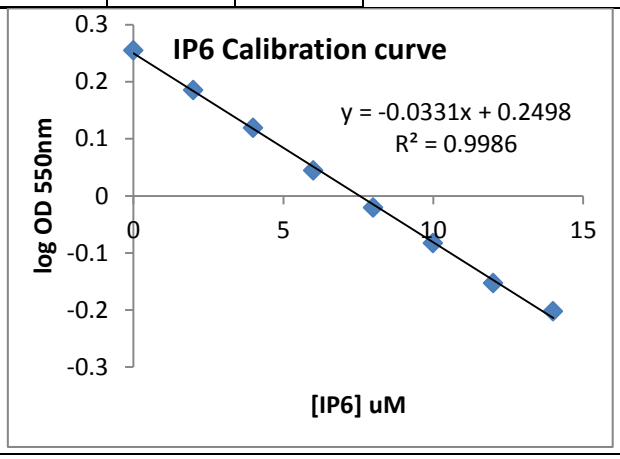


day10

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine	% recov
1	1.575	0.197	0.976	0.244	
2	1.503	0.177	1.609	0.402	
2+1Um	1.251	0.097	4.092	1.023	62.1
4	1.572	0.196	1.001	0.741	
5+6	1.552	0.191	1.175	1.034	

02-Oct-12

[phytate]uM	OD550nm	log OD550nm
0	1.797	0.255
2	1.532	0.185
4	1.316	0.119
6	1.107	0.044
8	0.953	-0.021
10	0.826	-0.083
12	0.703	-0.153
14	0.627	-0.203

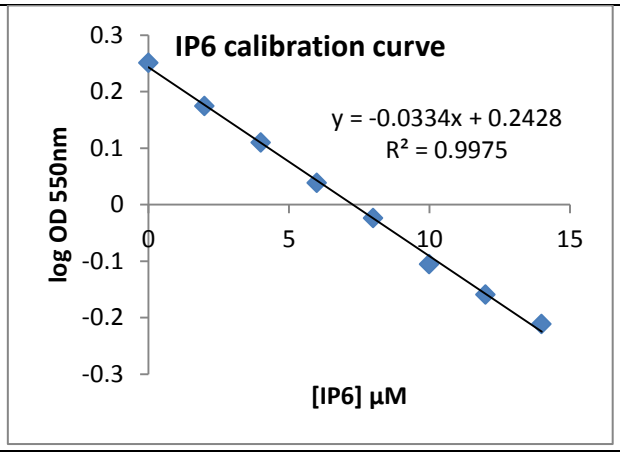


day14

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine	% recov
1	1.675	0.224	0.779	0.195	
2	1.706	0.232	0.538	0.135	
2 + 1um IP6	1.403	0.147	3.104	0.913	77.8
4	1.745	0.242	0.242	0.060	
5+6	1.712	0.234	0.492	0.123	

05-Oct-12

[phytate]uM	OD550nm	log OD550nm
0	1.781	0.251
2	1.493	0.174
4	1.287	0.110
6	1.092	0.038
8	0.946	-0.024
10	0.784	-0.106
12	0.693	-0.159
14	0.614	-0.212

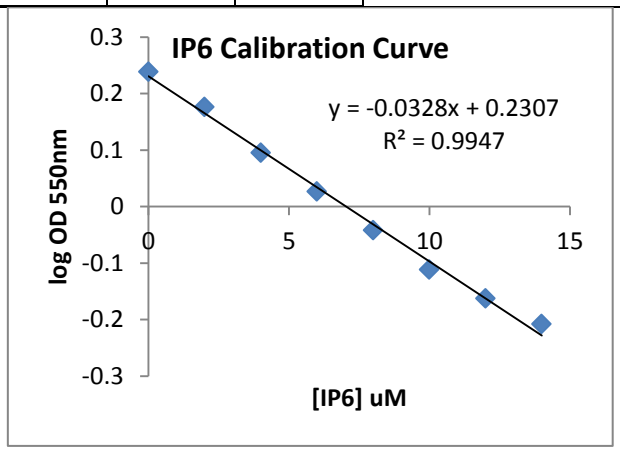


day17

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine	% recov
1	1.673	0.223	0.578	0.144	
2	1.659	0.220	0.687	0.172	
2 + 1um IP6	1.509	0.179	1.919	0.960	78.8
4	1.653	0.218	0.734	0.184	
5+6	1.659	0.220	0.687	0.286	

09-Oct-12

[IP6] uM	OD550nm	log OD550nm
0	1.73	0.238
2	1.499	0.176
4	1.245	0.095
6	1.062	0.026
8	0.908	-0.042
10	0.773	-0.112
12	0.687	-0.163
14	0.619	-0.208

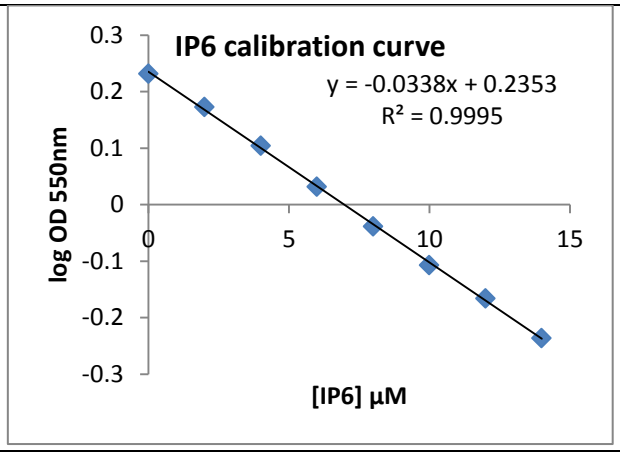


Urine of rats on UAR diet. Day 3

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine
1	1.464	0.166	1.987	0.497
2	1.422	0.153	2.372	0.593
4	1.535	0.186	1.360	0.654
5+6	1.534	0.186	1.368	0.342

11-Oct-12

[IP6] uM	OD 550nm	log OD 550nm
0	1.705	0.232
2	1.487	0.172
4	1.271	0.104
6	1.075	0.031
8	0.915	-0.039
10	0.781	-0.107
12	0.682	-0.166
14	0.58	-0.237

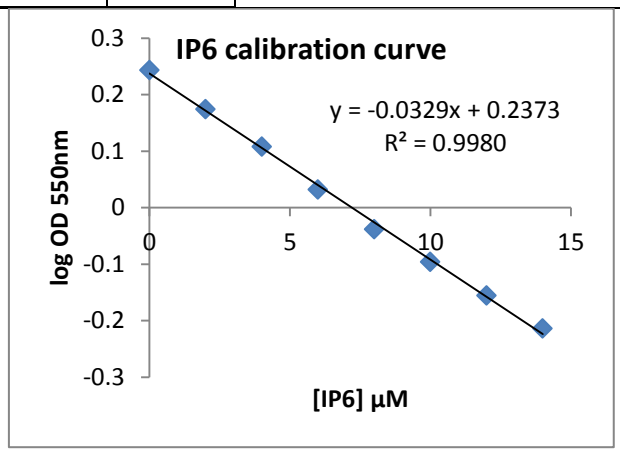


Day4 (UAR) diet

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine
1	1.387	0.142	2.758	0.690
2	1.541	0.188	1.405	0.351
4	1.573	0.197	1.141	0.357
5+6	1.358	0.133	3.030	0.757

16-Oct-12

[IP6] uM	OD 550nm	log OD 550nm
0	1.752	0.244
2	1.493	0.174
4	1.282	0.108
6	1.077	0.032
8	0.916	-0.038
10	0.802	-0.096
12	0.699	-0.156
14	0.611	-0.214

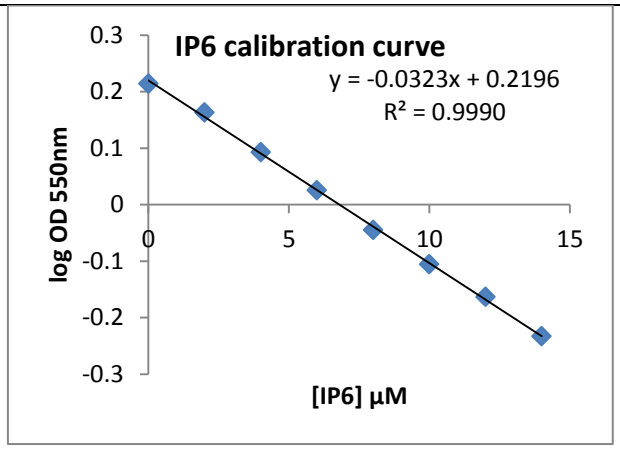


Day10 (UAR) diet

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine
1	1.287	0.110	3.882	1.142
2	0.859	-0.066	9.219	2.195
4	1.366	0.135	3.096	1.248
5+6	1.181	0.072	5.017	3.484

19-Oct-12

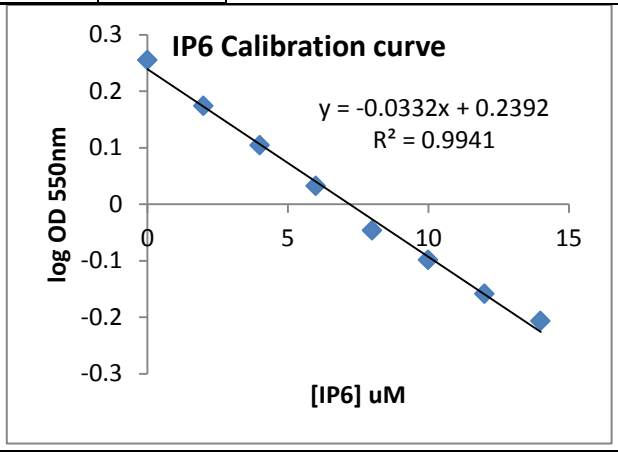
[IP6] uM	OD 550nm	log OD 550nm
0	1.637	0.214
2	1.456	0.163
4	1.238	0.093
6	1.06	0.025
8	0.901	-0.045
10	0.784	-0.106
12	0.686	-0.164
14	0.585	-0.233



Day13 (UAR) diet				
rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine
1	1.359	0.133	2.674	1.592
2	1.063	0.027	5.977	1.494
4	1.51	0.179	1.258	0.873
5+6	1.358	0.133	2.684	1.459

23-Oct-12

[IP6] uM	OD 550nm	log OD 550nm
0	1.797	0.255
2	1.492	0.174
4	1.271	0.104
6	1.077	0.032
8	0.898	-0.047
10	0.796	-0.099
12	0.694	-0.159
14	0.621	-0.207



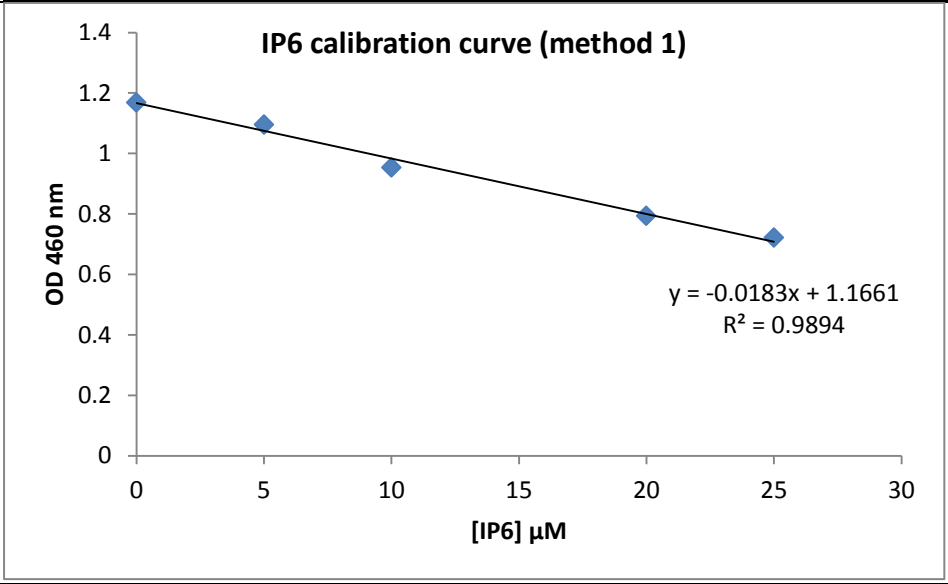
Day17 (UAR) diet

rat ##	OD 550nm	log OD 550nm	conc in eluate	conc in urine
1	1.185	0.074	4.984	1.246
2	1.247	0.096	4.317	1.079
4	1.473	0.168	2.138	0.764
5+6	0.819	-0.087	9.817	4.090

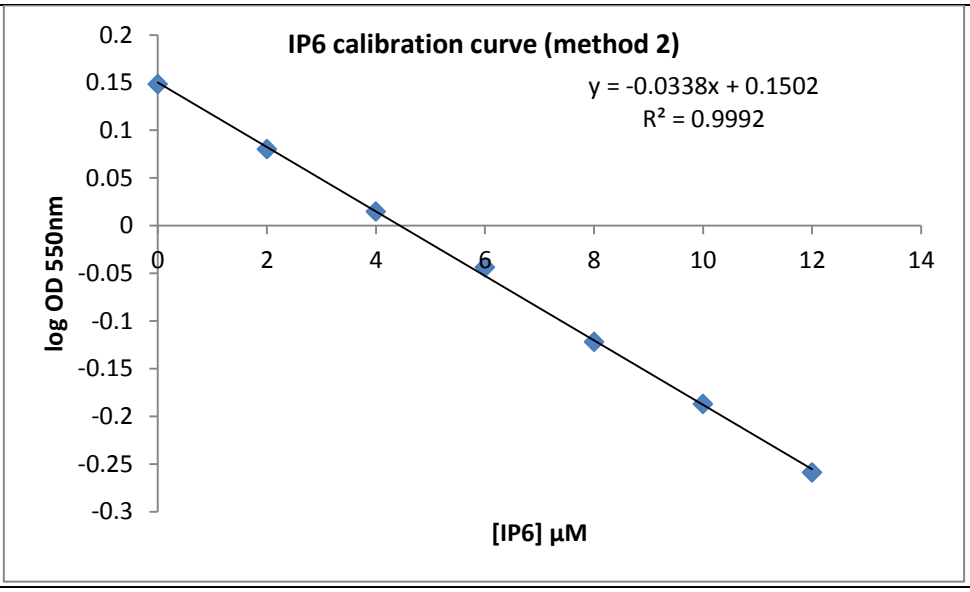
Appendix 4.2
Raw data for testing alternative resin

OD 460nm

[IP6] uM	1	2	3	4	5	average			
0	1.1616	1.1763	1.1677			1.1685			
5	1.0968	1.0941	1.0335			1.0955			
10	0.955	0.9515	0.9529			0.9531			
20	0.7868	0.7934	0.7984			0.7929			
25	0.7198	0.7222	0.721			0.7210	[IP6] uM in eluate	[IP6] uM in AU	% recovery
AU	1.1536	1.1586	1.1563	1.1557	1.1559	1.1560	0.55	0.11	
AU + 1uM	1.0669	1.0677	1.052	1.0558	1.0513	1.0587	5.87	1.17	106
AU + 2uM	0.9834	0.9853	1.0036	1.0105	1.004	0.9974	9.22	1.84	87

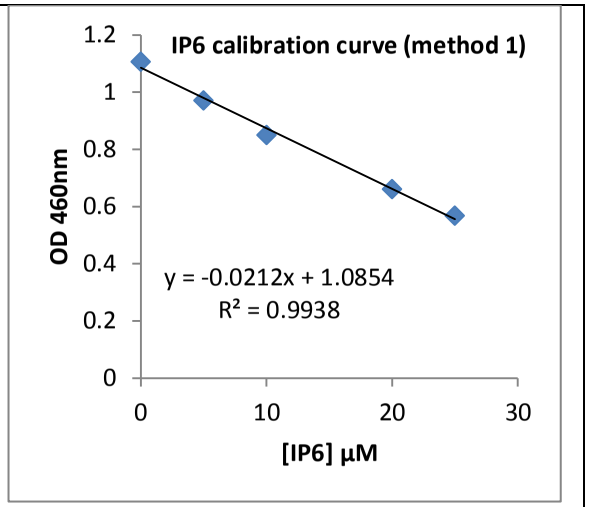


[IP6] uM	OD 550 nm 1	2	3	4	5	average	logOD			
0	1.432	1.383				1.4075	0.15			
2	1.208	1.197				1.2025	0.08			
4	1.004	1.065				1.0345	0.01			
6	0.9	0.908				0.904	-0.04			
8	0.734	0.776				0.755	-0.12			
10	0.651	0.649				0.65	-0.19			
12	0.55	0.552				0.551	-0.26	IP6 in eluate	IP6 in urine	% recovery
AU	1.296	1.342	1.376	1.208	1.215	1.2874	0.11	1.20	0.30	
AU+1	1.118	1.131	1.129	1.053	1.062	1.0986	0.04	3.24	0.81	51
AU+2	0.914	0.93	0.93	0.867	0.908	0.9098	-0.04	5.66	1.41	56


IP6 calibration curve (method 2)
y = -0.0338x + 0.1502
R² = 0.9992
log OD 550nm
[IP6] µM
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
0
2
4
6
8
10
12
14

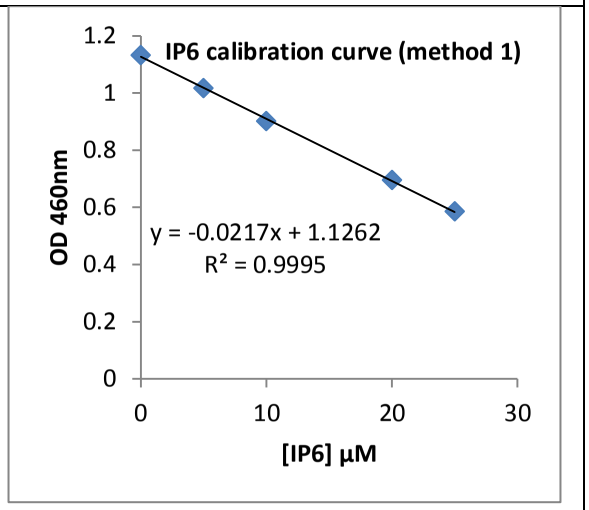
[IP6] uM	OD 460nm		
	1	2	average
0	1.107	1.106	1.1065
5	0.984	0.958	0.971
10	0.851	0.849	0.85
20	0.665	0.656	0.6605
25	0.573	0.562	0.5675

sample ID	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)					% recovery
	1	2	3	1	2	3	1	2	3	average	SE	
urine1	0.903	0.889	0.896	8.60	9.26	8.93	1.72	1.85	1.79	1.79	0.04	
urine1 + 1	0.798					13.56			2.71			92



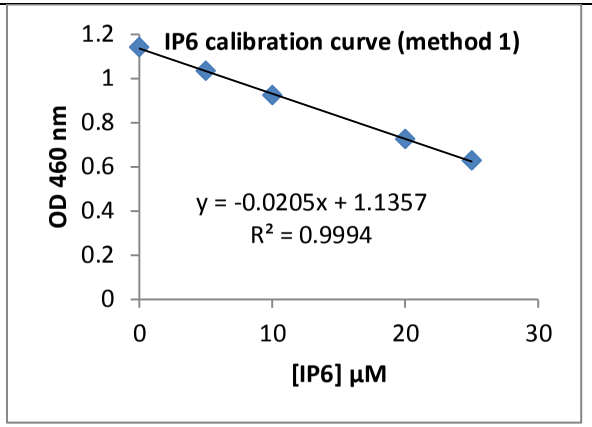
[IP6] uM	OD 460nm		
	1	2	average
0	1.126	1.137	1.132
5	1.013	1.02	1.017
10	0.908	0.894	0.901
20	0.701	0.688	0.695
25	0.593	0.577	0.585

sample ID	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				% recovery
	1	2	1	2	1	2	average	SE	
urine2	0.956	0.947	7.84	8.26	1.57	1.65	1.61	0.04	
urine2 + 1	0.84		13.19		2.64		2.64		103
urine3	0.988	0.958	6.37	7.75	1.27	1.55	1.41	0.14	
urine3 + 1	0.885		11.12		2.22		2.22		81



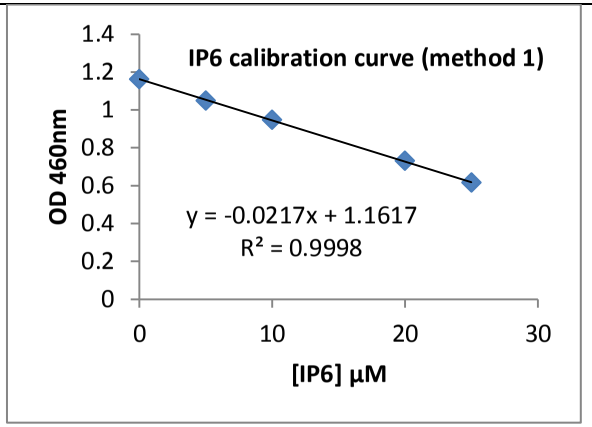
[IP6] uM	OD 460nm		
	1	2	average
0	1.14	1.142	1.141
5	1.033	1.033	1.033
10	0.923	0.923	0.923
20	0.715	0.734	0.725
25	0.623	0.633	0.628

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	1	2	1	2	1	2	average	SE	% recovery
urine4	1.097	1.093	1.89	2.08	0.38	0.42	0.40	0.02	
urine4 + 1	1.006		6.33		1.27		1.27		87
urine5	1.004	0.999	6.42	6.67	1.28	1.33	1.31	0.02	
urine5 + 1	0.918		10.62		2.12		2.12		81



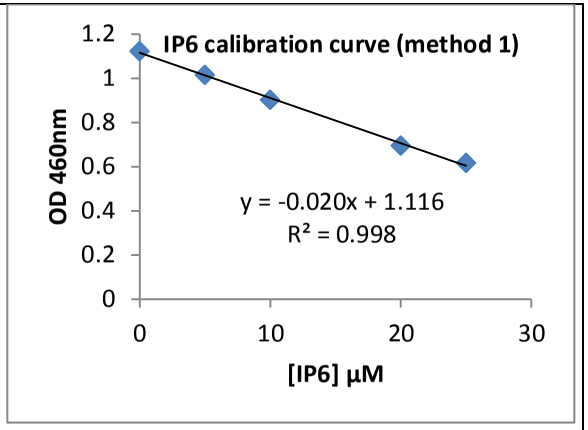
[IP6] uM	OD 460nm		
	1	2	average
0	1.155	1.17	1.163
5	1.047	1.051	1.049
10	0.942	0.953	0.948
20	0.733	0.728	0.731
25	0.619	0.612	0.616

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	1	2	1	2	1	2	average	SE	% recovery
urine6	1.073	1.069	4.09	4.27	0.82	0.85	0.84	0.02	
urine6 + 1	0.99		7.91		1.58		1.58		75
urine7	1.137	1.134	1.14	1.28	0.23	0.26	0.24	0.01	
urine7 + 1	1.043		5.47		1.09		1.09		85
urine8	1.013		6.85		1.37		1.37	nd	
urine8 + 1	0.931		10.63		2.13		2.13		76



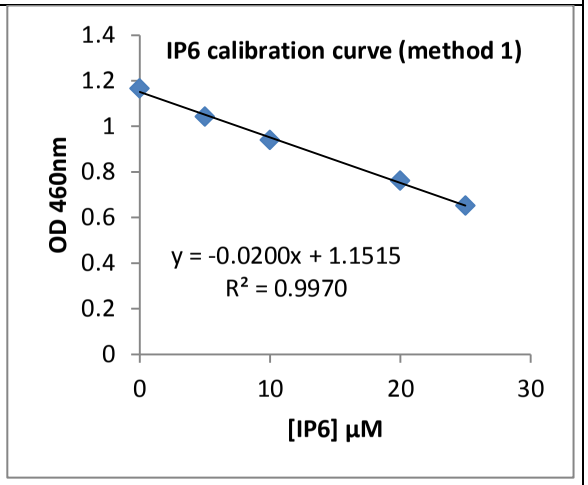
[IP6] uM	OD 460nm		
	1	2	average
0	1.1197	1.1242	1.122
5	1.0218	1.0075	1.015
10	0.9083	0.8956	0.902
20	0.6934	0.6961	0.695
25	0.6232	0.6081	0.616

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	1	2	1	2	1	2	average	SE	% recovery
urine9	0.9853	0.9713	6.54	7.24	1.31	1.45	1.38	0.07	
urine9 + 1	0.9029		10.66		2.13		2.13		75
urine10	1.0544	1.0473	3.08	3.44	0.62	0.69	0.65	0.04	
urine10 + 1	0.9519		8.21		1.64		1.64		99
urine11	1.0069	0.979	5.46	6.85	1.09	1.37	1.23	0.14	
urine11 + 1	0.9101		10.30		2.06		2.06		83



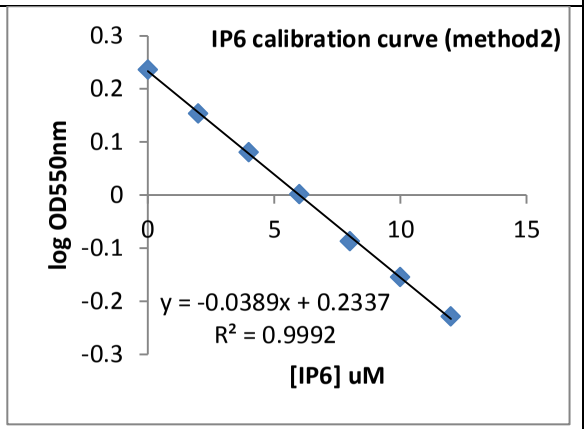
[IP6] uM	OD 460nm		
	1	2	average
0	1.1197	1.1242	1.122
5	1.0218	1.0075	1.015
10	0.9083	0.8956	0.902
20	0.6934	0.6961	0.695
25	0.6232	0.6081	0.616

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	1	2	1	2	1	2	average	SE	% recovery
urine12	1.073	1.069	3.925	4.125	0.79	0.83	0.81	0.02	



uriine12 + 1	0.984		8.375		1.68		1.68		87
urine13	1.051	1.057	5.025	4.725	1.01	0.95	0.98	0.03	
urine13 + 1	0.938		10.675		2.14		2.14		116
urine14	1.069	1.056	4.125	4.775	0.83	0.95	0.89	0.06	
urine14 + 1	0.956		9.775		1.96		1.96		107

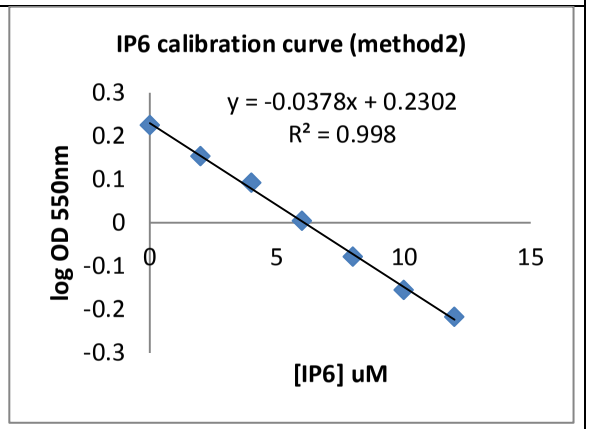
[IP6] (uM)	**absorbance @ 550nm**			
	OD1	**OD2**	**average**	**log**
0	1.734	1.7120	1.7230	0.2363
2	1.435	1.4140	1.4245	0.1537
4	1.21	1.1980	1.2040	0.0806
6	1.034	0.9730	1.0035	0.0015
8	0.815	0.8210	0.8180	-0.0872
10	0.71	0.6910	0.7005	-0.1546
12	0.59	0.5920	0.5910	-0.2284



	OD 550nm		Log OD		IP6 in eluate (uM)		IP6 in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
urine1	1.368	1.374	0.14	0.14	2.51	2.46	0.63	0.62	0.62	0.01	
urine1 +1	1.054		0.02		5.42		1.36		1.36		73
urine2	1.284	1.201	0.11	0.08	3.22	3.96	0.80	0.99	0.90	0.09	
urine2 + 1	0.993		0.00		6.09		1.52		1.52		62

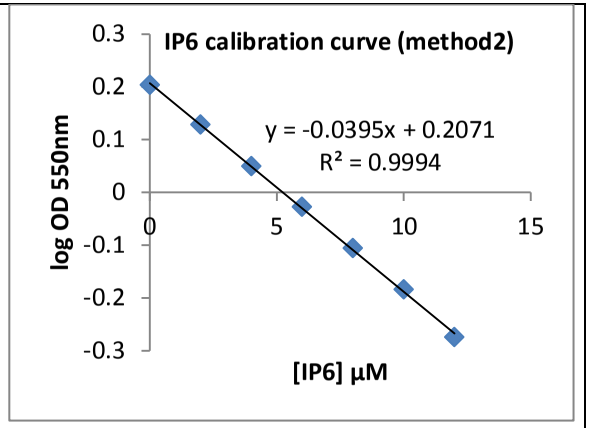
urine3	1.238	1.363	0.09	0.13	3.62	2.55	0.91	0.64	0.77	0.13	
urine3 + 1	1.077		0.03		5.18		1.29		1.29		52

[IP6] (uM)	absorbance @ 550nm			
	OD1	**OD2**	**average**	**log**
0	1.698	1.662	1.68	0.23
2	1.405	1.442	1.4235	0.15
4	1.179	1.295	1.237	0.09
6	0.98	1.039	1.0095	0.00
8	0.821	0.85	0.8355	-0.08
10	0.68	0.719	0.6995	-0.16
12	0.585	0.627	0.606	-0.22



	OD 550nm		Log OD		IP6 in eluate (uM)		IP6 in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	averag e	SE	% recover y
urine4	1.492	1.504	0.17	0.18	1.49	1.40	0.37	0.35	0.36	0.01	
urine4 + 1	1.101		0.04		4.98		1.25		1.25		88
urine5	1.313	1.388	0.12	0.14	2.96	2.32	0.74	0.58	0.66	0.08	
urine5 + 1	1.053		0.02		5.50		1.37		1.37		71

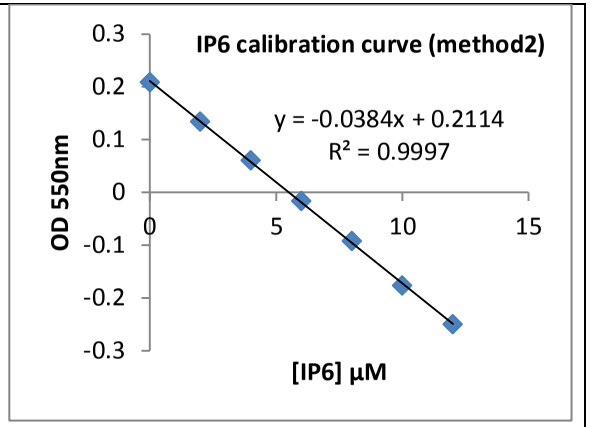
[IP6] (uM)	absorbance @ 550nm			
	OD1	OD2	average	log
0	1.602	1.596	1.599	0.20
2	1.349	1.338	1.3435	0.13
4	1.113	1.127	1.12	0.05
6	0.932	0.944	0.938	-0.03
8	0.771	0.797	0.784	-0.11
10	0.65	0.66	0.655	-0.18
12	0.531	0.532	0.5315	-0.27



sample ID	OD 550nm		Log OD		IP6 in eluate (uM)		IP6 in urine (uM)				
	1	2	1	2	1	2	1	2	average	SE	% recovery
urine6	1.232	1.186	0.09	0.07	2.95	3.37	0.74	0.84	0.79	0.05	
urine6 + 1	0.998		0.00		5.27		1.32		1.32		53
urine7	1.341	1.366	0.13	0.14	2.02	1.81	0.50	0.45	0.48	0.03	
urine7 + 1	1.094		0.04		4.26		1.06		1.06		58
urine8	1.062	1.111	0.03	0.05	4.58	4.09	1.15	1.02	1.08	0.06	
urine8 + 1	0.918		-0.04		6.18		1.55		1.55		46

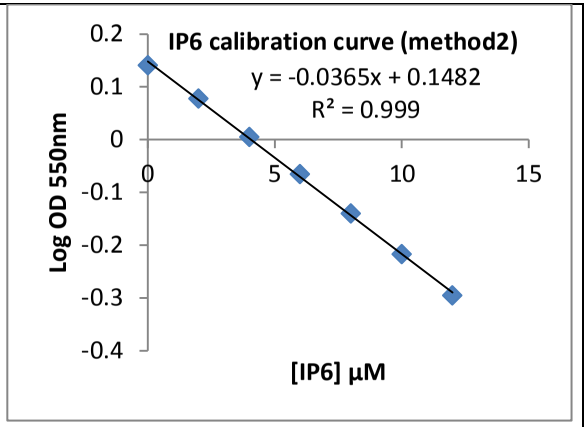
[IP6] (uM)	absorbance @ 550nm			
	OD1	OD2	average	log
0	1.62	1.613	1.6165	0.209
2	1.355	1.366	1.3605	0.134
4	1.149	1.151	1.15	0.061
6	0.962	0.963	0.9625	-0.017
8	0.814	0.803	0.8085	-0.092
10	0.663	0.669	0.666	-0.177
12	0.559	0.565	0.562	-0.250

	OD 550nm		Log OD		IP6 in eluate (uM)		IP6 in urine (uM)				
sample ID	1	2	1	2	1	2	1	2	average	SE	% recovery
urine9	1.24	1.212	0.09	0.08	3.07	3.33	0.77	0.83	0.80	0.03	
urine9 + 1	1.028		0.01		5.19		1.30		1.30		50
urine10	1.306	1.266	0.12	0.10	2.49	2.84	0.62	0.71	0.67	0.04	
urine10 +1	1.048		0.02		4.97		1.24		1.24		58
urine11	1.265	1.269	0.10	0.10	2.85	2.81	0.71	0.70	0.71	0.00	
urine11 + 1	1.035		0.01		5.12		1.28		1.28		57



[IP6] (uM)	absorbance @ 550nm			
	OD1	OD2	average	log
0	1.398	1.367	1.3825	0.14
2	1.186	1.204	1.195	0.08
4	1.014	1.009	1.0115	0.00
6	0.859	0.861	0.86	-0.07
8	0.723	0.724	0.7235	-0.14
10	0.61	0.602	0.606	-0.22
12	0.502	0.51	0.506	-0.30

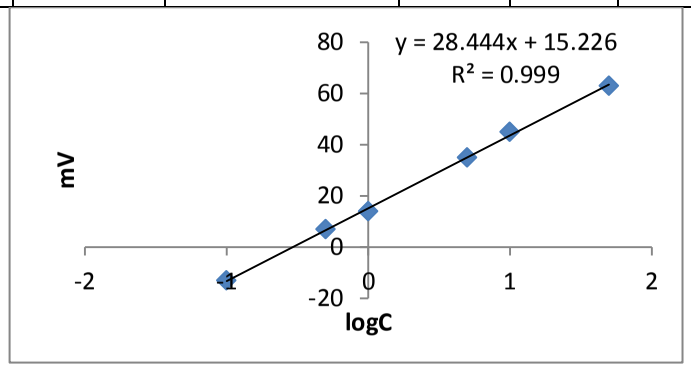
sample ID	OD 550nm		Log OD		IP6 in eluate (uM)		IP6 in urine (uM)				
	1	2	1	2	1	2	1	2	average	SE	% recovery
urine12	1.205	1.227	0.08	0.09	1.84	1.63	0.46	0.41	0.43	0.03	
urine12 + 1	1.008		0.00		3.97		0.99		0.99		56
urine13	1.127	1.128	0.05	0.05	2.64	2.63	0.66	0.66	0.66	0.00	
urine13 + 1	0.919		-0.04		5.07		1.27		1.27		61
urine14	1.234	1.225	0.09	0.09	1.56	1.65	0.39	0.41	0.40	0.01	
urine14 + 1	0.992		0.00		4.16		1.04		1.04		64

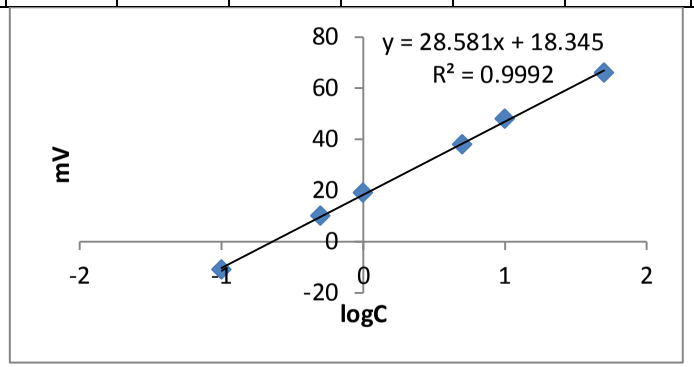


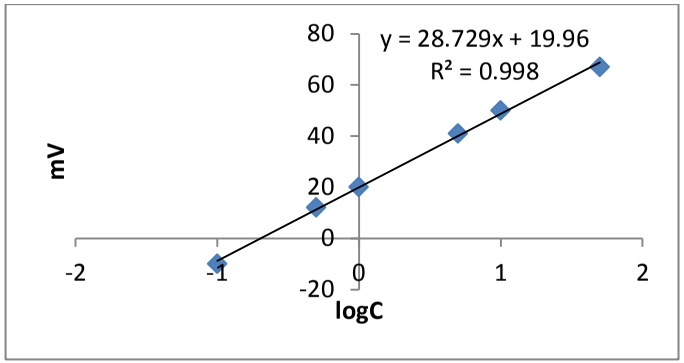
Appendix 5

Appendix 5.1
ionized calcium in AU

Date	Sample ID	Equation	R 2	Y(mV)			LogC			Ca2+ (mmol/L)			average	SE
				1	2	3	1	2	3	1	2	3		
04-May-13	AU0	28.444x + 15.226	0.999	20	22	23	0.167	0.238	0.273	1.47	1.73	1.87	1.69	
08-May-13	AU0	28.581x + 18.345	0.9992	26	27	26	0.267	0.303	0.267	1.85	2	1.85	1.9	0.07
"	AU1	"	"	28	27	26	0.337	0.303	0.267	2.18	2	1.85	2.01	0.10
	AU2	"	"	26	26	27	0.27	0.27	0.30	1.85	1.85	2.01	1.90	0.05
10-May	AU3	28.729x + 19.96	0.998	27	31	30	0.245	0.384	0.349	1.76	2.42	2.24	2.14	0.20







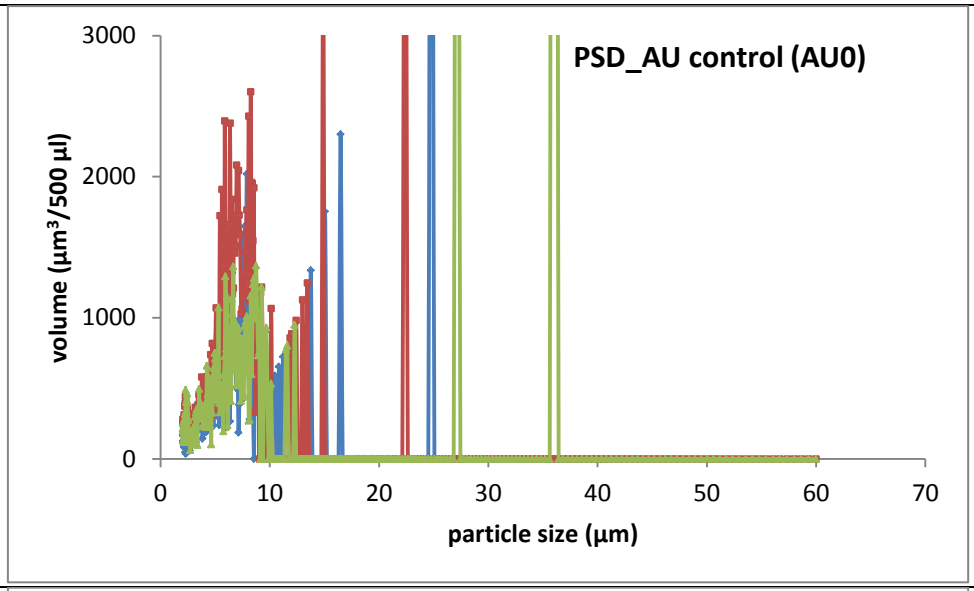
Appendix 5.2
CaOx MSL and rate of crystallisation in artificial urine

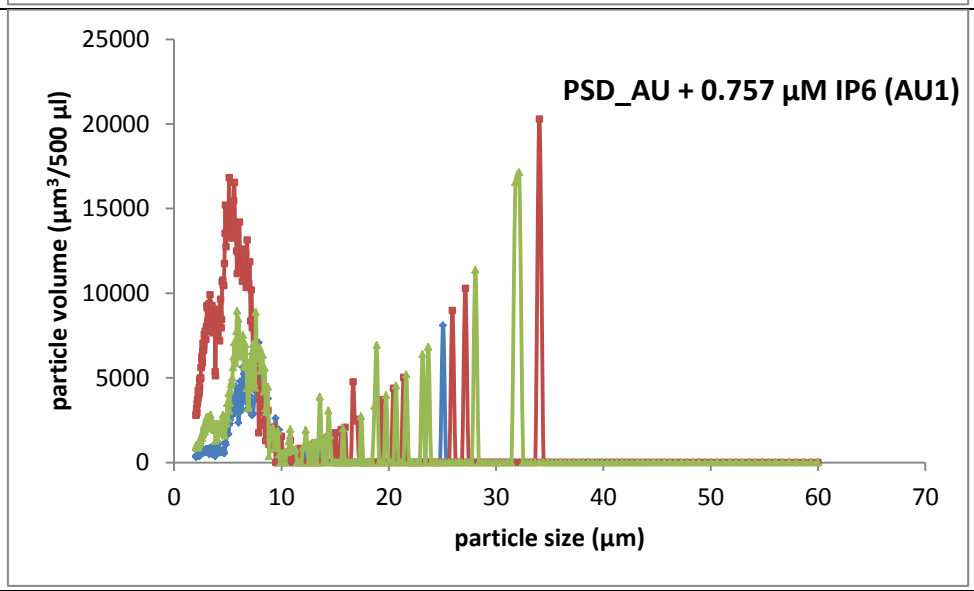
CaOx MSL in artificial urine															
	Absorbance @ 620nm			Absorbance @ 620nm			Absorbance @ 620nm			Absorbance @ 620nm			Absorbance @ 620nm		
	AU baseline (AU0)			**AU + 0.757 uM IP6 (AU1)**			**AU + 2.27 uM IP6 (AU2)**			**AU + 4.54 uM IP6 (AU3)**			**AU + 15uM IP6 (AU4)**		
[Na2Ox]mM	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3
0.15	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.30	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.45	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.60	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.90	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.05	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.20	**0.001**	**0.000**	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.35	0.002	0.004	0.010	**0.001**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000
1.50	0.008	0.012	0.015	0.004	0.004	0.006	0.004	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000
1.65	0.008	0.017	0.017	0.004	0.006	0.006	0.013	0.029	0.006	**0.000**	**0.001**	**0.000**	0.000	0.000	0.000
1.80	0.015	0.038	0.016	0.012	0.019	0.026	0.010	0.055	0.028	0.005	0.002	0.003	0.000	0.000	0.000

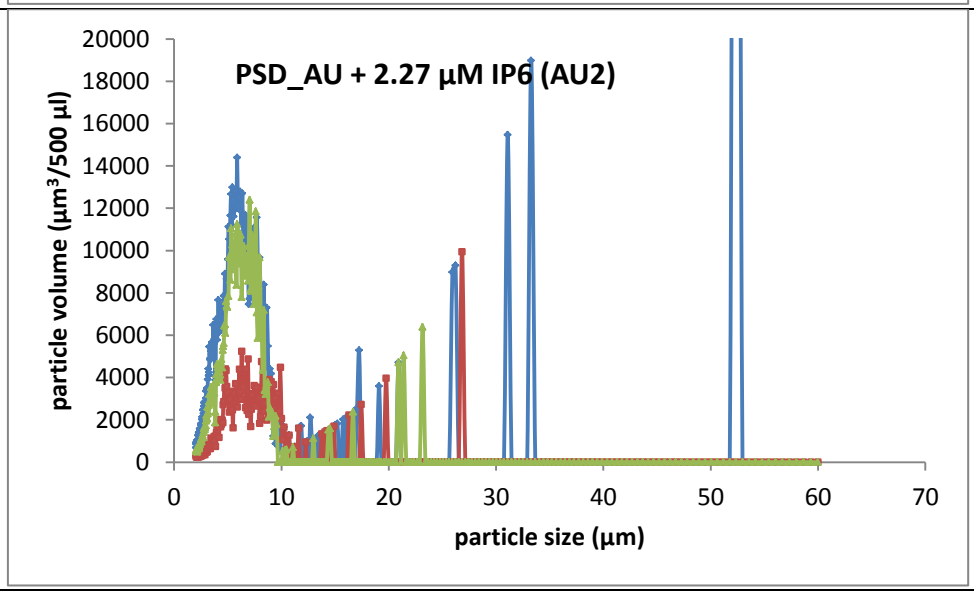
1.95	0.024	0.097	0.098	0.007	0.019	0.011	0.045	0.057	0.043	0.009	0.011	0.008	0.000	0.000	0.000

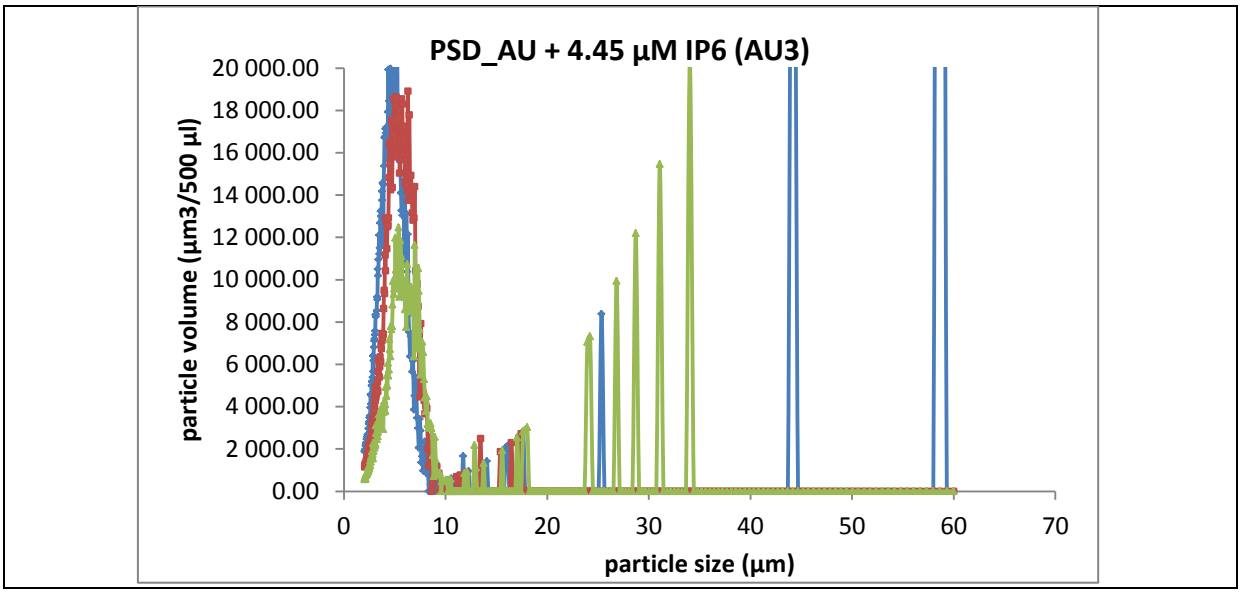
rate of CaOx crystallisation in artificial urine												
	Absorbance @ 620nm			Absorbance @ 620nm			Absorbance @ 620nm			Absorbance @ 620nm		
	AU baseline (AU0)			**AU + 0.757 uM IP6 (AU1)**			**AU + 2.27 uM IP6 (AU2)**			**AU + 4.54 uM IP6 (AU3)**		
time (mins)	**exp1**	**exp2**	**exp3**	**exp1**	**exp2**	**exp3**	**exp1**	**exp2**	**exp3**	exp1	exp2	exp3
0	0.001	0.001	0.001	0	0.001	0.001	0	0	0	0.001	0	0.001
15	0.002	0.002	0.002	0.004	0.004	0.004	0.001	0.001	0.001	0.001	0.002	0.002
30	0.005	0.004	0.005	0.004	0.004	0.004	0.005	0.005	0.007	0.001	0.002	0.002
45	0.011	0.009	0.01	0.007	0.009	0.008	0.007	0.007	0.008	0.002	0.004	0.005
60	0.016	0.015	0.015	0.012	0.011	0.011	0.008	0.009	0.011	0.004	0.007	0.009
75	0.017	0.019	0.019	0.014	0.015	0.014	0.007	0.009	0.012	0.007	0.009	0.011
90	0.018	0.019	0.018	0.015	0.012	0.009	0.007	0.008	0.013			
105	0.016	0.017	0.016				0.007	0.007	0.011	0.006	0.007	0.011
120	0.01	0.01	0.011	0.015	0.013	0.01	0.002	0.003	0.006	0.009	0.009	0.011

Appendix 5.3
Particle size distribution plots (artificial urine)





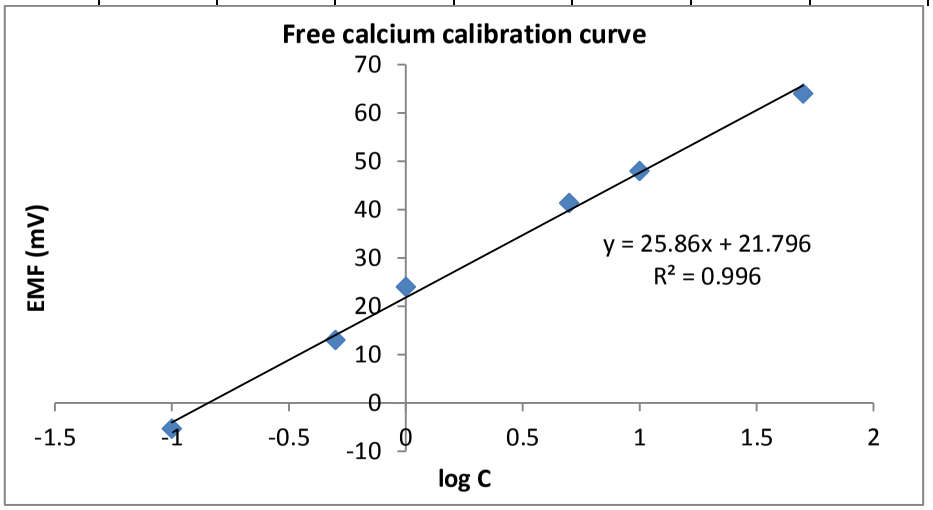



PSD_AU + 4.45 µM IP6 (AU3)
particle volume (µm3/500 µl)
20 000.00
18 000.00
16 000.00
14 000.00
12 000.00
10 000.00
8 000.00
6 000.00
4 000.00
2 000.00
0.00
0
10
20
30
40
50
60
70
particle size (µm)

Appendix 5.4

Effect of IP6 on free calcium in BPU and WPU

Sample ID	Equation	R 2	Y(mV)			LogC			Free Ca2+ (mmol/L)			average	SE
			1	2	3	1	2	3	1	2	3		
BPU (control)	25.86x + 21.796	0.996	21	20	22	-0.03	-0.07	0.01	0.93	0.85	1.02	0.93	0.05
BPU2	"	"	21	21	23	-0.03	-0.03	0.05	0.93	0.93	1.11	0.99	0.06
BPU3	"	"	21	20	20	-0.03	-0.07	-0.07	0.93	0.85	0.85	0.88	0.03
BPU4	"	"	20	22	22	-0.07	0.01	0.01	0.85	1.02	1.02	0.96	0.06
BPU5	"	"	23	23	22	0.05	0.05	0.01	1.11	1.11	1.02	1.08	0.03
WPU (control)	"	"	18	17	18	-0.15	-0.19	-0.15	0.71	0.65	0.71	0.69	0.02
WPU2	"	"	17	18	19	-0.19	-0.15	-0.11	0.65	0.71	0.78	0.72	0.04
WPU3	"	"	19	19	20	-0.11	-0.11	-0.07	0.78	0.78	0.85	0.80	0.02
WPU4	"	"	20	20	21	-0.07	-0.07	-0.03	0.85	0.85	0.93	0.88	0.03
WPU5	"	"	20	20	19	-0.07	-0.07	-0.11	0.85	0.85	0.78	0.83	0.02

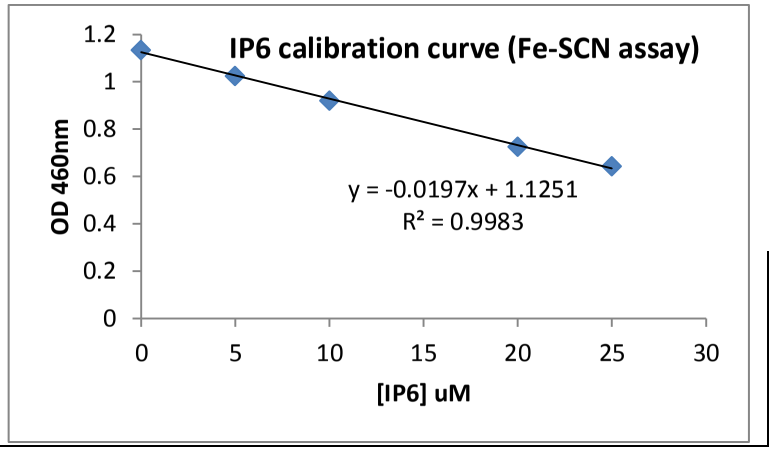


Appendix 5.5
Effect of IP6 on CaOx msl and kinetics in BPU & WPU

IP6 in BPU (baseline):

standard	OD1	OD2	average
0	1.13	1.137	1.1335
5	1.014	1.035	1.0245
10	0.921	0.919	0.92
20	0.726	0.724	0.725
25	0.646	0.64	0.643

sample	OD1	OD2	average	IP6 in eluate (uM)	IP6 in BPU (uM)	% recovery
BPU	1.024	1.025	1.0245	5.1	1.0	
BPU + 1uM	0.928		0.928	10.0	2.0	98



MSL															
[Na2Ox] mM	Baseline (BPU)			2.27 µM IP6 (BPU2)			4.45 µM IP6 (BPU3)			15 µM IP6 (BPU4)			30 µM IP6 (BPU5)		
	OD 620nm			OD 620nm			OD 620nm			OD 620nm			OD 620nm		
	exp1	exp2	epx3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3
0.15	0.017	0.016	0.015	0.017	0.017	0.017	0.017	0.017	0.017	0.020	0.021	0.021	0.022	0.022	0.022
0.3	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.020	0.02	0.02	0.022	0.022	0.022
0.45	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.020	0.02	0.02	0.022	0.022	0.021
0.6	0.017	0.017	0.017	0.019	0.018	0.017	0.017	0.017	0.017	0.021	0.02	0.02	0.022	0.022	0.022
0.75	0.021	0.023	0.017	0.021	0.02	0.022	0.021	0.022	0.021	0.023	0.022	0.023	0.024	0.024	0.023
0.9	0.026	0.028	0.025	0.025	0.025	0.026	0.025	0.026	0.026	0.026	0.027	0.025	0.027	0.026	0.027
1.05	0.036	0.040	0.038	0.030	0.031	0.031	0.028	0.028	0.028	0.027	0.025	0.026	0.029	0.028	0.027
1.2	0.076	0.079	0.078	0.040	0.041	0.04	0.034	0.033	0.034	0.029	0.028	0.029	0.028	0.028	0.028

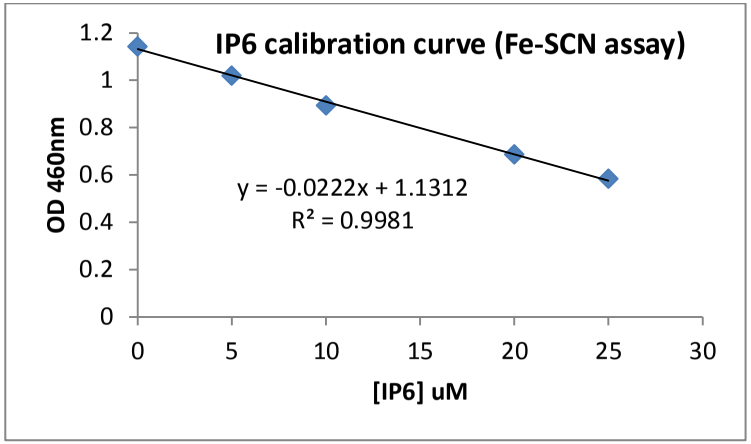
1.35	0.092	0.100	0.093	0.091	0.093	0.091	0.091	0.091	0.092	0.037	0.038	0.037	0.026	0.028	0.027
1.5	0.276	0.282	0.278	0.217	0.217	0.218	0.217	0.217	0.217	0.042	0.043	0.041	0.024	0.027	0.027
1.65	0.330	0.350	0.329	0.350	0.352	0.348	0.233	0.234	0.232	0.062	0.065	0.064	0.025	0.027	0.028
1.8	0.464	0.455	0.460	0.394	0.395	0.39	0.278	0.280	0.278	0.111	0.112	0.115	0.03	0.029	0.028
1.95	0.498	0.510	0.500	0.448	0.450	0.446	0.393	0.394	0.392	0.266	0.268	0.264	0.042	0.041	0.045

Kinetics															
time mins	**Baseline (BPU)**			**2.27 µM IP6 (BPU2)**			**4.45 µM IP6 (BPU3)**			**15 µM IP6 (BPU4)**			**30 µM IP6 (BPU5)**		
	OD 620nm			OD 620nm			OD 620nm			OD 620nm			OD 620nm		
	exp1	exp2	epx3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3
0	0.026	0.027	0.026	0.023	0.024	0.024	0.023	0.024	0.024	0.028	0.028	0.028	0.024	0.024	0.024
15	0.036	0.036	0.035	0.038	0.04	0.041	0.038	0.039	0.038	0.05	0.047	0.059	0.044	0.044	0.044
30	0.054	0.053	0.054	0.069	0.066	0.067	0.048	0.048	0.049	0.056	0.056	0.056	0.052	0.051	0.052
45	0.105	0.1	0.102	0.131	0.126	0.13	0.062	0.061	0.062	0.076	0.064	0.065	0.055	0.057	0.056
60	0.164	0.162	0.164	0.198	0.195	0.198	0.088	0.086	0.088	0.081	0.081	0.081	0.062	0.064	0.064
75	0.216	0.214	0.216	0.24	0.241	0.242	0.116	0.11	0.114	0.101	0.085	0.085	0.065	0.068	0.067
90	0.244	0.243	0.245	0.263	0.266	0.266	0.142	0.132	0.141	0.1	0.093	0.101	0.066	0.065	0.066
105	0.261	0.258	0.262	0.269	0.273	0.274	0.16	0.15	0.161	0.101	0.101	0.101	0.066	0.065	0.066
120	0.261	0.258	0.262	0.258	0.269	0.27	0.17	0.157	0.172	0.1	0.102	0.102	0.072	0.066	0.067

IP6 in WPU (baseline):

standard	OD1	OD2	average
0	1.14	1.144	1.142
5	1.012	1.025	1.0185
10	0.894	0.893	0.8935
20	0.684	0.687	0.6855
25	0.58	0.586	0.583

sample	OD1	OD2	average	IP6 in eluate (uM)	IP6 in WPU (uM)	% recovery
WPU	1.018	1.009	1.0135	5.3	1.1	
WPU + 1uM	0.894		0.894	10.7	2.1	108



MSL															
[Na2Ox] mM	Baseline(WPU)			2.27 µM IP6(WPU2)			4.45 µM IP6(WPU3)			15 µM IP6(WPU4)			30 µM IP6(WPU5)		
	OD 620nm			OD 620nm			OD 620nm			OD 620nm			OD 620nm		
	exp1	exp2	epx3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3
0.15	0.022	0.022	0.022	0.022	0.023	0.025	0.027	0.036		0.023	0.023	0.023	0.025	0.023	0.025
0.3	0.022	0.022	0.023	0.022	0.023	0.024	0.026	0.036		0.023	0.023	0.022	0.026	0.023	0.024
0.45	0.022	0.022	0.027	0.023	0.023	0.025	0.026	0.027		0.022	0.023	0.023	0.025	0.023	0.025
0.6	0.023	0.023	0.024	0.028	0.03	0.03	0.039	0.043		0.023	0.024	0.023	0.026	0.023	0.025
0.75	0.027	0.025	0.034	0.049	0.045	0.055	0.066	0.08		0.023	0.023	0.023	0.026	0.023	0.025
0.9	0.03	0.028	0.038	0.05	0.056	0.062	0.078	0.086		0.023	0.023	0.023	0.026	0.024	0.026
1.05	0.03	0.038	0.044	0.046	0.059	0.062	0.082	0.089		0.023	0.023	0.023	0.027	0.025	0.026
1.2	0.04	0.042	0.054	0.036	0.044	0.066	0.086	0.102		0.023	0.024	0.023	0.029	0.025	0.028
1.35	0.042	0.028	0.041	0.031	0.056	0.055	0.107	0.08		0.023	0.024	0.023	0.028	0.028	0.028
1.5	0.045	0.034	0.041	0.031	0.062	0.076	0.05	0.096		0.023	0.024	0.023	0.028	0.029	0.028

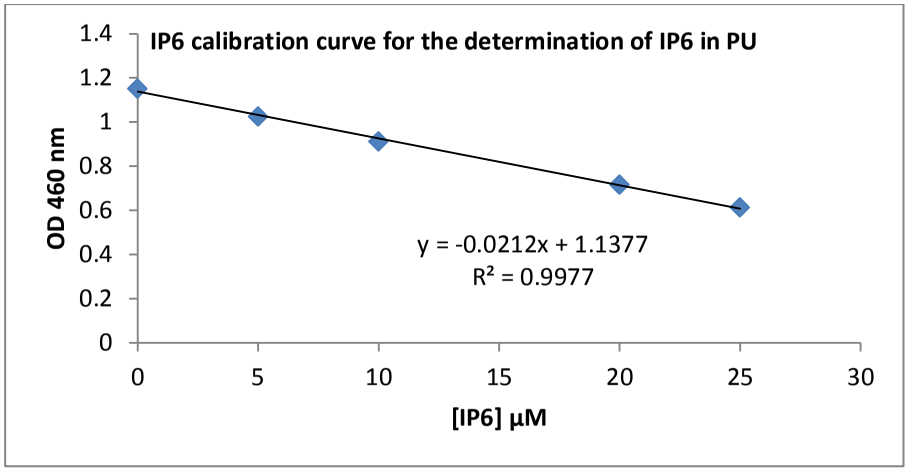
1.65	0.073	0.066	0.057	0.033	0.051	0.058	0.043	0.074		0.023	0.024	0.023	0.028	0.029	0.028
1.8	0.107	0.082	0.088	0.035	0.084	0.072	0.037	0.082		0.023	0.024	0.023	0.028	0.029	0.028
1.95	0.107	0.107	0.105	0.101	0.073	0.11	0.039	0.107		0.024	0.024	0.023	0.028	0.029	0.028

Kinetics															
time mins	**Baseline(WPU)**			**2.27 µM IP6(WPU2)**			**4.45 µM IP6(WPU3)**			**15 µM IP6(WPU4)**			**30 µM IP6(WPU5)**		
	OD 620nm			OD 620nm			OD 620nm			OD 620nm			OD 620nm		
	exp1	exp2	epx3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3	exp1	exp2	exp3
0	0.028	0.028	0.029	0.028	0.028	0.031	0.03	0.03	0.029						
15	0.032	0.036	0.037	0.034	0.032	0.033	0.04	0.039	0.039						
30	0.033	0.039	0.041	0.04	0.037	0.038	0.051	0.05	0.05						
45	0.035	0.039	0.043	0.045	0.041	0.042	0.058	0.058	0.057						
60	0.037	0.044	0.051	0.047	0.043	0.043	0.06	0.06	0.059						
75	0.039	0.048	0.056	0.046	0.042	0.043	0.058	0.057	0.057						
90	0.043	0.053	0.059	0.045	0.042	0.042	0.056	0.064	0.052						
105	0.046	0.053	0.058	0.05	0.039	0.04	0.051	0.053	0.048						
120	0.048	0.055	0.061	0.043	0.042	0.038	0.064	0.057	0.047	msl above 195			msl above 195		

IP6 concentration in PU:

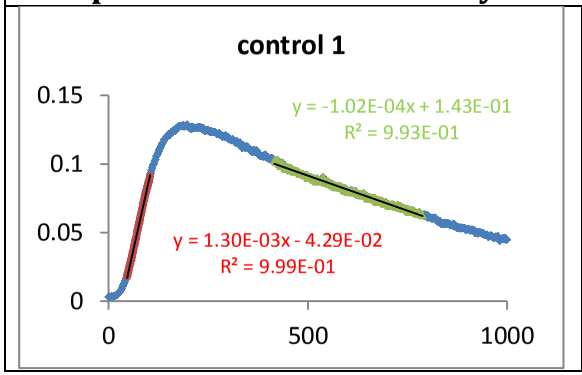
standard [IP6] uM	**OD1**	**OD2**	**average**
0	1.145	1.157	1.151
5	1.038	1.012	1.025
10	0.909	0.915	0.912
20	0.717	0.714	0.7155
25	0.617	0.61	0.6135

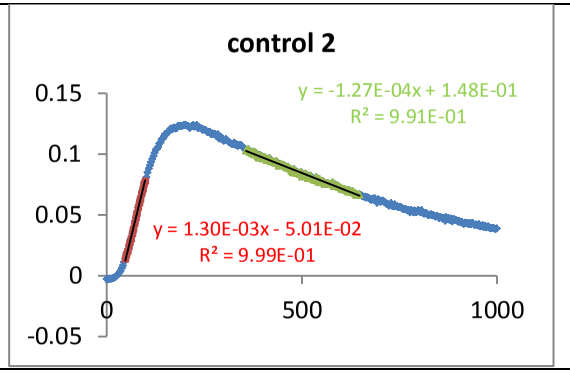
Sample	**OD1**	**OD2**		**[IP6] µM in eluate**	**[IP6] µM in urine**	**%recovery**
PU	1.049	1.077	1.063	3.538	**0.708**	
PU + 1µM	0.971	0.972	0.972	7.854	1.571	86

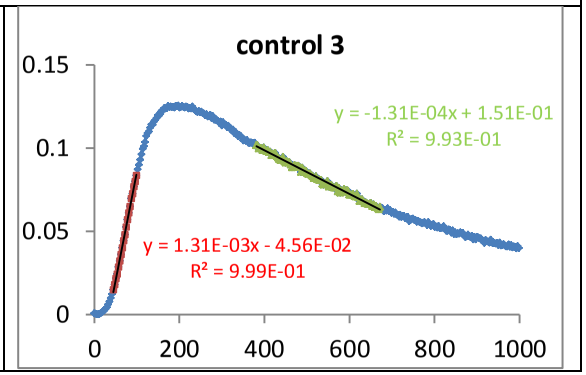


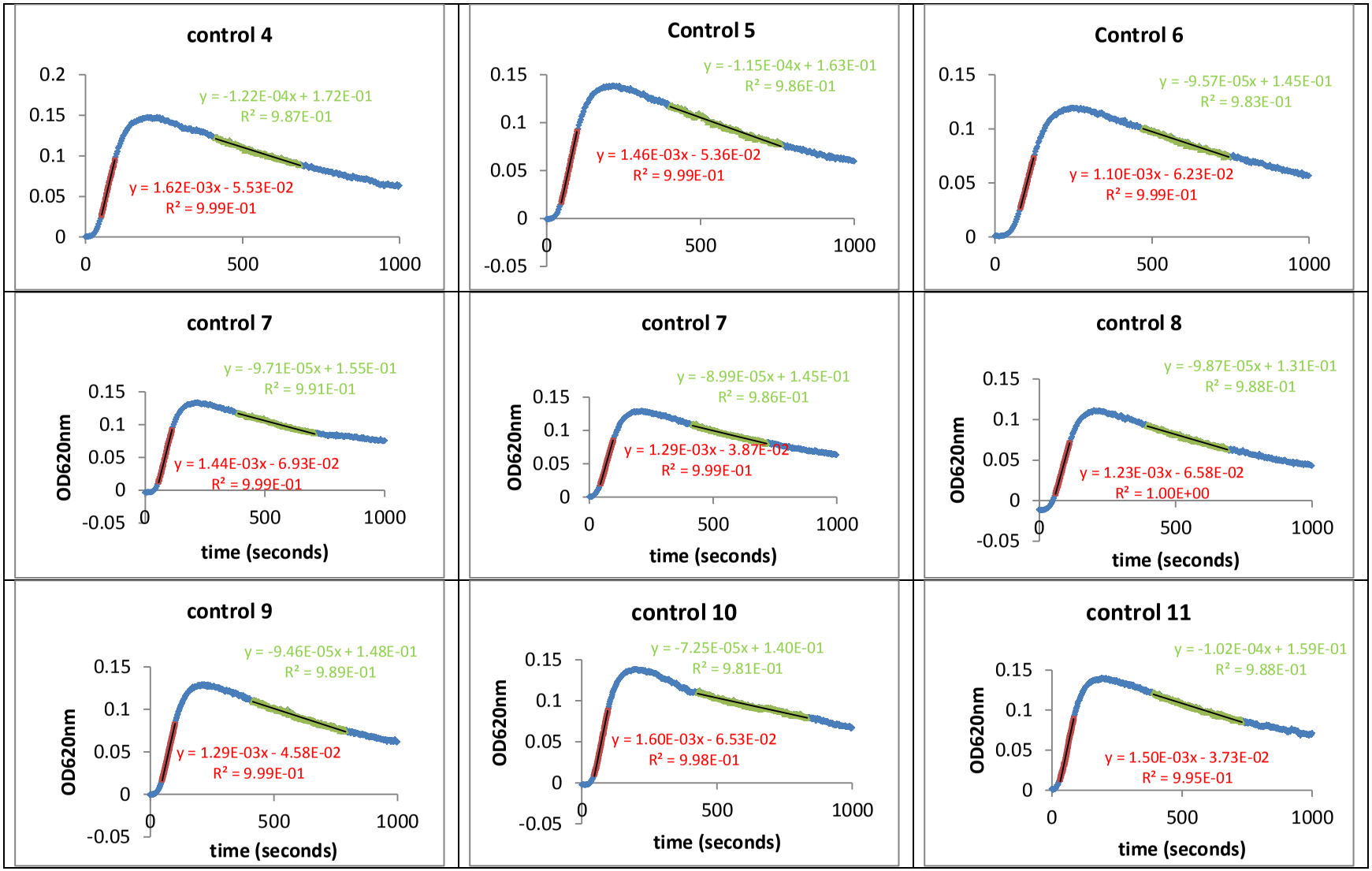
Appendix 5.6
nucleation and aggregation

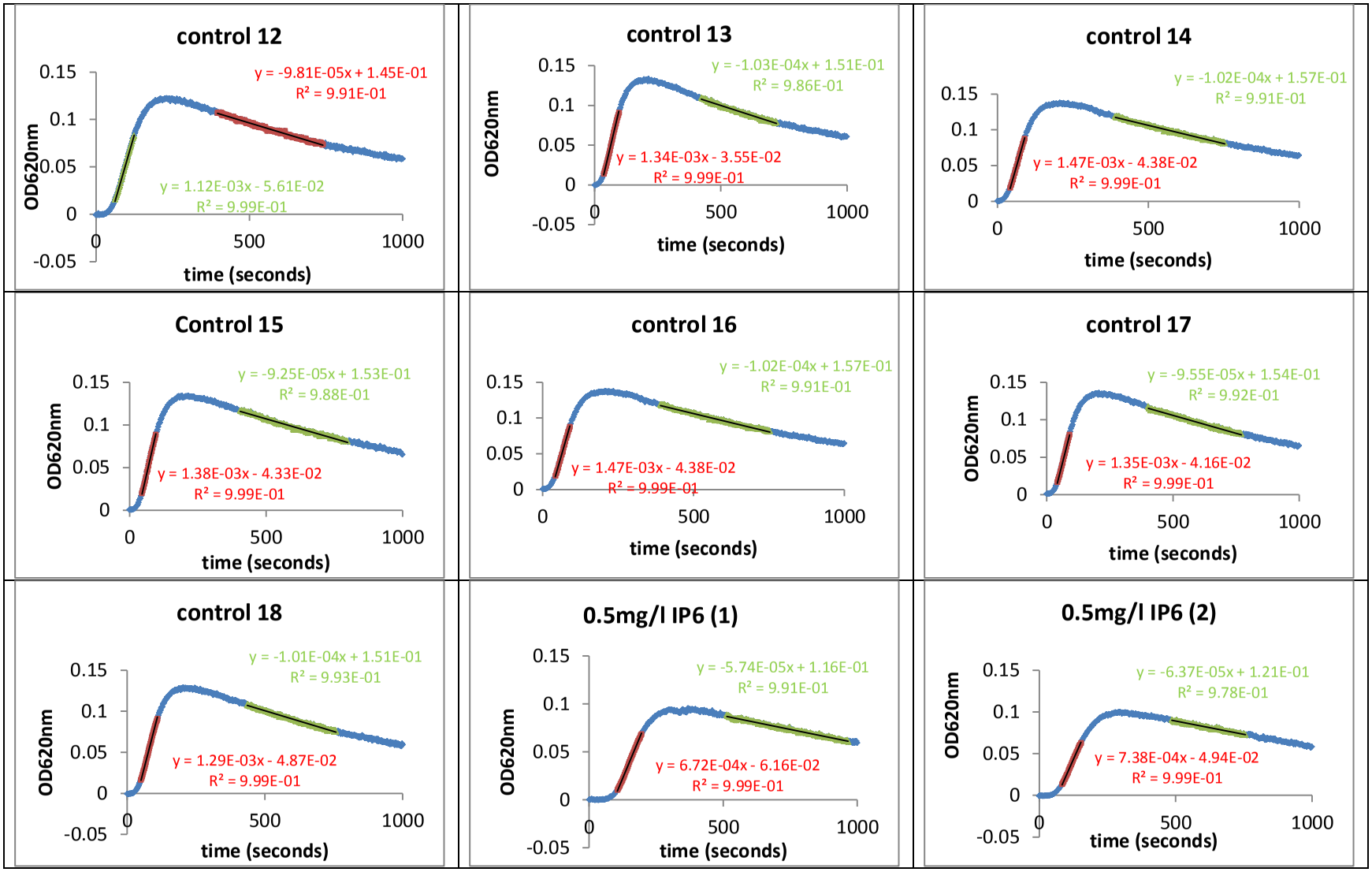
"simple salt solution" IP6 only

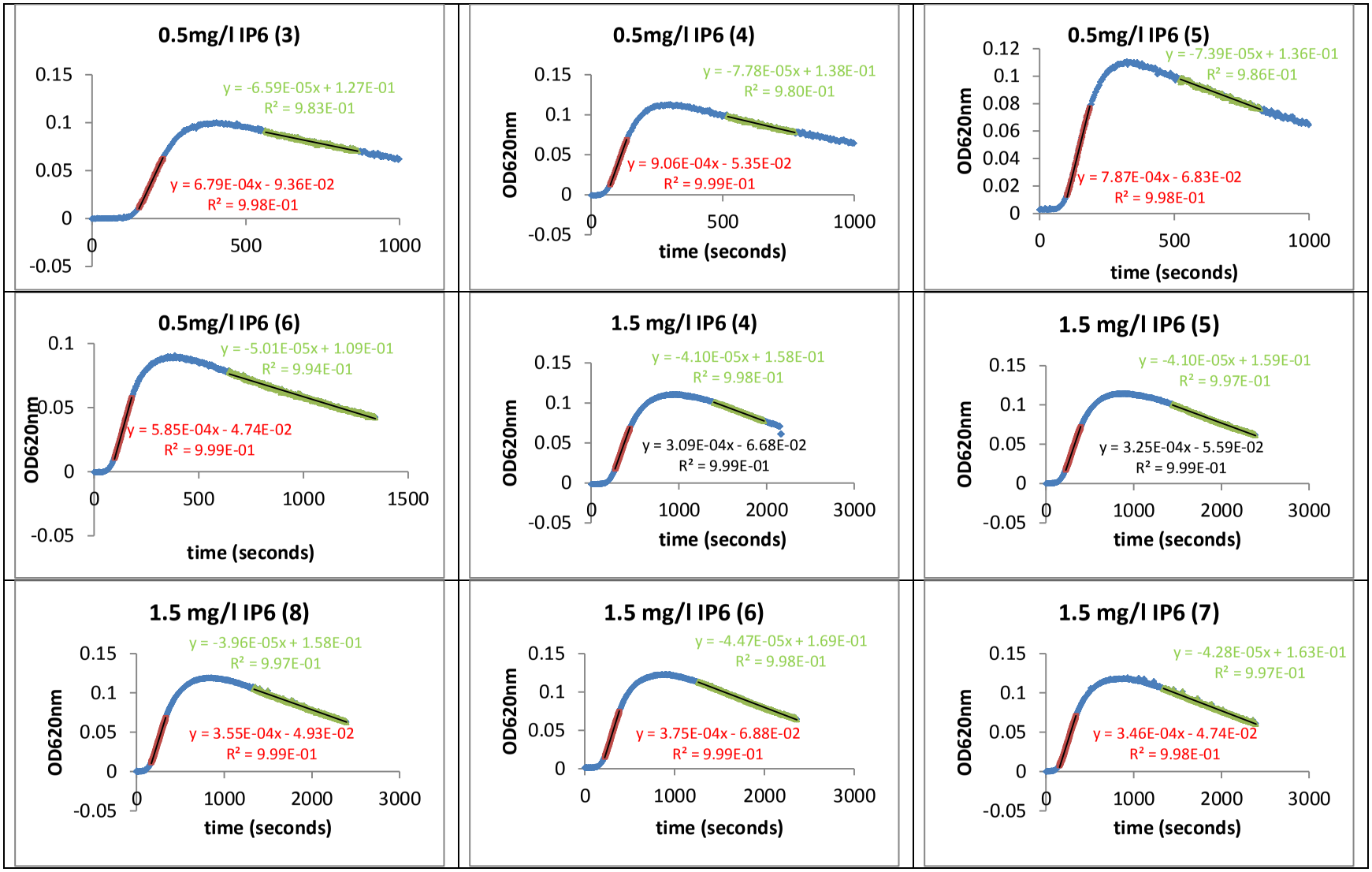


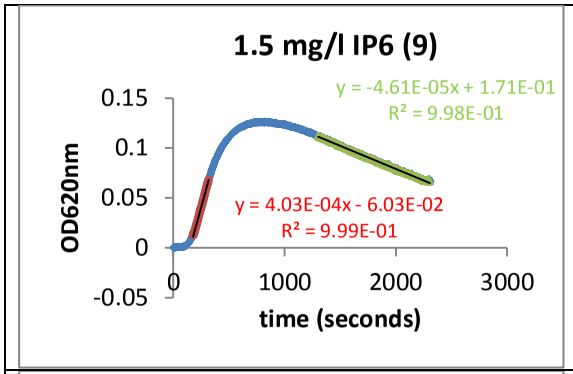


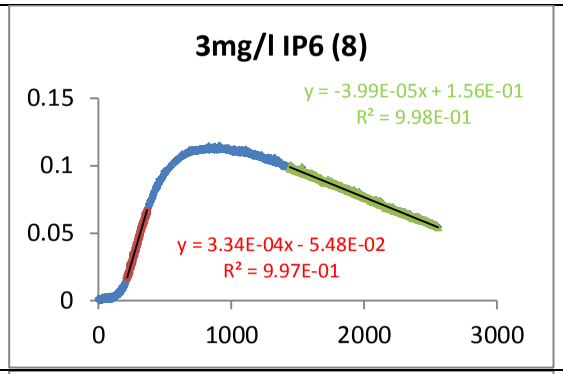


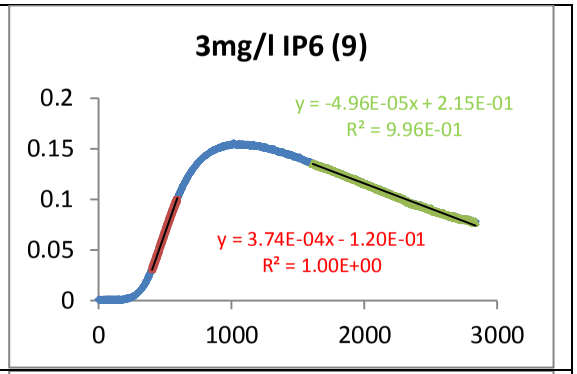

control 4
y = -1.22E-04x + 1.72E-01
R² = 9.87E-01
y = 1.62E-03x - 5.53E-02
R² = 9.99E-01
Control 5
y = -1.15E-04x + 1.63E-01
R² = 9.86E-01
y = 1.46E-03x - 5.36E-02
R² = 9.99E-01
Control 6
y = -9.57E-05x + 1.45E-01
R² = 9.83E-01
y = 1.10E-03x - 6.23E-02
R² = 9.99E-01
control 7
y = -9.71E-05x + 1.55E-01
R² = 9.91E-01
y = 1.44E-03x - 6.93E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 7
y = -8.99E-05x + 1.45E-01
R² = 9.86E-01
y = 1.29E-03x - 3.87E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 8
y = -9.87E-05x + 1.31E-01
R² = 9.88E-01
y = 1.23E-03x - 6.58E-02
R² = 1.00E+00
OD620nm
time (seconds)
control 9
y = -9.46E-05x + 1.48E-01
R² = 9.89E-01
y = 1.29E-03x - 4.58E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 10
y = -7.25E-05x + 1.40E-01
R² = 9.81E-01
y = 1.60E-03x - 6.53E-02
R² = 9.98E-01
OD620nm
time (seconds)
control 11
y = -1.02E-04x + 1.59E-01
R² = 9.88E-01
y = 1.50E-03x - 3.73E-02
R² = 9.95E-01
OD620nm
time (seconds)

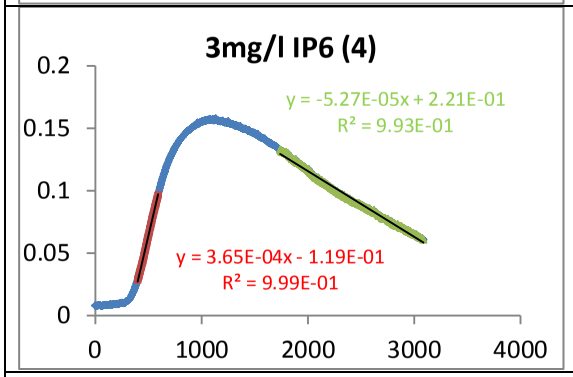

control 12
y = -9.81E-05x + 1.45E-01
R² = 9.91E-01
y = 1.12E-03x - 5.61E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 13
y = -1.03E-04x + 1.51E-01
R² = 9.86E-01
y = 1.34E-03x - 3.55E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 14
y = -1.02E-04x + 1.57E-01
R² = 9.91E-01
y = 1.47E-03x - 4.38E-02
R² = 9.99E-01
time (seconds)
Control 15
y = -9.25E-05x + 1.53E-01
R² = 9.88E-01
y = 1.38E-03x - 4.33E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 16
y = -1.02E-04x + 1.57E-01
R² = 9.91E-01
y = 1.47E-03x - 4.38E-02
R² = 9.99E-01
time (seconds)
control 17
y = -9.55E-05x + 1.54E-01
R² = 9.92E-01
y = 1.35E-03x - 4.16E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 18
y = -1.01E-04x + 1.51E-01
R² = 9.93E-01
y = 1.29E-03x - 4.87E-02
R² = 9.99E-01
OD620nm
time (seconds)
0.5mg/l IP6 (1)
y = -5.74E-05x + 1.16E-01
R² = 9.91E-01
y = 6.72E-04x - 6.16E-02
R² = 9.99E-01
OD620nm
time (seconds)
0.5mg/l IP6 (2)
y = -6.37E-05x + 1.21E-01
R² = 9.78E-01
y = 7.38E-04x - 4.94E-02
R² = 9.99E-01
OD620nm
time (seconds)

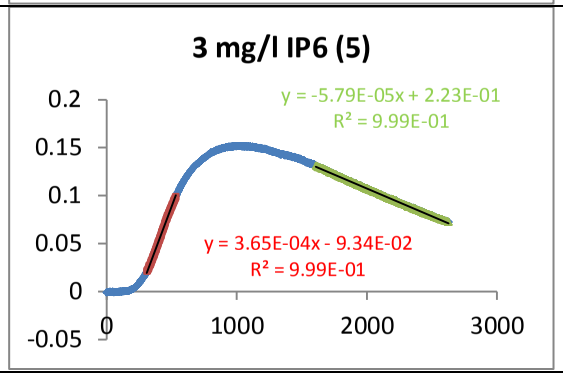

0.5mg/l IP6 (3)
y = -6.59E-05x + 1.27E-01
R² = 9.83E-01
y = 6.79E-04x - 9.36E-02
R² = 9.98E-01
0.5mg/l IP6 (4)
y = -7.78E-05x + 1.38E-01
R² = 9.80E-01
y = 9.06E-04x - 5.35E-02
R² = 9.99E-01
OD620nm
time (seconds)
0.5mg/l IP6 (5)
y = -7.39E-05x + 1.36E-01
R² = 9.86E-01
y = 7.87E-04x - 6.83E-02
R² = 9.98E-01
0.5mg/l IP6 (6)
y = -5.01E-05x + 1.09E-01
R² = 9.94E-01
y = 5.85E-04x - 4.74E-02
R² = 9.99E-01
1.5 mg/l IP6 (4)
y = -4.10E-05x + 1.58E-01
R² = 9.98E-01
y = 3.09E-04x - 6.68E-02
R² = 9.99E-01
1.5 mg/l IP6 (5)
y = -4.10E-05x + 1.59E-01
R² = 9.97E-01
y = 3.25E-04x - 5.59E-02
R² = 9.99E-01
1.5 mg/l IP6 (8)
y = -3.96E-05x + 1.58E-01
R² = 9.97E-01
y = 3.55E-04x - 4.93E-02
R² = 9.99E-01
1.5 mg/l IP6 (6)
y = -4.47E-05x + 1.69E-01
R² = 9.98E-01
y = 3.75E-04x - 6.88E-02
R² = 9.99E-01
1.5 mg/l IP6 (7)
y = -4.28E-05x + 1.63E-01
R² = 9.97E-01
y = 3.46E-04x - 4.74E-02
R² = 9.98E-01

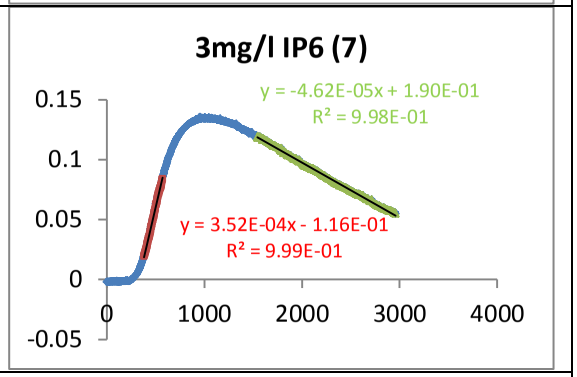




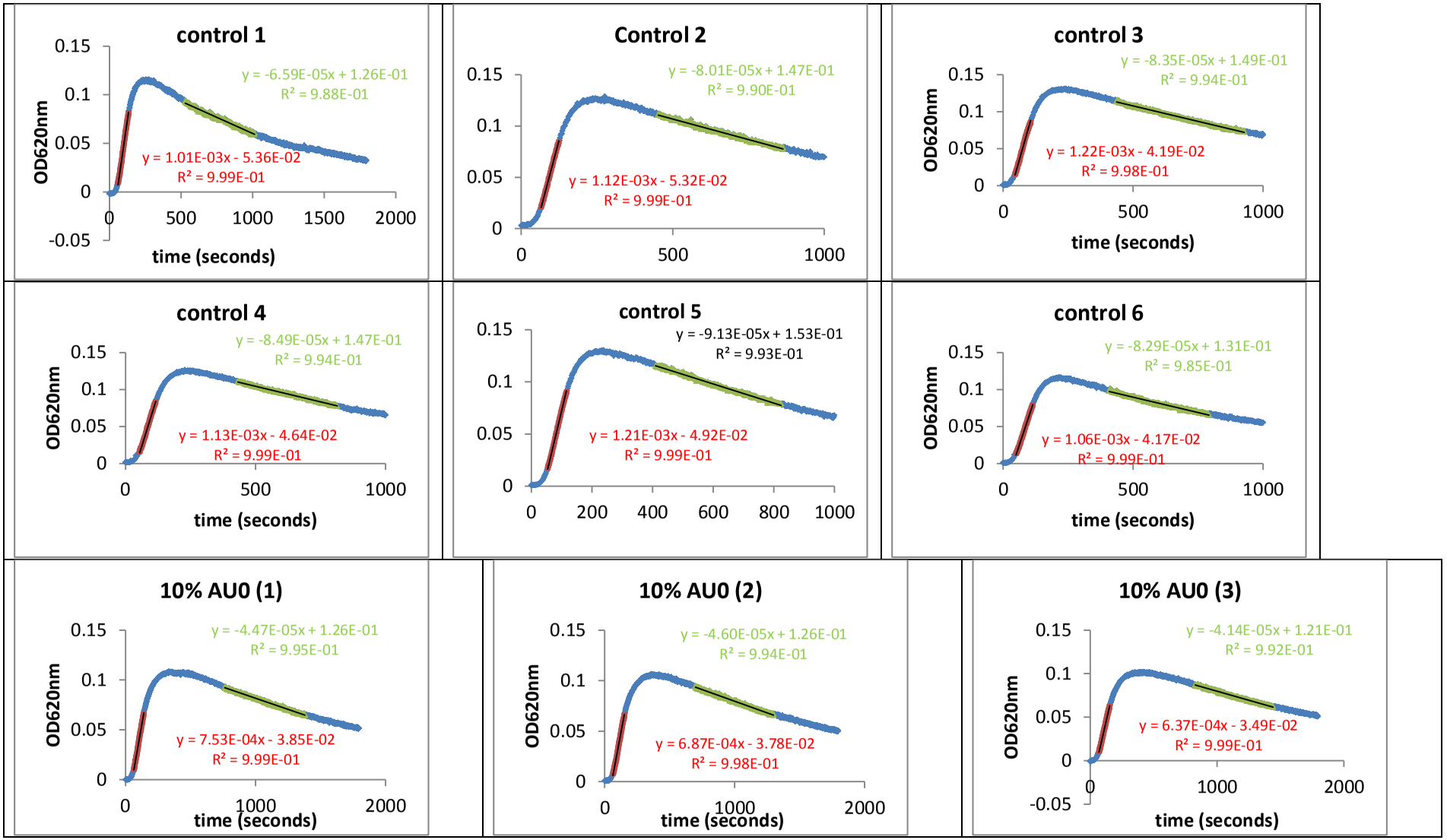


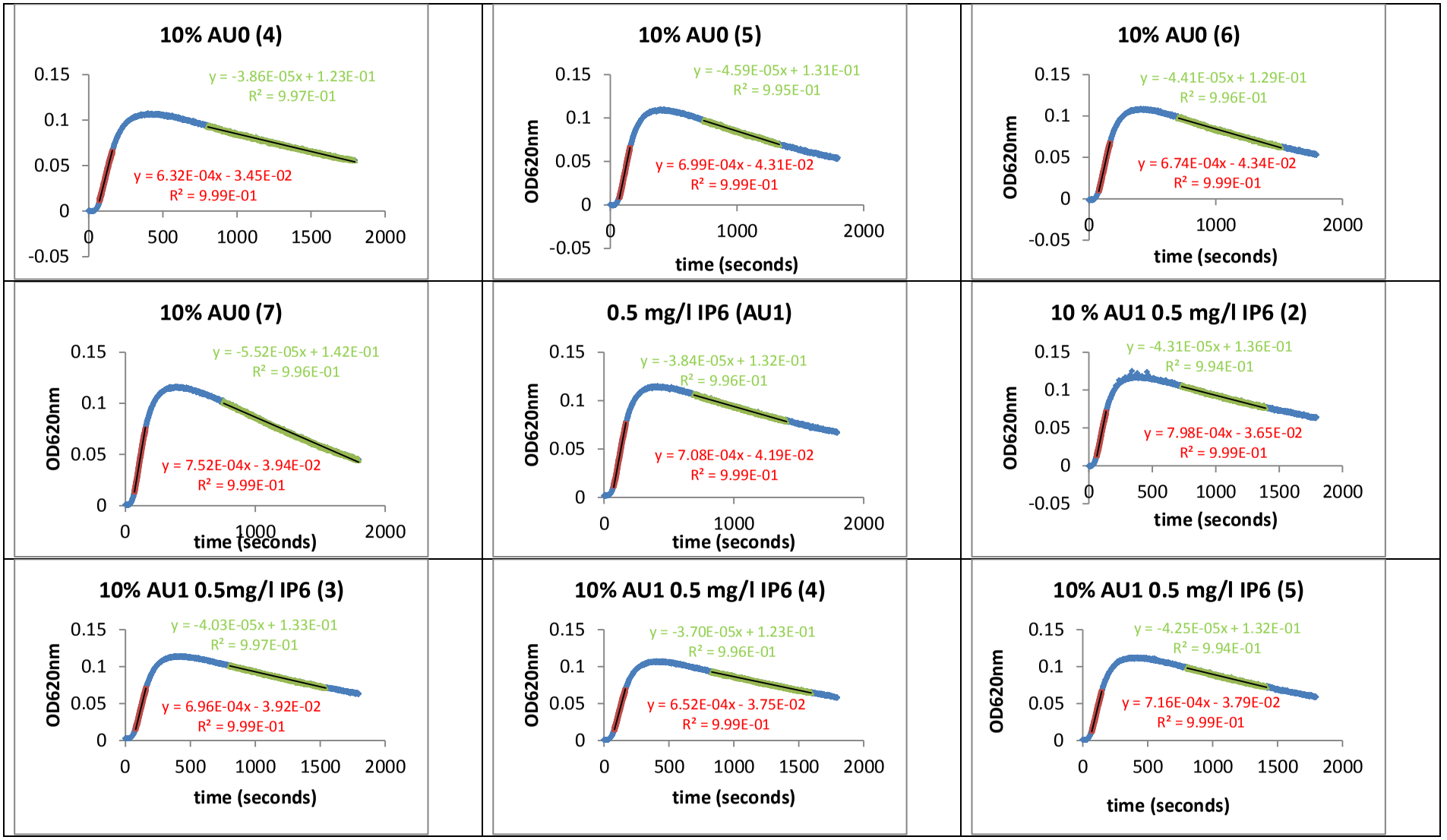


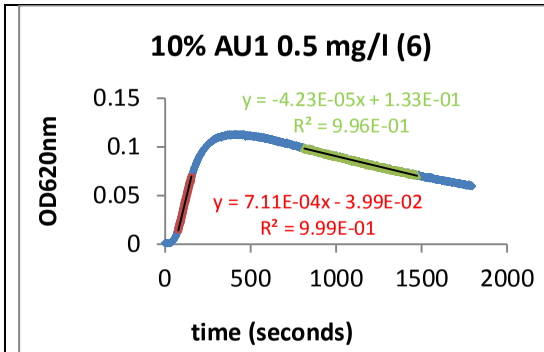


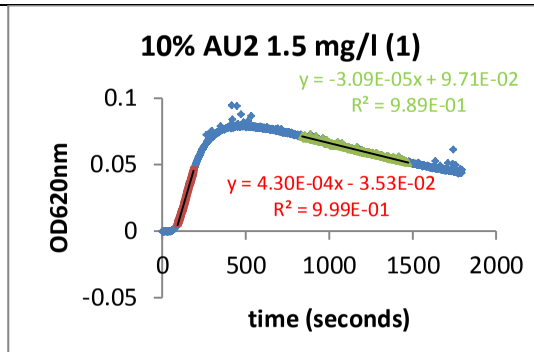


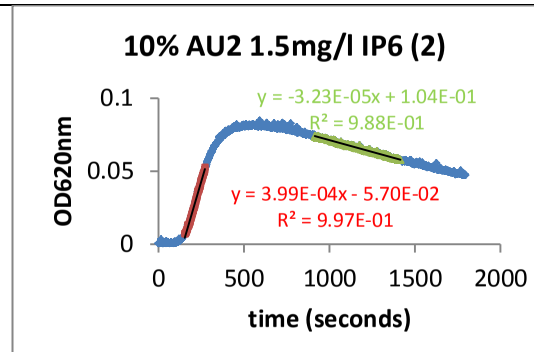
CaOx nucleation and aggregation (effect of artificial urine)

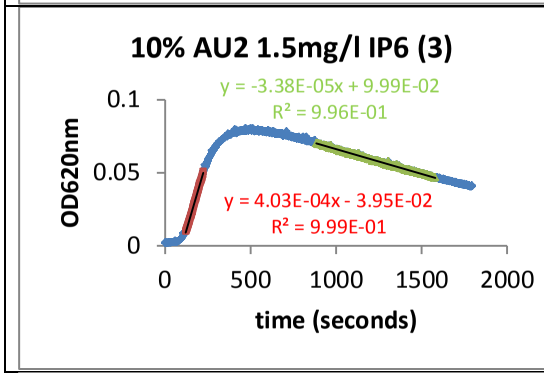

control 1
y = -6.59E-05x + 1.26E-01
R² = 9.88E-01
y = 1.01E-03x - 5.36E-02
R² = 9.99E-01
OD620nm
time (seconds)
Control 2
y = -8.01E-05x + 1.47E-01
R² = 9.90E-01
y = 1.12E-03x - 5.32E-02
R² = 9.99E-01
control 3
y = -8.35E-05x + 1.49E-01
R² = 9.94E-01
y = 1.22E-03x - 4.19E-02
R² = 9.98E-01
control 4
y = -8.49E-05x + 1.47E-01
R² = 9.94E-01
y = 1.13E-03x - 4.64E-02
R² = 9.99E-01
control 5
y = -9.13E-05x + 1.53E-01
R² = 9.93E-01
y = 1.21E-03x - 4.92E-02
R² = 9.99E-01
control 6
y = -8.29E-05x + 1.31E-01
R² = 9.85E-01
y = 1.06E-03x - 4.17E-02
R² = 9.99E-01
10% AU0 (1)
y = -4.47E-05x + 1.26E-01
R² = 9.95E-01
y = 7.53E-04x - 3.85E-02
R² = 9.99E-01
10% AU0 (2)
y = -4.60E-05x + 1.26E-01
R² = 9.94E-01
y = 6.87E-04x - 3.78E-02
R² = 9.98E-01
10% AU0 (3)
y = -4.14E-05x + 1.21E-01
R² = 9.92E-01
y = 6.37E-04x - 3.49E-02
R² = 9.99E-01

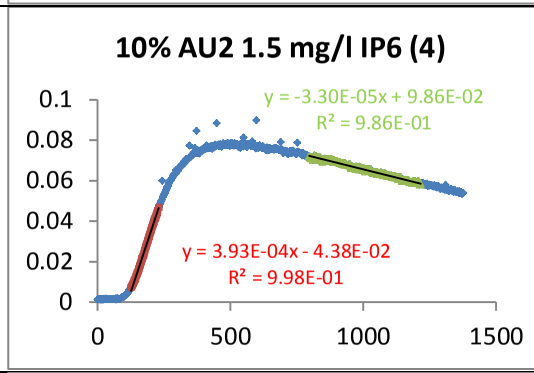

10% AU0 (4)
y = -3.86E-05x + 1.23E-01
R² = 9.97E-01
y = 6.32E-04x - 3.45E-02
R² = 9.99E-01
10% AU0 (5)
y = -4.59E-05x + 1.31E-01
R² = 9.95E-01
y = 6.99E-04x - 4.31E-02
R² = 9.99E-01
10% AU0 (6)
y = -4.41E-05x + 1.29E-01
R² = 9.96E-01
y = 6.74E-04x - 4.34E-02
R² = 9.99E-01
10% AU0 (7)
y = -5.52E-05x + 1.42E-01
R² = 9.96E-01
y = 7.52E-04x - 3.94E-02
R² = 9.99E-01
0.5 mg/l IP6 (AU1)
y = -3.84E-05x + 1.32E-01
R² = 9.96E-01
y = 7.08E-04x - 4.19E-02
R² = 9.99E-01
10 % AU1 0.5 mg/l IP6 (2)
y = -4.31E-05x + 1.36E-01
R² = 9.94E-01
y = 7.98E-04x - 3.65E-02
R² = 9.99E-01
10% AU1 0.5mg/l IP6 (3)
y = -4.03E-05x + 1.33E-01
R² = 9.97E-01
y = 6.96E-04x - 3.92E-02
R² = 9.99E-01
10% AU1 0.5 mg/l IP6 (4)
y = -3.70E-05x + 1.23E-01
R² = 9.96E-01
y = 6.52E-04x - 3.75E-02
R² = 9.99E-01
10% AU1 0.5 mg/l IP6 (5)
y = -4.25E-05x + 1.32E-01
R² = 9.94E-01
y = 7.16E-04x - 3.79E-02
R² = 9.99E-01
OD620nm
time (seconds)

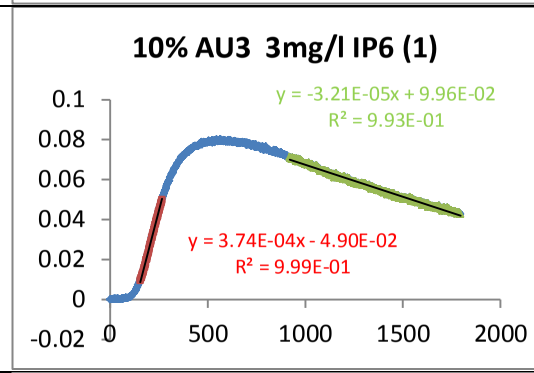

10% AU1 0.5 mg/l (6)
y = -4.23E-05x + 1.33E-01
R² = 9.96E-01
y = 7.11E-04x - 3.99E-02
R² = 9.99E-01
OD620nm
time (seconds)

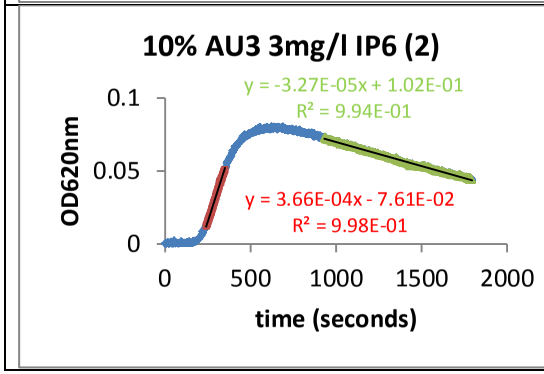

10% AU2 1.5 mg/l (1)
y = -3.09E-05x + 9.71E-02
R² = 9.89E-01
y = 4.30E-04x - 3.53E-02
R² = 9.99E-01
OD620nm
time (seconds)

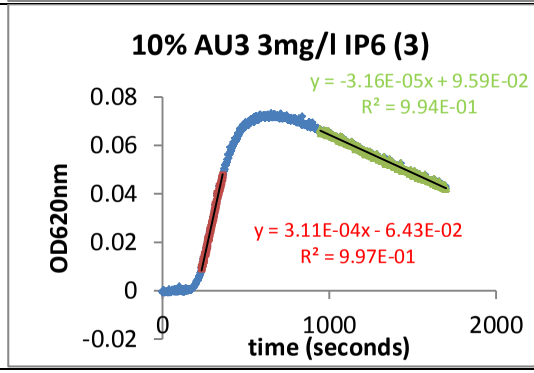

10% AU2 1.5mg/l IP6 (2)
y = -3.23E-05x + 1.04E-01
R² = 9.88E-01
y = 3.99E-04x - 5.70E-02
R² = 9.97E-01
OD620nm
time (seconds)

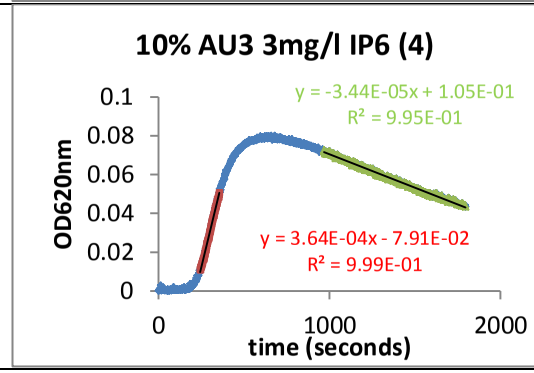

10% AU2 1.5mg/l IP6 (3)
y = -3.38E-05x + 9.99E-02
R² = 9.96E-01
y = 4.03E-04x - 3.95E-02
R² = 9.99E-01
OD620nm
time (seconds)

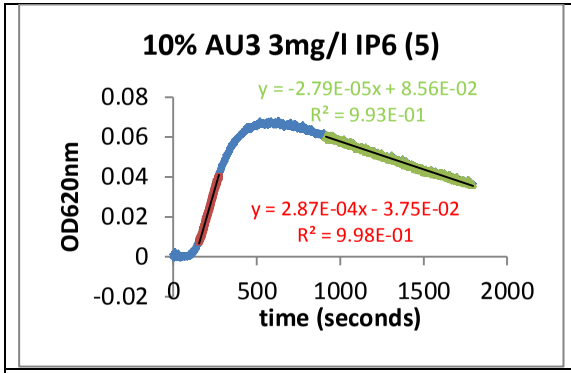

10% AU2 1.5 mg/l IP6 (4)
y = -3.30E-05x + 9.86E-02
R² = 9.86E-01
y = 3.93E-04x - 4.38E-02
R² = 9.98E-01

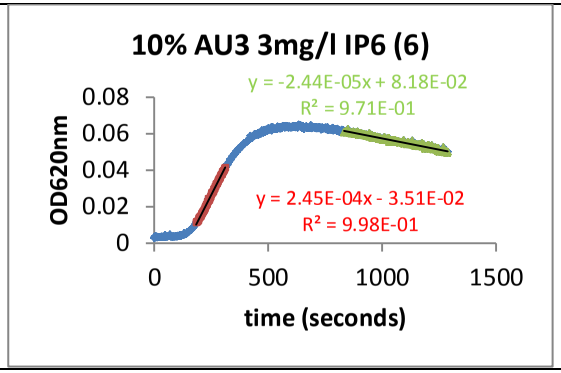

10% AU3 3mg/l IP6 (1)
y = -3.21E-05x + 9.96E-02
R² = 9.93E-01
y = 3.74E-04x - 4.90E-02
R² = 9.99E-01


10% AU3 3mg/l IP6 (2)
y = -3.27E-05x + 1.02E-01
R² = 9.94E-01
y = 3.66E-04x - 7.61E-02
R² = 9.98E-01
OD620nm
time (seconds)

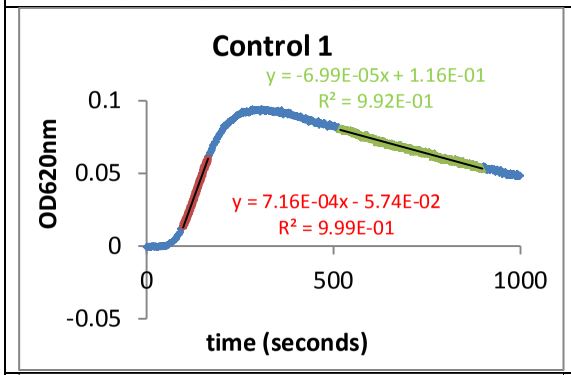

10% AU3 3mg/l IP6 (3)
y = -3.16E-05x + 9.59E-02
R² = 9.94E-01
y = 3.11E-04x - 6.43E-02
R² = 9.97E-01
OD620nm
time (seconds)

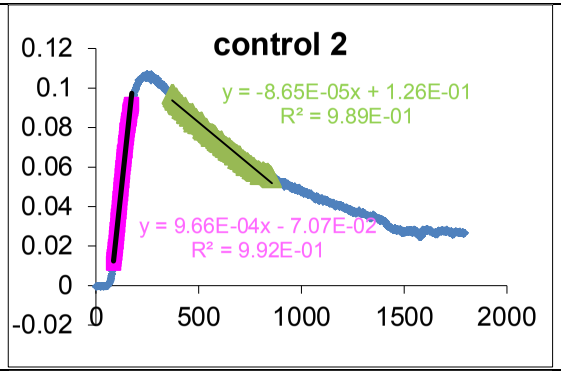

10% AU3 3mg/l IP6 (4)
y = -3.44E-05x + 1.05E-01
R² = 9.95E-01
y = 3.64E-04x - 7.91E-02
R² = 9.99E-01
OD620nm
time (seconds)

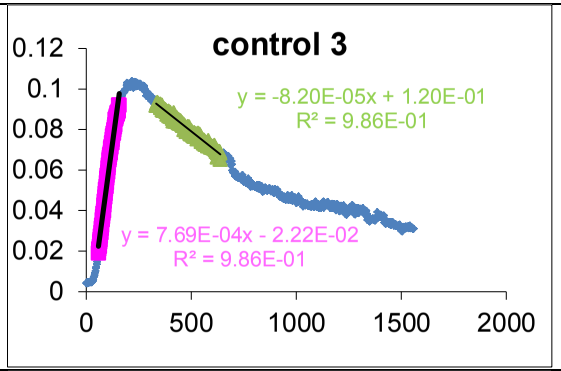


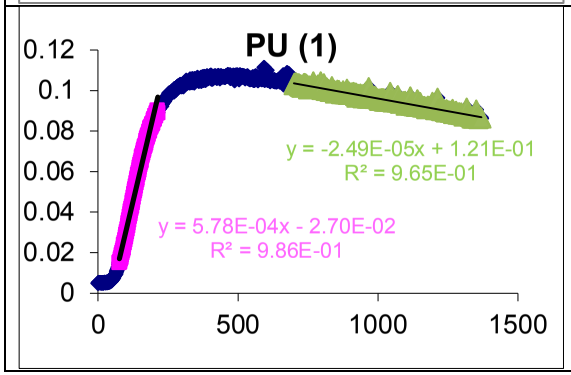


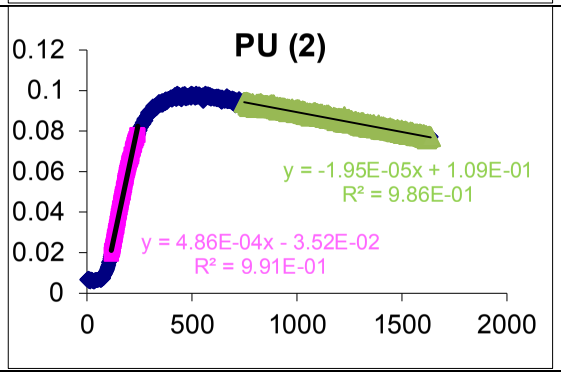
Effect of Pooled Urine

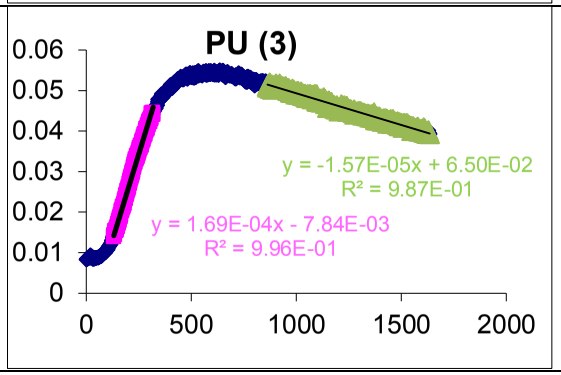


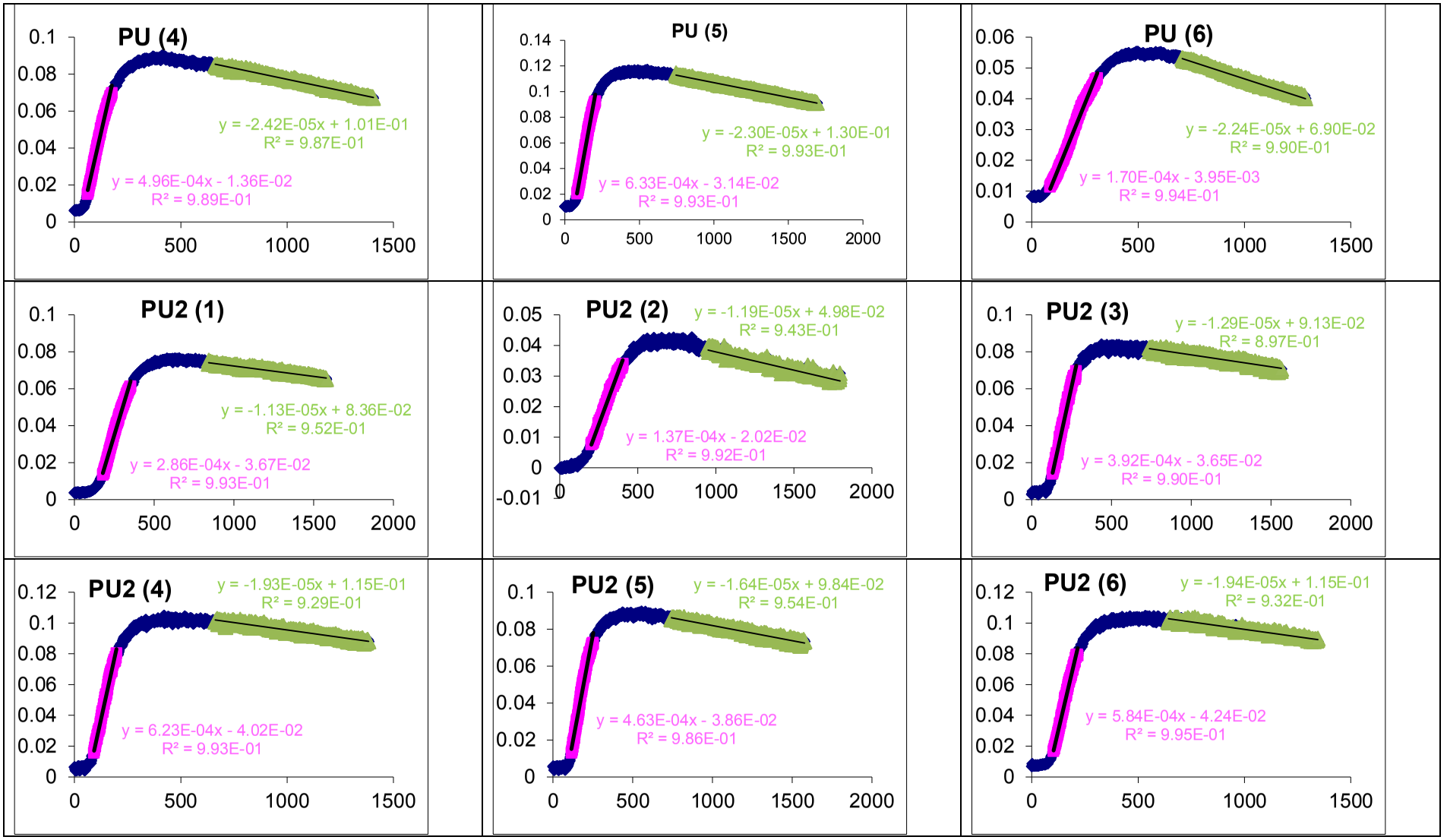






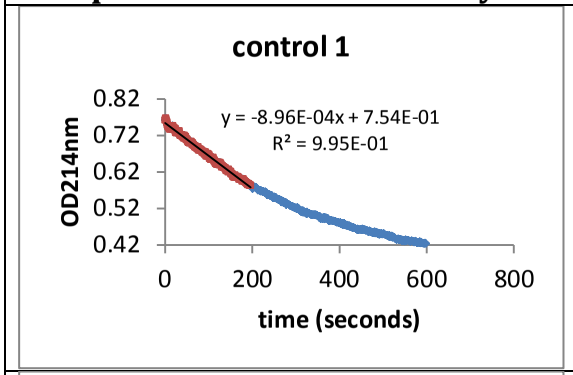


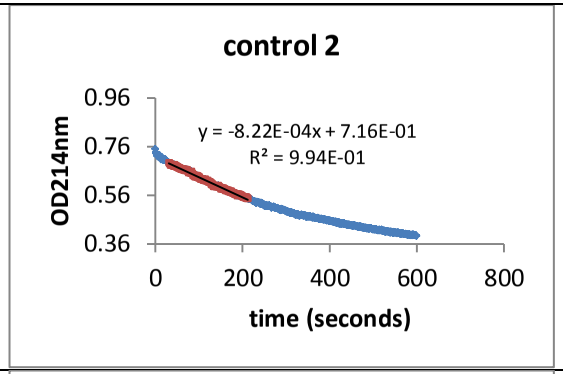


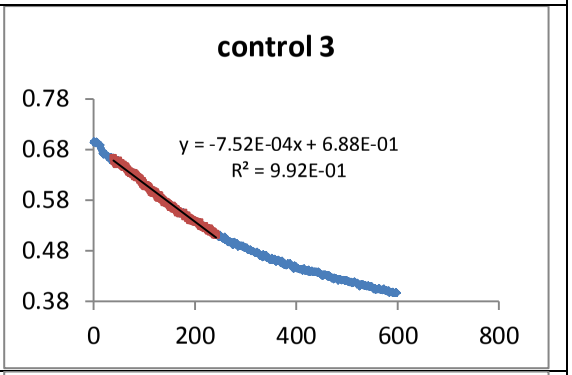

PU (4)
y = -2.42E-05x + 1.01E-01
R² = 9.87E-01
y = 4.96E-04x - 1.36E-02
R² = 9.89E-01
PU (5)
y = -2.30E-05x + 1.30E-01
R² = 9.93E-01
y = 6.33E-04x - 3.14E-02
R² = 9.93E-01
PU (6)
y = -2.24E-05x + 6.90E-02
R² = 9.90E-01
y = 1.70E-04x - 3.95E-03
R² = 9.94E-01
PU2 (1)
y = -1.13E-05x + 8.36E-02
R² = 9.52E-01
y = 2.86E-04x - 3.67E-02
R² = 9.93E-01
PU2 (2)
y = -1.19E-05x + 4.98E-02
R² = 9.43E-01
y = 1.37E-04x - 2.02E-02
R² = 9.92E-01
PU2 (3)
y = -1.29E-05x + 9.13E-02
R² = 8.97E-01
y = 3.92E-04x - 3.65E-02
R² = 9.90E-01
PU2 (4)
y = -1.93E-05x + 1.15E-01
R² = 9.29E-01
y = 6.23E-04x - 4.02E-02
R² = 9.93E-01
PU2 (5)
y = -1.64E-05x + 9.84E-02
R² = 9.54E-01
y = 4.63E-04x - 3.86E-02
R² = 9.86E-01
PU2 (6)
y = -1.94E-05x + 1.15E-01
R² = 9.32E-01
y = 5.84E-04x - 4.24E-02
R² = 9.95E-01

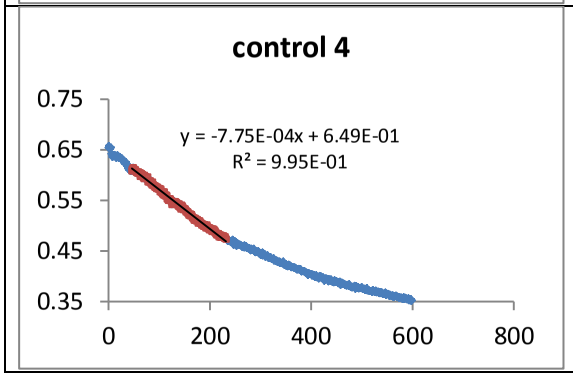
Appendix 5.7
CaOx growth

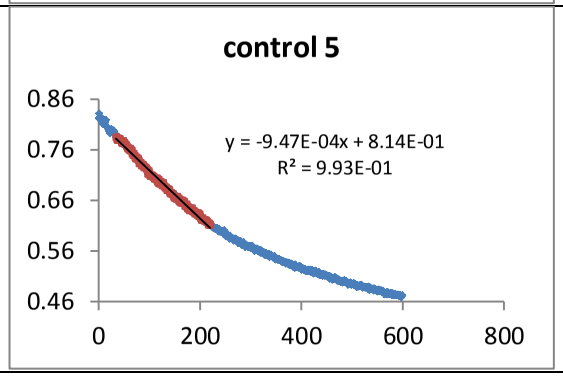
"simple salt solution" IP6 only

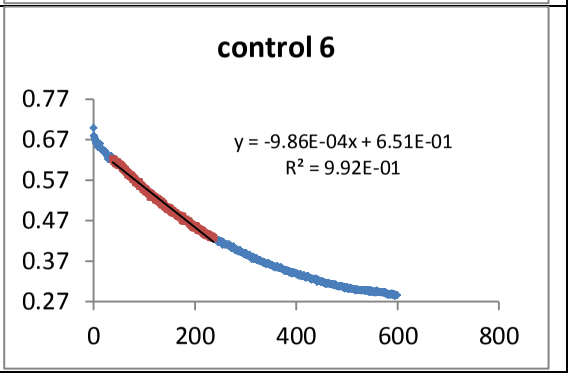


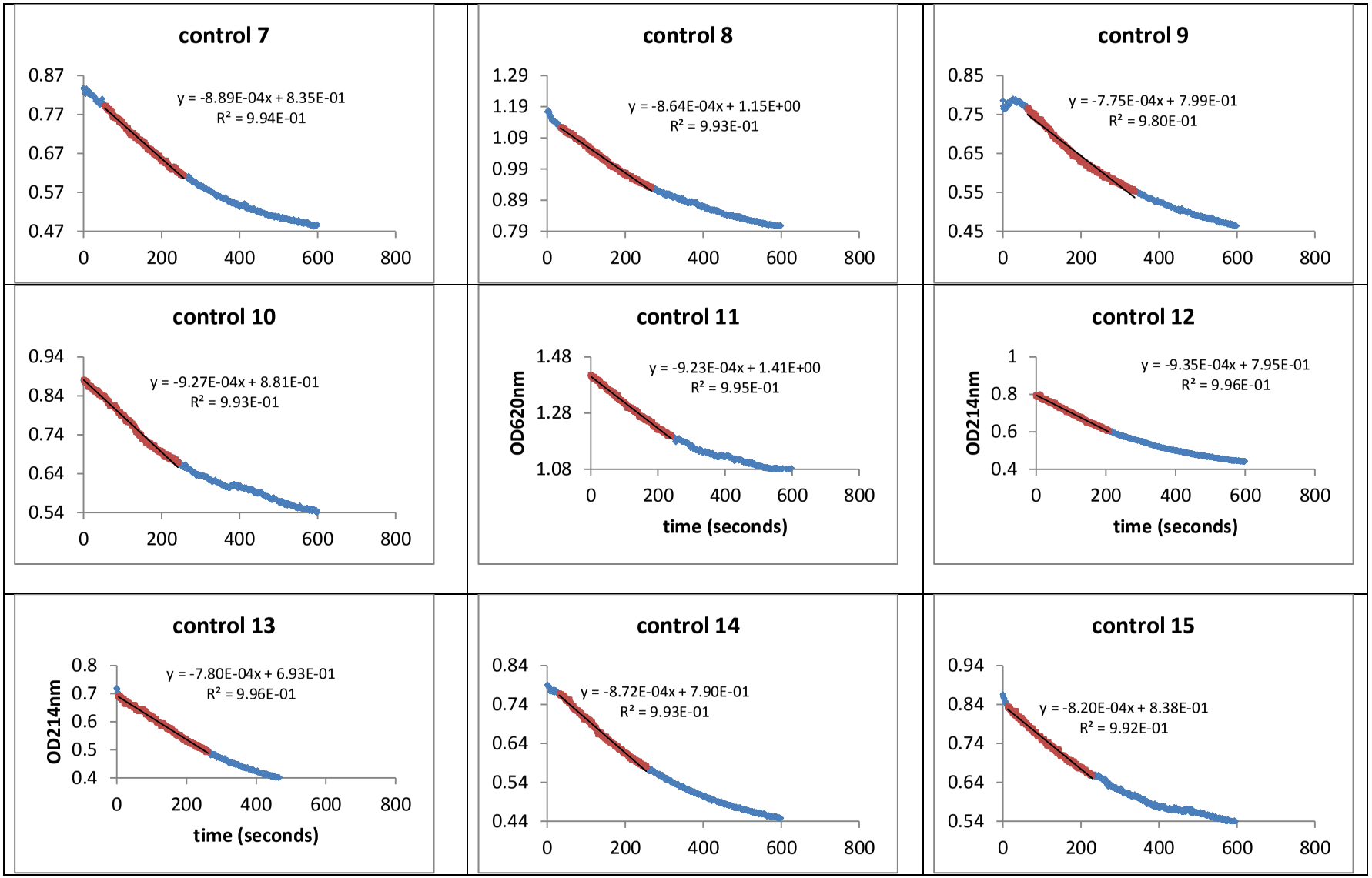


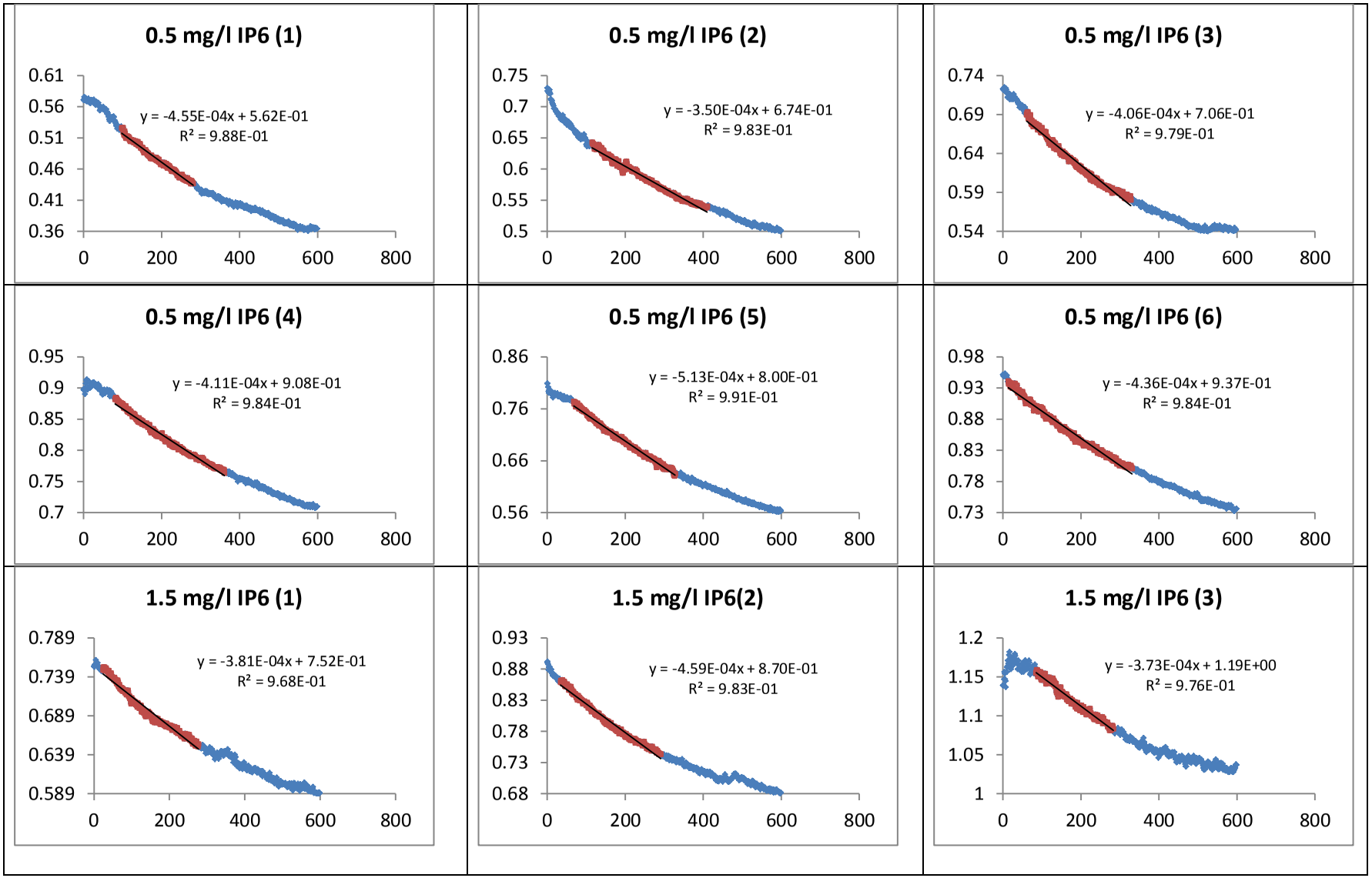


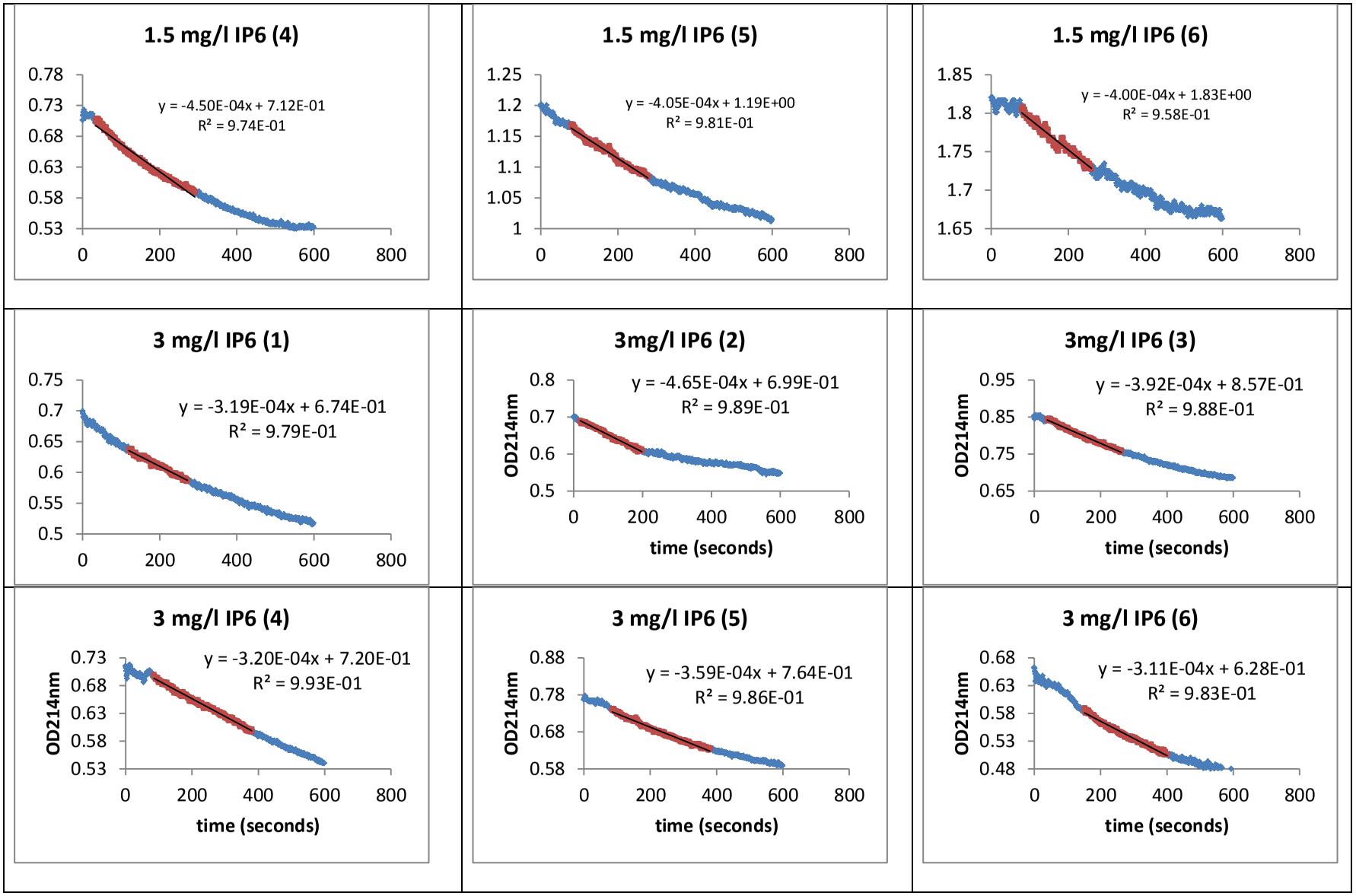




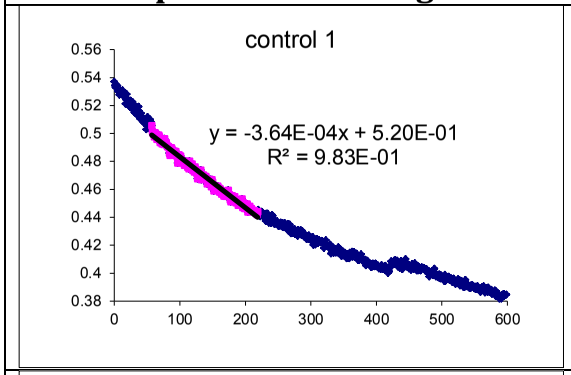


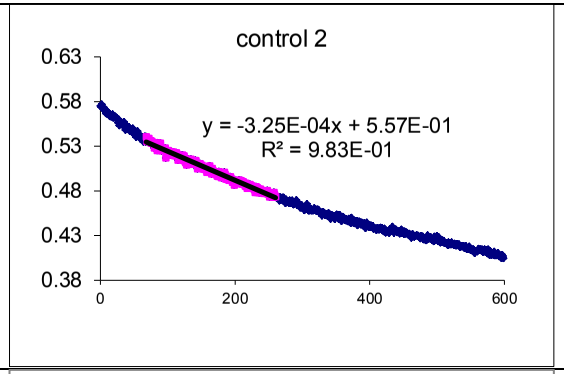

control 7
y = -8.89E-04x + 8.35E-01
R² = 9.94E-01
control 8
y = -8.64E-04x + 1.15E+00
R² = 9.93E-01
control 9
y = -7.75E-04x + 7.99E-01
R² = 9.80E-01
control 10
y = -9.27E-04x + 8.81E-01
R² = 9.93E-01
control 11
y = -9.23E-04x + 1.41E+00
R² = 9.95E-01
OD620nm
time (seconds)
control 12
y = -9.35E-04x + 7.95E-01
R² = 9.96E-01
OD214nm
time (seconds)
control 13
y = -7.80E-04x + 6.93E-01
R² = 9.96E-01
OD214nm
time (seconds)
control 14
y = -8.72E-04x + 7.90E-01
R² = 9.93E-01
control 15
y = -8.20E-04x + 8.38E-01
R² = 9.92E-01

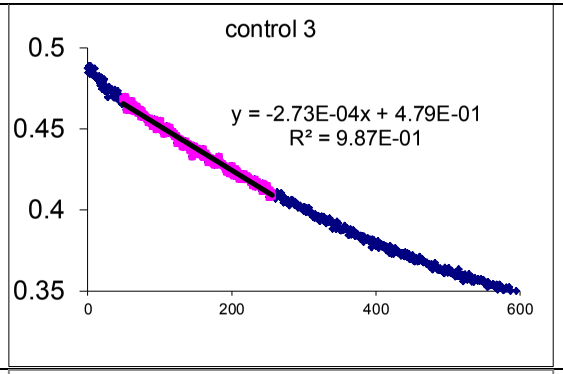

0.5 mg/l IP6 (1)
y = -4.55E-04x + 5.62E-01
R² = 9.88E-01
0.5 mg/l IP6 (2)
y = -3.50E-04x + 6.74E-01
R² = 9.83E-01
0.5 mg/l IP6 (3)
y = -4.06E-04x + 7.06E-01
R² = 9.79E-01
0.5 mg/l IP6 (4)
y = -4.11E-04x + 9.08E-01
R² = 9.84E-01
0.5 mg/l IP6 (5)
y = -5.13E-04x + 8.00E-01
R² = 9.91E-01
0.5 mg/l IP6 (6)
y = -4.36E-04x + 9.37E-01
R² = 9.84E-01
1.5 mg/l IP6 (1)
y = -3.81E-04x + 7.52E-01
R² = 9.68E-01
1.5 mg/l IP6(2)
y = -4.59E-04x + 8.70E-01
R² = 9.83E-01
1.5 mg/l IP6 (3)
y = -3.73E-04x + 1.19E+00
R² = 9.76E-01

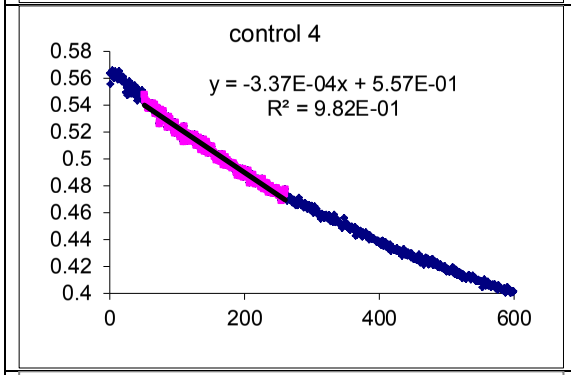

1.5 mg/l IP6 (4)
y = -4.50E-04x + 7.12E-01
R² = 9.74E-01
1.5 mg/l IP6 (5)
y = -4.05E-04x + 1.19E+00
R² = 9.81E-01
1.5 mg/l IP6 (6)
y = -4.00E-04x + 1.83E+00
R² = 9.58E-01
3 mg/l IP6 (1)
y = -3.19E-04x + 6.74E-01
R² = 9.79E-01
3mg/l IP6 (2)
y = -4.65E-04x + 6.99E-01
R² = 9.89E-01
3mg/l IP6 (3)
y = -3.92E-04x + 8.57E-01
R² = 9.88E-01
3 mg/l IP6 (4)
y = -3.20E-04x + 7.20E-01
R² = 9.93E-01
3 mg/l IP6 (5)
y = -3.59E-04x + 7.64E-01
R² = 9.86E-01
3 mg/l IP6 (6)
y = -3.11E-04x + 6.28E-01
R² = 9.83E-01
OD214nm
time (seconds)

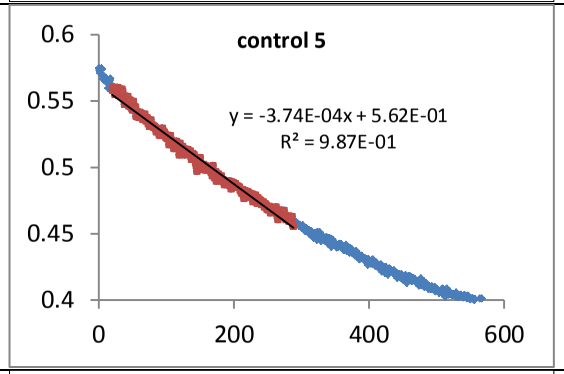
Effect of pooled urine on growth

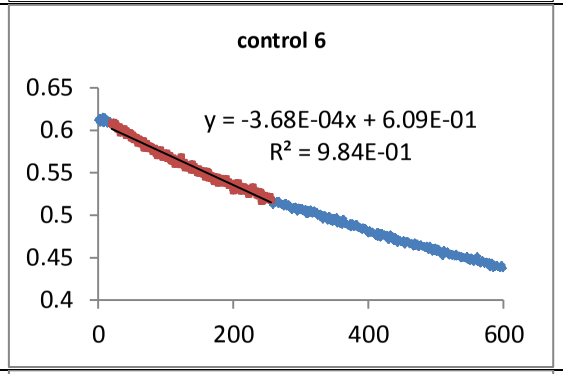


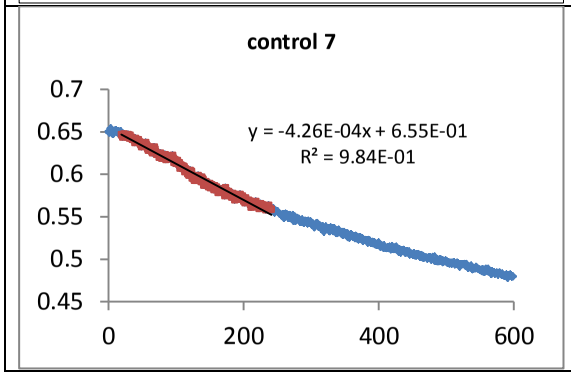


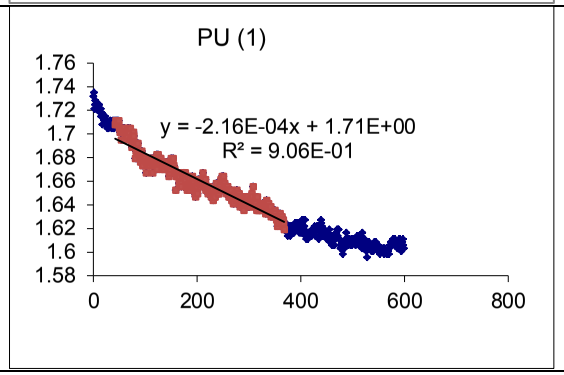


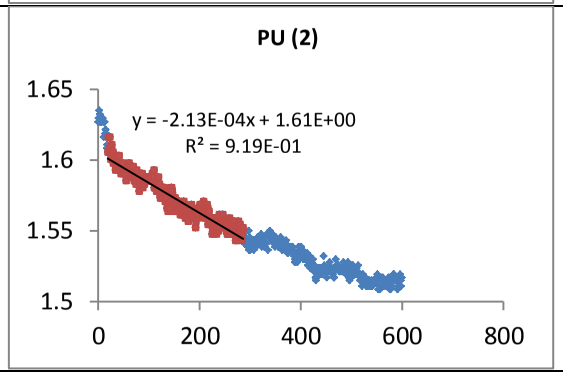


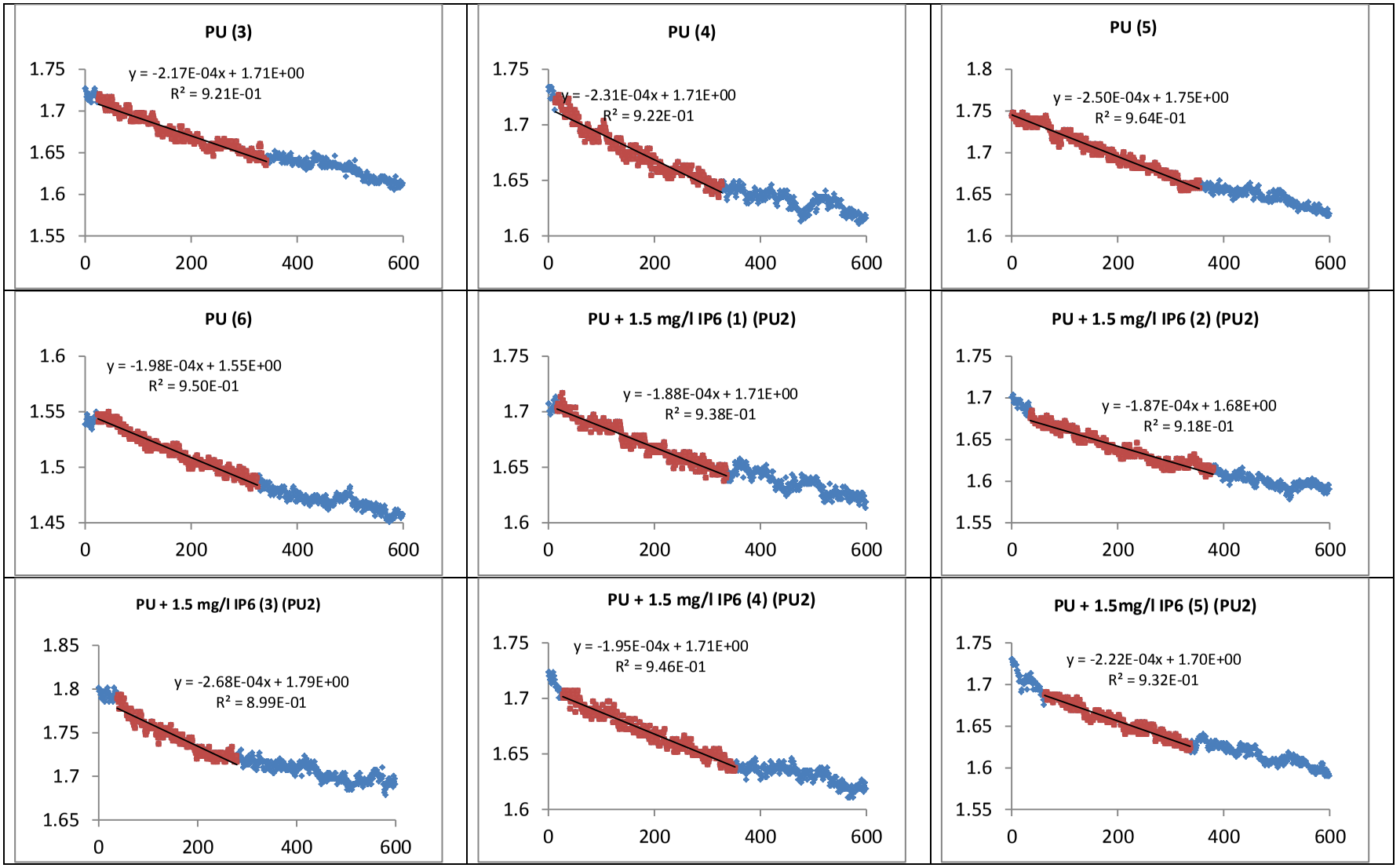


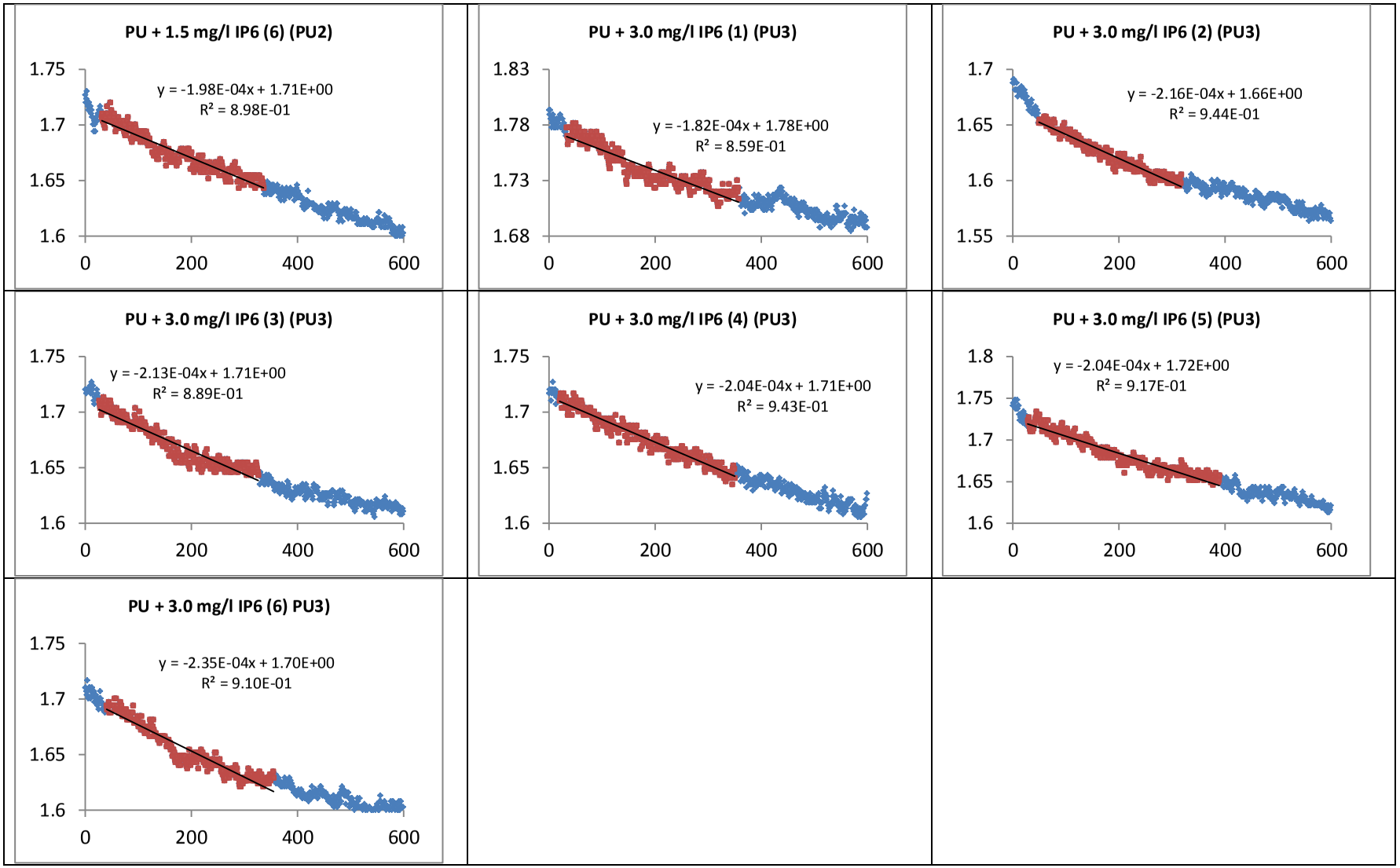









PU (3)
y = -2.17E-04x + 1.71E+00
R² = 9.21E-01
PU (4)
y = -2.31E-04x + 1.71E+00
R² = 9.22E-01
PU (5)
y = -2.50E-04x + 1.75E+00
R² = 9.64E-01
PU (6)
y = -1.98E-04x + 1.55E+00
R² = 9.50E-01
PU + 1.5 mg/l IP6 (1) (PU2)
y = -1.88E-04x + 1.71E+00
R² = 9.38E-01
PU + 1.5 mg/l IP6 (2) (PU2)
y = -1.87E-04x + 1.68E+00
R² = 9.18E-01
PU + 1.5 mg/l IP6 (3) (PU2)
y = -2.68E-04x + 1.79E+00
R² = 8.99E-01
PU + 1.5 mg/l IP6 (4) (PU2)
y = -1.95E-04x + 1.71E+00
R² = 9.46E-01
PU + 1.5mg/l IP6 (5) (PU2)
y = -2.22E-04x + 1.70E+00
R² = 9.32E-01


PU + 1.5 mg/l IP6 (6) (PU2)
y = -1.98E-04x + 1.71E+00
R² = 8.98E-01
PU + 3.0 mg/l IP6 (1) (PU3)
y = -1.82E-04x + 1.78E+00
R² = 8.59E-01
PU + 3.0 mg/l IP6 (2) (PU3)
y = -2.16E-04x + 1.66E+00
R² = 9.44E-01
PU + 3.0 mg/l IP6 (3) (PU3)
y = -2.13E-04x + 1.71E+00
R² = 8.89E-01
PU + 3.0 mg/l IP6 (4) (PU3)
y = -2.04E-04x + 1.71E+00
R² = 9.43E-01
PU + 3.0 mg/l IP6 (5) (PU3)
y = -2.04E-04x + 1.72E+00
R² = 9.17E-01
PU + 3.0 mg/l IP6 (6) PU3)
y = -2.35E-04x + 1.70E+00
R² = 9.10E-01

Appendix 6

Appendix 6.1
General questionnaire

General Questionnaire

General

Name: _____ Tel:_____

Date:_____ Cell:_____

Date of birth:_____ Gender: Male Race: _____

Nutritional

Weight:_____ Height:_____

What foods are you allergic to?_____

Are you taking any vitamin supplements? _____

If yes: Name of supplement_____ Dose_____

Frequency_____

Do you take any medication?_____

If yes, please list: prescription over-the counter

_____ _____

Lifestyle

Do you smoke? _____ How many a day?_____

Do you drink alcohol? _____ How many a day?_____

How many a week?_____

(drink = 1 can beer or 1 glass wine or tot spirits)

Medical history

Have you had any illnesses recently? _____

Have you undergone any recent surgery? _____

What kind of exercise do you do? _____

Hours per day? _____

Hours per week? _____

Appendix 6.2
Ethics approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

20 October 2011

HREC REF: 174/2011

Prof A Rodgers
Department of Chemistry
6.33.1 PD Hahn Building
Upper Campus

Dear Prof Rodgers

PROJECT TITLE: EFFECTS OF PHYTATE INGESTION ON THE URINARY RISK FACTORS FOR CALCIUM OXALATE UROLITHIASIS IN POPULATION GROUPS WITH DIFFERENT RISK PROFILES: COMPUTER MODELLING AND IN VITRO STUDIES.

Thank you for responding to the issues raised by the Faculty of Health Sciences Human Research Ethics Committee in your letter dated 13th October 2011.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 28 October 2012.

Please submit a progress form, using the standardised Annual Report Form (FHS016), if the study continues beyond the approval period. Please submit a Standard Closure form (FHS010) if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

s.thomas

Appendix 6.3
urine collection

Instructions for 24hr urine collections

- On the day on which urine collection begins, do ***not*** collect the 1st urine specimen upon waking.
- Thereafter, ***every*** urine voiding for the next 24 hours, must be passed into the collection bottle.
- The collection ends when the ***first*** voiding upon waking is passed into the bottle, on the next day.
- Because ***all*** urine for 24 hours is required, please remember to void into the bottle prior to defaecation.
- During the period of urine collection, the specimen bottle should be kept in a cool place. If possible, it should be refrigerated.
- If you have any queries, please phone: 021 650 2534.

Appendix 6.4
IP6 in oats

Raw data for determination of IP6 in oats

[IP6] uM	OD1 (500nm)	OD2 (500nm)	average	Calibration curve: equation of line
0	0.478	0.47	0.474	
7.57	0.425	0.432	0.4285	
15.15	0.385	0.391	0.388	
22.72	0.35	0.351	0.3505	$y = -0.0048x + 0.4645$; $R^2 = 0.9961$
30.3	0.31	0.304	0.307	
37.88	0.277	0.281	0.279	
45.45	0.245	0.258	0.2515	
53.03	0.212	0.212	0.212	
60.6	0.176	0.173	0.1745	

	Absorbance @ 500nm				
sample	OD1	OD2	OD3	OD4	OD5
Oats	0.393	0.392	0.393	0.395	0.392
Oats + 600 uM IP6	0.38	0.373	0.372	0.378	0.383

Appendix 6.5
Raw data for determination of IP6 in urine samples

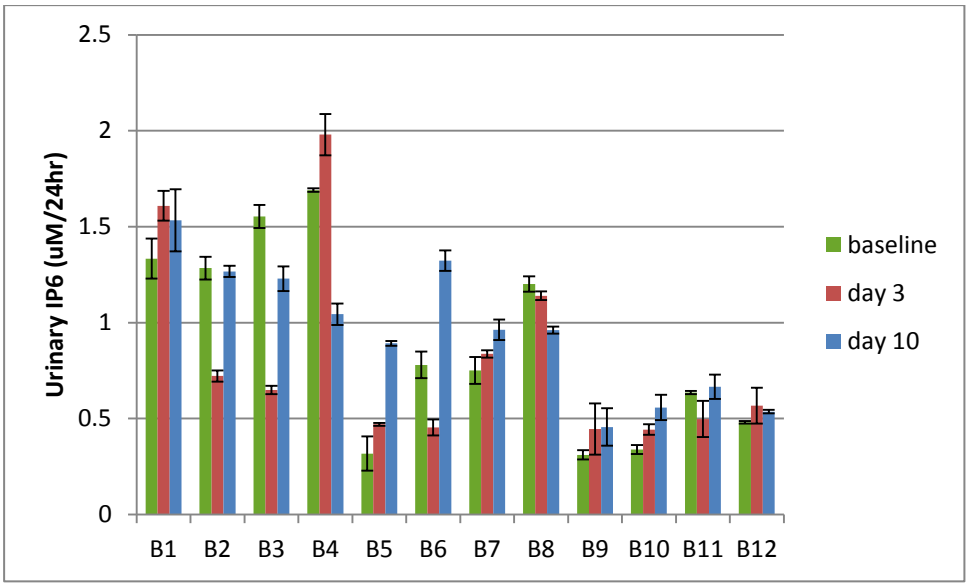
[IP6] uM	**OD1**	**OD2**	**OD3**	**average**	equation of line						
0	1.2117	1.2300	1.2543	1.242							
5	1.0767	1.0669	1.0779	1.077							
10	1.0022	0.7318	0.9968	1.036	$y = -0.0253x + 1.2404$; $R^2 = 0.9862$						
20	0.7388	0.7195	0.7128	0.724							
25	0.6024	0.6069	0.5997	0.603							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
BU1a	1.095	1.0497	1.0701	5.75	7.54	6.73	1.15	1.51	1.35	1.33	0.10
BU2a	1.091	1.065	1.078	5.91	6.93	6.42	1.18	1.39	1.28	1.28	0.06
BU3a	1.0908	1.0514	1.0362	5.91	7.47	8.07	1.18	1.49	1.61	1.55	0.06
BU4a	1.003	1.0278	1.0253	9.38	8.40	8.50	1.88	1.68	1.70	1.69	0.01
BU1b	1.027	1.0466		8.43	7.66		1.69	1.53		1.61	0.08
BU2b	1.0782	1.1528	1.1454	6.41	3.46	3.75	1.28	0.69	0.75	0.72	0.03
BU3b	1.1611	1.1555		3.13	3.36		0.63	0.67		0.65	0.02
BU4b	1.009	0.9973	0.9636	9.15	9.61	10.94	1.83	1.92	2.19	1.98	0.11
BU1c	1.0483	1.081	1.0101	7.59	6.30	9.10	1.52	1.26	1.82	1.53	0.16
BU2c	1.0838	1.1209	1.0765	6.19	4.72	6.48	1.24	0.94	1.30	1.27	0.03
BU3c	1.0259	1.0931	1.0768	8.48	5.82	6.47	1.70	1.16	1.29	1.23	0.06
BU4c	1.1472	1.1013	1.1154	3.68	5.50	4.94	0.74	1.10	0.99	1.04	0.06

[IP6] uM	OD1	OD2	OD3	average	equation of line
0	1.14	1.1461	1.1551	1.147	
5	1.0461	1.045	1.023	1.046	
10	0.9335	0.934	0.9277	0.932	$y = -0.0196 + 1.1419$; $R^2 = 0.9981$
20	0.7618	0.7563	0.7422	0.759	
25	0.642	0.6535	0.652	0.649	

	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
BU5a	1.1195	1.102	1.0669	1.14	2.04	3.83	0.23	0.41	0.77	0.32	0.09
BU6a	1.0587	1.0915	1.0722	4.24	2.57	3.56	0.85	0.51	0.71	0.78	0.07
BU7a	1.0615	1.0752	1.1088	4.10	3.40	1.69	0.82	0.68	0.34	0.75	0.07
BU8a	1.0281	0.9833	1.0203	5.81	8.09	6.20	1.16	1.62	1.24	1.20	0.04
BU5b	1.0963	1.0947		2.33	2.41		0.47	0.48		0.47	0.01
BU6b	1.1016	1.0933		2.06	2.48		0.41	0.50		0.45	0.04
BU7b	1.1156	1.0618	1.058	1.34	4.09	4.28	0.27	0.82	0.86	0.84	0.02
BU8b	1.0312	1.0334	1.0259	5.65	5.54	5.92	1.13	1.11	1.18	1.14	0.02
BU5c	1.0532	1.0558		4.53	4.39		0.91	0.88		0.89	0.01
BU6c	1.007	1.0175		6.88	6.35		1.38	1.27		1.32	0.05
BU7c	1.0423	1.0528	0.8996	5.08	4.55	12.36	1.02	0.91	2.47	0.96	0.05
BU8c	1.0459	1.0623	1.0495	4.90	4.06	4.71	0.98	0.81	0.94	0.96	0.02

[IP6] uM	OD1	OD2	OD3	average	equation of line
0	1.1519	1.1591	1.1571	1.156	
5	1.0487	1.0575	1.0306	1.053	
10	0.9457	0.9452	0.9592	0.945	$y = -0.0184 + 1.1454$; $R^2 = 0.9969$
20	0.7938	0.7596	0.7727	0.775	
25	0.6913	0.6979	0.6945	0.695	

	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
BU9a	1.1146	1.141	1.119	1.67	0.24	1.43	0.33	0.05	0.29	0.31	0.02
BU10a	1.1146	1.1177	1.1103	1.67	1.51	1.91	0.33	0.30	0.38	0.34	0.02
BU11a	1.0861	1.0876		3.22	3.14		0.64	0.63		0.636	0.01
BU12a	1.1018	1.1005		2.37	2.44		0.47	0.49		0.48	0.01
BU9b	1.1166	1.1418	1.0921	1.57	0.20	2.90	0.31	0.04	0.58	0.45	0.13
BU10b	1.1021	1.1071	1.1363	2.35	2.08	0.49	0.47	0.42	0.10	0.44	0.03
BU11b	1.1083	1.0909		2.02	2.96		0.40	0.59		0.50	0.09
BU12b	1.1051	1.0981	1.0766	2.19	2.57	3.74	0.44	0.51	0.75	0.57	0.09
BU9c	1.1153	1.1092	1.0859	1.64	1.97	3.23	0.33	0.39	0.65	0.46	0.10
BU10c	1.0997	1.1006	1.0819	2.48	2.43	3.45	0.50	0.49	0.69	0.56	0.07
BU11c	1.0782	1.09	1.12	3.65	3.01	1.38	0.73	0.60	0.28	0.666	0.06
BU12c	1.0947	1.0976	1.096	2.76	2.60	2.68	0.55	0.52	0.54	0.54	0.01



[IP6] uM	OD1	OD2	OD3	average	equation of line
0	1.065	1.1342	1.12	1.127	
5	1.0085	1.0214	0.9874	1.015	
10	0.921	0.9501	1.0593	0.977	$y = -0.0175x + 1.1239$; $R^2 = 0.9893$
20	0.7587	0.7615	0.7639	0.761	
25	0.6965	0.685	0.7358	0.691	

	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
W1a	1.1082	1.1082	1.104	0.90	0.90	1.14	0.18	0.18	0.23	0.195	0.02
W2a	1.0943	1.0746	1.0959	1.69	2.82	1.60	0.34	0.56	0.32	0.329	0.01
W3a	1.1029	1.0617	1.1052	1.20	3.55	1.07	0.24	0.71	0.21	0.227	0.01
W1b	1.0611	1.099	1.0978	3.59	1.42	1.49	0.72	0.28	0.30	0.291	0.01
W3b	1.0966	1.0693	1.0716	1.56	3.12	2.99	0.31	0.62	0.60	0.611	0.01
W3c	1.0362	1.0739	1.0305	5.01	2.86	5.34	1.00	0.57	1.07	1.035	0.03
W1c	1.0547	1.1457	1.0513	3.95	-1.25	4.15	0.79	-0.25	0.83	0.810	0.02

[IP6] uM	OD1	OD2	OD2	average	equation of line
0	1.138	1.126	1.141	1.140	
5	1.065	1.064	1.069	1.066	
10	0.969	0.972	0.973	0.971	$y = -0.0185x + 1.1497$; $R^2 = 0.9983$
20	0.778	0.756	0.773	0.776	
25	0.687	0.685	0.696	0.686	

	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
w4a	1.093	1.105	1.1	3.06	2.42	2.69	0.61	0.48	0.54	0.545	0.04

w5a	1.103	1.101	1.092	2.52	2.63	3.12	0.50	0.53	0.62	0.552	0.04
w7a	1.124	1.126	1.12	1.39	1.28	1.61	0.28	0.26	0.32	0.285	0.02
w4b	1.006	1.044	1.034	7.77	5.71	6.25	1.55	1.14	1.25	1.197	0.05
w5b	1.138	1.131	1.134	0.63	1.01	0.85	0.13	0.20	0.17	0.166	0.02
w7b	1.136	1.132	1.136	0.74	0.96	0.74	0.15	0.19	0.15	0.163	0.01
w4c	1.128	1.118	1.14	1.17	1.71	0.52	0.23	0.34	0.10	0.289	0.05
w5c	1.108	1.141	1.105	2.25	0.47	2.42	0.45	0.09	0.48	0.467	0.02
w7c	1.104	1.105	1.108	2.47	2.42	2.25	0.49	0.48	0.45	0.476	0.01

[IP6] uM	**OD1**	**OD2**	**OD2**	**average**	**equation of line**						
0	1.168	1.178	1.176	1.177							
5	1.07	1.077	1.069	1.072							
10	0.948	0.945	0.936	0.947	$y = -0.0219 + 1.1741$; $R^2 = 0.997$						
20	0.736	0.72	0.722	0.721							
25	0.64	0.645	0.625	0.643							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
w6a	1.126	1.06	1.144	2.20	5.21	1.37	0.44	1.04	0.27	0.357	0.08

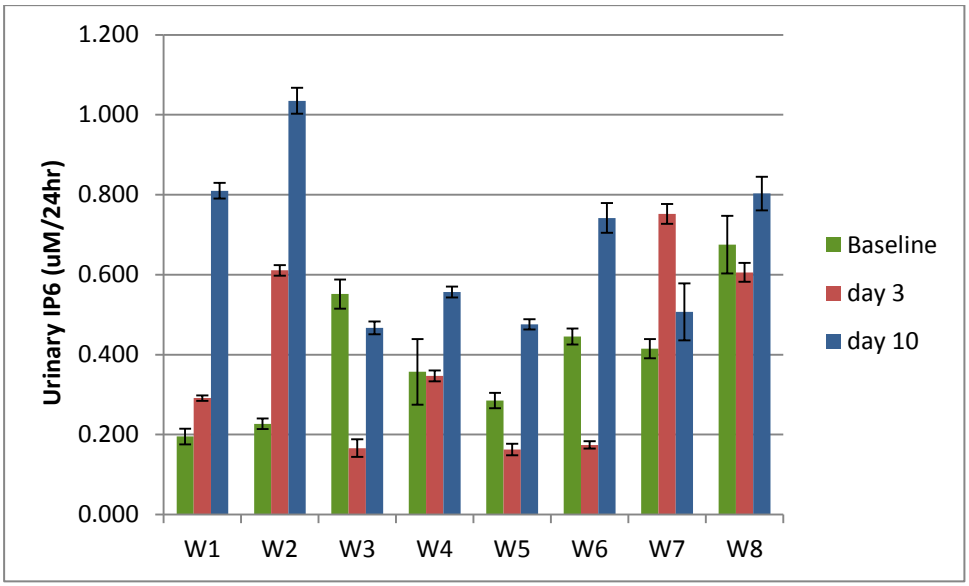
[IP6] uM	**OD1**	**OD2**	**OD2**	**average**	**equation of line**						
0	1.185	1.154	1.161	1.158							
5	1.054	1.041	1.01	1.048							
10	0.902	0.916	0.908	0.909	$y = -0.0226x + 1.1517$; $R^2 = 0.9965$						
20	0.712	0.689	0.687	0.688							
25	0.583	0.601	0.603	0.602							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
W6b	1.145	1.114	1.111	0.30	1.67	1.80	0.06	0.33	0.36	0.347	0.01
W8b	1.131	1.119	1.133	0.92	1.45	0.83	0.18	0.29	0.17	0.174	0.01

[IP6] uM	**OD1**	**OD2**	**OD2**	**average**	**equation of line**						
0	1.19	1.288	1.201	1.196							
5	1.075	1.082	1.074	1.075							
10	0.962	0.961		0.962	$y = -0.0213x + 1.185$; $R^2 = 0.9983$						
20	0.756	0.746	0.766	0.756							
25	0.662	0.658	0.664	0.661							
sample ID	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
	1	2	3	1	2	3	1	2	3	average	SE
W6c	1.128	1.126	1.123	2.68	2.77	2.91	0.54	0.55	0.58	0.557	0.01
W8c	1.11	1.11	1.098	3.52	3.52	4.08	0.70	0.70	0.82	0.742	0.04

[IP6] uM	OD1	OD2	OD2	average	equation of line						
0	1.2	1.184	1.19	1.1913							
5	1.074	1.082	1.09	1.0780							
10	0.973	0.97	0.968	0.9703	$y = -0.0208 + 1.1837$; $R^2 = 0.9977$						
20	0.758	0.777	0.754	0.7560							
25	0.679	0.679	0.675	0.6777							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
W9A	1.143	1.138	1.119	1.96	2.20	3.11	0.39	0.44	0.62	0.415	0.02
W10A	1.106	1.121	1.084	3.74	3.01	4.79	0.75	0.60	0.96	0.675	0.07
W11A	1.117	1.123	1.098	3.21	2.92	4.12	0.64	0.58	0.82	0.613	0.03

[IP6] uM	OD1	OD2	OD2	average	equation of line						
0	1.235	1.219	1.200	1.210							
5	1.084	1.082	1.078	1.081							
10	0.967	0.963	1.001	0.965	$y = -0.0212x + 1.1937$; $R^2 = 0.9962$						
20	0.760	0.760	0.770	0.763							
25	0.672	0.678	0.654	0.675							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
W8A	1.156	1.148	1.145	1.78	2.16	2.30	0.36	0.43	0.46	0.445	0.01
W9B	1.113	1.110	1.119	3.81	3.95	3.52	0.76	0.79	0.70	0.752	0.02
W10B	1.132	1.127	1.151	2.91	3.15	2.01	0.58	0.63	0.40	0.606	0.02
W11B	1.063	1.058	1.045	6.17	6.40	7.01	1.23	1.28	1.40	1.257	0.02

[IP6] uM	OD1	OD2	OD2	average	equation of line						
0	1.241	1.239	1.245	1.240							
5	1.122	1.121	1.140	1.122							
10	1.009	1.018	1.017	1.018	$y = -0.0215x + 1.2348$; $R^2 = 0.9996$						
20	0.806	0.845	0.810	0.808							
25	0.696	0.729	0.697	0.697							
	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)				
sample ID	1	2	3	1	2	3	1	2	3	average	SE
W9C	1.166	1.183	1.192	3.20	2.41	1.99	0.64	0.48	0.40	0.507	0.07
W10C	1.153	1.144	1.187	3.80	4.22	2.22	0.76	0.84	0.44	0.803	0.04
W11C	1.125	1.110	1.097	5.11	5.80	6.41	1.02	1.16	1.28	1.221	0.06


1.200
1.000
0.800
0.600
0.400
0.200
0.000
Urinary IP6 (uM/24hr)
W1
W2
W3
W4
W5
W6
W7
W8
Baseline
day 3
day 10

Appendix 6.6
urine composition and physicochemical properties

Urine data (black males): All units in mmol/24 hr except for IP6 (µM/24hr)

Date	Lab No	pH	Vol,ml	Citrate	Ox	Ca	Mg	Na	K	Urate	Creat	Phos	Cl	IP6
20-Feb-12	BU1a	5.58	610	1.03	0.11	2.10	1.76	103.1	32.1	2.7	14.0	21.5	96	1.330
"	BU2a	7.89	2190	0.96	0.27	2.18	2.19	93.2	46.8	3.0	12.0	19.9	85	1.28
"	BU3a	6.05	1420	1.54	0.20	2.32	1.98	81.5	27.6	2.5	13.1	15.2	76	1.550
"	BU4a	6.20	1439	2.38	0.33	2.42	2.03	87.7	41.0	4.4	17.7	20.7	103	1.690
23-Feb	BU1b	5.92	1260	1.62	0.26	2.28	2.03	141.6	46.2	2.5	13.4	13.2	195	1.610
"	BU2b	6.34	2430	0.95	0.42	2.22	2.31	97.8	50.0	2.9	11.4	19.2	97	0.720
"	BU3b	6.05	2000	1.40	0.47	2.82	2.44	150.2	40.6	3.0	14.4	22.2	198	0.650
"	BU4b	6.16	2149	1.37	0.40	2.96	2.54	133.7	47.9	3.2	16.8	22.8	182	1.980
01-Mar	BU1c	6.34	1239	2.04	0.15	2.61	2.14	100.2	47.6	2.4	13.5	14.6	144	1.530
"	BU2c	6.09	2340	2.57	0.33	2.98	2.56	143.7	51.9	4.6	14.3	20.4	184	1.270
""	BU3c	5.57	1400	2.29	0.25	3.21	2.84	125.0	49.3	2.6	14.7	17.2	148	1.230
"	BU4c	6.10	3040	5.22	0.26	3.00	2.78	119.2	39.8	3.0	14.6	13.1	136	1.040
12-Mar	BU5a	6.70	2349	1.36	0.24	1.79	1.35	115.2	43.4	3.0	17.1	20.7	124.0	0.320
"	BU6a	6.67	720	0.92	0.16	1.24	1.70	67.3	22.8	2.0	9.9	11.9	57	0.780
"	BU7a	8.36	2280	1.48	0.25	2.33	2.56	139.7	45.5	2.0	10.9	8.9	148	0.750
"	BU8a	7.71	1000	1.01	0.26	1.46	1.21	81.3	37.2	3.1	14.1	15.5	74	1.200
15-Mar	BU5b	6.98	1900	1.86	0.43	1.38	2.00	100.2	37.5	3.0	18.8	23.6	96	0.470
"	BU6b	5.76	610	0.60	0.19	1.19	1.52	85.7	40.1	2.3	14.8	18.0	128	0.450
"	BU7b	7.04	2300	2.33	0.40	2.88	2.73	155.6	52.2	2.9	15.2	18.6	170	0.840
"	BU8b	6.74	840	1.49	0.29	1.51	1.67	131.4	36.2	2.6	16.0	21.1	141	1.140
22-Mar	BU5c	6.78	2190	2.85	0.55	1.94	1.77	73.6	25.4	2.8	18.4	21.0	96	0.890
"	BU6c	5.87	380	0.51	0.18	0.77	0.98	69.6	18.9	1.5	8.0	11.0	61	1.320
"	BU7c	6.25	1920	3.22	0.52	2.79	2.68	136.4	37.8	3.0	17.3	12.1	136	0.960

"	BU8c	6.11	1220	1.74	0.43	2.16	1.94	94.5	36.3	2.4	16.1	15.7	118	0.960
21-May	BU9a	7.06	1640	3.02	0.22	1.96	2.28	137.0	31.5	3.2	14.1	27.2	165	0.310
"	BU10a	5.87	1070	1.19	0.18	1.30	1.07	89.8	42.2	3.5	15.4	25.3	110	0.340
"	BU11a	6.68	1420	2.69	0.20	2.18	2.01	131.5	39.7	4.9	21.7	46.7	156	0.636
"	BU12a	5.62	1299	1.06	0.19	2.40	2.19	92.6	35.4	3.3	15.6	32.6	146	0.480
24-May	BU11b	5.82	900	1.79	0.16	2.36	2.50	140.2	21.6	3.2	14.9	33.8	103	0.500
"	BU9b	6.86	850	1.74	0.19	2.11	2.58	156.1	40.8	2.1	13.2	15.1	108	0.450
"	BU12b	6.61	1780	3.25	0.21	2.31	3.50	140.5	42.0	3.3	17.8	31.7	229	0.570
"	BU10b	5.56	729	0.93	0.17	1.92	2.40	127.2	36.5	2.5	13	27.2	102	0.440
31-May	BU11c	5.89	1290	2.80	0.19	2.23	2.71	139.5	37.6	3.4	17.3	27.2	118	0.666
"	BU10c	5.83	1449	2.90	0.19	2.14	2.76	142.9	27.2	3.3	13.2	23.9	128	0.560
"	BU9c	6.12	1340	1.95	0.17	2.22	2.85	146.2	33.3	2.2	10.6	24.5	96	0.460
"	BU12c	6.42	2430	3.77	0.23	2.22	3.68	151.3	36.1	3.1	16	28.9	170	0.540

Physicochemical factors (black males)

Date	Lab No	MSL	SS CaOx	SS Brush	SS Uric	TRI	agg	$\%I_A$	nuc	$\%I_N$
20-Feb-12	BU1a	0.45	3.606	0.910	4.519	91.1	-1.29E-05	38.37	4.62E-04	32.95
"	BU2a	n+	2.133	1.722	0.012	281.4	n/a	n/a	n/a	n/a
"	BU3a	1.65	5.129	1.089	0.877	185.3	-1.41E-05	32.61	6.10E-04	11.47
"	BU4a	n+	6.339	1.349	1.096	193.1	-1.68E-08	19.42	5.20E-04	24.53
23-Feb-12	BU1b	1.35	5.888	0.736	1.153	228.2	-1.10E-05	48.7	2.03E-04	60.89
"	BU2b	1.65	5.082	0.957	0.340	475.2	-1.99E-05	7.44	2.13E-04	58.96
"	BU3b	0.9	7.228	0.951	0.716	426.7	-7.77E-06	63.86	1.49E-04	71.39
"	BU4b	0.9	5.943	1.125	0.579	298.6	-2.00E-05	6.98	1.27E-04	75.53
01-Mar-12	BU1c	1.65	3.690	1.663	0.498	139.0	-2.24E-05	-44.52	1.67E-04	51.17
"	BU2c	1.65	3.917	0.684	0.887	294.3	-1.65E-05	-6.45	1.97E-04	42.54
""	BU3c	0.75	6.730	0.608	2.023	225.0	-1.22E-05	21.52	1.47E-04	57.02

"	BU4c	0.6	1.811	0.228	0.463	208.1	-1.05E-05	32.39	1.60E-04	53.22
12-Mar-12	BU5a	1.65	1.995	1.005	0.164	133.5	-1.94E-05	-20.18	1.82E-04	43.39
"	BU6a	n+	4.853	2.438	0.352	154.2	-1.06E-05	32.7	8.26E-05	70.46
"	BU7a	>1.95	1.459	0.507	0.003	294.8	-1.43E-05	3.17	2.00E-04	3.17
"	BU8a	>1.95	3.090	1.702	0.037	173.9	-1.70E-05	32.22	1.55E-04	42.38
15-Mar-12	BU5b	>1.95	3.251	1.361	0.107	158.7	-8.00E-06	47.37	1.37E-04	44.53
"	BU6b	0.6	1.552	0.037	2.483	104.4	-1.08E-05	-35.44	7.98E-05	41.79
"	BU7b	>1.95	3.516	1.337	0.072	318.5	-1.36E-05	-70.31	1.47E-04	-7.3
"	BU8b	1.2	4.529	2.046	0.321	151.7	-8.61E-06	-7.62	1.25E-04	9.12
22-Mar-12	BU5c	1.8	3.622	0.851	0.141	260.1	-2.27E-05	45.74	3.34E-04	19.61
"	BU6c	n/a	10.990	1.538	2.438	193.9	n/a	n/a	n/a	n/a
"	BU7c	0.9	7.161	0.632	0.509	324.9	-2.11E-05	53.53	2.27E-04	34.54
"	BU8c	0.9	10.375	1.199	0.828	277.0	-2.02E-05	51.62	2.81E-04	32.37
21-May-12	BU9a	0.6	0.995	0.933	0.103	148.8	-3.66E-05	4.18	5.88E-04	15.09
"	BU10a	1.35	3.573	0.899	2.143	97.5	-3.62E-05	5.24	6.18E-04	10.83
"	BU11a	1.5	1.406	1.862	0.414	79.7	-5.45E-06	85.73	5.87E-04	15.31
"	BU12a	n+	5.058	1.079	2.564	142.8	n/a	n/a	n/a	n/a
24-May-12	BU11b	1.2	5.236	2.203	2.472	118.4	-3.36E-05	6.28	2.28E-04	72.65
"	BU9b	0.75	4.375	2.483	0.191	154.9	-4.12E-05	-14.9	4.56E-04	45.25
"	BU12b	>1.95	1.510	1.330	0.236	105.9	-4.59E-05	-28.03	3.26E-04	60.82
"	BU10b	1.5	7.079	1.521	3.614	142.6	-4.83E-05	-34.59	4.54E-04	45.49
31-May-12	BU11c	0.3	3.508	1.072	1.663	102.5	-2.65E-05	-6.43	1.42E-04	55.08
"	BU10c	0.3	3.048	0.719	1.626	147.3	-1.01E-05	59.55	2.24E-04	29.05
"	BU9c	0.9	3.221	1.521	0.671	193.4	-1.29E-05	48.26	1.00E-04	68.25
"	BU12c	>1.95	1.387	0.785	1.54E-09	129.0	-2.00E-05	19.72	2.25E-04	28.57

Urine data (white males): All units in mmol/24 hr except for IP6 (µM/24hr)

Date	Lab No	pH	Vol,ml	Citrate	Ox	Ca	Mg	Na	K	Urate	Creat	Phos	Cl	IP6
16-Apr-12	W1a	6.05	844	0.83	0.14	1.3	1.12	88.5	24.6	4.1	15.1	34.8	97	0.195
16-Apr-12	W3a	5.64	659	1.1	0.09	1.45	1.21	99.3	29.2	7.1	16.5	29.8	73	0.227
16-Apr-12	W2a	5.68	300	0.51	0.01	0.71	0.6	43.2	9.5	1.0	6.0	15.7	36	0.329
19-Apr-12	W1b	5.56	1180	1.66	0.26	1.13	1.1	88.3	30.2	3.4	18.4	32.0	109	0.291
19-Apr-12	W3b	5.89	1170	0.63	0.1	1.2	1.07	102.1	40.0	1.6	11.3	19.8	92	0.611
26-Apr-12	W1C	5.60	1740	1.50	0.11	1.53	1.27	115.7	36.8	4.3	20.9	36.0	106	0.810
26-Apr-12	W2C	5.74	1520	1.63	0.22	1.14	0.98	81.7	31.2	5.3	17.6	41.6	188	n/d
26-Apr-12	W3C	5.53	999	2.00	0.24	1.21	1.16	121.2	41.3	4.3	17.0	34.0	119	1.035
30-Jul-12	W4a	6.78	560	0.96	0.10	0.75	0.51	30.2	9.7	2.6	12.2	8.6	162	0.545
30-Jul-12	W5a	6.08	1260	2.39	0.15	1.13	1.02	66.5	21.8	2.6	10.3	15.2	148	0.552
30-Jul-12	W7a	6.17	1020	1.85	0.22	1.21	0.98	89.4	27.6	3.2	14.9	38.9	146	0.285
02-Aug-12	W4b	5.80	300	0.80	0.04	0.85	0.92	30.3	7.6	0.7	8.5	23.7	12	1.197
02-Aug-12	W5b	6.33	580	1.21	0.05	1.06	1.00	37.6	18.7	1.4	8.0	6.0	68	0.166
02-Aug-12	W7b	6.04	1000	2.08	0.26	1.41	1.36	86.2	23.5	4.0	17.7	37.4	189	0.163
09-Aug-12	W4c	5.89	300	0.67	0.12	0.80	0.86	31.6	10.2	1.3	7.5	19.7	16	0.289
09-Aug-12	W5c	5.92	860	1.75	0.43	1.40	1.33	47.2	23.8	3.0	16.5	39.4	160	0.467
09-Aug-12	W7c	5.94	1160	2.32	0.53	1.61	1.39	86.3	31.5	4.5	21.1	38.9	165	0.476
29-Jul-13	W6A	6.29	1700	2.25	0.33	3.76	3.15	103.3	34.4	3.5	19.7	35.4	119	0.357
01-Aug-13	W6B	5.89	1600	3.70	0.49	4.21	3.44	177.6	42.8	6.2	27.0	49.9	209	0.347
01-Aug-13	W8B	6.43	2350	3.04	0.33	6.27	4.23	116.9	28.0	3.7	17.4	30.8	152	0.174
08-Aug-13	W6C	6.56	2265	4.07	0.28	4.46	3.28	144.1	41.0	4.0	19.3	41.4	158	0.557
08-Aug-13	W8C	5.67	1860	4.35	0.25	6.30	2.67	51.80	22.60	4.0	15.4	35.2	72	0.742
12-Aug-13	W9A	5.89	1220	1.54	0.20	3.56	3.05	99.40	20.30	2.6	12.2	21.5	119	0.415
12-Aug-13	W11A	6.75	2052	4.90	0.25	2.11	4.31	210.40	37.60	4.3	18.9	26.3	219	0.613
12-Aug-13	W10A	5.96	830	2.41	0.20	2.96	1.41	97.60	30.30	2.2	11.0	21.2	141	0.675

15-Aug-13	W8A	6.07	1580	2.65	0.30	2.40	2.45	98.10	20.20	4.2	18.3	39.7	93	0.445
15-Aug-13	W9B	5.45	1360	4.14	0.29	2.55	2.74	104.30	27.30	3.5	15.8	28.4	112	0.752
15-Aug-13	W10B	5.90	2180	3.38	0.17	2.40	2.51	129.60	34.80	3.0	10.0	11.1	163	0.606
15-Aug-13	W11B	5.82	1720	2.88	0.28	3.69	4.64	134.90	33.10	4.6	20.6	53.1	141	1.257
22-Aug-13	W9C	6.70	2300	3.75	0.29	2.08	2.51	114.50	22.80	5.0	16.3	33.4	147	0.507
22-Aug-13	W10C	6.11	1600	3.80	0.33	2.31	2.04	82.10	12.50	2.7	7.5	14.7	68	0.803
22-Aug-13	W11C	6.41	1210	5.20	0.32	2.88	2.98	151.70	26.60	3.6	16.2	43.1	165	1.221

Data in red excluded from calculations as explained in thesis, chapter 4

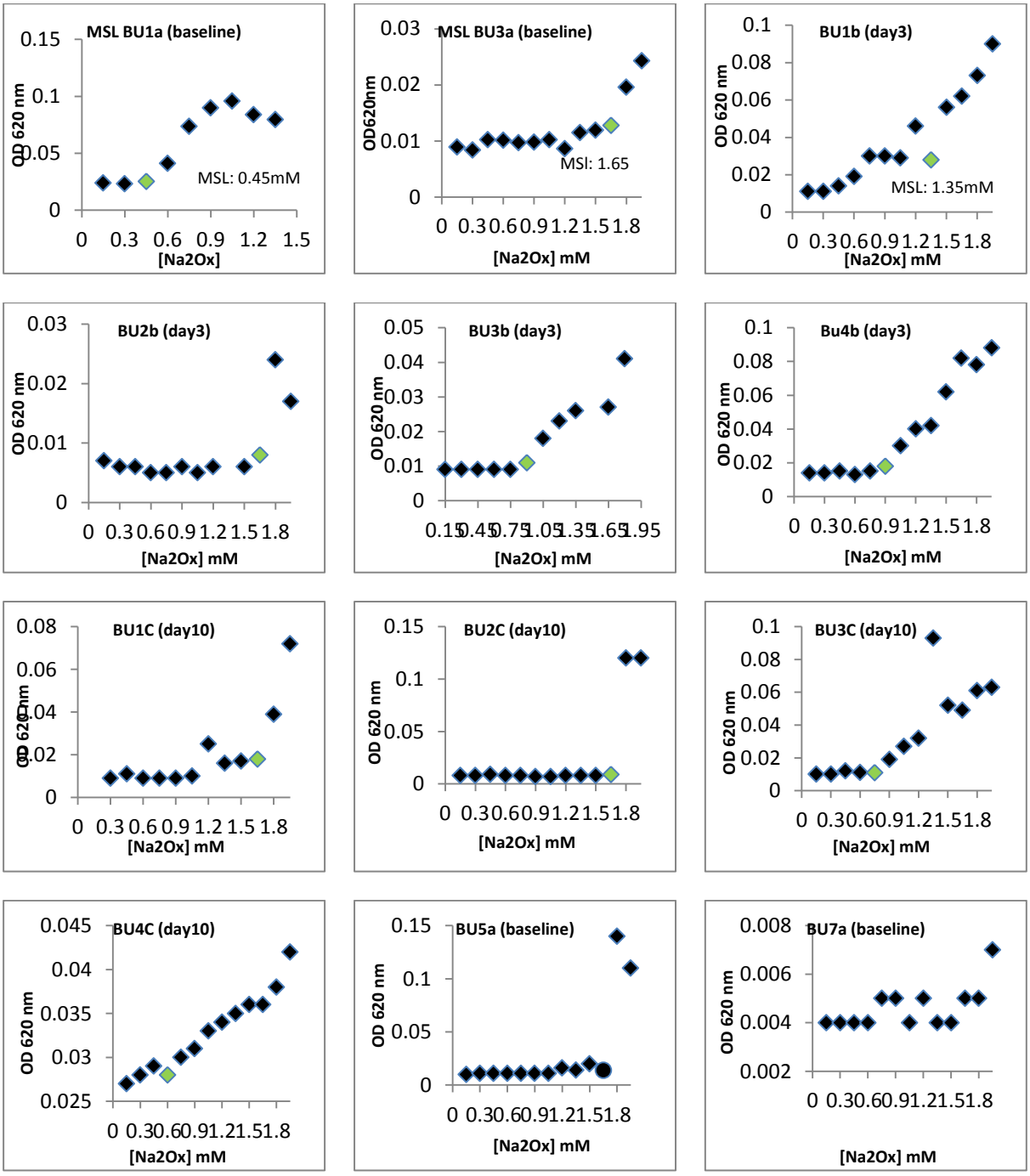
Physicochemical factors (white males)

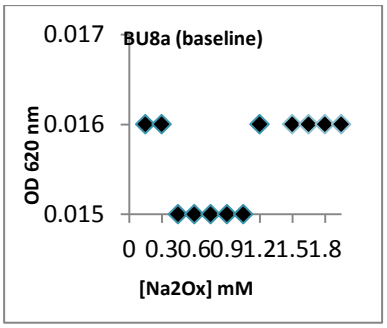
Date	Lab No	MSL	RS CaOx	RS Brush	RS Uric	TRI	CaOx agg.	% inhib	CaOx nuc.	% inhib
16-Apr-12	W1a	1.2	3.319	2.014	2.213	80.38	-1.85E-05	28.12	2.32E-04	6.2
16-Apr-12	W3a	0.45	3.715	1.581	10.139	47.14	-2.38E-05	7.27	2.75E-04	-11.18
16-Apr-12	W2a	1.2				16.63	n/a	n/a	n/a	n/a
19-Apr-12	W1b	1.2	4.036	0.439	3.214	94.53	-1.81E-05	56.81	3.66E-04	40.13
19-Apr-12	W3b	1.05	2.037	0.767	0.881	85.93	-1.70E-05	59.45	3.83E-04	37.26
26-Apr-12	W1C	0.75	1.413	0.433	2.661	40.72	-1.01E-05	51.33	2.05E-04	8.89
26-Apr-12	W2C					87.48	n/a	n/a	n/a	n/a
26-Apr-12	W3C	0.75	4.188	0.550	4.853	100.25	-2.41E-05	-16.71	2.19E-04	2.67
30-Jul-12	W4a	<0.15				58.49	-1.63E-05	31.22	1.25E-04	84.55
30-Jul-12	W5a	1.65	1.648	0.406	0.927	124.70	-1.55E-05	34.66	1.57E-04	80.66
30-Jul-12	W7a	1.65	2.535	1.253	1.117	115.11	n/a	n/a	n/a	n/a
02-Aug-12	W4b	1.05				40.78	-1.81E-05	-4.32	2.15E-04	32.18
02-Aug-12	W5b	1.05	2.188	1.146	0.640	61.82	-1.29E-05	25.94	2.53E-04	20.35
02-Aug-12	W7b	1.05	3.673	1.256	1.820	111.15	-1.49E-05	14.12	2.51E-04	20.82
09-Aug-12	W4c					144.74	n/a	n/a	n/a	n/a
09-Aug-12	W5c	1.5	8.299	1.393	2.198	206.95	-1.59E-05	25.70	4.06E-04	-108.2
09-Aug-12	W7c	1.8	7.621	1.076	0.295	189.61	-1.42E-05	33.64	1.45E-04	25.64

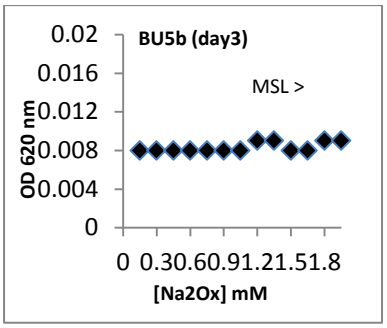
29-Jul-13	W6A	0.45	6.668			213.33	3.36E-05	15.6	2.50E-04	33.0
01-Aug-13	W6B	0.9	9.311	2.032	2.393	202.95	2.44E-05	43.3	1.99E-04	12.9
01-Aug-13	W8B	0.9	6.622	3.459	0.356	342.76	2.65E-04	38.6	2.30E-04	-0.7
08-Aug-13	W6C	1.65	3.119	2.489	0.295	197.35	2.21E-05	38.5	1.93E-04	15.0
08-Aug-13	W8C	1.8	8.570	1.633	2.168	320.82	2.03E-05	43.6	1.99E-04	12.7
12-Aug-13	W9A	1.05	7.499	2.061	1.365	262.46	3.24E-05	46.9	3.10E-04	-4.21
12-Aug-13	W11A	>1.95				100.91	3.04E-05	50.3	3.01E-04	-1.2
12-Aug-13	W10A	0.9	8.933	2.427	1.406	285.57	3.05E-05	50.3	3.01E-04	-1.2
15-Aug-13	W8A	1.05	4.529	1.483	1.250	160.13	3.14E-05	49.2	3.27E-04	-12.6
15-Aug-13	W9B	0.6	5.875	0.484	3.373	188.81	3.68E-05	40.3	3.78E-04	-30.2
15-Aug-13	W10B	0.9	1.837	0.237	0.904	214.56	3.27E-05	47.1	2.33E-04	19.7
15-Aug-13	W11B	>1.95				154.56	2.89E-04	53.2	1.99E-04	31.6
22-Aug-13	W9C	1.35	1.337	0.887	0.273	159.57	3.18E-05	46.9	2.92E-04	-26.2
22-Aug-13	W10C	0.9				629.52	3.46E-05	42.1	2.67E-04	-15.4
22-Aug-13	W11C	1.5	5.164	0.612	0.767	211.51	2.77E-05	53.6	2.39E-04	-3.5

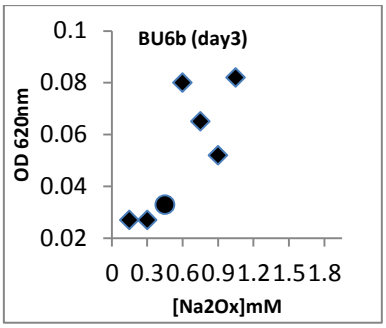
Appendix 6.7

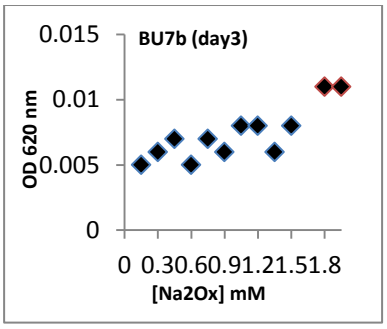
CaOx MSLs

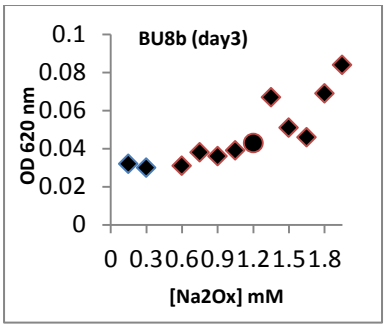


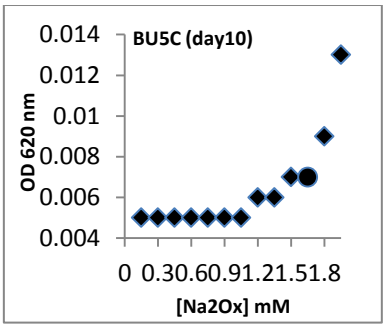

BU8a (baseline)
OD 620 nm
0.017
0.016
0.015
0 0.30.60.91.21.51.8
[Na2Ox] mM

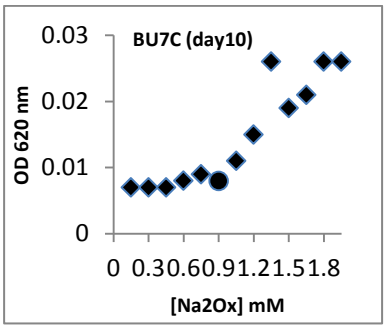

BU5b (day3)
MSL >
OD 620 nm
0.02
0.016
0.012
0.008
0.004
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

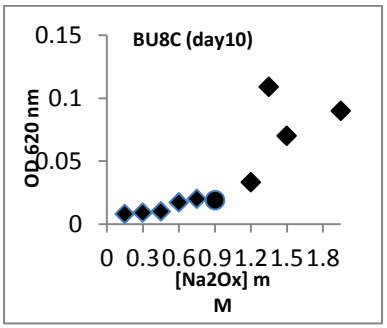

BU6b (day3)
OD 620nm
0.1
0.08
0.06
0.04
0.02
0 0.30.60.91.21.51.8
[Na2Ox]mM

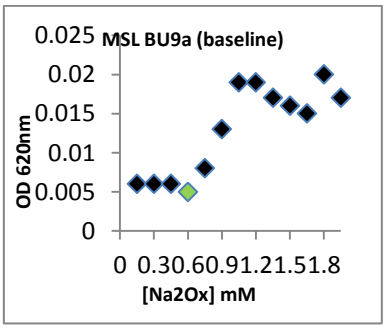

BU7b (day3)
OD 620 nm
0.015
0.01
0.005
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

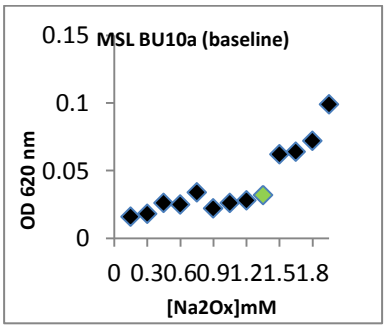

BU8b (day3)
OD 620 nm
0.1
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

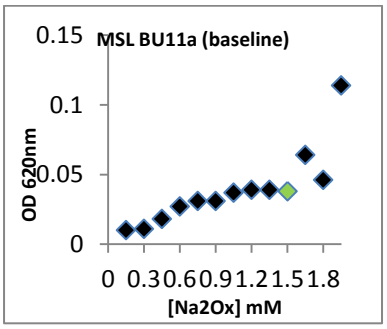

BU5C (day10)
OD 620 nm
0.014
0.012
0.01
0.008
0.006
0.004
0 0.30.60.91.21.51.8
[Na2Ox] mM

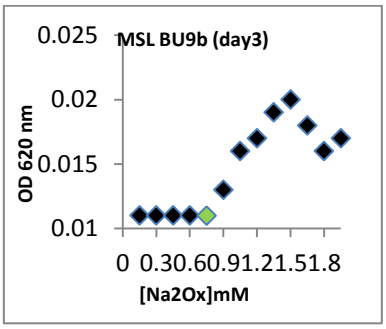

BU7C (day10)
OD 620 nm
0.03
0.02
0.01
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

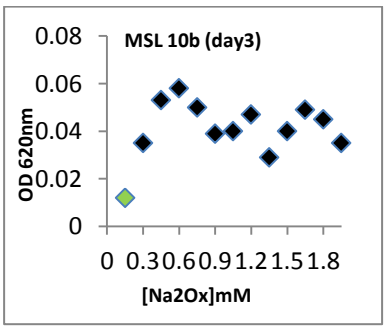

BU8C (day10)
OD620 nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] m
M

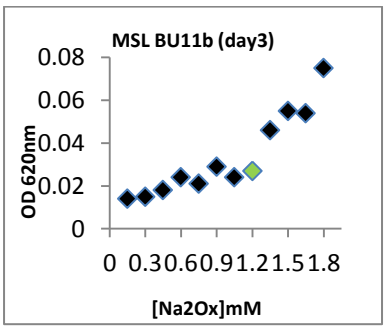

MSL BU9a (baseline)
OD 620nm
0.025
0.02
0.015
0.01
0.005
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

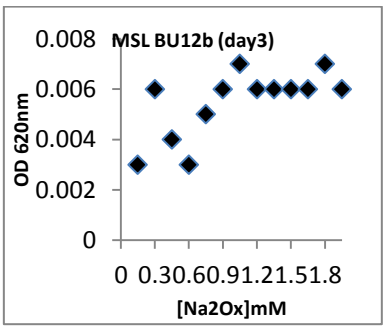

MSL BU10a (baseline)
OD 620 nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox]mM

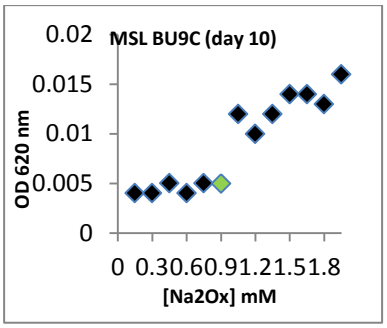

MSL BU11a (baseline)
OD 620nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

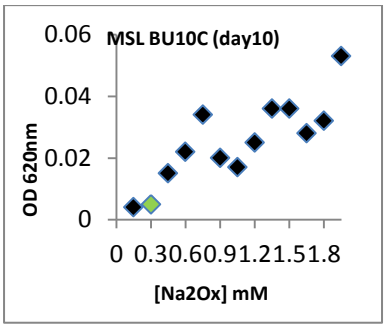

MSL BU9b (day3)
OD 620 nm
0.025
0.02
0.015
0.01
0 0.30.60.91.21.51.8
[Na2Ox]mM

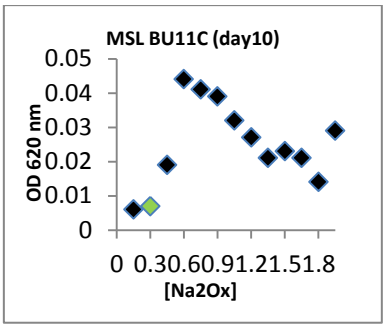

MSL 10b (day3)
OD 620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox]mM

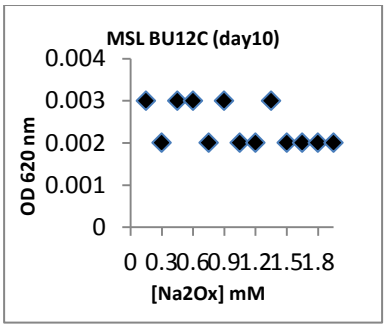

MSL BU11b (day3)
OD 620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox]mM

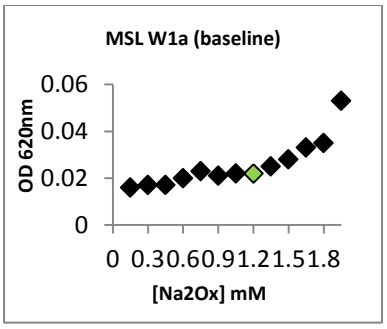

MSL BU12b (day3)
OD 620nm
0.008
0.006
0.004
0.002
0
0 0.30.60.91.21.51.8
[Na2Ox]mM

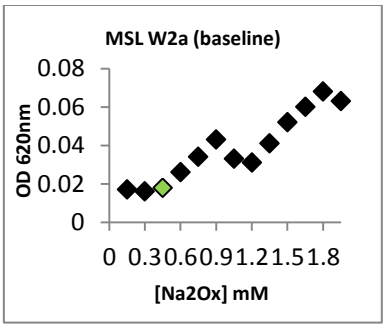

MSL BU9C (day 10)
OD 620 nm
0.02
0.015
0.01
0.005
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

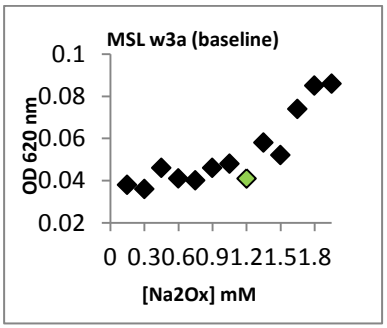

MSL BU10C (day10)
OD 620nm
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

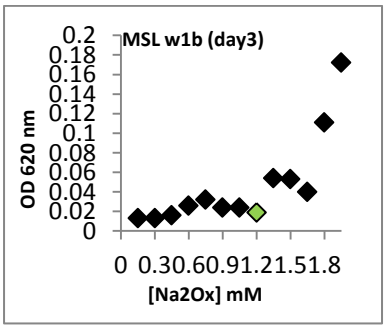

MSL BU11C (day10)
OD 620 nm
0.05
0.04
0.03
0.02
0.01
0
0 0.30.60.91.21.51.8
[Na2Ox]

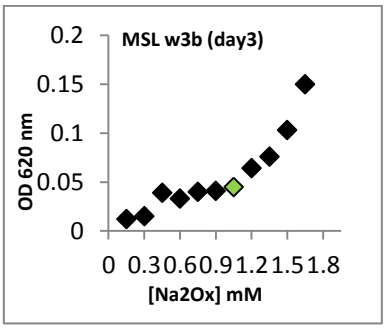

MSL BU12C (day10)
OD 620 nm
0.004
0.003
0.002
0.001
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

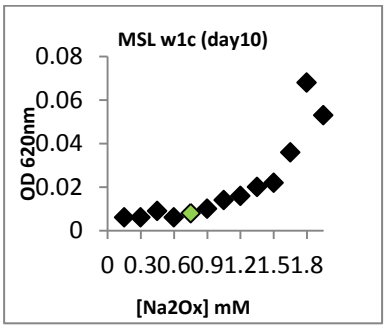

MSL W1a (baseline)
OD 620nm
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

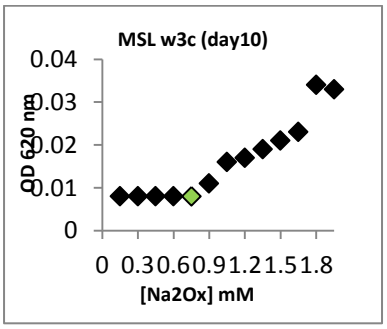

MSL W2a (baseline)
OD 620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

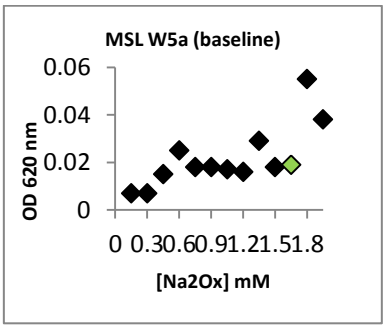

MSL w3a (baseline)
OD 620 nm
0.1
0.08
0.06
0.04
0.02
0 0.30.60.91.21.51.8
[Na2Ox] mM

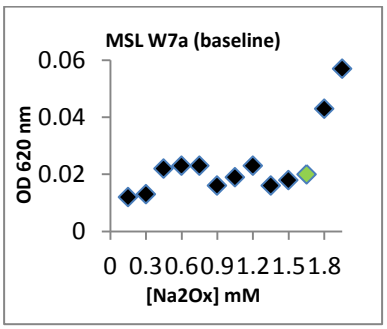

MSL w1b (day3)
OD 620 nm
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

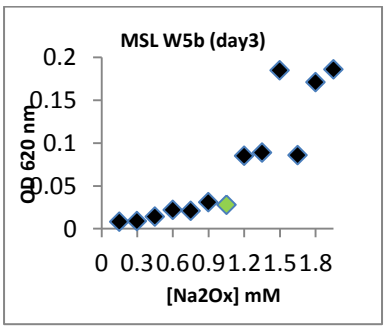

MSL w3b (day3)
OD 620 nm
0.2
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

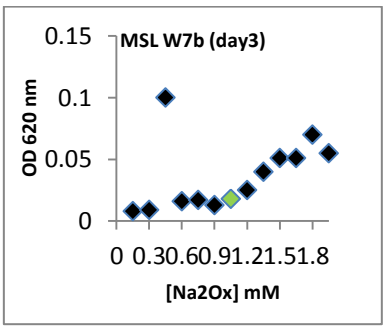

MSL w1c (day10)
OD 620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

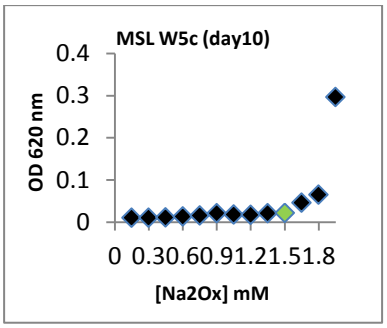

MSL w3c (day10)
OD 620 nm
0.04
0.03
0.02
0.01
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

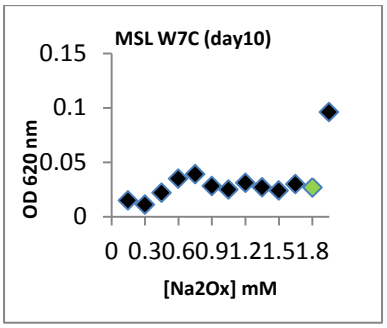

MSL W5a (baseline)
OD 620 nm
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

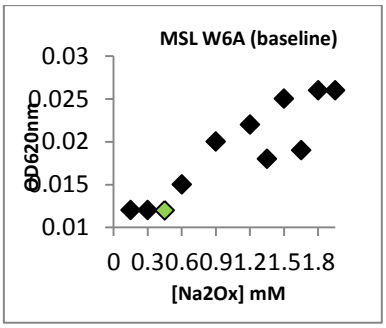

MSL W7a (baseline)
OD 620 nm
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

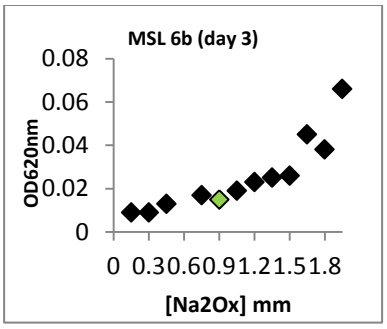

MSL W5b (day3)
OD 620 nm
0.2
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

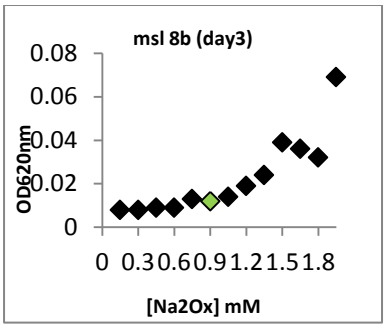

MSL W7b (day3)
OD 620 nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

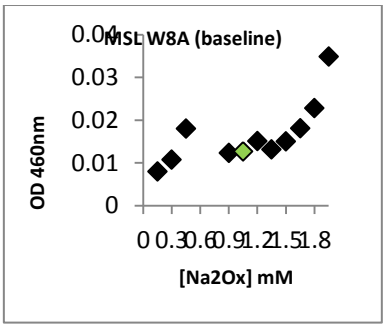

MSL W5c (day10)
OD 620 nm
0.4
0.3
0.2
0.1
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

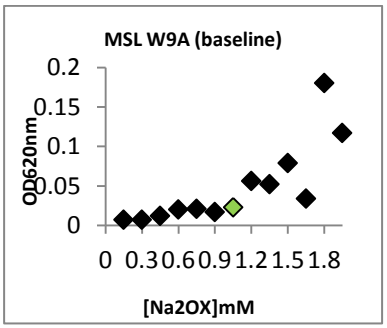

MSL W7C (day10)
OD 620 nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

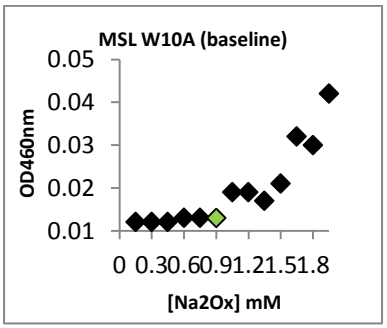

MSL W6A (baseline)
OD620nm
0.03
0.025
0.02
0.015
0.01
0 0.30.60.91.21.51.8
[Na2Ox] mM

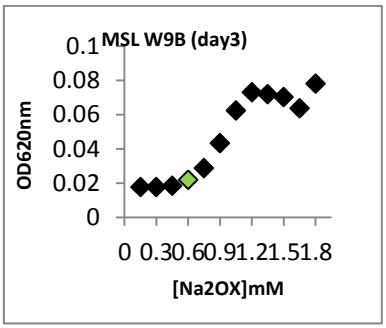

MSL 6b (day 3)
OD620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mm

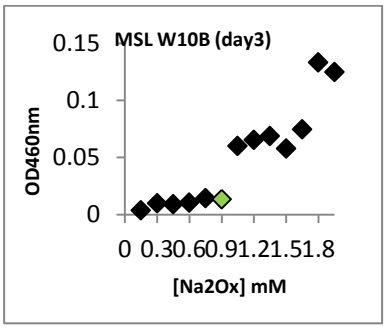

msl 8b (day3)
OD620nm
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2Ox] mM


MSL W8A (baseline)
OD 460nm
0.04
0.03
0.02
0.01
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

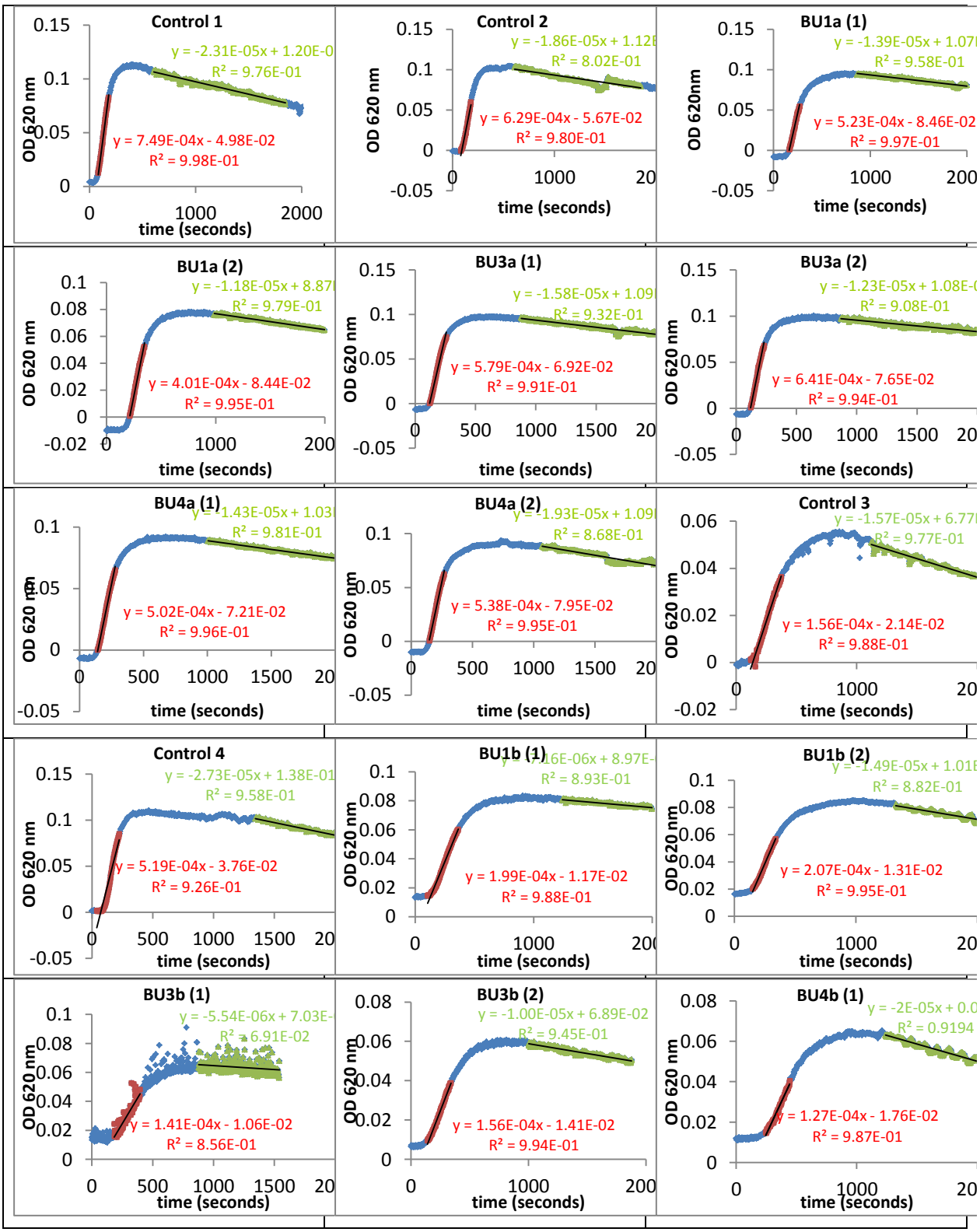

MSL W9A (baseline)
OD620nm
0.2
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2OX]mM

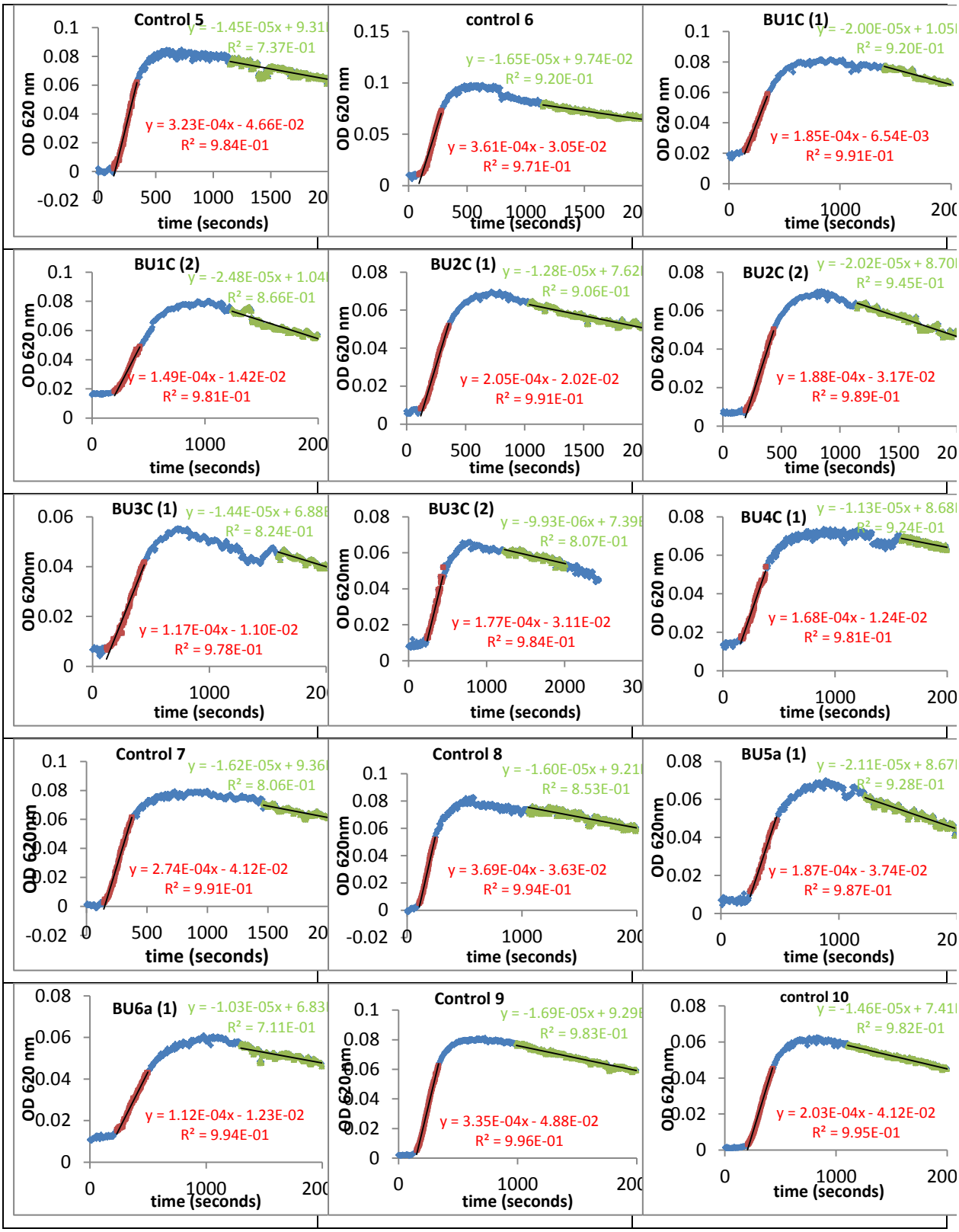

MSL W10A (baseline)
OD460nm
0.05
0.04
0.03
0.02
0.01
0 0.30.60.91.21.51.8
[Na2Ox] mM

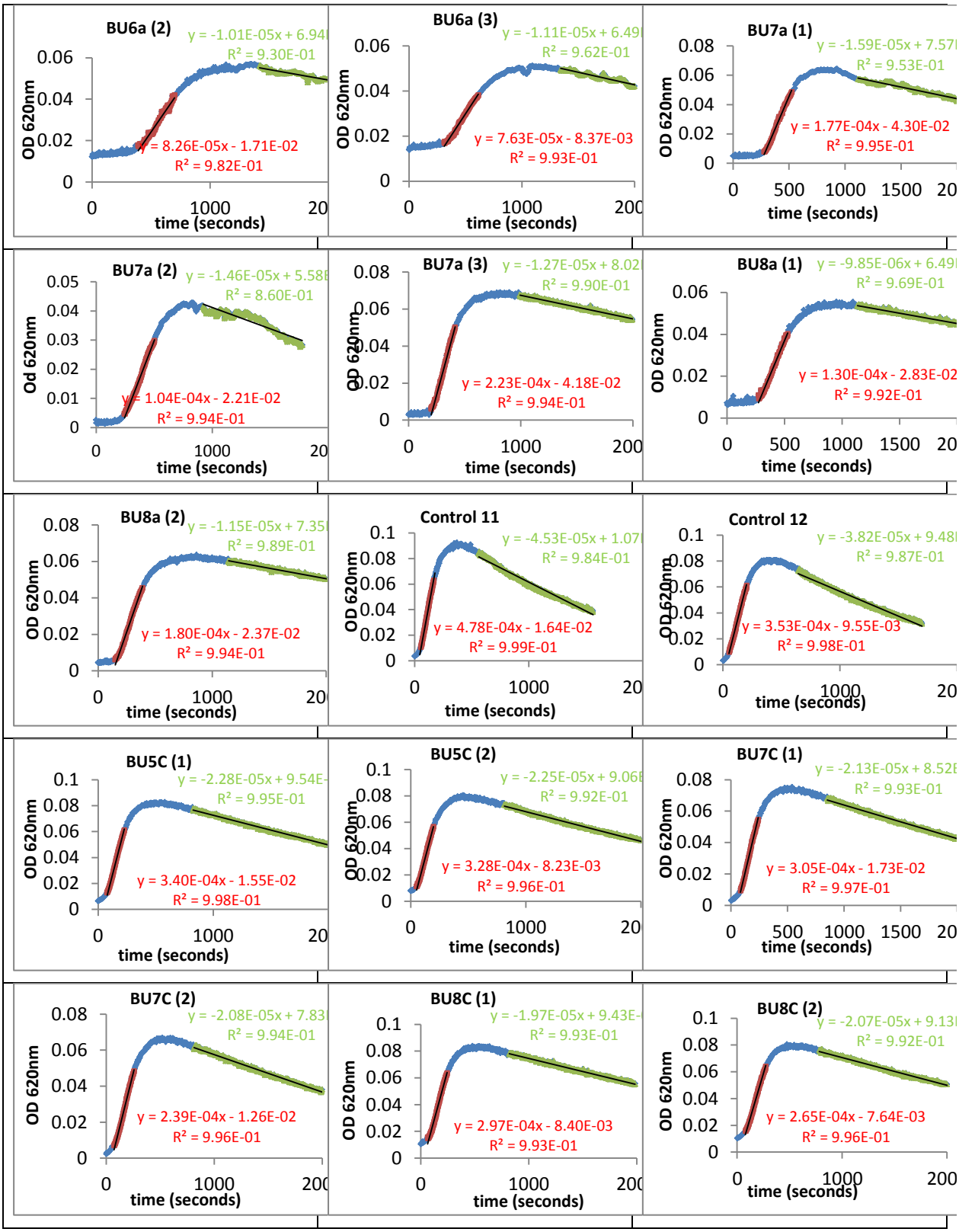

MSL W9B (day3)
OD620nm
0.1
0.08
0.06
0.04
0.02
0
0 0.30.60.91.21.51.8
[Na2OX]mM

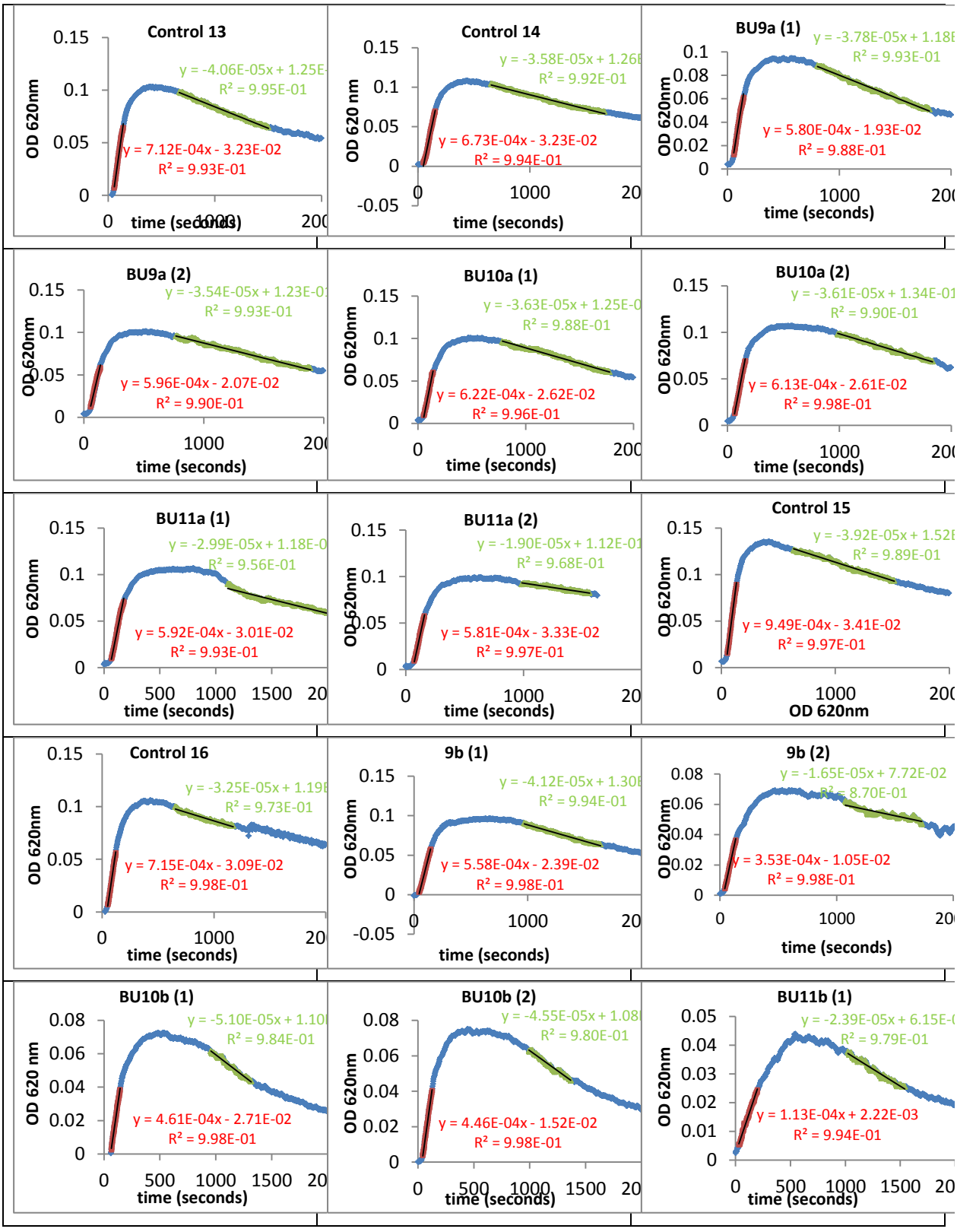

MSL W10B (day3)
OD460nm
0.15
0.1
0.05
0
0 0.30.60.91.21.51.8
[Na2Ox] mM

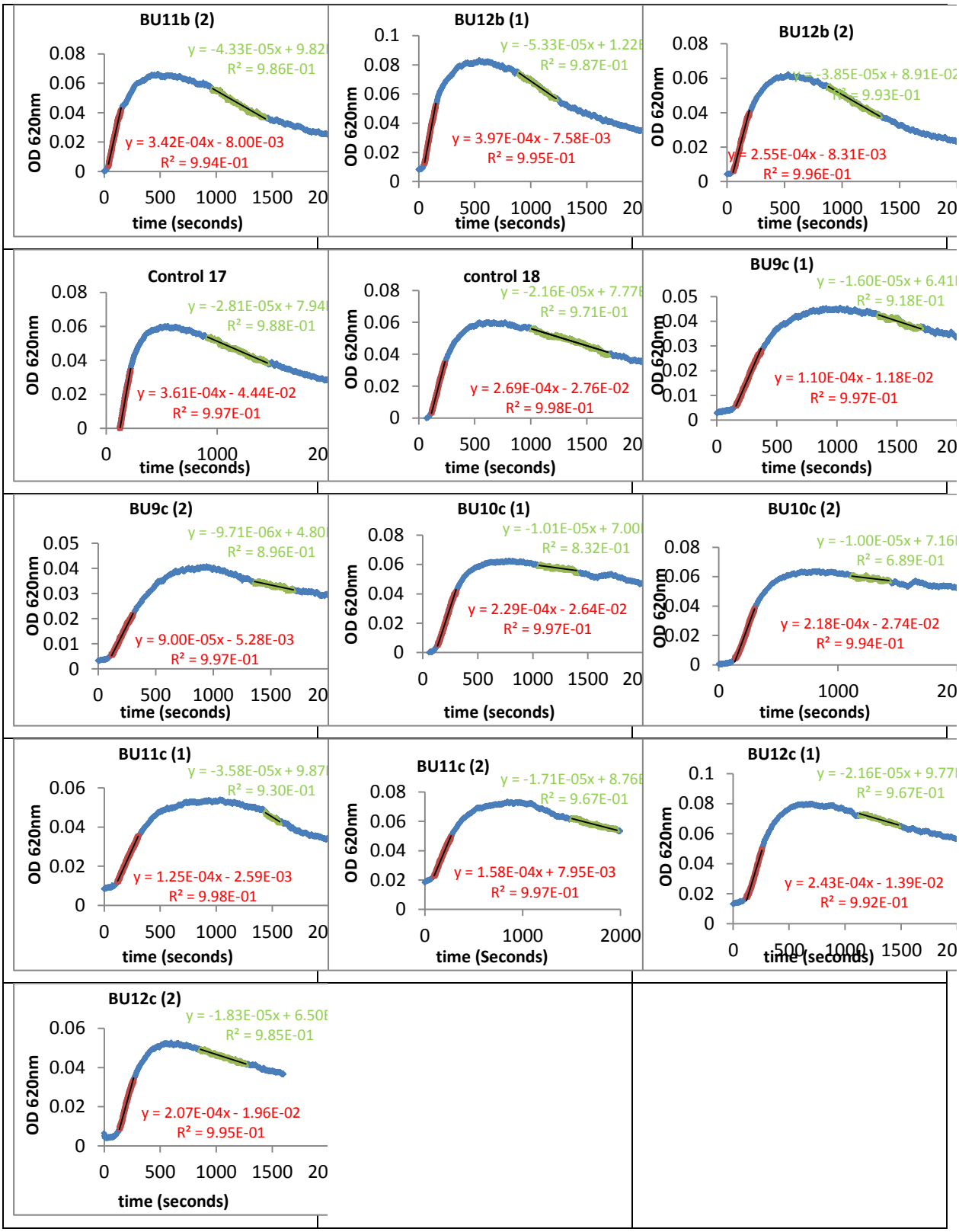
Appendix 6.8
CaOx nucleation and aggregation

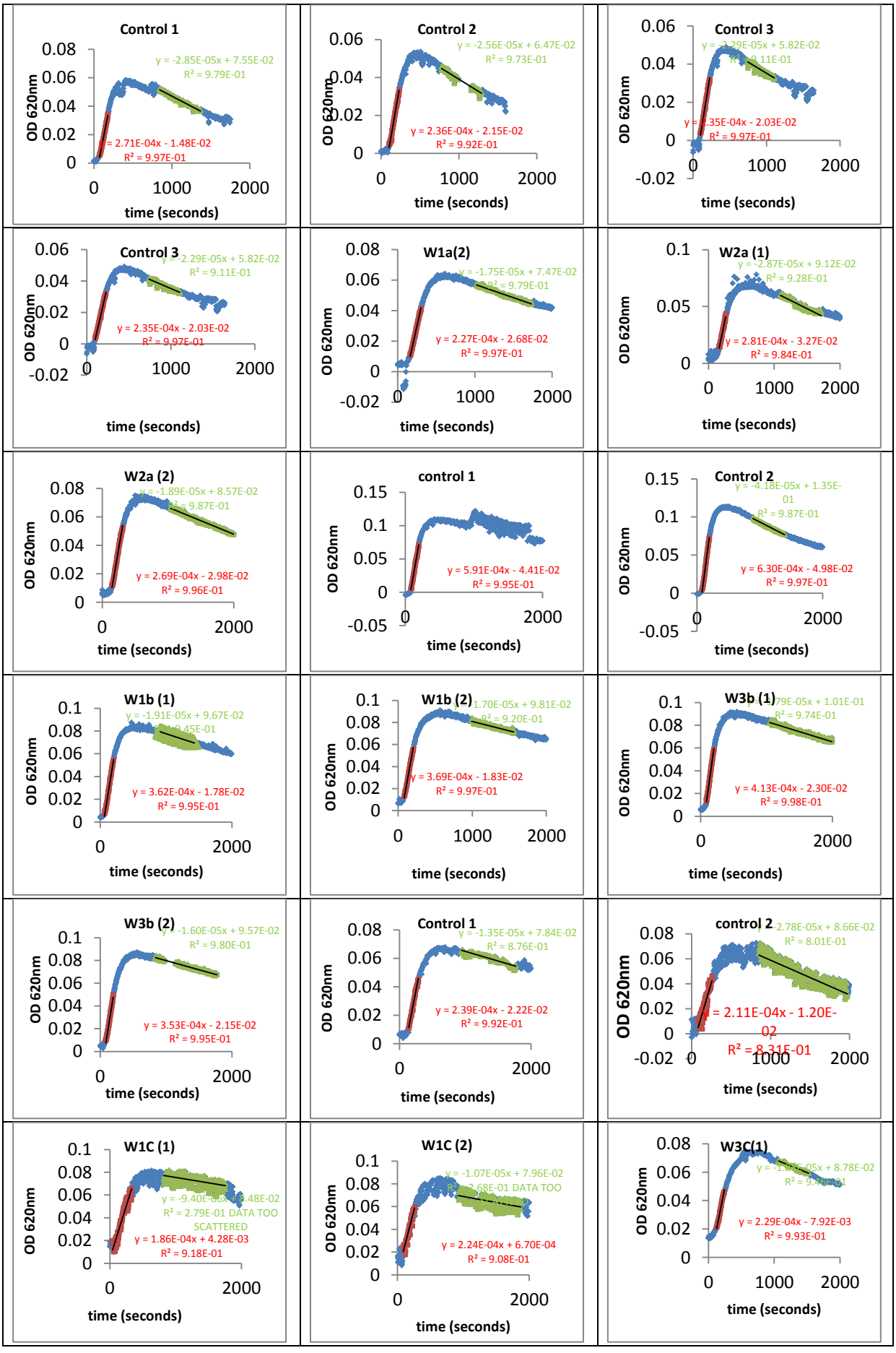


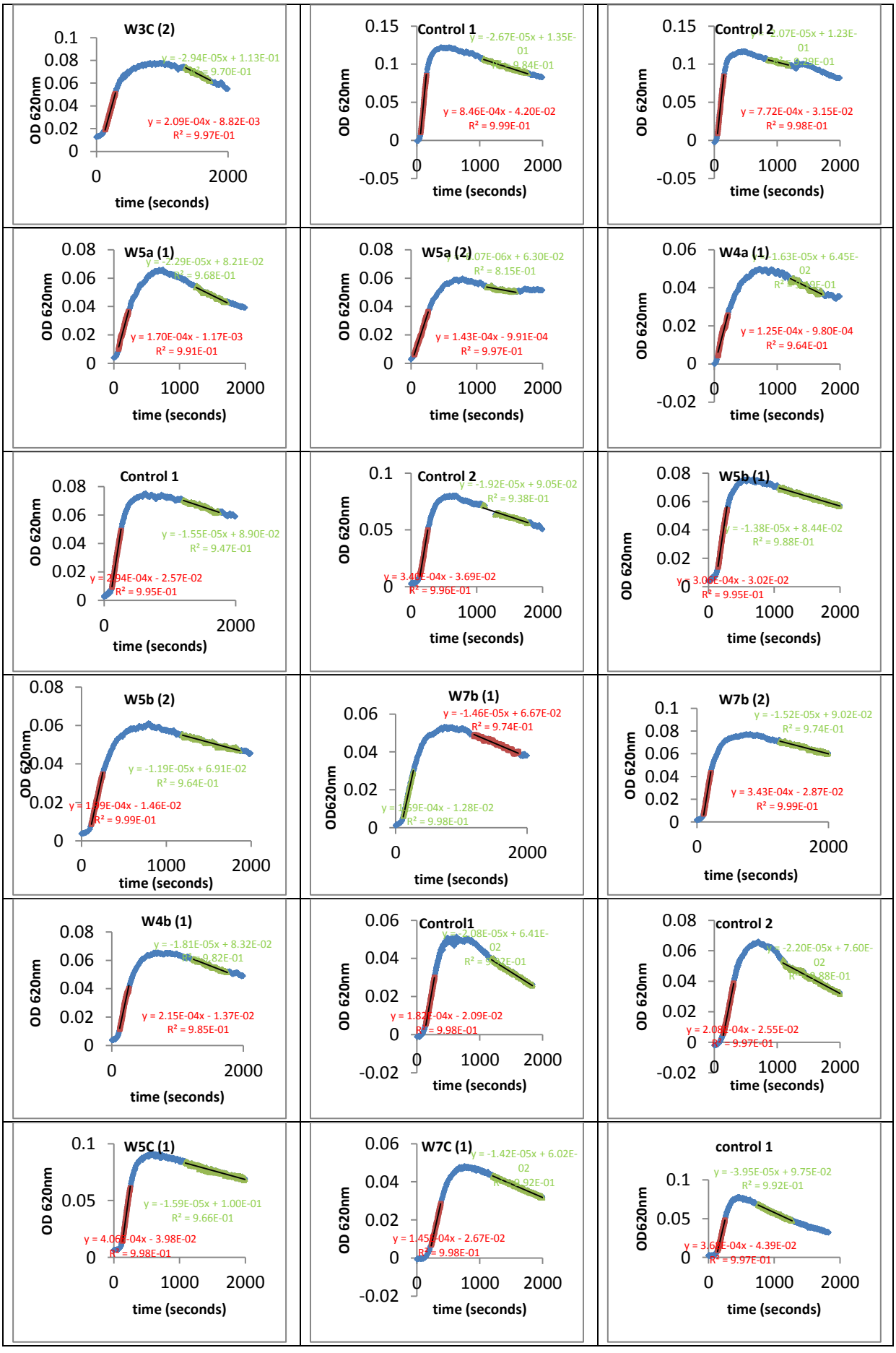

Control 5
y = 3.23E-04x - 4.66E-02
R² = 9.84E-01
control 6
y = -1.65E-05x + 9.74E-02
R² = 9.20E-01
y = 3.61E-04x - 3.05E-02
R² = 9.71E-01
BU1C (1)
y = 1.85E-04x - 6.54E-03
R² = 9.91E-01
BU1C (2)
y = 1.49E-04x - 1.42E-02
R² = 9.81E-01
BU2C (1)
y = 2.05E-04x - 2.02E-02
R² = 9.91E-01
BU2C (2)
y = 1.88E-04x - 3.17E-02
R² = 9.89E-01
BU3C (1)
y = 1.17E-04x - 1.10E-02
R² = 9.78E-01
BU3C (2)
y = 1.77E-04x - 3.11E-02
R² = 9.84E-01
BU4C (1)
y = 1.68E-04x - 1.24E-02
R² = 9.81E-01
Control 7
y = 2.74E-04x - 4.12E-02
R² = 9.91E-01
Control 8
y = 3.69E-04x - 3.63E-02
R² = 9.94E-01
BU5a (1)
y = 1.87E-04x - 3.74E-02
R² = 9.87E-01
BU6a (1)
y = 1.12E-04x - 1.23E-02
R² = 9.94E-01
Control 9
y = 3.35E-04x - 4.88E-02
R² = 9.96E-01
control 10
y = 2.03E-04x - 4.12E-02
R² = 9.95E-01
OD 620 nm
time (seconds)

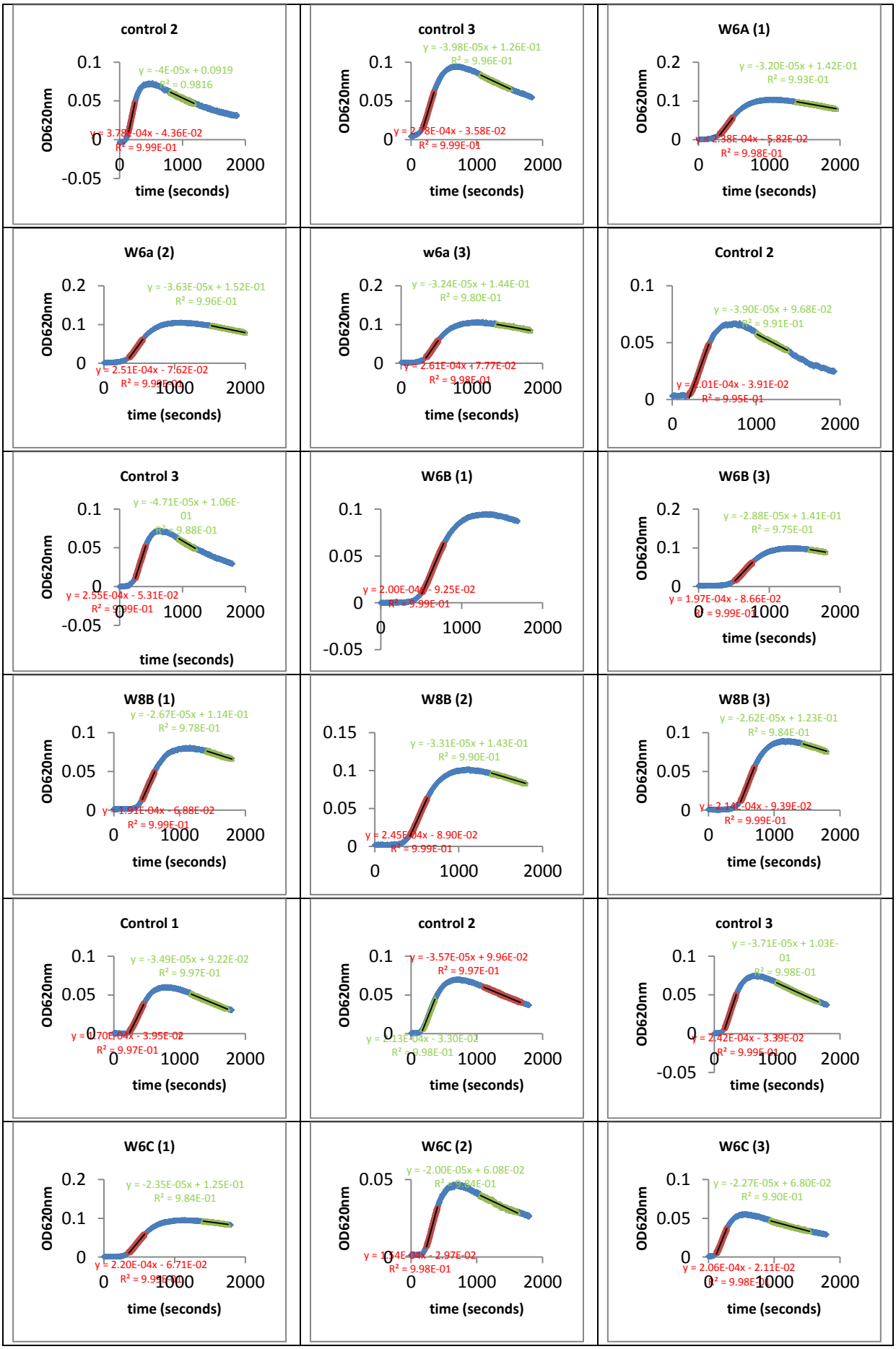

BU6a (2)
y = -1.01E-05x + 6.94
R² = 9.30E-01
y = 8.26E-05x - 1.71E-02
R² = 9.82E-01
OD 620nm
time (seconds)
BU6a (3)
y = -1.11E-05x + 6.49
R² = 9.62E-01
y = 7.63E-05x - 8.37E-03
R² = 9.93E-01
OD 620nm
time (seconds)
BU7a (1)
y = -1.59E-05x + 7.57
R² = 9.53E-01
y = 1.77E-04x - 4.30E-02
R² = 9.95E-01
OD 620nm
time (seconds)
BU7a (2)
y = -1.46E-05x + 5.58
R² = 8.60E-01
y = 1.04E-04x - 2.21E-02
R² = 9.94E-01
Od 620nm
time (seconds)
BU7a (3)
y = -1.27E-05x + 8.02
R² = 9.90E-01
y = 2.23E-04x - 4.18E-02
R² = 9.94E-01
OD 620nm
time (seconds)
BU8a (1)
y = -9.85E-06x + 6.49
R² = 9.69E-01
y = 1.30E-04x - 2.83E-02
R² = 9.92E-01
OD 620nm
time (seconds)
BU8a (2)
y = -1.15E-05x + 7.35
R² = 9.89E-01
y = 1.80E-04x - 2.37E-02
R² = 9.94E-01
OD 620nm
time (seconds)
Control 11
y = -4.53E-05x + 1.07
R² = 9.84E-01
y = 4.78E-04x - 1.64E-02
R² = 9.99E-01
OD 620nm
time (seconds)
Control 12
y = -3.82E-05x + 9.48
R² = 9.87E-01
y = 3.53E-04x - 9.55E-03
R² = 9.98E-01
OD 620nm
time (seconds)
BU5C (1)
y = -2.28E-05x + 9.54E-
R² = 9.95E-01
y = 3.40E-04x - 1.55E-02
R² = 9.98E-01
OD 620nm
time (seconds)
BU5C (2)
y = -2.25E-05x + 9.06E
R² = 9.92E-01
y = 3.28E-04x - 8.23E-03
R² = 9.96E-01
OD 620nm
time (seconds)
BU7C (1)
y = -2.13E-05x + 8.52E
R² = 9.93E-01
y = 3.05E-04x - 1.73E-02
R² = 9.97E-01
OD 620nm
time (seconds)
BU7C (2)
y = -2.08E-05x + 7.83
R² = 9.94E-01
y = 2.39E-04x - 1.26E-02
R² = 9.96E-01
OD 620nm
time (seconds)
BU8C (1)
y = -1.97E-05x + 9.43E-
R² = 9.93E-01
y = 2.97E-04x - 8.40E-03
R² = 9.93E-01
OD 620nm
time (seconds)
BU8C (2)
y = -2.07E-05x + 9.13
R² = 9.92E-01
y = 2.65E-04x - 7.64E-03
R² = 9.96E-01
OD 620nm
time (seconds)

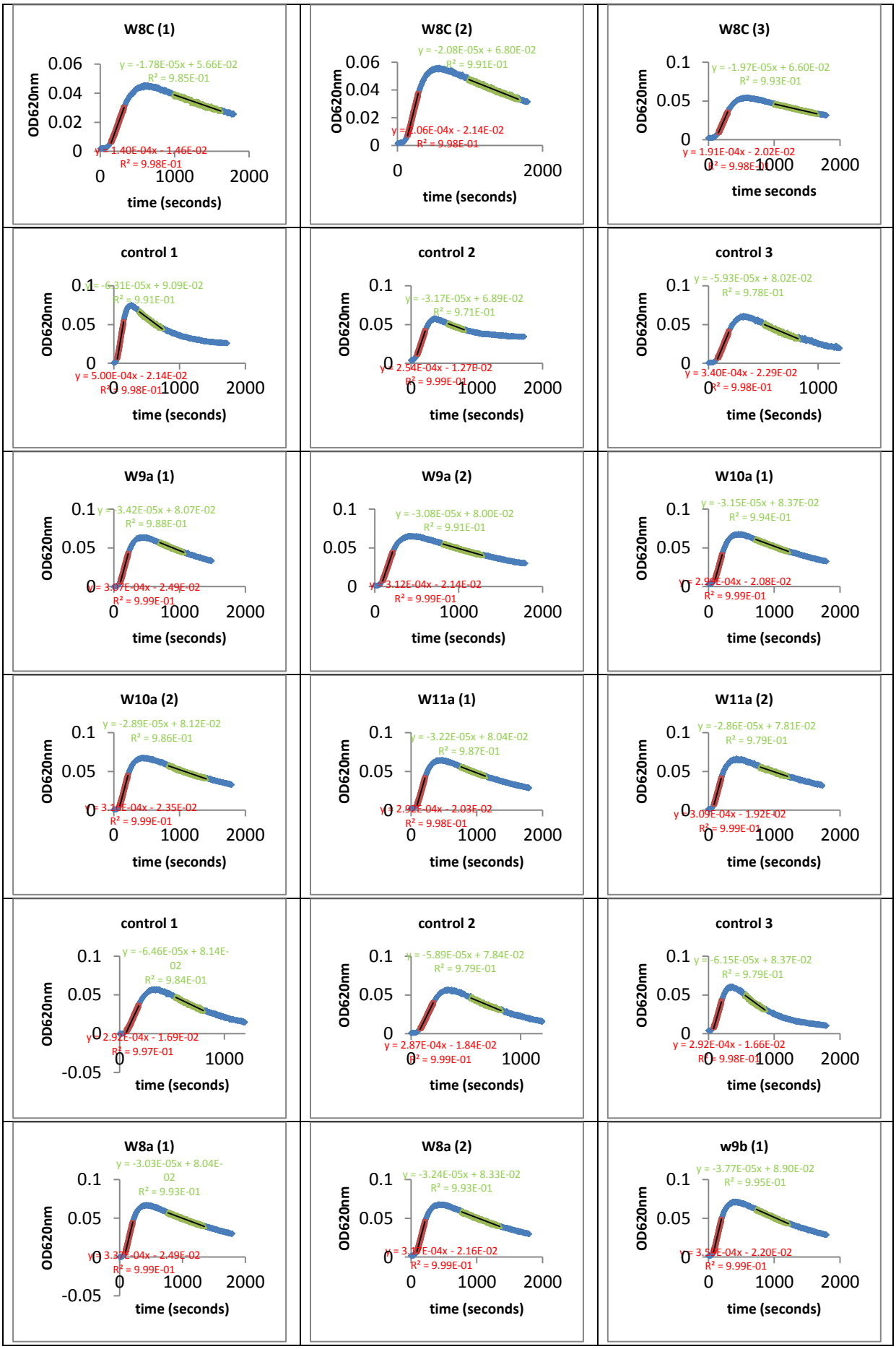

Control 13
y = -4.06E-05x + 1.25E-
R² = 9.95E-01
y = 7.12E-04x - 3.23E-02
R² = 9.93E-01
OD 620nm
time (seconds)
Control 14
y = -3.58E-05x + 1.26E
R² = 9.92E-01
y = 6.73E-04x - 3.23E-02
R² = 9.94E-01
OD 620 nm
time (seconds)
BU9a (1)
y = -3.78E-05x + 1.18E
R² = 9.93E-01
y = 5.80E-04x - 1.93E-02
R² = 9.88E-01
OD 620nm
time (seconds)
BU9a (2)
y = -3.54E-05x + 1.23E-01
R² = 9.93E-01
y = 5.96E-04x - 2.07E-02
R² = 9.90E-01
OD 620nm
time (seconds)
BU10a (1)
y = -3.63E-05x + 1.25E-0
R² = 9.88E-01
y = 6.22E-04x - 2.62E-02
R² = 9.96E-01
OD 620nm
time (seconds)
BU10a (2)
y = -3.61E-05x + 1.34E-01
R² = 9.90E-01
y = 6.13E-04x - 2.61E-02
R² = 9.98E-01
OD 620nm
time (seconds)
BU11a (1)
y = -2.99E-05x + 1.18E-0
R² = 9.56E-01
y = 5.92E-04x - 3.01E-02
R² = 9.93E-01
OD 620nm
time (seconds)
BU11a (2)
y = -1.90E-05x + 1.12E-01
R² = 9.68E-01
y = 5.81E-04x - 3.33E-02
R² = 9.97E-01
OD 620nm
time (seconds)
Control 15
y = -3.92E-05x + 1.52E
R² = 9.89E-01
y = 9.49E-04x - 3.41E-02
R² = 9.97E-01
OD 620nm
OD 620nm
Control 16
y = -3.25E-05x + 1.19E
R² = 9.73E-01
y = 7.15E-04x - 3.09E-02
R² = 9.98E-01
OD 620nm
time (seconds)
9b (1)
y = -4.12E-05x + 1.30E
R² = 9.94E-01
y = 5.58E-04x - 2.39E-02
R² = 9.98E-01
OD 620nm
time (seconds)
9b (2)
y = -1.65E-05x + 7.72E-02
R² = 8.70E-01
y = 3.53E-04x - 1.05E-02
R² = 9.98E-01
OD 620nm
time (seconds)
BU10b (1)
y = -5.10E-05x + 1.10
R² = 9.84E-01
y = 4.61E-04x - 2.71E-02
R² = 9.98E-01
OD 620 nm
time (seconds)
BU10b (2)
y = -4.55E-05x + 1.08
R² = 9.80E-01
y = 4.46E-04x - 1.52E-02
R² = 9.98E-01
OD 620nm
time (seconds)
BU11b (1)
y = -2.39E-05x + 6.15E-(
R² = 9.79E-01
y = 1.13E-04x + 2.22E-03
R² = 9.94E-01
OD 620nm
time (seconds)

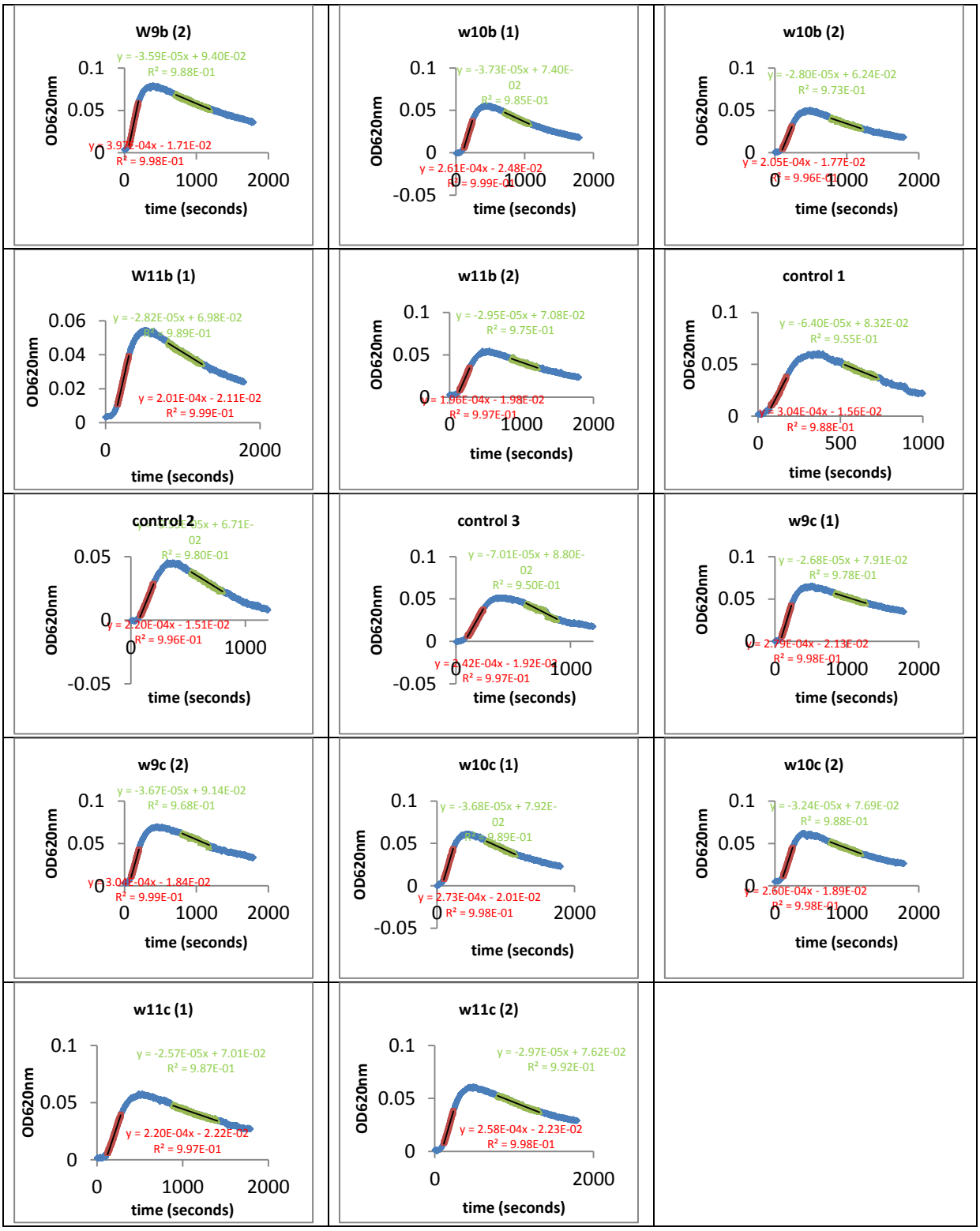

BU11b (2)
y = -4.33E-05x + 9.82
R² = 9.86E-01
y = 3.42E-04x - 8.00E-03
R² = 9.94E-01
BU12b (1)
y = -5.33E-05x + 1.22
R² = 9.87E-01
y = 3.97E-04x - 7.58E-03
R² = 9.95E-01
BU12b (2)
y = -3.85E-05x + 8.91E-02
R² = 9.93E-01
y = 2.55E-04x - 8.31E-03
R² = 9.96E-01
Control 17
y = -2.81E-05x + 7.94
R² = 9.88E-01
y = 3.61E-04x - 4.44E-02
R² = 9.97E-01
control 18
y = -2.16E-05x + 7.77
R² = 9.71E-01
y = 2.69E-04x - 2.76E-02
R² = 9.98E-01
BU9c (1)
y = -1.60E-05x + 6.41
R² = 9.18E-01
y = 1.10E-04x - 1.18E-02
R² = 9.97E-01
BU9c (2)
y = -9.71E-06x + 4.80
R² = 8.96E-01
y = 9.00E-05x - 5.28E-03
R² = 9.97E-01
BU10c (1)
y = -1.01E-05x + 7.00
R² = 8.32E-01
y = 2.29E-04x - 2.64E-02
R² = 9.97E-01
BU10c (2)
y = -1.00E-05x + 7.16
R² = 6.89E-01
y = 2.18E-04x - 2.74E-02
R² = 9.94E-01
BU11c (1)
y = -3.58E-05x + 9.87
R² = 9.30E-01
y = 1.25E-04x - 2.59E-03
R² = 9.98E-01
BU11c (2)
y = -1.71E-05x + 8.76
R² = 9.67E-01
y = 1.58E-04x + 7.95E-03
R² = 9.97E-01
BU12c (1)
y = -2.16E-05x + 9.77
R² = 9.67E-01
y = 2.43E-04x - 1.39E-02
R² = 9.92E-01
BU12c (2)
y = -1.83E-05x + 6.50
R² = 9.85E-01
y = 2.07E-04x - 1.96E-02
R² = 9.95E-01
OD 620nm
time (seconds)
time (Seconds)


Control 1
y = -2.85E-05x + 7.55E-02
R² = 9.79E-01
y = 2.71E-04x - 1.48E-02
R² = 9.97E-01
OD 620nm
time (seconds)
Control 2
y = -2.56E-05x + 6.47E-02
R² = 9.73E-01
y = 2.36E-04x - 2.15E-02
R² = 9.92E-01
OD 620nm
time (seconds)
Control 3
y = -2.29E-05x + 5.82E-02
R² = 9.11E-01
y = 2.35E-04x - 2.03E-02
R² = 9.97E-01
OD 620nm
time (seconds)
Control 3
y = -2.29E-05x + 5.82E-02
R² = 9.11E-01
y = 2.35E-04x - 2.03E-02
R² = 9.97E-01
OD 620nm
time (seconds)
W1a(2)
y = -1.75E-05x + 7.47E-02
R² = 9.79E-01
y = 2.27E-04x - 2.68E-02
R² = 9.97E-01
OD 620nm
time (seconds)
W2a (1)
y = -2.87E-05x + 9.12E-02
R² = 9.28E-01
y = 2.81E-04x - 3.27E-02
R² = 9.84E-01
OD 620nm
time (seconds)
W2a (2)
y = -1.89E-05x + 8.57E-02
R² = 9.87E-01
y = 2.69E-04x - 2.98E-02
R² = 9.96E-01
OD 620nm
time (seconds)
control 1
y = 5.91E-04x - 4.41E-02
R² = 9.95E-01
OD 620nm
time (seconds)
Control 2
y = -4.18E-05x + 1.35E-01
R² = 9.87E-01
y = 6.30E-04x - 4.98E-02
R² = 9.97E-01
OD 620nm
time (seconds)
W1b (1)
y = -1.91E-05x + 9.67E-02
y = 3.62E-04x - 1.78E-02
R² = 9.95E-01
OD 620nm
time (seconds)
W1b (2)
y = 3.69E-04x - 1.83E-02
R² = 9.97E-01
OD 620nm
time (seconds)
W3b (1)
y = 4.13E-04x - 2.30E-02
R² = 9.98E-01
OD 620nm
time (seconds)
W3b (2)
y = -1.60E-05x + 9.57E-02
R² = 9.80E-01
y = 3.53E-04x - 2.15E-02
R² = 9.95E-01
OD 620nm
time (seconds)
Control 1
y = -1.35E-05x + 7.84E-02
R² = 8.76E-01
y = 2.39E-04x - 2.22E-02
R² = 9.92E-01
OD 620nm
time (seconds)
control 2
y = 2.11E-04x - 1.20E-02
R² = 8.31E-01
OD 620nm
time (seconds)
W1C (1)
y = 1.86E-04x + 4.28E-03
R² = 9.18E-01
OD 620nm
time (seconds)
W1C (2)
y = -1.07E-05x + 7.96E-02
y = 2.24E-04x + 6.70E-04
R² = 9.08E-01
OD 620nm
time (seconds)
W3C(1)
y = 2.29E-04x - 7.92E-03
R² = 9.93E-01
OD 620nm
time (seconds)


W3C (2)
y = -2.94E-05x + 1.13E-01
R² = 9.70E-01
y = 2.09E-04x - 8.82E-03
R² = 9.97E-01
OD 620nm
time (seconds)
Control 1
y = -2.67E-05x + 1.35E-01
R² = 9.84E-01
y = 8.46E-04x - 4.20E-02
R² = 9.99E-01
OD 620nm
time (seconds)
Control 2
y = -2.07E-05x + 1.23E-01
R² = 9.98E-01
y = 7.72E-04x - 3.15E-02
R² = 9.98E-01
OD 620nm
time (seconds)
W5a (1)
y = -2.29E-05x + 8.21E-02
R² = 9.68E-01
y = 1.70E-04x - 1.17E-03
R² = 9.91E-01
OD 620nm
time (seconds)
W5a (2)
y = -1.07E-06x + 6.30E-02
R² = 8.15E-01
y = 1.43E-04x - 9.91E-04
R² = 9.97E-01
OD 620nm
time (seconds)
W4a (1)
y = -1.63E-05x + 6.45E-02
R² = 9.89E-01
y = 1.25E-04x - 9.80E-04
R² = 9.64E-01
OD 620nm
time (seconds)
Control 1
y = -1.55E-05x + 8.90E-02
R² = 9.47E-01
y = 2.94E-04x - 2.57E-02
R² = 9.95E-01
OD 620nm
time (seconds)
Control 2
y = -1.92E-05x + 9.05E-02
R² = 9.38E-01
y = 3.44E-04x - 3.69E-02
R² = 9.96E-01
OD 620nm
time (seconds)
W5b (1)
y = -1.38E-05x + 8.44E-02
R² = 9.88E-01
y = 3.05E-04x - 3.02E-02
R² = 9.95E-01
OD 620nm
time (seconds)
W5b (2)
y = -1.19E-05x + 6.91E-02
R² = 9.64E-01
y = 1.89E-04x - 1.46E-02
R² = 9.99E-01
OD 620nm
time (seconds)
W7b (1)
y = -1.46E-05x + 6.67E-02
R² = 9.74E-01
y = 1.29E-04x - 1.28E-02
R² = 9.98E-01
OD620nm
time (seconds)
W7b (2)
y = -1.52E-05x + 9.02E-02
R² = 9.74E-01
y = 3.43E-04x - 2.87E-02
R² = 9.99E-01
OD 620nm
time (seconds)
W4b (1)
y = -1.81E-05x + 8.32E-02
R² = 9.82E-01
y = 2.15E-04x - 1.37E-02
R² = 9.85E-01
OD 620nm
time (seconds)
Control1
y = -2.08E-05x + 6.41E-02
R² = 9.92E-01
y = 1.82E-04x - 2.09E-02
R² = 9.98E-01
OD 620nm
time (seconds)
control 2
y = -2.20E-05x + 7.60E-02
R² = 9.88E-01
y = 2.08E-04x - 2.55E-02
R² = 9.97E-01
OD 620nm
time (seconds)
W5C (1)
y = -1.59E-05x + 1.00E-01
R² = 9.66E-01
y = 4.06E-04x - 3.98E-02
R² = 9.98E-01
OD 620nm
time (seconds)
W7C (1)
y = -1.42E-05x + 6.02E-02
R² = 9.92E-01
y = 1.45E-04x - 2.67E-02
R² = 9.98E-01
OD 620nm
time (seconds)
control 1
y = -3.95E-05x + 9.75E-02
R² = 9.92E-01
y = 3.62E-04x - 4.39E-02
R² = 9.97E-01
OD620nm
time (seconds)


control 2
y = -4E-05x + 0.0919
R² = 0.9816
y = 3.78E-04x - 4.36E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 3
y = -3.98E-05x + 1.26E-01
R² = 9.96E-01
y = 2.78E-04x - 3.58E-02
R² = 9.99E-01
OD620nm
time (seconds)
W6A (1)
y = -3.20E-05x + 1.42E-01
R² = 9.93E-01
y = 2.38E-04x - 5.82E-02
R² = 9.98E-01
OD620nm
time (seconds)
W6a (2)
y = -3.63E-05x + 1.52E-01
R² = 9.96E-01
y = 2.51E-04x - 7.62E-02
R² = 9.99E-01
OD620nm
time (seconds)
w6a (3)
y = -3.24E-05x + 1.44E-01
R² = 9.80E-01
y = 2.61E-04x + 7.77E-02
R² = 9.98E-01
OD620nm
time (seconds)
Control 2
y = -3.90E-05x + 9.68E-02
R² = 9.91E-01
y = 2.01E-04x - 3.91E-02
R² = 9.95E-01
time (seconds)
Control 3
y = -4.71E-05x + 1.06E-01
R² = 9.88E-01
y = 2.55E-04x - 5.31E-02
R² = 9.99E-01
OD620nm
time (seconds)
W6B (1)
y = 2.00E-04x - 9.25E-02
R² = 9.99E-01
time (seconds)
W6B (3)
y = -2.88E-05x + 1.41E-01
R² = 9.75E-01
y = 1.97E-04x - 8.66E-02
R² = 9.99E-01
OD620nm
time (seconds)
W8B (1)
y = -2.67E-05x + 1.14E-01
R² = 9.78E-01
y = 1.91E-04x - 6.88E-02
R² = 9.99E-01
OD620nm
time (seconds)
W8B (2)
y = -3.31E-05x + 1.43E-01
R² = 9.90E-01
y = 2.45E-04x - 8.90E-02
R² = 9.99E-01
time (seconds)
W8B (3)
y = -2.62E-05x + 1.23E-01
R² = 9.84E-01
y = 2.14E-04x - 9.39E-02
R² = 9.99E-01
OD620nm
time (seconds)
Control 1
y = -3.49E-05x + 9.22E-02
R² = 9.97E-01
y = 2.70E-04x - 3.95E-02
R² = 9.97E-01
OD620nm
time (seconds)
control 2
y = -3.57E-05x + 9.96E-02
R² = 9.97E-01
y = 2.13E-04x - 3.30E-02
R² = 9.98E-01
OD620nm
time (seconds)
control 3
y = -3.71E-05x + 1.03E-01
R² = 9.98E-01
y = 2.42E-04x - 3.39E-02
R² = 9.99E-01
OD620nm
time (seconds)
W6C (1)
y = -2.35E-05x + 1.25E-01
R² = 9.84E-01
y = 2.20E-04x - 6.71E-02
R² = 9.99E-01
OD620nm
time (seconds)
W6C (2)
y = -2.00E-05x + 6.08E-02
R² = 9.84E-01
y = 1.64E-04x - 2.97E-02
R² = 9.98E-01
OD620nm
time (seconds)
W6C (3)
y = -2.27E-05x + 6.80E-02
R² = 9.90E-01
y = 2.06E-04x - 2.11E-02
R² = 9.98E-01
OD620nm
time (seconds)


W8C (1)
y = -1.78E-05x + 5.66E-02
R² = 9.85E-01
y = 1.40E-04x - 1.46E-02
R² = 9.98E-01
OD620nm
time (seconds)
W8C (2)
y = -2.08E-05x + 6.80E-02
R² = 9.91E-01
y = 1.06E-04x - 2.14E-02
R² = 9.98E-01
OD620nm
time (seconds)
W8C (3)
y = -1.97E-05x + 6.60E-02
R² = 9.93E-01
y = 1.91E-04x - 2.02E-02
R² = 9.98E-01
OD620nm
time seconds
control 1
y = -6.31E-05x + 9.09E-02
R² = 9.91E-01
y = 5.00E-04x - 2.14E-02
R² = 9.98E-01
OD620nm
time (seconds)
control 2
y = -3.17E-05x + 6.89E-02
R² = 9.71E-01
y = 2.54E-04x - 1.27E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 3
y = -5.93E-05x + 8.02E-02
R² = 9.78E-01
y = 3.40E-04x - 2.29E-02
R² = 9.98E-01
OD620nm
time (Seconds)
W9a (1)
y = -3.42E-05x + 8.07E-02
R² = 9.88E-01
R² = 9.99E-01
OD620nm
time (seconds)
W9a (2)
y = -3.08E-05x + 8.00E-02
R² = 9.91E-01
y = 3.12E-04x - 2.14E-02
R² = 9.99E-01
OD620nm
time (seconds)
W10a (1)
y = -3.15E-05x + 8.37E-02
R² = 9.94E-01
R² = 9.99E-01
OD620nm
time (seconds)
W10a (2)
y = -2.89E-05x + 8.12E-02
R² = 9.86E-01
R² = 9.99E-01
OD620nm
time (seconds)
W11a (1)
y = -3.22E-05x + 8.04E-02
R² = 9.87E-01
R² = 9.98E-01
OD620nm
time (seconds)
W11a (2)
y = -2.86E-05x + 7.81E-02
R² = 9.79E-01
R² = 9.99E-01
OD620nm
time (seconds)
control 1
y = -6.46E-05x + 8.14E-02
R² = 9.84E-01
y = 2.92E-04x - 1.69E-02
R² = 9.97E-01
OD620nm
time (seconds)
control 2
y = -5.89E-05x + 7.84E-02
R² = 9.79E-01
y = 2.87E-04x - 1.84E-02
R² = 9.99E-01
OD620nm
time (seconds)
control 3
y = -6.15E-05x + 8.37E-02
R² = 9.79E-01
y = 2.92E-04x - 1.66E-02
R² = 9.98E-01
OD620nm
time (seconds)
W8a (1)
y = -3.03E-05x + 8.04E-02
R² = 9.93E-01
R² = 9.99E-01
OD620nm
time (seconds)
W8a (2)
y = -3.24E-05x + 8.33E-02
R² = 9.93E-01
R² = 9.99E-01
OD620nm
time (seconds)
w9b (1)
y = -3.77E-05x + 8.90E-02
R² = 9.95E-01
R² = 9.99E-01
OD620nm
time (seconds)


W9b (2)
y = -3.59E-05x + 9.40E-02
R² = 9.88E-01
y = 3.97E-04x - 1.71E-02
R² = 9.98E-01
w10b (1)
y = -3.73E-05x + 7.40E-02
R² = 9.85E-01
y = 2.61E-04x - 2.48E-02
R² = 9.99E-01
w10b (2)
y = -2.80E-05x + 6.24E-02
R² = 9.73E-01
y = 2.05E-04x - 1.77E-02
R² = 9.96E-01
W11b (1)
y = -2.82E-05x + 6.98E-02
R² = 9.89E-01
y = 2.01E-04x - 2.11E-02
R² = 9.99E-01
w11b (2)
y = -2.95E-05x + 7.08E-02
R² = 9.75E-01
y = 1.96E-04x - 1.98E-02
R² = 9.97E-01
control 1
y = -6.40E-05x + 8.32E-02
R² = 9.55E-01
y = 3.04E-04x - 1.56E-02
R² = 9.88E-01
control 2
y = 2.20E-04x - 1.51E-02
R² = 9.96E-01
control 3
y = -7.01E-05x + 8.80E-02
R² = 9.50E-01
y = 2.42E-04x - 1.92E-02
R² = 9.97E-01
w9c (1)
y = -2.68E-05x + 7.91E-02
R² = 9.78E-01
y = 2.79E-04x - 2.13E-02
R² = 9.98E-01
w9c (2)
y = -3.67E-05x + 9.14E-02
R² = 9.68E-01
y = 3.04E-04x - 1.84E-02
R² = 9.99E-01
w10c (1)
y = -3.68E-05x + 7.92E-02
R² = 9.89E-01
y = 2.73E-04x - 2.01E-02
R² = 9.98E-01
w10c (2)
y = -3.24E-05x + 7.69E-02
R² = 9.88E-01
y = 2.60E-04x - 1.89E-02
R² = 9.98E-01
w11c (1)
y = -2.57E-05x + 7.01E-02
R² = 9.87E-01
y = 2.20E-04x - 2.22E-02
R² = 9.97E-01
w11c (2)
y = -2.97E-05x + 7.62E-02
R² = 9.92E-01
y = 2.58E-04x - 2.23E-02
R² = 9.98E-01
OD620nm
time (seconds)

Appendix 7

Appendix 7.1

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

22 April 2014

HREC REF: 072/2014

Prof A Rodgers
Chemistry Department
Room 6.33.4, level 1
PD Hahn Building
Upper Campus

Dear Prof Rodgers

PROJECT TITLE: THE EFFECT OF AN IP-6 SUPPLEMENT ON URINARY RISK FACTORS ASSOCIATED WITH CaOx UROLITHIASIS IN SOUTH AFRICAN POPULATION GROUPS WITH DIFFERENT RISK PROFILES (PhD Saajidah Fakier)

Thank you for your response letter to the Faculty of Health Sciences Human Research Ethics Committee dated 16 April 2014.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2015

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

We acknowledge that the PhD student, Saajidah Fakier is also involved in this study.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

HREC 072/2014

Appendix 7.2
raw data for the determination of urinary IP6

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.13	1.137	1.1335	
5	1.041	1.035	1.038	
10	0.921	0.919	0.92	y = -0.0199x + 1.1305; R2 = 0.998
20	0.726	0.724	0.725	
25	0.646	0.64	0.643	

sample ID	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				% recovery
	1	2	1	2	1	2	average	SE	
B1a	1.035	1.029	4.80	5.10	0.96	1.02	0.99	0.030	
B1a +1		0.933		9.92		1.98	1.98		99

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.107	1.106	1.1065	
5	0.984	0.958	0.971	
10	0.851	0.849	0.85	y = -0.0212x + 1.0854; R2 = 0.9938
20	0.665	0.656	0.6605	
25	0.573	0.562	0.5675	

sample ID	Absorbance at 460nm			[IP6] in eluate (uM)			[IP6] in urine (uM)					% recovery
	1	2	3	1	2	3	1	2	3	average	SE	
B2a	1.022	0.994	0.986	2.99	4.31	4.69	0.60	0.86	0.94	0.90	0.04	
B3a	0.941	0.97	0.954	6.81	5.44	6.20	1.36	1.09	1.24	1.30	0.06	
B3a + 1			0.847			11.25			2.25			95
B4a	0.903	0.889	0.896	8.60	9.26	8.93	1.72	1.85	1.79	1.79	0.04	

B4a + 1			0.798			13.56			2.71			92

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.126	1.137	1.132	
5	1.013	1.02	1.017	
10	0.908	0.894	0.901	y = -0.0217 + 1.1262; R2 = 0.9995
20	0.701	0.688	0.695	
25	0.593	0.577	0.585	

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
B1b	1.112	1.118	0.65	0.38	0.13	0.08	0.10	0.03	
B1b + 1uM	0.966		7.38		1.48		1.48		137
B2b	1.091	1.051	1.62	3.47	0.32	0.69	0.51	0.18	
B2b + 1uM	0.959		7.71		1.54		1.54		103
B3b	0.956	0.947	7.84	8.26	1.57	1.65	1.61	0.04	
B3b + 1uM	0.84		13.19		2.64		2.64		103
B4b	0.988	0.958	6.37	7.75	1.27	1.55	1.41	0.14	
B4b + 1uM	0.885		11.12		2.22		2.22		81

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.14	1.142	1.141	
5	1.033	1.033	1.033	
10	0.923	0.923	0.923	y = -0.0205x + 1.1357; R2 = 0.9994
20	0.715	0.734	0.725	
25	0.623	0.633	0.628	

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**

B1c	1.142	1.096		1.94		0.39	0.39		
B1c + 1uM	1.034		4.96		0.99		0.99		60
B2c	1.097	1.093	1.89	2.08	0.38	0.42	0.40	0.02	
B2c + 1uM	1.006		6.33		1.27		1.27		87
B3c	1.06	1.108	3.69	1.35	0.74	0.27	0.50	0.23	
B3c + 1uM	0.955		8.81		1.76		1.76		126
B4c	1.004	0.999	6.42	6.67	1.28	1.33	1.31	0.02	
B4c + 1uM	0.918		10.62		2.12		2.12		81

[IP6] uM	**Absorbance at 460nm**			**equation of line (calibration curve)**
	OD1	**OD2**	**average**	
0	1.155	1.17	1.163	
5	1.047	1.051	1.049	
10	0.942	0.953	0.948	y = -0.0217x + 1.1617
20	0.733	0.728	0.731	
25	0.619	0.612	0.616	

	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
B1d	1.073	1.069	4.09	4.27	0.82	0.85	0.84	0.02	
B1d + 1uM	0.99		7.91		1.58		1.58		75
B2d	1.137	1.134	1.14	1.28	0.23	0.26	0.24	0.01	
B2d + 1uM	1.043		5.47		1.09		1.09		85
B3d	1.056	1.081	4.87	3.72	0.97	0.74	0.86	0.12	
B3d+ 1uM	0.946		9.94		1.99		1.99		113
B4d	1.013		6.85		1.37		1.37		
B4d + 1uM	0.931		10.63		2.13		2.13		76

[IP6] uM	**Absorbance at 460nm**	**equation of line (calibration curve)**

	OD1	OD2	average	
0	1.1197	1.1242	1.122	
5	1.0218	1.0075	1.015	
10	0.9083	0.8956	0.902	y = -0.020x + 1.116; R2 = 0.998
20	0.6934	0.6961	0.695	
25	0.6232	0.6081	0.616	

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
B1e	0.9853	0.9713	6.54	7.24	1.31	1.45	1.38	0.07	
B1e + 1uM	0.9029		10.66		2.13		2.13		75
B2e	1.0381	1.0683	3.90	2.39	0.78	0.48	0.63	0.15	
B2e + 1uM	0.921		9.75		1.95		1.95		132
B3e	1.0544	1.0473	3.08	3.44	0.62	0.69	0.65	0.04	
B3e + 1uM	0.9519		8.21		1.64		1.64		99
B4e	1.0069	0.979	5.46	6.85	1.09	1.37	1.23	0.14	
B4e + 1uM	0.9101		10.30		2.06		2.06		83

[IP6] uM	**OD1**	**OD2**	**average**	**equation of line (callibration curve)**
0	1.13	1.137	1.1335	
5	1.041	1.035	1.038	
10	0.921	0.919	0.92	y = -0.0199x + 1.1305; R2 = 0.998
20	0.726	0.724	0.725	
25	0.646	0.64	0.643	

	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
7a	1.014	1.02	5.85	5.55	1.17	1.11	1.14	0.03	
7a +1	0.922		10.48			2.10			95

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.155	1.175	1.165	
5	1.038	1.045	1.0415	
10	0.919	0.96	0.9395	
20	0.75	0.772	0.761	
25	0.659	0.644	0.6515	

sample ID	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				%recovery
	1	2	1	2	1	2	average	SE	
5a	1.073	1.069	3.93	4.13	0.79	0.83	0.81	0.03	
5a + 1	0.984			8.38		1.68			87
6a	1.051	1.057	5.03	4.73	1.01	0.95	0.98	0.04	
6a + 1	0.938			10.68		2.14			116
SP1a	1.069	1.056	4.13	4.78	0.83	0.95	0.89	0.09	
SP1a + 1	0.956			9.78		1.96			107

[IP6] uM	Absorbance at 460nm			equation of line (calibration curve)
	OD1	OD2	average	
0	1.145	1.157	1.151	
5	1.038	1.012	1.025	
10	0.909	0.915	0.912	y = -0.0212x + 1.1377; R2 = 0.9977
20	0.717	0.714	0.7155	
25	0.617	0.61	0.6135	

sample ID	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				%recovery
	1	2	1	2	1	2	average	SE	
B5b	1.161	1.136		0.08		0.02	0.02		
B5b + 1um	1.011		5.98		1.20		1.20		118

B7b	1.099	1.1	1.83	1.78	0.37	0.36	0.36	0.00	
B7b + 1um	0.971		7.86		1.57		1.57		121
SP1b	1.048	1.042	4.23	4.51	0.85	0.90	0.87	0.03	
SP1b + 1um	0.97		7.91		1.58		1.58		71
B6b	1	1.028	6.50	5.17	1.30	1.03	1.17	0.13	
B6b + 1um	0.933		9.66		1.93		1.93		76

[IP6] uM	**OD1**	**OD2**	**average**	**equation of line (calibration curve)**
0	1.137	1.107	1.137	
5	0.997	0.993	0.995	
10	0.897	0.89	0.8935	y = -0.0224x + 1.1212; R2 = 0.99667
20	0.683	0.646	0.6645	
25	0.576	0.572	0.574	

	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
B5c	1.056	1.048	2.91	3.27	0.58	0.65	0.62	0.02	
B5c + 1um	0.929		8.58		1.72		1.72		110
B7c	1.019	0.993	4.56	5.72	0.91	1.14	1.03	0.06	
B7c + 1um	0.92		8.98		1.80		1.80		77
SP1c	1.015	0.992	4.74	5.77	0.95	1.15	1.05	0.05	
SP1c + 1um	0.937		8.22		1.64		1.64		59
B6c	1.076	1.081	2.02	1.79	0.40	0.36	0.38	0.01	
B6c + 1um	0.976		6.48		1.30		1.30		92

	Absorbance at 460nm			
[IP6] uM	**OD1**	**OD2**	**average**	**equation of line (calibration curve)**
0	1.13	1.137	1.1335	

5	1.041	1.035	1.038						
10	0.921	0.919	0.92	y = -0.0199x + 1.1305; R2 = 0.998					
20	0.726	0.724	0.725						
25	0.646	0.64	0.643						
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
B5d	1.074	1.078	2.84	2.64	0.57	0.53	0.55	0.020	
B5d+1uM		0.995		6.81		1.36			81

	Absorbance at 460nm			**equation of line (calibration curve)**					
[IP6] uM	**OD1**	**OD2**	**average**						
0	1.178	1.178	1.178						
5	1.067	1.058	1.0625						
10	0.941	0.948	0.9445	y = -0.0213x + 1.1699; R2 = 0.9987					
20	0.746	0.743	0.7445						
25	0.644	0.641	0.6425						
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
B6d	1.113	1.114	2.67	2.62	0.53	0.52	0.53	0.00	
B6d + 1um	1.014		7.32		1.46		1.46		93
B7d	1.085	1.013	3.99	7.37	0.80	1.47	1.14	0.34	
B7d + 1um	0.981		8.87		1.77		1.77		64
SP1d	1.076	1.081	4.41	4.17	0.88	0.83	0.86	0.02	
SP1d + 1um	0.981		8.87		1.77		1.77		92

	Absorbance at 460nm			**equation of line (calibration curve)**
[IP6] uM	**OD1**	**OD2**	**average**	
0	1.188	1.192	1.19	

5	1.068	1.075	1.0715	
10	0.971	0.971	0.971	y = -0.0226x + 1.1902; R2 = 0.9994
20	0.758	0.728	0.743	
25	0.621	0.62	0.6205	

	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
B5e	1.096	1.083	4.17	4.74	0.83	0.95	0.89	0.06	
B5e + 1um	0.983		9.17		1.83		1.83		94
B6e	1.088	1.053	4.52	6.07	0.90	1.21	1.06	0.15	
B6e + 1um	0.975		9.52		1.90		1.90		85
SP1e	1.091	1.058	4.39	5.85	0.88	1.17	1.02	0.15	
SP1e + 1um	0.959		10.23		2.05		2.05		102

	Absorbance at 460nm			
[IP6] uM	**OD1**	**OD2**	**average**	**equation of line (calibration curve)**
0	1.16	1.167	1.1635	
5	1.044	1.036	1.04	
10	0.938	0.93	0.934	y = -0.022x + 1.1563; R2 = 0.9994
20	0.719	0.709	0.714	
25	0.616	0.607	0.6115	

	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**%recovery**
B7f	1.042	0.992	5.20	7.47	1.04	1.49	1.27	0.23	
B7f + 1um	0.964		8.74		1.75		1.75		48

[IP6] uM	**OD1**	**OD2**	**average**	**SE**	**Equation of line (equation of line)**
0	1.148	1.147	1.1475	0.000	
5	1.006	1.01	1.008	0.002	

10	0.891	0.891	0.891	0.000	y = -0.0229 + 1.1317 R2 = 0.997				
20	0.681	0.65	0.6655	0.016					
25	0.571	0.572	0.5715	0.001					
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
SP2a	1.052	1.038	3.48	4.09	0.70	0.82	0.76	0.06	
SP2a + 1	0.926		8.98		1.80		1.80		104
SP3a	1.089	1.098	1.86	1.47	0.37	0.29	0.33	0.04	
SP3a + 1	0.962		7.41		1.48		1.48		115
SP4a	0.995	0.993	5.97	6.06	1.19	1.21	1.20	0.01	
SP4a +1	0.888		10.64		2.13		2.13		93
SP5a	1.022		4.79		0.96		0.96		
SP5a +1	0.896		10.29		2.06		2.06		110

[IP6] uM	**OD1**	**OD2**	**average**	**SE**	**Equation of line (calibration curve)**				
0	1.175	1.181	1.178	0.003					
5	1.044	1.017	1.0305	0.014					
10	0.939	0.94	0.9395	0.001	y = -0.0221x + 1.1612				
20	0.722	0.716	0.719	0.003					
25	0.616	0.612	0.614	0.002					
	Absorbance at 460nm		[IP6] in eluate (uM)		[IP6] in urine (uM)				
sample ID	1	2	1	2	1	2	average	SE	% recovery
SP6a	1.066	1.064	4.31	4.40	0.86	0.88	0.87	0.01	
SP6a + 1		0.962		9.01		1.80	1.80		93
SP7a	1.134	1.132	1.23	1.32	0.25	0.26	0.26	0.01	
SP7a + 1		1.03		5.94		1.19	1.19		93

[IP6] uM	**OD1**	**OD2**	**average**	**SE**	**Equation of line**

0	1.134	1.128	1.131	0.003					
5	1	1.023	1.0115	0.012					
10	0.907	0.912	0.9095	0.003	y = -0.0233x + 1.131; R2 = 0.9967				
20	0.64	0.648	0.644	0.004					
25	0.56	0.565	0.5625	0.002					
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
SP2b	1.083	1.078	2.06	2.27	0.41	0.45	0.43	0.02	
SP2b + 1		0.962		7.25		1.45			102
SP3b	1.108	1.103	0.99	1.20	0.20	0.24	0.22	0.02	
SP3b + 1		0.983		6.35		1.27			105
SP4b	1.032	1.044	4.25	3.73	0.85	0.75	0.80	0.05	
SP4b +1		0.92		9.06		1.81			101
SP5b	1.038	1.028	3.99	4.42	0.80	0.88	0.84	0.04	
SP5b +1		0.904		9.74		1.95			111
SP6b	0.974	0.977	6.74	6.61	1.35	1.32	1.33	0.01	
SP7b	0.99	1.014	6.05	5.02	1.21	1.00	1.11	0.05	

[IP6] uM	**OD1**	**OD2**	**average**	**SE**	**Equation of line**				
0	1.14	1.144	1.142	0.002					
5	1.012	1.025	1.0185	0.006					
10	0.894	0.893	0.8935	0.001	y = -0.0222x + 1.1312; R2 = 0.9981				
20	0.684	0.687	0.6855	0.002					
25	0.58	0.586	0.583	0.003					
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
SP2C	1.063	1.041	3.07	4.06	0.61	0.81	0.71	0.10	
SP2C + 1		0.958		7.80		1.56			85

SP3C	1.098	1.092	1.50	1.77	0.30	0.35	0.33	0.03	
SP3C + 1		0.988		6.45		1.29			96
SP4C	1.022	1.008	4.92	5.55	0.98	1.11	1.05	0.06	
SP4C +1		0.925		9.29		1.86			81
SP6C	1.012	0.993	5.37	6.23	1.07	1.25	1.16	0.04	
SP6C + 1		0.91		9.96		1.99			83
SP7C	1.027	1.028	4.69	4.65	0.94	0.93	0.93	0.002	
SP7C + 1		0.939		8.66		1.73			80

[IP6] uM	**OD1**	**OD2**	**average**	**SE**	**Equation of line**
0	1.13	1.137	1.1335	0.004	
5	1.041	1.035	1.038	0.003	
10	0.921	0.919	0.92	0.001	y = -0.0199x + 1.1305; R2 = 0.998
20	0.726	0.724	0.725	0.001	
25	0.646	0.64	0.643	0.003	

	Absorbance at 460nm			**[IP6] in eluate (uM)**			**[IP6] in urine (uM)**					
sample ID	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**average**	**SE**	**% recovery**
SP2d	1.035	1.045	1.057	4.80	4.30	3.69	0.96	0.86	0.74	0.85	0.06	
SP2d + 1	0.951			9.02			1.80			1.80		95
SP3d	1.089	1.069	1.08	2.09	3.09	2.54	0.42	0.62	0.51	0.51	0.06	
SP3d + 1	0.99			7.06			1.41			1.41		90
SP4d	1.038	1.055	1.024	4.65	3.79	5.35	0.93	0.76	1.07	0.92	0.09	
SP4d +1	0.948			9.17			1.83			1.83		91
SP5d	1.093	1.104		1.88	1.33		0.38	0.27		0.32	0.06	
SP5d + 1	0.987			7.21			1.44			1.44		112
SP6d	1.04	1.052		4.55	3.94		0.91	0.79		0.85	0.06	
SP6d + 1	0.98			7.56			1.51			1.51		66
SP7d	1.036	1.057		4.75	3.69		0.95	0.74		0.84	0.11	106

SP7d +1	0.941			9.52			1.90			1.90		

[IP6] uM	OD1	OD2	average	SE	Equation of line (calibration curve)				
0	1.158	1.171	1.1645	0.007					
5	1.051	1.052	1.0515	0.001					
10	0.942	0.944	0.943	0.001	y = -0.0207x + 0.9993				
20	0.737	0.745	0.741	0.004					
25	0.643	0.646	0.6445	0.002					
	Absorbance at 460nm		**[IP6] in eluate (uM)**		**[IP6] in urine (uM)**				
sample ID	**1**	**2**	**1**	**2**	**1**	**2**	**average**	**SE**	**% recovery**
SP2e	1.052	1.068	5.11	4.33	1.02	0.87	0.94	0.08	
SP3e	1.067	1.061	4.38	4.67	0.88	0.93	0.91	0.03	
SP4e	1.042	1.047	5.59	5.35	1.12	1.07	1.09	0.02	
SP5e	1.012	1.014	7.04	6.94	1.41	1.39	1.40	0.01	
SP5e + 1	0.91		11.97		2.39		2.39		100
SP6e	1.021	0.986	6.60	8.29	1.32	1.66	1.49	0.17	
SP6e + 1	0.936		10.71		2.14		2.14		65
SP7e	1.011	0.985	7.09	8.34	1.42	1.67	1.54	0.13	
SP7e + 1	0.912		11.87		2.37		2.37		83

Appendix 7.3
Urine composition and physicochemical risk factors

Black subjects

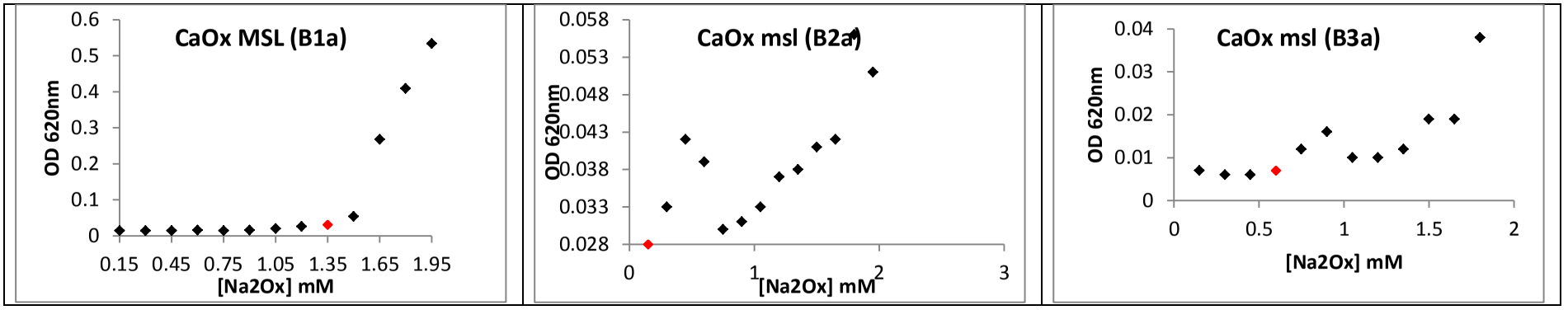
Lab no	pH	vol, ml	Cit	Ox	Ca	Mg	Na	K	Urate	Creat	Phos	Cl	IP6	SS CaOx	SS Brush	SS UA	TRI
B0F(b1a)	6.5	880	2.1	0.11	1.87	0.98	151.3	20.5	3.9	17.7	31	136	0.99	1.845	2.427	0.757	60
B2a	5.74	520	1.05	0.21	1.61	1.18	60.3	15.8	1.9	13.1	15.4	53	0.9	15.849	2.018	2.972	168
B3a	6.32	1230	2.52	0.24	1.89	1.4	138.7	49.2	3.6	13.4	28.9	204	1.3	2.965	1.393	0.752	186
B4a	6.29	650	1.22	0.23	1.93	1.51	103.6	30.8	3.7	12.5	25.6	115	1.79	8.531	3.926	1.510	213
B5a	6.81	1080	1.87	0.22	2.31	1.26	87.8	27.5	1.6	10.7	10.9	108	0.81	5.702	1.941	0.138	286
B6a	7.19	690	1.29	0.13	3.04	2.02	119.6	44.6	2.2	10.6	11	184	0.98	1.279	0.184	0.117	202
BB4 (B7a)	6.21	1000	2.39	0.26	1.67	0.71	138.2	23.6	3.6	17.6	36.8	103	1.14	4.046	1.738	1.178	137
B1d	6.65	760	1.19	0.17	2.11	1.72	89.6	23.5	3.8	14.7	36.4	115	0.84	4.009	5.035	0.618	130
B2d	6.47	1360	2.5	0.21	2.17	1.99	91.3	22.6	2.4	12.4	17.4	118	0.24	3.491	1.432	0.355	191
B3d	6.41	1040	1.86	0.36	2.07	2.23	96.5	27.3	3.1	13.8	32.1	128	0.86	6.501	2.667	0.646	275
B4d	6.31	500	0.8	0.17	2.12	1.75	93.7	25.5	2.7	10.4	28.6	102	1.37	9.354	6.982	1.346	225
B5F (B5d)	6.42	600	1.11	0.27	0.67	0.92	79.6	9.94	1.4	10.8	11.8	69	0.55	5.559	1.081	0.494	158
B6d	7.23	1380	2.5	0.27	1.92	1.55	133.1	46.5	2.3	11.2	23	209	0.53	1.282	0.841	0.057	271
B7d	6.36	650	1.13	0.12	1.88	1.49	87.5	27.6	2.7	13.2	25.9	97	1.14	4.477	4.285	0.964	102
B1e	6.34	760	1.24	0.27	2.29	2.31	103.6	31	2.8	13.8	25.4	169	1.38	9.036	4.001	0.875	230
B2e	6.66	1760	3.07	0.3	2.16	2.23	98.5	27.4	2.6	13.4	19.2	130	0.63	2.844	1.072	0.201	235
B3e	6.65	1050	1.76	0.24	2.41	2.29	136.6	38.5	3.6	12.1	29.7	220	0.65	4.046	3.027	0.419	248
B4e	5.91	1000	1.54	0.29	2.33	2.27	151.4	39.2	4	13.6	28	175	1.23	7.889	1.832	2.307	250
B5e	6.81	1120	1.52	0.24	3.44	1.04	126.1	29.8	2.1	14.1	15.8	138	0.89	8.492	3.936	0.169	289
B6e	7.41	860	1.68	0.36	2.76	0.93	131.9	47.9	2.3	12.5	25.5	185	1.06	7.261	3.926	0.057	445
B7f (B7e)	5.97	790	1.83	0.16	1.51	1.3	86.8	23.2	3.0	15	23.8	127	1.27	4.315	1.479	1.991	93

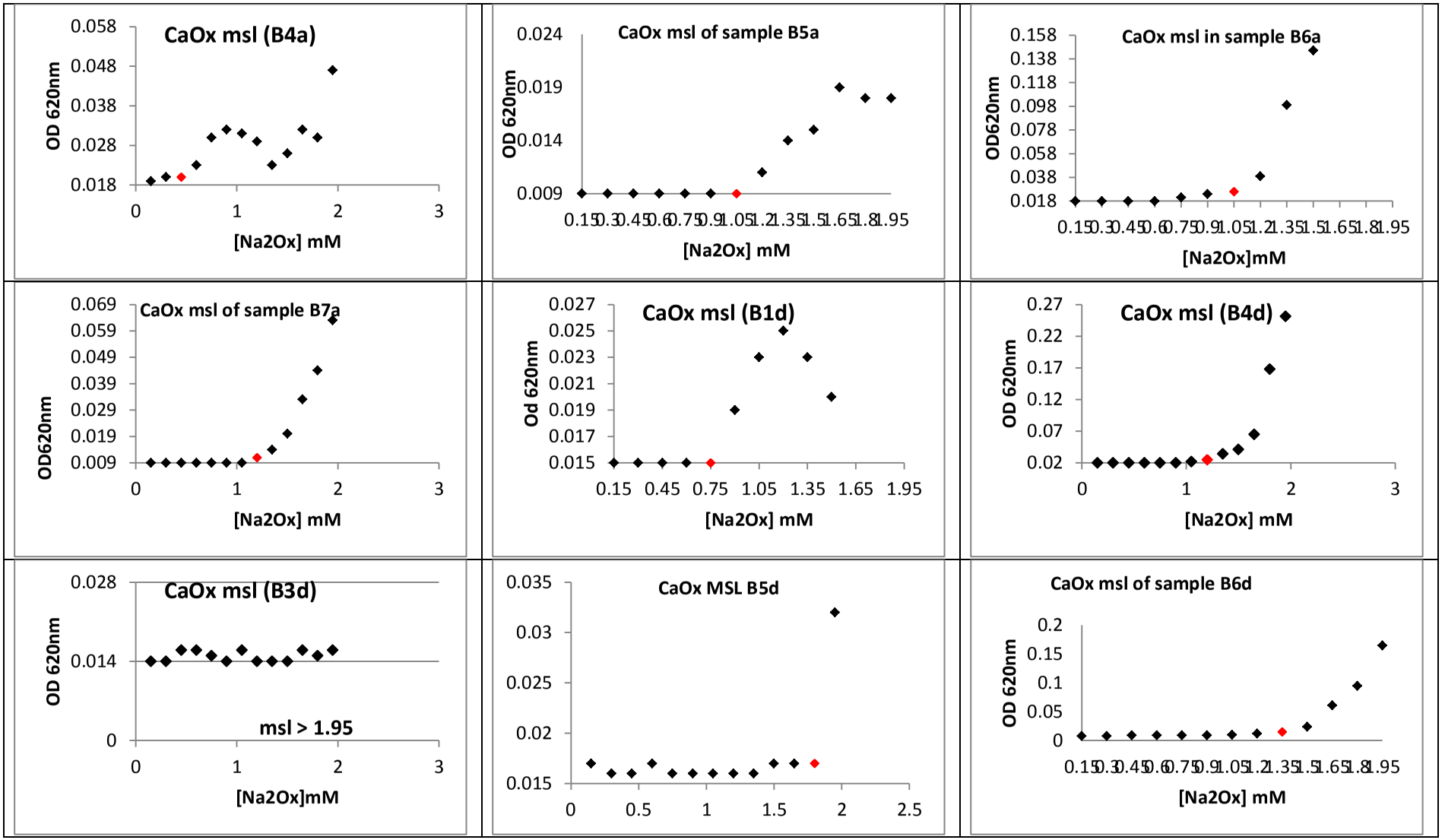
White subjects

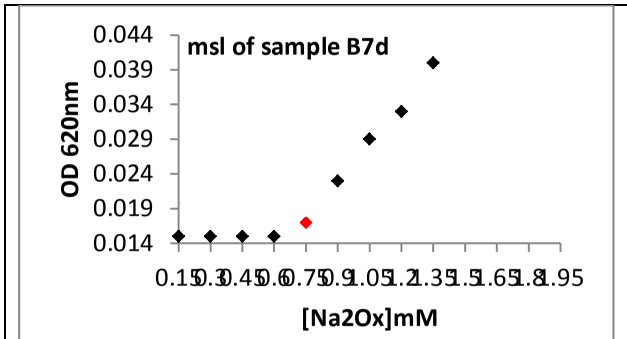
Lab no	pH	vol, ml	Cit	Ox	Ca	Mg	Na	K	Urate	Creat	Phos	Cl	IP6	SS CaOx	SS Brush	SS UA	TRI
SP1a	6.12	2420	3.37	0.17	2.74	1.81	84.4	25.3	3.8	17.7	39	125	0.89	1.641	0.889	0.697	107
SP2a	6.19	1180	2.32	0.19	0.96	1.55	231.2	32.2	4.6	19.5	33	218	0.76	1.294	0.733	1.276	52
SP3a	7.49	2650	3.58	0.29	2.37	1.23	275.6	35.4	4.5	17.2	27.8	246	0.33	0.575	0.429	0.034	180
SP4a	6.07	710	1.52	0.18	0.69	0.84	77.3	13.4	2.3	12.3	24	110	1.2	2.564	0.838	1.403	87
SP5a	6.25	1080	2.23	0.2	2.24	1.61	248.1	33.8	4.7	16.2	43	194	0.96	3.112	2.438	1.242	131
SP6a	6.8	1160	1.99	0.2	1.5	1.56	146.9	24.5	3.7	14.2	29	105	0.87	1.671	1.510	0.294	122
SP7a	6.79	700	1.11	0.13	0.72	0.67	151.6	7.01	1.6	6.4	5.4	141	0.26	2.094	0.504	0.204	181
SP1d	6.58	2830	3.55	0.35	3.16	2.88	117.3	37.2	3.6	14.4	19.5	169	0.86	2.877	0.875	0.213	307
SP2d	6.25	1800	3.32	0.23	1.6	1.65	199.4	41.3	5.5	21.4	40	207	0.85	1.371	0.785	0.938	76
SP3d	7.05	2600	4.89	0.15	1.55	1.87	264.4	45.7	3.9	19.2	32.5	262	0.51	0.204	0.307	0.081	53
SP4d	5.85	1025	1.95	0.16	3.55	1.53	185.5	11.9	3	14.6	29	159	0.92	6.324	2.506	1.901	173
SP5d	5.84	1080	1.84	0.17	2.53	2.24	163.7	15.6	4.9	17.9	37.8	146	0.32	4.487	1.968	3.034	102
SP6d	6.84	1180	1.91	0.16	0.88	1.88	155.7	29	3.5	17.1	28.2	151	0.85	0.631	0.716	0.246	50
SP7d	6.00	640	1.43	0.12	0.63	0.43	53	9.26	1.2	6.1	7.9	54	0.84	2.884	0.394	0.993	169
SP1e	6.46	2410	4.73	0.34	2.58	2.33	126.5	33.8	3.6	15.7	31.1	149	0.84	2.065	0.807	0.317	227
SP2e	5.88	1100	2.38	0.14	1.44	1.99	122.1	23.8	5.1	21.6	32.7	101	1.02	2.193	0.962	2.972	42
Sp3e	6.61	2480	4.05	0.51	2.06	1.59	225.3	31.5	4.4	20.8	37.2	220	0.94	2.004	0.733	0.259	206
SP4e	6.36	1910	2.99	0.43	2.36	1.85	186.6	26.4	3	13.8	30.4	189	0.91	4.046	1.140	0.391	353
SP5e	6.1	1180	2.69	0.15	3.03	1.71	329.5	45.1	5.4	20.4	47	245	1.09	2.661	2.594	1.750	85
SP6e	6.18	1060	2	0.12	2.14	1.8	186.8	35.1	4.5	16.9	40.3	210	1.4	2.032	2.291	1.416	71
SP7e	6.69	940	2.12	0.21	1.18	1.2	210.2	24.9	5.9	15.5	32.6	183	1.49	1.406	1.172	0.689	98

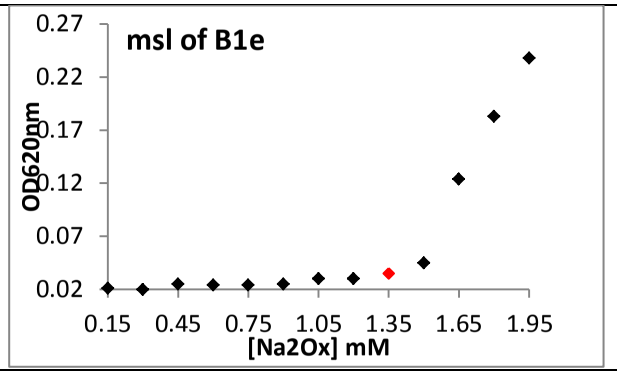
Appendix 7.4
CaOx MSLs black subjects

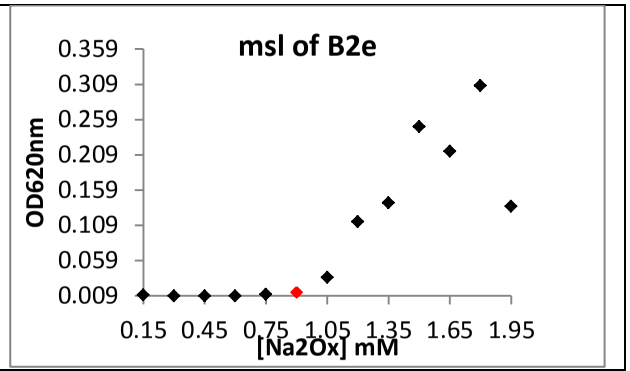
CaOx MSL			
	Day		
sample	0	15	18
B1	1.2	0.75	1.35
B2	0.15	0.75	0.9
B3	0.6	>1.95	1.05
B4	0.45	1.2	0.75
B5	1.05	1.8	1.2
B6	1.05	1.35	<0.15
B7	0.6	0.75	0.75

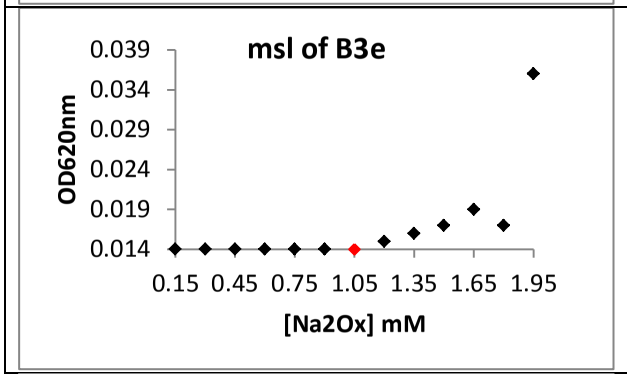


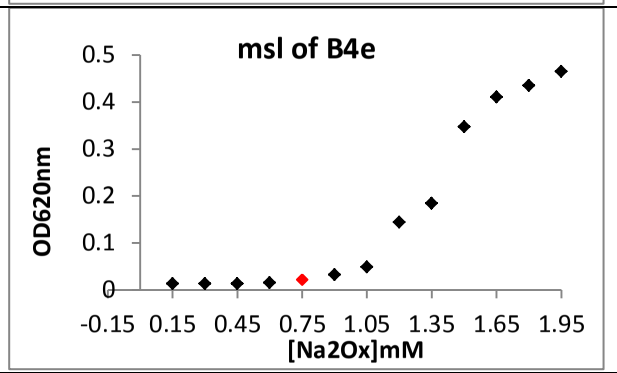

CaOx msl (B4a)
OD 620nm
0.058
0.048
0.038
0.028
0.018
0
1
2
3
[Na2Ox] mM
CaOx msl of sample B5a
OD 620nm
0.024
0.019
0.014
0.009
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox] mM
CaOx msl in sample B6a
OD620nm
0.158
0.138
0.118
0.098
0.078
0.058
0.038
0.018
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox]mM
CaOx msl of sample B7a
OD620nm
0.069
0.059
0.049
0.039
0.029
0.019
0.009
0
1
2
3
[Na2Ox] mM
CaOx msl (B1d)
Od 620nm
0.027
0.025
0.023
0.021
0.019
0.017
0.015
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (B4d)
OD 620nm
0.27
0.22
0.17
0.12
0.07
0.02
0
1
2
3
[Na2Ox] mM
CaOx msl (B3d)
OD 620nm
0.028
0.014
0
msl > 1.95
0
1
2
3
[Na2Ox]mM
CaOx MSL B5d
0.035
0.03
0.025
0.02
0.015
0
0.5
1
1.5
2
2.5
CaOx msl of sample B6d
OD 620nm
0.2
0.15
0.1
0.05
0
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox] mM

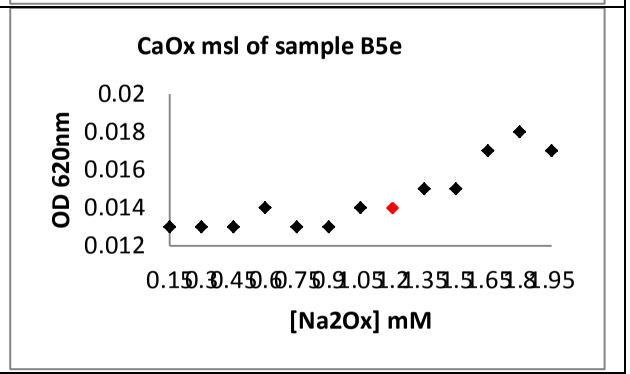

msl of sample B7d
OD 620nm
0.044
0.039
0.034
0.029
0.024
0.019
0.014
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox]mM

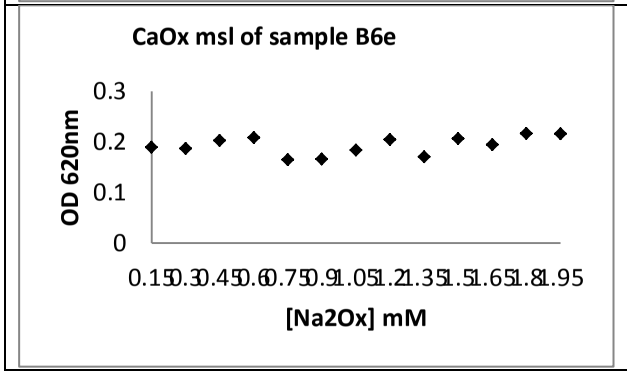

msl of B1e
OD620nm
0.27
0.22
0.17
0.12
0.07
0.02
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM

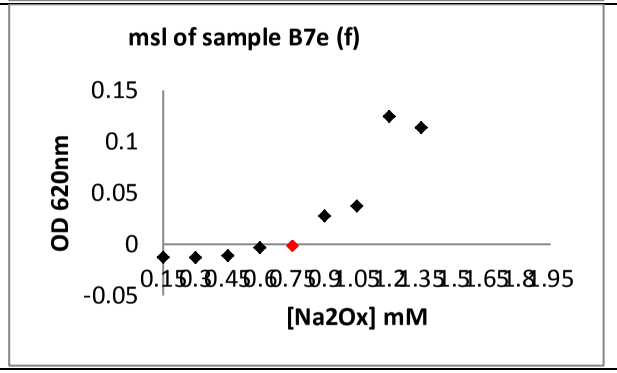

msl of B2e
OD620nm
0.359
0.309
0.259
0.209
0.159
0.109
0.059
0.009
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM


msl of B3e
OD620nm
0.039
0.034
0.029
0.024
0.019
0.014
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM


msl of B4e
OD620nm
0.5
0.4
0.3
0.2
0.1
0
-0.15 0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox]mM

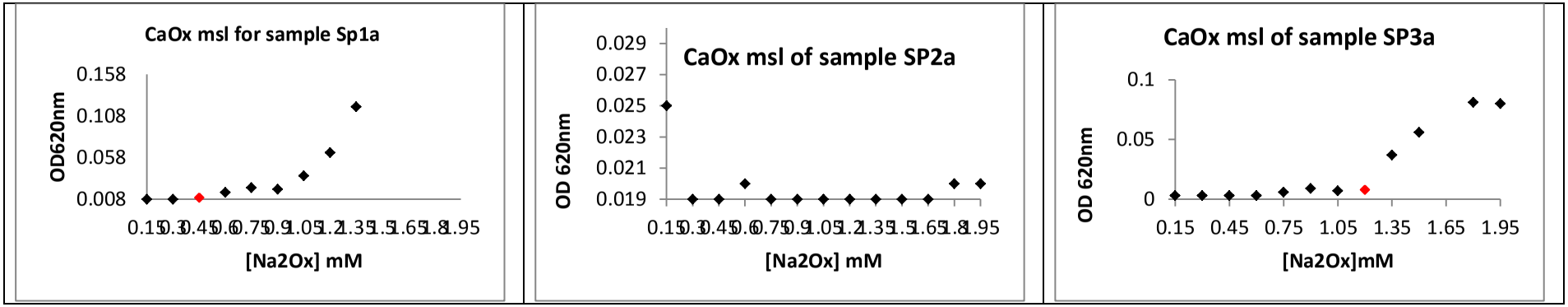

CaOx msl of sample B5e
OD 620nm
0.02
0.018
0.016
0.014
0.012
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox] mM

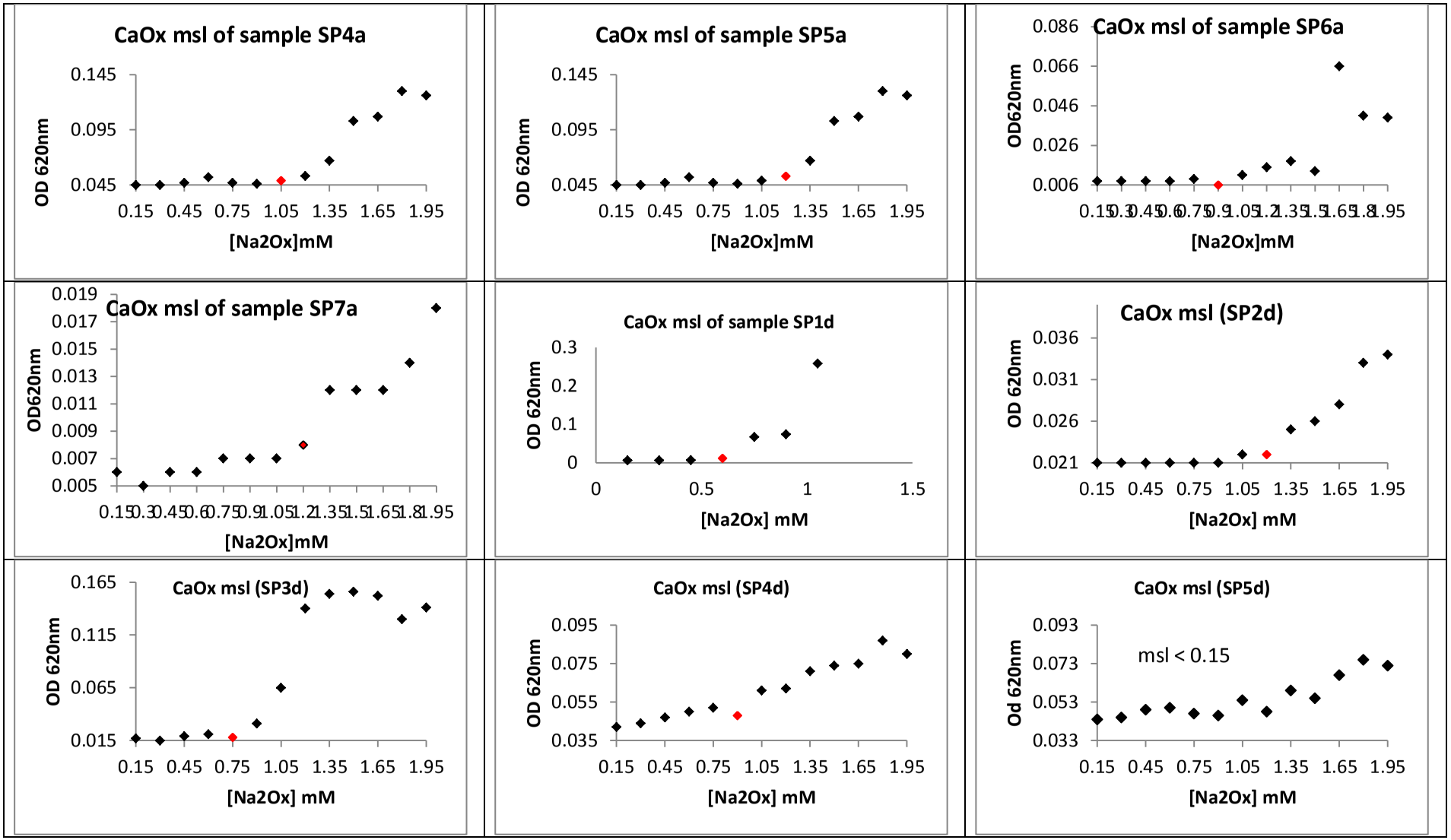

CaOx msl of sample B6e
OD 620nm
0.3
0.2
0.1
0
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox] mM

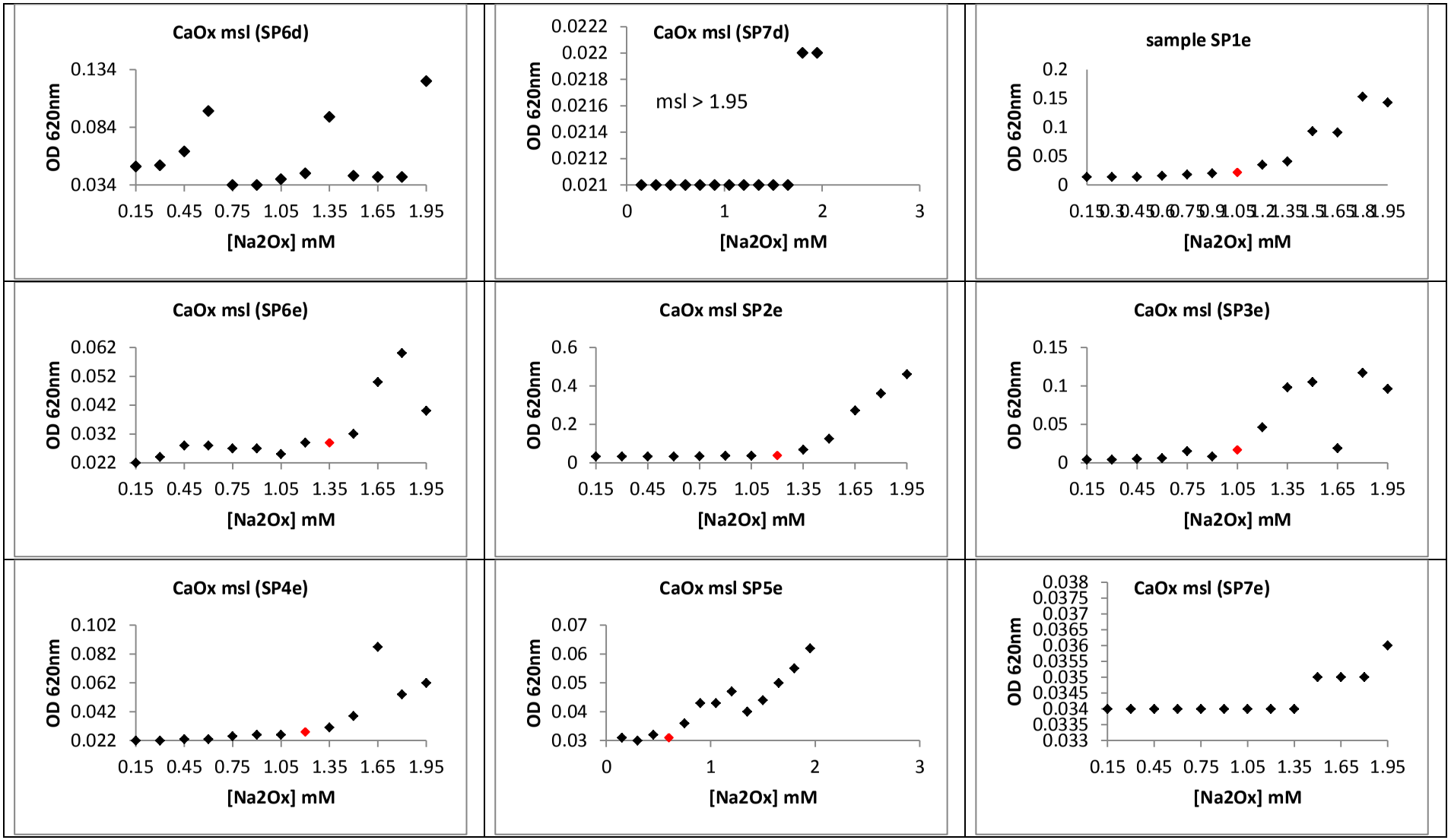

msl of sample B7e (f)
OD 620nm
0.15
0.1
0.05
0
-0.05
0.15 0.3 0.45 0.6 0.75 0.9 1.05 1.2 1.35 1.5 1.65 1.8 1.95
[Na2Ox] mM

White subjects

CaOx MSL			
	Day		
sample	0	15	18
SP1	0.45	0.6	1.05
SP2	>1.95	1.2	1.2
SP3	1.2	0.75	1.05
SP4	1.05	0.9	1.2
SP5	1.2	<0.15	0.6
SP6	0.9	<0.15	1.35
SP7	1.2	>1.95	>1.95



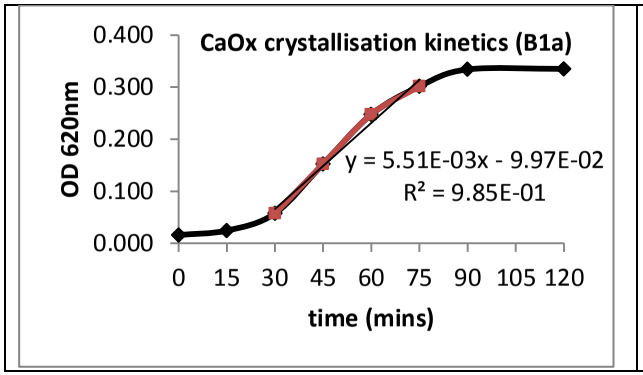

CaOx msl of sample SP4a
OD 620nm
0.145
0.095
0.045
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox]mM
CaOx msl of sample SP5a
OD 620nm
0.145
0.095
0.045
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox]mM
CaOx msl of sample SP6a
OD620nm
0.086
0.066
0.046
0.026
0.006
[Na2Ox]mM
CaOx msl of sample SP7a
OD620nm
0.019
0.017
0.015
0.013
0.011
0.009
0.007
0.005
[Na2Ox]mM
CaOx msl of sample SP1d
OD 620nm
0.3
0.2
0.1
0
0 0.5 1 1.5
[Na2Ox] mM
CaOx msl (SP2d)
OD 620nm
0.036
0.031
0.026
0.021
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP3d)
OD 620nm
0.165
0.115
0.065
0.015
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP4d)
OD 620nm
0.095
0.075
0.055
0.035
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP5d)
Od 620nm
0.093
0.073
0.053
0.033
msl < 0.15
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM

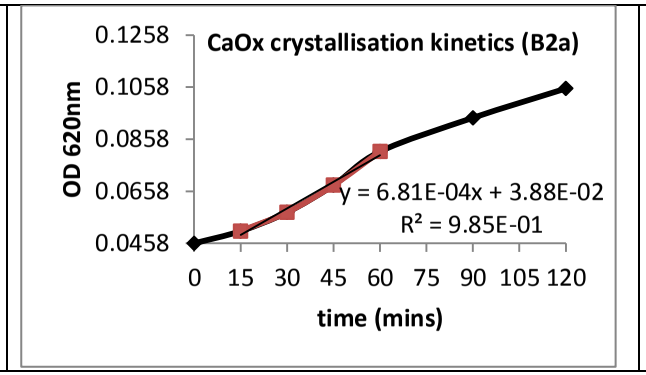

CaOx msl (SP6d)
OD 620nm
0.134
0.084
0.034
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP7d)
OD 620nm
0.0222
0.022
0.0218
0.0216
0.0214
0.0212
0.021
msl > 1.95
0 1 2 3
[Na2Ox] mM
sample SP1e
OD 620nm
0.2
0.15
0.1
0.05
0
[Na2Ox] mM
CaOx msl (SP6e)
OD 620nm
0.062
0.052
0.042
0.032
0.022
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl SP2e
OD 620nm
0.6
0.4
0.2
0
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP3e)
OD 620nm
0.15
0.1
0.05
0
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl (SP4e)
OD 620nm
0.102
0.082
0.062
0.042
0.022
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM
CaOx msl SP5e
OD 620nm
0.07
0.06
0.05
0.04
0.03
0 1 2 3
[Na2Ox] mM
CaOx msl (SP7e)
OD 620nm
0.038
0.0375
0.037
0.0365
0.036
0.0355
0.035
0.0345
0.034
0.0335
0.033
0.15 0.45 0.75 1.05 1.35 1.65 1.95
[Na2Ox] mM

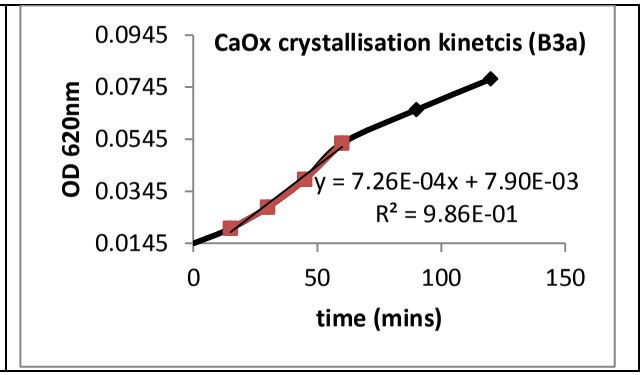
Appendix 7.5
Rate of urinary CaOx crystallisation

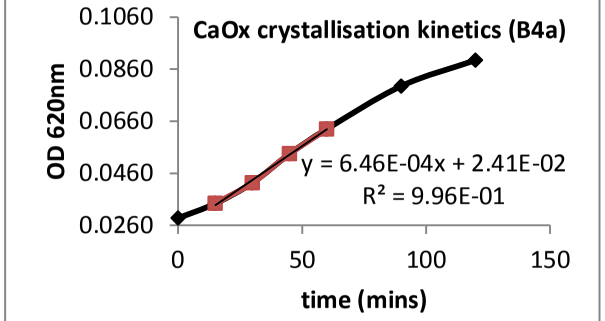
Black subjects

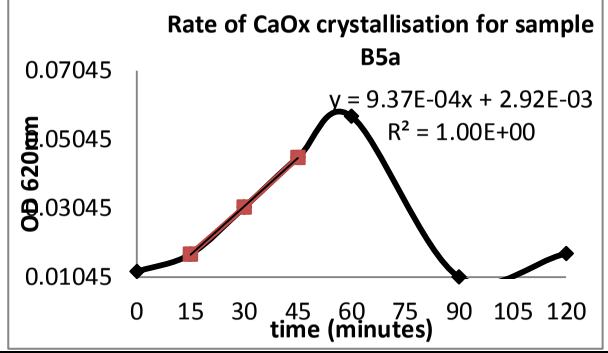
	rate of CaOx crystallisation x 10^{-3} min^{-1}		
	Day		
sample	**0**	**15**	**18**
B1	5.51	0.15	2.05
B2	0.68	0.47	1.33
B3	0.73	0	2.04
B4	0.65	3.02	1.42
B5	0.94	0.77	0.045
B6	2.67	3.43	ss
B7	1.91	0.49	2.57

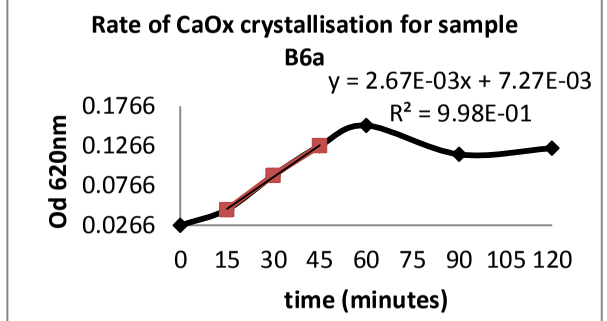


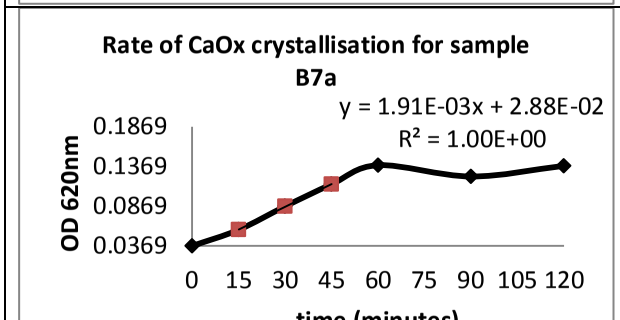


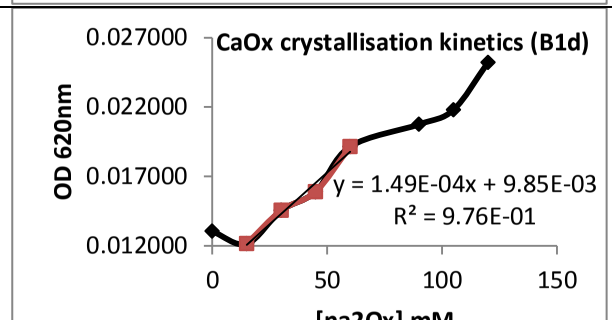


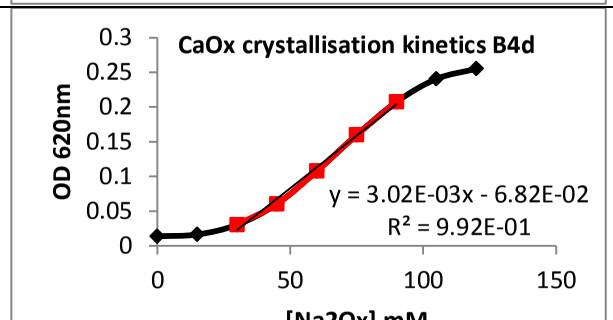

CaOx crystallisation kinetics (B4a)
OD 620nm
0.1060
0.0860
0.0660
0.0460
0.0260
y = 6.46E-04x + 2.41E-02
R² = 9.96E-01
0
50
100
150
time (mins)

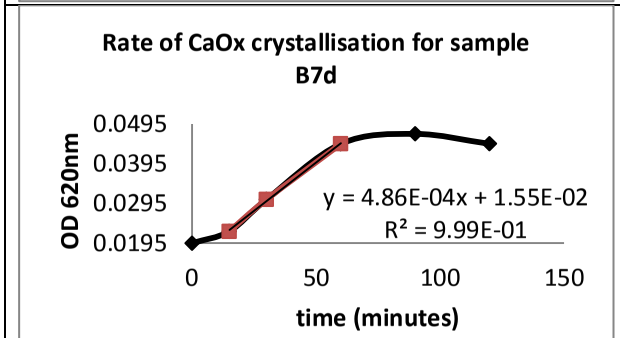

Rate of CaOx crystallisation for sample B5a
OD 620nm
0.07045
0.05045
0.03045
0.01045
y = 9.37E-04x + 2.92E-03
R² = 1.00E+00
0 15 30 45 60 75 90 105 120
time (minutes)

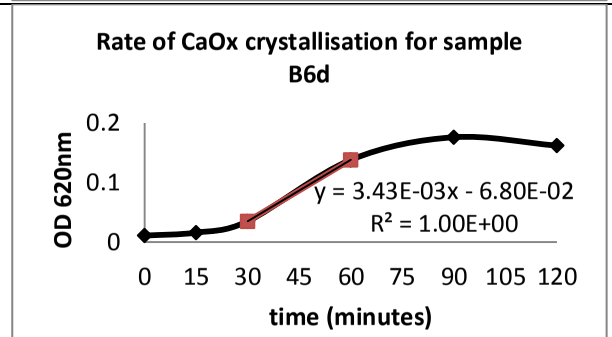

Rate of CaOx crystallisation for sample B6a
Od 620nm
0.1766
0.1266
0.0766
0.0266
y = 2.67E-03x + 7.27E-03
R² = 9.98E-01
0 15 30 45 60 75 90 105 120
time (minutes)

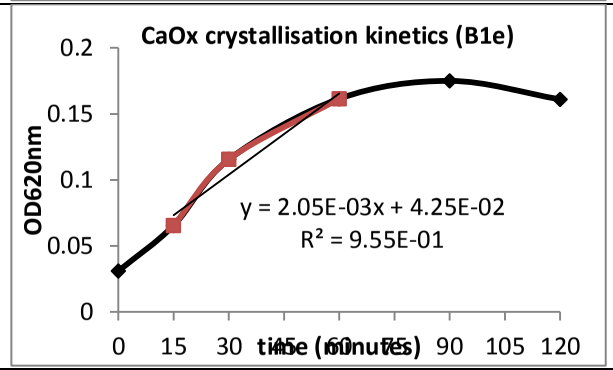

Rate of CaOx crystallisation for sample B7a
OD 620nm
0.1869
0.1369
0.0869
0.0369
y = 1.91E-03x + 2.88E-02
R² = 1.00E+00
0 15 30 45 60 75 90 105 120
time (minutes)

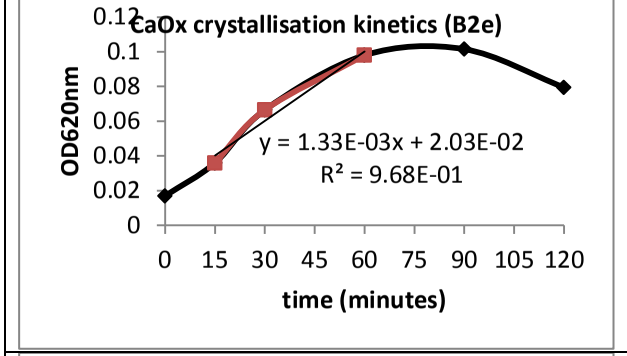

CaOx crystallisation kinetics (B1d)
OD 620nm
0.027000
0.022000
0.017000
0.012000
y = 1.49E-04x + 9.85E-03
R² = 9.76E-01
0
50
100
150
[na2Ox] mM

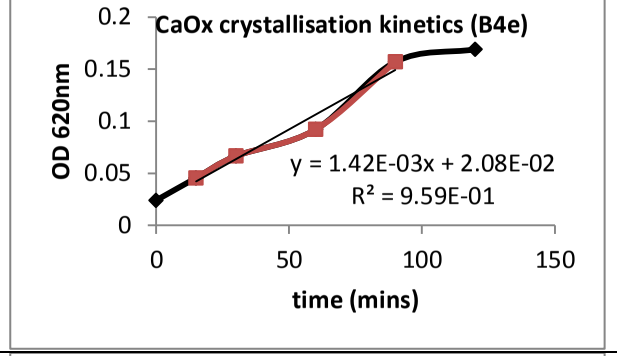

CaOx crystallisation kinetics B4d
OD 620nm
0.3
0.25
0.2
0.15
0.1
0.05
0
y = 3.02E-03x - 6.82E-02
R² = 9.92E-01
0
50
100
150
[Na2Ox] mM

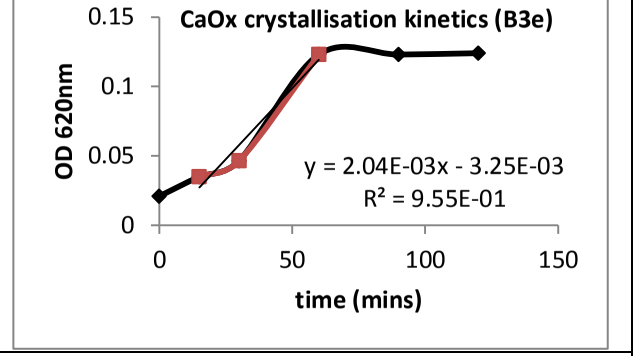

Rate of CaOx crystallisation for sample B7d
OD 620nm
0.0495
0.0395
0.0295
0.0195
y = 4.86E-04x + 1.55E-02
R² = 9.99E-01
0
50
100
150
time (minutes)

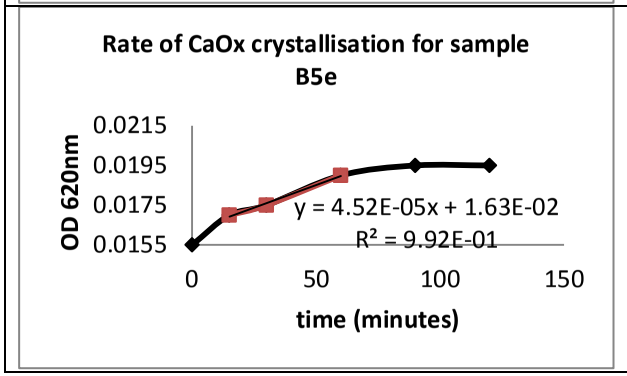

Rate of CaOx crystallisation for sample B6d
OD 620nm
0.2
0.1
0
y = 3.43E-03x - 6.80E-02
R² = 1.00E+00
0 15 30 45 60 75 90 105 120
time (minutes)

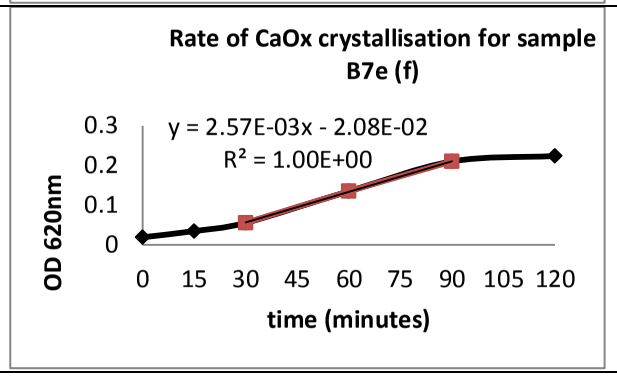

CaOx crystallisation kinetics (B1e)
OD620nm
0.2
0.15
0.1
0.05
0
y = 2.05E-03x + 4.25E-02
R² = 9.55E-01
0 15 30 45 60 75 90 105 120
time (minutes)


CaOx crystallisation kinetics (B2e)
OD620nm
0.12
0.1
0.08
0.06
0.04
0.02
0
y = 1.33E-03x + 2.03E-02
R² = 9.68E-01
0 15 30 45 60 75 90 105 120
time (minutes)


CaOx crystallisation kinetics (B4e)
OD 620nm
0.2
0.15
0.1
0.05
0
y = 1.42E-03x + 2.08E-02
R² = 9.59E-01
0 50 100 150
time (mins)

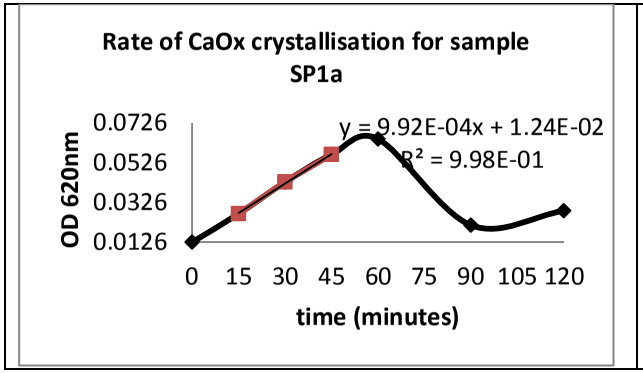

CaOx crystallisation kinetics (B3e)
OD 620nm
0.15
0.1
0.05
0
y = 2.04E-03x - 3.25E-03
R² = 9.55E-01
0 50 100 150
time (mins)

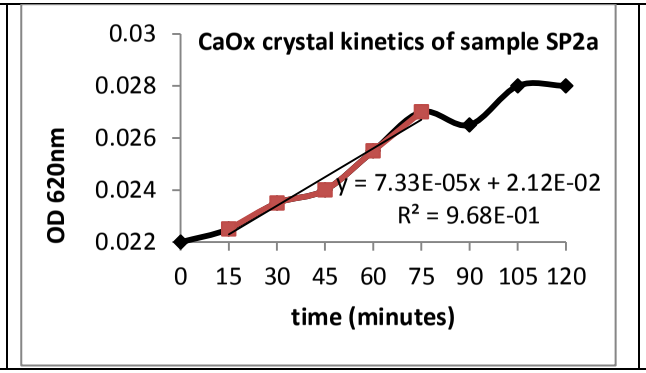

Rate of CaOx crystallisation for sample B5e
OD 620nm
0.0215
0.0195
0.0175
0.0155
y = 4.52E-05x + 1.63E-02
R² = 9.92E-01
0 50 100 150
time (minutes)

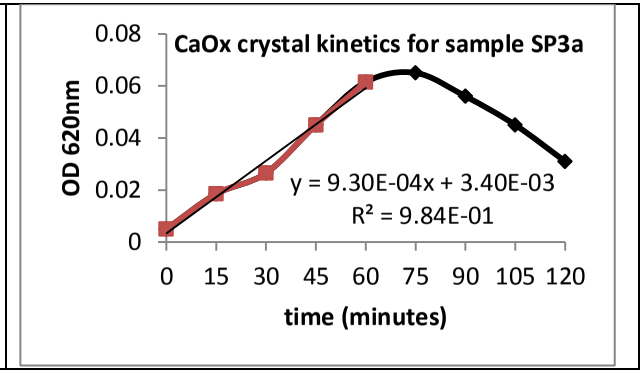

Rate of CaOx crystallisation for sample B7e (f)
OD 620nm
0.3
0.2
0.1
0
y = 2.57E-03x - 2.08E-02
R² = 1.00E+00
0 15 30 45 60 75 90 105 120
time (minutes)

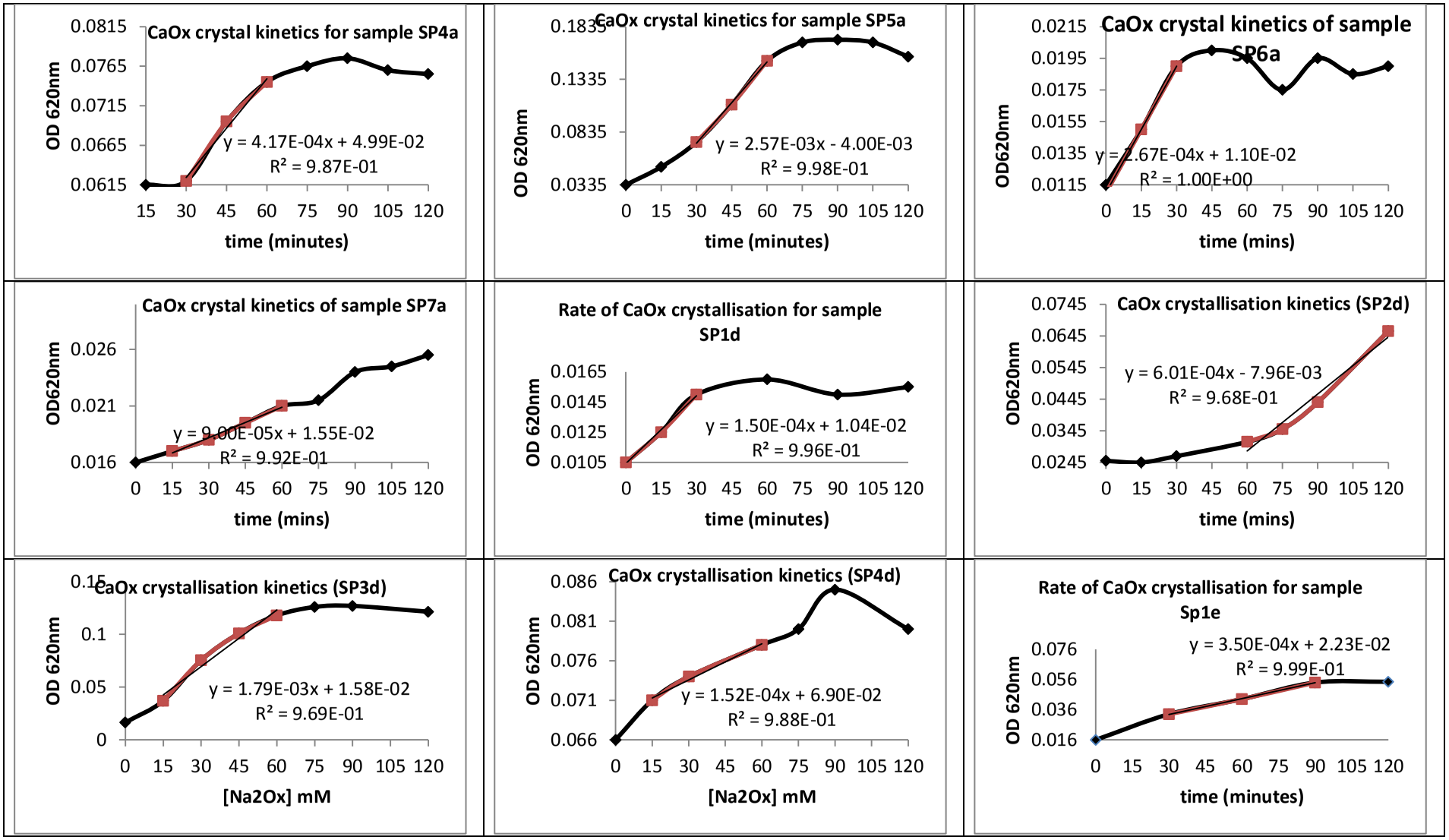
White subjects

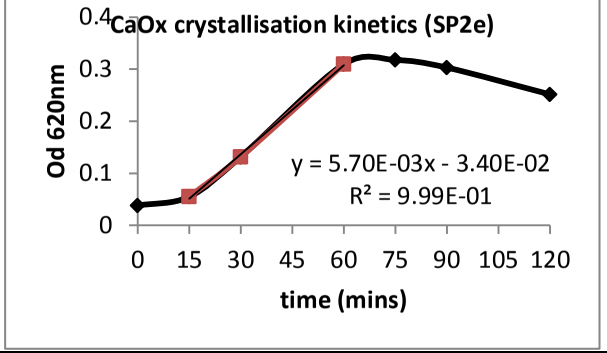
	rate of CaOx crystallisation x 10^{-3} min^{-1}		
	Day		
sample	**0**	**15**	**18**
SP1	0.992	0.15	0.35
SP2	0.0733	0.601	5.7
SP3	0.93	1.79	1.2
SP4	0.417	0.152	0.392
SP5	2.57	ss	0.102
SP6	0.267	ss	1.05
SP7	0.09	0	0

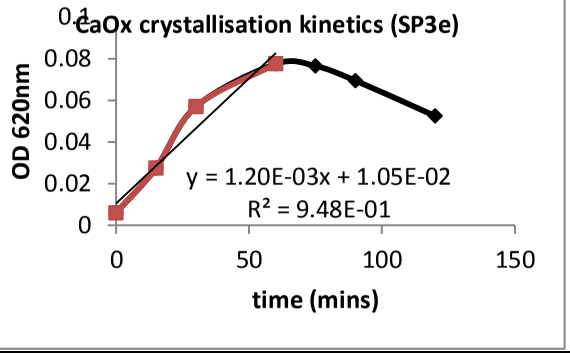


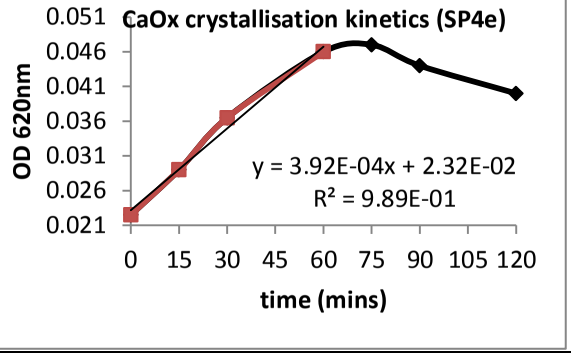


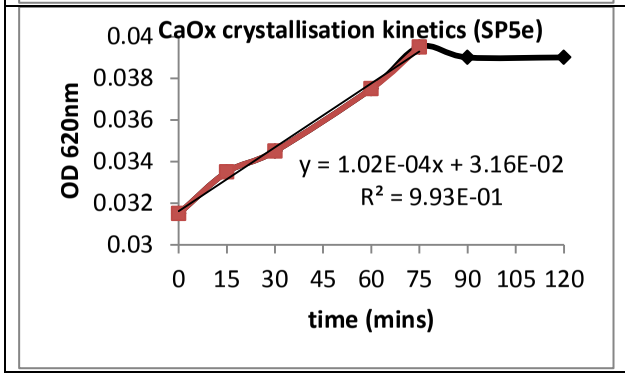


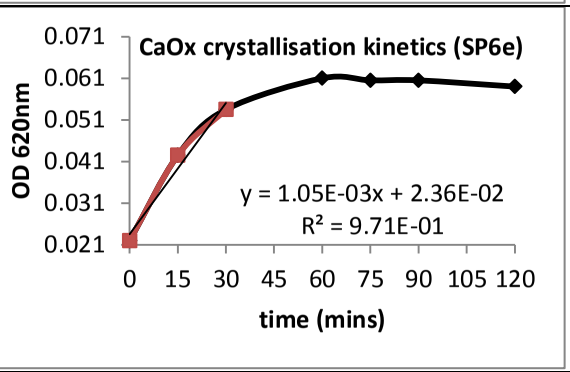

CaOx crystal kinetics for sample SP4a
OD 620nm
0.0815
0.0765
0.0715
0.0665
0.0615
y = 4.17E-04x + 4.99E-02
R² = 9.87E-01
15 30 45 60 75 90 105 120
time (minutes)
CaOx crystal kinetics for sample SP5a
OD 620nm
0.1835
0.1335
0.0835
0.0335
y = 2.57E-03x - 4.00E-03
R² = 9.98E-01
0 15 30 45 60 75 90 105 120
time (minutes)
CaOx crystal kinetics of sample SP6a
OD620nm
0.0215
0.0195
0.0175
0.0155
0.0135
0.0115
y = 2.67E-04x + 1.10E-02
R² = 1.00E+00
0 15 30 45 60 75 90 105 120
time (mins)
CaOx crystal kinetics of sample SP7a
OD620nm
0.026
0.021
0.016
y = 9.00E-05x + 1.55E-02
R² = 9.92E-01
0 15 30 45 60 75 90 105 120
time (mins)
Rate of CaOx crystallisation for sample SP1d
OD 620nm
0.0165
0.0145
0.0125
0.0105
y = 1.50E-04x + 1.04E-02
R² = 9.96E-01
0 15 30 45 60 75 90 105 120
time (minutes)
CaOx crystallisation kinetics (SP2d)
OD620nm
0.0745
0.0645
0.0545
0.0445
0.0345
0.0245
y = 6.01E-04x - 7.96E-03
R² = 9.68E-01
0 15 30 45 60 75 90 105 120
time (mins)
CaOx crystallisation kinetics (SP3d)
OD 620nm
0.15
0.1
0.05
0
y = 1.79E-03x + 1.58E-02
R² = 9.69E-01
0 15 30 45 60 75 90 105 120
[Na2Ox] mM
CaOx crystallisation kinetics (SP4d)
OD 620nm
0.086
0.081
0.076
0.071
0.066
y = 1.52E-04x + 6.90E-02
R² = 9.88E-01
0 15 30 45 60 75 90 105 120
[Na2Ox] mM
Rate of CaOx crystallisation for sample Sp1e
OD 620nm
0.076
0.056
0.036
0.016
y = 3.50E-04x + 2.23E-02
R² = 9.99E-01
0 15 30 45 60 75 90 105 120
time (minutes)


CaOx crystallisation kinetics (SP2e)
Od 620nm
0.4
0.3
0.2
0.1
0
y = 5.70E-03x - 3.40E-02
R² = 9.99E-01
0 15 30 45 60 75 90 105 120
time (mins)


CaOx crystallisation kinetics (SP3e)
OD 620nm
0.1
0.08
0.06
0.04
0.02
0
y = 1.20E-03x + 1.05E-02
R² = 9.48E-01
0 50 100 150
time (mins)


CaOx crystallisation kinetics (SP4e)
OD 620nm
0.051
0.046
0.041
0.036
0.031
0.026
0.021
y = 3.92E-04x + 2.32E-02
R² = 9.89E-01
0 15 30 45 60 75 90 105 120
time (mins)


CaOx crystallisation kinetics (SP5e)
OD 620nm
0.04
0.038
0.036
0.034
0.032
0.03
y = 1.02E-04x + 3.16E-02
R² = 9.93E-01
0 15 30 45 60 75 90 105 120
time (mins)


CaOx crystallisation kinetics (SP6e)
OD 620nm
0.071
0.061
0.051
0.041
0.031
0.021
y = 1.05E-03x + 2.36E-02
R² = 9.71E-01
0 15 30 45 60 75 90 105 120
time (mins)