THE DESIGN, DEVELOPMENT AND TESTING OF A NON-INVASIVE CONTINUOUS CRYSTALLISER SYSTEM FOR THE STUDY OF CALCIUM OXALATE CRYSTALLISATION PROCESSES

by

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Dissertation presented to the
University of Cape Town
in fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

August 1996

ACKNOWLEDGEMENTS

I am deeply indebted to the following people without whom this work would not have been possible or enjoyable:

Prof A.L.Rodgers for always giving of his time, for his incessant enthusiasm and not least of all for his sense of humour;

Prof A.L.Rodgers for obtaining financial support from the Foundation for Research and Development and the Medical Research Council;

Mr D.Lensen and Mr A.Joseph in the workshop for machining the teflon flow cell and other sections of the system;

Mr C.Ledger, the glassblower, for his enthusiasm and innovations when making the numerous glassware designs;

Prof K.R.Koch (Chemistry Department UCT) and Dr S.Harrison (Chemical Engineering Department UCT) for generously allowing the use of their peristaltic pumps for the duration of this study;

Dr D.Ball (Physics Department UCT) for generously allowing the Malvern Particle Sizer to be relocated in the Chemistry Department for the duration of this study;

Dr J.Kavanagh (Urology Department University Hospitals South Manchester) for his hospitality and useful discussions when I visited;

Dr M.J.Hounslow and Mr A.S.Bramley (Chemical Engineering Department Cambridge University) for their hospitality and constructive criticisms when I visited their laboratory;

Mrs D.Pinnock for the routine urinalysis;

Mrs U.Seemann and Miss K.Gifford Nash for proofreading;

Mrs G.Baretta, Mrs J.Durbach, Mrs D.Pinnock, Miss B.Hibbert and Miss D.Jappie for providing an enjoyable and unique laboratory environment;

All my friends with special mention of Miss N.Hitchins, Miss K.Gifford Nash and Mrs F.Pocock for their continual reminders that there is more to life;

Jeffrey for his advice, support and continual patience;

My parents for their support and encouragement and for teaching me that life is dictated by the choices we make and that anything and everything is possible.

ABSTRACT

This thesis describes the design, development and testing of a mixed suspension, mixed product removal (MSMPR) crystalliser and an on-line flow cell for use in conjunction with a Malvern Particle Size Analyser. In addition, *in vitro* experiments are described in which this system was used to determine the rate of calcium oxalate crystallisation in minimally diluted urine.

The MSMPR was designed according to chemical engineering principles and tests showed that it conforms to the assumptions necessary for conventional MSMPR analysis. Various crystalliser offtakes and six system designs were developed and tested to allow measurements using the thermostatted flow cell. The flow cell allows for continuous measurement of the sample dispersion using a non-invasive monitoring instrument - a Malvern Particle Size Analyser. In this technique, measurement of the steady state crystal size distribution is based on a diffraction pattern produced by the particles in a parallel beam of monochromatic light. Comparative measurements were obtained by sampling directly from the crystalliser and analysing the product in a Coulter Counter. In this technique, the steady state crystal size distribution is measured by the change in resistance as particles, in an electrically conductive liquid, pass through the Coulter aperture.

Due to the large volumes required by the 200cm^3 crystalliser, it was necessary to pool urines. Aliquots of volume 1.5 dm^3 from the 24 hour urines of each of 8 male controls were pooled. The first series of experiments were performed to investigate the effect on calcium oxalate crystallisation of varying the concentration of sodium oxalate solutions used to initiate crystallisation, and also to investigate the effect of varying the temperature. A further series of experiments was performed to determine the effect of adding inhibitors individually (citrate, magnesium, chondroitin sulphate A) and in combination (citrate and magnesium).

The inhibitor experiments were performed using two different approaches. In the first, the pooled urine was pre-treated with the inhibitor prior to being introduced into the crystalliser. In the second set of experiments, the inhibitor was introduced into the crystalliser via a separate, independent feed. It is suggested that these approaches represent crude models respectively of (a) the

endogenous presence of inhibitors and (b) the oral administration of such inhibitors.

Each individual experiment was performed using three different urine pools. The steady state crystal size distributions obtained from both the Coulter and the Malvern instruments were used to determine nucleation and growth rates in each urine. Scanning electron microscopy was used to obtain qualitative and semi-quantitative data related to crystal morphology, number, size and degree of aggregation.

Both nucleation and growth rates increased as the concentrations of the initiating sodium oxalate solutions increased. Nucleation and growth rates also increased with increasing temperatures. In the latter series of experiments, different calcium oxalate crystal phases precipitated at the various temperatures.

Citrate inhibited nucleation and growth of calcium oxalate, irrespective of how it was admitted to the crystalliser. Magnesium was found to inhibit growth only and, like citrate, this role was independent of how it was introduced into the crystalliser. Although both inhibited growth, the effect of the citrate was greater. When both components were added in combination, inhibition was observed to be additive. In all cases, inhibitory effects increased with increasing concentration.

Chondroitin sulphate A was found to inhibit nucleation; inhibition of growth was minimal at the concentrations tested. Aggregation of calcium oxalate crystals was found to be inhibited by chondroitin sulphate A.

The results of the calcium oxalate crystallisation kinetic studies, obtained by using Malvern particle sizing and Coulter Counter techniques independently of each other, showed general agreement. These results, in turn, were in agreement with the published results of others, thereby lending confidence to the findings and highlighting the potential application of the non-invasive continuous crystalliser in urolithiasis research.

PUBLICATIONS

Publications:

Bretherton,T.; Rodgers,A.L.
"Design, development and testing of a non-invasive continuous crystalliser system with an on-line Malvern particle sizer: preliminary results".
In: *Urolithiasis: Consensus and Controversies, Proceedings of the 6th European Symposium on Urolithiasis* eds: Rao,P.; Kavanagh,J.; Tiselius,H. Manchester, South Manchester University Hospitals, (1995), 376-378

Bretherton,T.; Rodgers,A.L.
"Crystallisation experiments in urine - a possible key to understanding kidney stone formation"
In: *Proceedings of the 19th Congress of the Urological Association of South Africa*
Sun City, Bophuthatswana, (1992), 60

Conferences attended:

XIXth Congress of the Urological Association of South Africa. 8-13 March 1992, Sun City, Bophuthatswana.
Oral Presentation: "Crystallisation experiments in urine - a possible key to understanding kidney stone formation"

Vth European Urolithaisis Symposium. 21-23 April 1994, Manchester, England
Oral Presentation: "Design, development and testing of a non-invasive continuous crystalliser system with an on-line Malvern particle sizer: preliminary results".

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CHAPTER I

INTRODUCTION

I.1 EPIDEMIOLOGY OF URINARY STONE DISEASE

Stone disease can occur in various organs and glands in the human body, including the kidney, bladder, prostrate, salivary gland and pancreas [Lonsdale 1968a; Rodgers and Spector 1981]. Of the different kinds that can occur, urinary calculi are probably the most clinically important. As a result there has been continual development of research techniques in an attempt to gain insight into the aetiology and physiochemical mechanisms of urinary stone formation.

Bladder stones can be traced back as far as Egypt 4800BC [Clarke 1968]. Differentiation between vesical (bladder) and renal (kidney) stones was noted by Hippocrates (460 - 370BC) [Prien 1971; Joly 1931 cited by Blacklock 1982]. Also known at this time was the technique of *lithotomy* (method of cutting for stone); mention was made in the Hippocratic oath that such operations should be carried out by specialised lithotomists only [Prien 1971]. Surgical improvements were made in the 3rd century with the technique of *lithotrity* used by the Greek physician Ammonios. The process involved mechanically breaking up the stone so that it could be passed harmlessly in the urine. The disease afflicted so many people that exploitation up to the 17th century by a wandering band of lithotomists was inevitable. Unfortunately, they often caused more harm than good.

In an attempt to alleviate the necessity for the difficult, painful and dangerous operations, herbal remedies for stone disease were concocted. The most famous of these was that of the 18th century charlatan Joanna Stevens [Prien 1971; Rodgers and Spector 1981]. The popular remedy, certified by physicians and supported by the prime minister at the

time Sir Horace Walpole, was later found to contain no stone relieving substances at all [Joly 1931, cited by Prien 1971].

Early insight into the causation of stone disease was shown by Celsus (23BC - 45AD) who attributed its formation to bile, mucus and wind. Modern day variables such as nutrition, heredity and climate were later described by Galenus (131 - 201AD) [Lonsdale 1968a].

In more contemporary times, the incidence of bladder stones has been reported to be on the decline in technically developed countries [Andersen 1973]. Today, they occur mainly in endemic stone regions. These are regions in which the incidence of stone disease per capita is higher than the rest of the country. Examples of such areas include northwest India, southern China, Japan and Thailand. These were identified long ago by Joly 1939 (cited by [Prien 1971]) who also noted a prevalence of the disease in children from agricultural regions.

In contrast to vesical calculus [Norlin *et al.* 1976], there has been an increase in renal calculus in first world countries since the late 19th century. Afflicted areas include Europe, North America and Japan [Blacklock 1982]. The disease has shown a predominance in males, who have double the stone incidence of females [Hesse *et al.* 1986a]. Recurrence rates in both sexes have been quoted as high as 75% [Williams 1963] and 67% [Blacklock 1969].

A definite correlation has been made between stone belts and climate. Exposure to UV radiation has been shown to increase calcium uptake in the intestine [Robertson and Peacock 1983; Schwille and Hermann 1992] while an increase in atmospheric temperature has also been directly related to stone incidence. The latter factor causes an increased frequency of dehydration leading to concentration in the urinary tract of stone forming salts which are unable to pass in the urine [Embon *et al.* 1990; Schwille and Hermann 1992]. Stone occurrence in workers exposed to high

temperatures [Blacklock 1969,1982; Rose 1982] (eg cooks, desert troops) are testimony to the necessity of a high fluid intake in such enviroments [Better *et al.* 1978; Fuss *et al.* 1979; Milvy *et al.* 1981; Robertson and Peacock 1983; Ferrie and Scott 1984; Pak *et al.* 1980,1984; Vahlensieck 1986; Rodgers 1991]. Mates (1969) also noted a link between sedentary work and increased stone risk [Blacklock 1969].

Several studies have addressed the question of whether tap water composition (with particular reference to hard and soft water) is related to the incidence of kidney stones or not. However, the results of such investigations have been inconclusive. Some studies have reported a higher incidence of urolithiasis in soft water areas [Rose and Westbury 1975; Churchill *et al.* 1978], and others have found that water hardness is correlated with a low incidence of urinary calculi [Sierakowsky *et al.* 1976; Juuti and Heinonen 1980]. However, several investigators have failed to find any correlation between water hardness and urinary stones [Donaldson *et al.* 1979; Shuster *et al.* 1982; Power *et al.* 1987; Singh *et al.* 1989].

To maintain a high fluid intake, many people drink bottled water rather than tap water. Mayne and Edwards (1990) conducted tests on bottled water in the United Kingdom and have warned of the possible increase in calcium intake from some brands of bottled water. On the other hand, Jaeger *et al.* (1984) and Trinchieri *et al.* (1995) conclude that the calcium content of water is not of primary importance in either causing or preventing urinary calculi.

Another contributing factor in urolithiasis is diet [Robertson 1985; Breslau *et al.* 1988; Trinchieri *et al.* 1991; Hesse *et al.* 1993; Goldfarb 1994; Hesse 1995; Siener and Hesse 1995]. In modern day western diets, there is an ever increasing consumption of foodstuffs containing lithogenic substances [Vahlensieck 1986]. Dairy products contain calcium, phosphorous and lactose, all of which have been regarded for some time as being lithogenic. The

importance of diet is discussed in more detail in Chapter I.3 under "Diet".

Besides diet, genetic, racial and heredity factors [McGeown 1960] also play a role in urolithiasis. For example, South African blacks, Eskimos and the Indians of Peru and Mexico appear to be immune to this disease.

The multifactorial aetiology of stone disease [King 1967; Robertson *et al.* 1981; Blacklock 1982] illustrates the challenge faced by investigators in this field. Stone incidence figures quoted in the literature highlight the increasing necessity for the continuation of urinary stone research in attempts to identify possible therapeutic and/or prophylactic measures.

I.2 PHYSIOCHEMICAL ASPECTS

I.2.1 PROCESSES INVOLVED IN CRYSTALLISATION

Crystallisation from solution refers to the formation of solid particles and therefore to the creation of a new phase. In urine several distinct crystallisation processes must occur in order for a stone to form. These are supersaturation, nucleation, growth, and aggregation [Finlayson 1978]. It is the understanding of the mechanisms which govern these processes and their interrelation, using physicochemical principles [Nancollas *et al.* 1991] and modelling [Ramanarayan *et al.* 1984], that is the goal of stone research today.

Supersaturation

For crystallisation to occur in any medium, the solution must be supersaturated with respect to the mineral phase [Ryall *et al.* 1985]. Early research by Andrews *et al.* (1955) using equilibration of urine with excess pure salt and by Miller *et al.* (1958) using a precipitation test, both demonstrated that urine is normally supersaturated with respect to calcium oxalate (CaOx). This has been

confirmed by more recent studies which have shown that there is a high level of CaOx supersaturation in urines from healthy controls and an even higher level in recurrent stone formers [Robertson *et al*. 1971,1976a].

It has been recognised that spontaneous crystallisation does not occur in "just-saturated" urines [Nancollas *et al*. 1991]. Indeed, at certain levels of supersaturation nucleation will not occur. Even at levels where nucleation would be expected, the introduction of seed crystals is sometimes necessary to induce precipitation [Robertson and Nordin 1976].

The significance of a urinary metastable region in urine with respect to urolithiasis risk factors was first highlighted by Robertson and Nordin (1976). This state occurs when the salt (eg CaOx) is in equilibrium with its surrounding medium (urine). It is in this region that spontaneous "homogeneous" nucleation may not take place (ie endogenous crystals are not present). However, "heterogeneous" nucleation can be initiated by the addition of seed crystals (which are of different composition to that of the endogenous material).

Thermodynamically, supersaturation can be expressed as free energy change (ΔG):

$$\Delta G = RT\ln(A_i/A_o) \quad \text{.....1}$$

where R is the gas constant, T is the temperature and A_i,A_o are the activities of the unionized salt species in solution under any given conditions and at equilibrium respectively.

Equation 1 shows that varying degrees of saturation depend upon the relationship between the activities of the unionised salt species in solution [Finlayson 1978].

Thus, supersaturation in urine can be calculated using activities of the ionic species present. The method, first

developed by Robertson [Robertson *et al.* 1968; Robertson 1969b; Finlayson 1978], uses *ab initio* calculations of mass action and conservation of mass equations. Measurements of the pH and total concentrations of all the main ionisable species in urine enable the calculation of the activity of ionised calcium to be made. From this, the concentrations of all other ions in the system can be calculated. Relative supersaturation for each stone forming salt is then determined by comparison of the activity products with known solubility and formation products.

Approximations involving this method were introduced by Pak and Chu (1973) and later by Marshall and Robertson (1976) and arose as a consequence of the impractibility of the calculations for routine analysis. A further semi-empirical approach was introduced by Gill *et al.* (1974).

Calculation of the supersaturation ratio for the important mineral components in kidney stones was first achieved with the Fortran program EQUIL developed by Finlayson (1977). The program was subsequently rewritten by Werness *et al.* (1985) in the Basic language as EQUIL2. Ackermann *et al.* (1989) extended the EQUIL program with respect to charge balance, temperature dependence and range of chemical species to permit *a priori* estimation of pH or computation of the composition at a particular pH.

More recently, an updated version of EQUIL2, called EQUIL93 has been developed [Brown *et al.* 1994]. Improvements in the program include the incorporation of the interfacial chemistry of calcium oxalate monohydrate (COM) crystals and the extension of the number of solutes considered.

The calculation of the driving force has allowed researchers to identify differences in supersaturation levels of healthy subjects and stone formers [Robertson *et al.* 1968,1976a] and to identify patients at risk of stone recurrence [Robert *et al.* 1994].

Vermuelen (1962) [cited by Finlayson *et al.* 1984] first postulated the role of kinetics as follows:

precipitate + matrix + time = stone 2

where the time factor encompasses the kinetic effects. The concept of a matrix is discussed in Chapter I.2.4. under "Role of the matrix".

Finlayson *et al.* (1984) also reported that stone forming potential is a function of both supersaturation and time, with long periods of high supersaturation being of greater risk than short periods at the same level. These considerations led to the concept of retention in stone formation.

The geometry of the urinary tract is such that there may be some regions that have poor flow characteristics and which therefore form "dead volume sites". With a decreased urine flow through these areas, crystals present in the supply urine have a greater chance of being retained at these sites. An increase in retention time would allow for longer growth periods giving rise to a possible pathological condition.

The transit time of urine through the duct of Bellini is 3 minutes [Finlayson and Reid 1978b], while that through the whole kidney is less than 10 minutes [Finlayson 1978]. This transit time determines the mean crystal residence time; therefore significant deviations in the average transit time could occur due to these "dead volume sites". Measurements by Burns *et al.* (1983) of retention time in humans showed no clear trends. Experimental modelling in rats by Khan (1991) has illustrated that the renal papilla is the primary site for crystal retention in the kidneys. Khan (1991) demonstrated that crystal formation, growth and retention could occur in the upper nephron or papillary collecting ducts, or in the bladder, depending upon the intensity of hyperoxaluria, duration of the hyperoxaluric

state, and the crystallisation inhibitory activity of the urine.

Nucleation

Nucleation refers to the formation of new particles and is a consequence of rapid local fluctuations on the molecular scale in a homogeneous phase that is in a state of metastable equilibrium [Finlayson 1978].

In general, nucleation is a non-linear function of the amount of solids present, while the number of particles smaller than 5μm is relatively independent of supersaturation [Rodgers and Garside 1981].

The initial event in the creation of new particles can occur as a result of three mechanisms [Botsaris 1980 cited by Garside 1985]: homogeneous nucleation, heterogeneous nucleation, or secondary nucleation.

Homogeneous nucleation is the formation of new crystals from the liquid phase as a result of supersaturation only. However, Robertson (1969) [cited by Finlayson 1978] has reported that the number concentration of particles in normal urine is well below the critical value of $10^6/cm^3$ required for homogeneous nucleation, thereby suggesting that primary nucleation probably occurs via some other mechanism. Heterogeneous nucleation seems to be the likely alternative.

Heterogeneous nucleation is induced by foreign insoluble material present in the solution. Indeed, urine is rich in cellular debris which points to heterogeneous nucleation of the stone salt species [Trump *et al.* 1972]. In support of this hypothesis is the fact that the kidney is incapable of creating sufficiently high enough supersaturation levels for homogeneous nucleation [Finlayson 1978].

An example of heterogeneous nucleation is illustrated by the concept of epitaxy which may play a role in the

formation and growth of urinary calculi [Lonsdale 1968a,b; Mandel and Mandel 1981]. A common feature of many kidney and bladder stones is the co-existence of alternating layers of differing composition around a nucleus [Achilles 1991]. Using x-ray crystallographic data of the components of urinary stones, possible epitaxial relationships have been demonstrated [Lonsdale 1968a].

After homogeneous or heterogeneous nucleation has occurred, solute particles will be present in the solution. This may lead to secondary nucleation. This is defined as nucleation which takes place only because of the prior presence of crystals of the deposited material [Garside 1985]. The process can be classified into apparent, true and contact secondary nucleation [Rodgers and Garside 1981] and is discussed in further detail in Chapter I.2.2. under "*In vitro* crystallisation".

Growth

Growth is the deposition of material from solution onto the surface of existing crystals [Dirksen and Ring 1991]. Crystal growth depends upon physiochemical factors such as supersaturation, temperature and concentration of the solution. Many crystal properties such as size, shape, purity and strength depend upon growth mechanisms [Garside 1985].

Growth is a diffusional process which takes place in the microscopic molecular and macroscopic size ranges, but will not occur without supersaturation.

At the molecular level, diffusion of particles (or "growth units") to the crystal surface with subsequent attachment will either result in their staying at that location or their being incorporated into the surface by further diffusion [Nielsen and Christoffersen 1982; Garside 1985]. Integration into the crystal structure then follows. It is therefore reasonable to expect the nature of the crystal

surface to play a large role in the diffusion process [Dirksen and Ring 1991].

More bonding sites are available on a rough surface than on a smooth surface [Garside 1985]. A rough surface not only increases the chances of contact, but also leads to a stronger bond (higher energy of bonding) [Garside 1985; Dirksen and Ring 1991]. A surface will remain rough after particle attachment due to the presence of new corners (from the newly attached particles) which then become preferred sites for further particle additions. It therefore follows that a higher rate of growth would be expected on a rough surface.

In addition, there exists a rate limiting step when growth occurs on a smooth surface. This step involves the addition of the first particle to the smooth surface which then provides a site for further additions. The second smooth layer after the initial addition has a higher activation energy. If a chemical process is used to induce precipitation, it's reaction rate will also be a rate determining factor in any growth process. These distinctions between growth on a smooth and rough surface have led to the terms "layered" and "continuous growth" respectively [Garside 1985].

Microscopically, there are two different sites where addition onto a crystal surface can be made. These are termed "steps" (often occurring in bunches) and "kinks" [Elwell and Scheel 1975; Nielsen and Christoffersen 1982; Garside 1985]. Steps are areas of layering on the crystal surface while kinks are indentations occurring on edges; therefore, kinks often occur on the edges of steps. Once diffusion onto the surface has taken place at these sites, the solvent molecules that surround the new attached solute particle diffuse away from the surface. Thereafter, the heat of crystallisation is released and the particle is incorporated into the crystal lattice.

Other factors determining growth rates include solute concentration and the presence of impurities. The increase in growth rates due to high concentrations of small particles in solution have been calculated by Jaganathan and Wey (1981). Experiments by Garside and Jancic (1976) on single crystals have shown a new effect termed "growth rate dispersion" which could be due to dislocations in the crystal or the presence of impurities.

Aggregation

Aggregation is the attachment of crystals to each other to form a collection of crystals joined at edges, corners and parallel faces [Nielsen and Christoffersen 1982]. The process is dominant in the presence of small particles (<1μm) due to the relatively greater strength of adhesional forces in comparison to gravitational force [Finlayson 1978].

It has been suggested that if aggregation could be prevented, urolithiasis would cease because stones are aggregates of smaller particles [Murphy and Pyrah 1962; Boyce 1969; Catalina and Cifuentes 1970]. This implies that aggregation is a key factor in stone formation.

The extent to which aggregation takes place is dependent upon particle surface potential. Those particles with low surface potentials will minimize their surface area by forming larger aggregates. Secondly, aggregation is also dependent upon the number density of "growth units" in the suspension. The number of these growth units could be limited by controlling nucleation. In such a way the formation of nuclei clusters and therefore aggregates are also controlled. Finlayson (1978) displayed scepticism about the importance of aggregation in urolithiasis or crystalluria, while Robertson *et al.* (1976a) have used aggregation inhibition in their evaluation of the stone forming potential of urine. Kok *et al.* (1990) recently measured the effect of agglomeration in urines from controls and stone formers. Their observations suggested

that agglomeration played a major role in the formation of large particles in the urines of stone formers.

I.2.2 IN VITRO CRYSTALLISATION

In *in vitro* systems, heterogeneous nucleation can be induced by foreign surfaces including the crystalliser walls. Walton (1965) suggested that submicroscopic particles entangled in the walls of a crystalliser affect nucleation. Due to reduced energy requirements, these foreign bodies provide sites for crystals to form at a lower critical supersaturation.

To minimize the possibility of heterogeneous nucleation from foreign insoluble material, rigorous cleaning and filtering procedures are necessary since even clean solutions have been reported to contain submicroscopic particles of concentration 10^6 - $10^8/cm^3$ [Walton 1961].

The physical difference between homogeneous and heterogeneous nucleation is that once heteronuclei are consumed within the crystalliser, heterogeneous nucleation ceases, thus limiting the maximum possible nucleation rate for this latter process [Robertson and Nordin 1976]. Homogeneous and heterogeneous nucleation are not likely to take place simultaneously [Dirksen and Ring 1991].

As with heterogeneous nucleation, secondary nucleation involves nucleation on solute particles already present in solution. However, it can also occur as a result of the contact of crystals with any available surfaces such as the crystalliser wall. However, the effects of this process have proved negligible in most small scale isothermal crystallisation experiments [Uhlmann and Chalmers 1965].

"Apparent secondary nucleation" refers to small fragments washed from the surface of seed crystals. True secondary nucleation occurs when the current level of supersaturation is higher than the critical level for the solute particles present in solution. "Contact secondary nucleation" occurs

when a growing particle contacts the walls of the crystalliser, stirrer or other particles thus leaving behind residual solute particles [Garside 1985; Dirksen and Ring 1991].

Contact nucleation rates depend upon factors such as stirrer rate, particle mass density, saturation ratios and the geometry of the crystalliser [Garside and Davey 1980]. The energy of contact is therefore the limiting factor of nuclei creation, and as such, secondary nucleation has been reported to be of lesser interest in urine where the maximum crystal concentrations are $10^4/cm^3$ [Robertson 1969, cited by Finlayson 1978].

Secondary nucleation studies have been carried out by Garside and Larson [Garside and Larson 1978; Garside *et al.* 1979] who investigated micro attrition on crystal surfaces. They observed the formation of large numbers of particles in the size range 1-10μm.

Attrition is the chipping or breaking by mechanical means (eg stirrer) of existing crystals (formed by homo- or heterogeneous nucleation) into smaller pieces which themselves begin to grow. The process is dependent upon the mechanics of the system with agitation of the fluid by an impeller playing a major role [Garside 1985].

Because there are three main nucleation mechanisms which can occur, investigators have to account for them in their design of *in vitro* crystallisation systems. Research has shown that defining the specific mechanisms in any one situation is difficult. In addition, data from nucleation experiments are difficult to interpret due to the growth period of new nuclei prior to their detection. However, it is important to understand the mechanisms of nucleation if surface active inhibitors are to be developed.

The most informative studies on growth rates in urinary precipitation systems have been experimentally calculated

using the relationship (equation 3) of Finlayson *et al.* (1984):

$$dc/dt = sk(C-C_o)^2 \quad \text{.....3}$$

where C is the solution concentration, C_o is the saturation concentration, s is the surface area and k is a rate constant.

Unfortunately, most growth studies have been unable to distinguish between the growth of a crystal and growth by aggregation.

The methods used to study growth have been conducted using spontaneous precipitation [Markovic and Komunjer 1979], seeded precipitation [Werness *et al.* 1979; Will *et al.* 1983], gel growth [Achilles 1991] and continuous crystallisers [Miller *et al.* 1977; Rodgers and Garside 1981]. These will be discussed in more detail in Chapter I.4. under "*In vitro* crystallisation studies".

I.2.3 ROLE OF INHIBITORS

Urinary inhibitors are naturally occurring components of the urine which have been shown in model crystallisation systems to prevent or retard the nucleation, growth or aggregation of various crystals [Ryall and Marshall 1990].

Interest in the possible role of inhibitors in CaOx stone formation began with Howard and Thomas (1958) who were the first to propose that inhibitors of mineralisation existed in urine. They further postulated that CaOx stone sufferers had a deficiency of these inhibitors (supported by [Robertson and Peacock 1972; Nakagawa *et al.* 1985]). Since then, the isolation and characterisation of these inhibitors has been the main aim of many experimentalists [Lewis *et al.* 1966; Goldberg and Cotlier 1972; Fleisch 1978; Kitamura *et al.* 1982; Nakagawa *et al.* 1983,1987; Atmani *et al.* 1993]. Robertson *et al.* (1971), supported qualitatively by the studies of Dent and Sutor (1971)

ascribed the larger sized crystals in the urine of recurrent stone formers relative to controls in *in vitro* studies, to the absence of inhibitors.

Identification of the principle urinary inhibitor is still a high priority. Most information on inhibitors has been derived from model aqueous crystallisation systems using dilute or fractionated urine [Meyer and Smith 1975a; Bowyer *et al.* 1979; Resnick *et al.* 1982; Drach *et al.* 1982; Scurr and Robertson 1986; Gohel *et al.* 1992; Shum and Gohel 1993; Rodgers *et al.* 1993,1994; Rodgers and Jappie 1996]. These systems offer reproducibility and permit quantification [Nancollas 1970; Nancollas and Mohan 1970; Meyer and Smith 1975a]. There are however large numbers of inorganic and organic compounds in urine (some in trace amounts) which have been reported to affect crystallisation, whose effects will not be detected in systems using dilute urine [Hallson and Rose 1979; Ryall *et al.* 1985] or ultrafiltered urine [Rodgers *et al.* 1994].

Urinary constituents which have been reported as being possible inhibitors of CaOx crystallisation include citrate, magnesium, pyrophosphate, ATP, ADP, and certain trace metals and some anionic macromolecules such as GAGs, RNA, acidic glycoproteins and non-polymerised Tamm Horsfall mucoprotein (THM, also known as uromucoid).

Due to their low urinary concentrations [Felix *et al.* 1977; Meyer and Smith 1975a; Nakagawa *et al.* 1985] the inhibitory role of small ions such as citrate, magnesium and pyrophosphate is not as significant as that of macromolecular polyanions. Growth and aggregation inhibition by the latter within urinary concentrations has been shown to be significant [Bowyer *et al.* 1979; Ryall *et al.* 1981b; Gjaldbaek 1982; Resnick *et al.* 1982; Scurr and Robertson 1986; Edyvane *et al.* 1987a; Shum and Gohel 1993; Ebisuno *et al.* 1993; Rodgers and Jappie 1996]. A review on the role of urinary GAGs has been published by Hesse *et al.* (1991).

Results of studies on the inhibitory or promotory role of urinary macromolecules (UMM) in urolithiasis have been inconclusive. Studies using aqueous solutions [Pak *et al.* 1979; Gjaldbaek and Robertson 1980; Nakagawa *et al.* 1985; Atmani *et al.* 1993], synthetic urine [Randolph *et al.* 1981; Scurr and Robertson 1986; Yoshioka *et al.* 1989; Ebisuno *et al.* 1993] and real urine [Azoury *et al.* 1985; Edyvane *et al.* 1987a; Gohel *et al.* 1992; Rodgers *et al.* 1994] have shown no agreement on the effects of addition or removal of UMM's. The collective and singular roles of UMM's have been shown to promote nucleation [Rose and Sulaiman 1984; Gohel *et al.* 1992; Schum and Gohel 1993], inhibit growth [Sallis and Lumley 1979; Resnick *et al.* 1982; Bek-Jensen and Tiselius 1991; Schum and Gohel 1993] and inhibit aggregation [Edyvane *et al.* 1987a; Koide *et al.* 1981; Ryall *et al.* 1991]. These findings have however been inconsistent. A possible reason for the inconsistencies may be related to the phase in which the macromolecules [Cambell *et al.* 1989] are present in the urine. The results of Cambell *et al.* (1989), indicated that polyelectrolytes and proteins behaved as inhibitors when free in solution, but also as promoters of crystal growth when immobilized on the crystal surface.

There has been considerable disagreement in establishing whether the urinary concentrations of GAGs is different in stone formers and controls. Various workers have reported higher concentrations in controls [Robertson *et al.* 1978,1984; Baggio *et al.* 1982; Michelacci *et al.* 1989; Nesse *et al.* 1992], while others have reported no difference [Sallis *et al.* 1981; Ryall *et al.* 1983,1986b; Caudarella *et al.* 1983; Hesse *et al.* 1986b; Hwang *et al.* 1988; Gianotti *et al.* 1989].

Chondroitin sulphate is the principle constituent of urinary GAGs and has been reported to inhibit aggregation [Ryall *et al.* 1981b; Gjaldbaek 1982; Robertson and Scurr 1986] and also growth [Bowyer *et al.* 1979; Fellström *et al.* 1986; Kok *et al.* 1988]. However, Ryall *et al.* (1981b) have suggested that growth inhibition is due to additive or

synergistic effects of a number of urinary constituents. Heparin has been found to be a more potent inhibitor of growth and aggregation than chondroitin sulphate [Crawford *et al.* 1968; Robertson *et al.* 1973], but it is virtually absent from urine [Goldberg and Cotlier 1972].

Another controversial urinary component is THM which has been reported as being a promoter [Drach *et al.* 1980; Rose and Sulaiman 1982; Yoshioka *et al.* 1989; Grover *et al.* 1990a], and an inhibitor [Kitamura and Pak 1982; Scurr and Robertson 1986; Yoshioka *et al.* 1989; Grover *et al.* 1990a] of CaOx crystallisation. The role of this macromolecule has been reviewed by Hess (1992).

Inhibitors exert their effects by two mechanisms. Firstly, they are able to "poison" the crystal surface by blocking growth sites. The binding of glycoproteins and other inhibitors by this method appears to be irreversible and follows the Langmuir theory of adsorption equilibrium [Meyer and Smith 1975a; Sheehan and Nancollas 1980; Wilson *et al.* 1985]. The second mechanism involves the complexation of inhibitor substances with Ca^{2+} or $C_2O_4^{2-}$ ions to decrease the level of supersaturation; examples include citrate [Hastings *et al.* 1934; Scott *et al.* 1943 cited by Berg and Tiselius 1989; Ryall and Marshall 1990; Tiselius *et al.* 1993b] and magnesium [Desmars and Tawashi 1973; Robertson *et al.* 1973; Scurr and Robertson 1986; Ryall and Marshall 1990].

As has already been stated, different interpretations concerning the identification of inhibitors and promoters and the mechanisms by which they operate, abound in the literature. This is mostly due to the different measurement techniques which have been employed. As a result, some studies have focussed on nucleation [Thomas *et al.* 1963; Fleisch 1965; Crawford *et al.* 1968; Sutor 1969; Fraser *et al.* 1972], while others have addressed growth [Francis 1969; Dent and Sutor 1971; Ryall *et al.* 1985], or aggregation [Robertson and Peacock 1972; Ryall *et al.* 1981b]. It has been shown that inhibitors in controls

act on growth and aggregation rather than nucleation [Sutor 1969; Robertson and Peacock 1972; Robertson *et al.* 1969,1973; Kok *et al.* 1986,1987,1990]. Werness *et al.* (1981) however found that their stone formers had a greater number of crystals in their urine than normal subjects. This suggests a lower nucleation rate in the healthy subjects which could possibly be due to the presence of endogenous inhibitors in their urine.

Methods of assessing crystal growth while nucleation is simultaneously occurring in the presence of inhibitors include measurements of mass increase [Dent and Sutor 1971], measurements of decreases in solute concentration [Meyer and Smith 1975a], measurements of increase in crystal diameter [Miller *et al.* 1977] and increase in crystal volume [Ryall *et al.* 1981a].

The necessity for standardising the investigative methods of inhibitor activity in real urine is obvious from the above discussion. The two most promising techniques which take into account most of the main controversial factors are the constant composition system [Sheehan and Nancollas 1980] and the continuous crystalliser method [Randolph and Drach 1981; Rodgers and Garside 1981]. These will both be discussed in more detail in Chapter I.4 under "Constant supersaturation".

I.2.4 ROLE OF THE MATRIX

All urinary stones consist of a crystalline component and an amorphous organic matrix [Khan and Hackett 1987]. The role of the matrix in stone formation is unclear. Some workers believe that it is a framework for deposition of the nucleating crystals that leads to growth of the calculus. Other workers however suggest that the matrix is organic debris that becomes entrapped during the crystallisation process [Rodgers and Spector 1981].

The concept of an organic matrix occurs in aetiological theories of different kinds of calculi [Harrill *et al.*

1959; Fox 1963; Boyce 1968; Sarles 1974]. Its presence has been demonstrated by removal of the crystalline components from stones [Carr 1953; Iwata *et al.* 1992]. In urinary stones, the organic matrix accounts for 2.5% of the dry weight, but in some calculi it could be as high as 60% [Mall *et al.* 1975; Bommer *et al.* 1979]. Boyce (1968) and Spector *et al.* (1976) were the earliest investigators to suggest that the organic matrix might play a role as a nucleating agent.

Chemically, the matrix is made up of approximately 65% protein [Boyce and King 1962 cited by Roberts and Resnick 1990], 10% non-amino sugars, 5% hexosamines [Boyce 1968; Finlayson *et al.* 1961], and 10% bound water with the remainder being inorganic substances [Thorne and Resnick 1983; Morse and Resnick 1988]. Nishio *et al.* (1985) analysed matrix GAG from urinary stones using 2 dimensional electrophoresis. They found that the principal matrix GAG content of CaOx stones was heparan sulphate and hyaluronic acid. This was supported by Roberts and Resnick (1986) and Wakatsuki *et al.* (1985), who demonstrated that hyaluronic acid was excreted in the urine of stone forming rabbits and that it was the major GAG in their matrices.

THM is the most abundant natural urinary macromolecule in whole human urine [Lynn *et al.* 1982; Grover *et al.* 1994]. It is produced only by the kidney and comes from the ascending limb of the Loop of Henle and the macula densa region of the distal tubule [Samuell 1979; Dawnay *et al.* 1982]. It is present in varying amounts in renal stones [Grant *et al.* 1973] which has prompted studies to investigate whether a difference in THM levels exists between stone formers and controls. These studies have however been unable to detect such a difference [Bichler *et al.* 1976; Samuell 1979; Thornley *et al.* 1985].

Results of *in vitro* studies performed to determine the role of THM on CaOx crystallisation have been contradictory. In inorganic solutions, THM has been shown to be an inhibitor of CaOx nucleation [Kitamura and Pak 1982], growth

[Kitamura and Pak 1982; Fellström *et al.* 1985a,1986; Scurr and Robertson 1986], and aggregation [Scurr and Robertson 1986; Hess *et al.* 1989,1991; Hess 1991]. Deganello (1993) on the other hand, concluded that THM neither nucleates calcium oxalate dihydrate (COD) crystals nor influences growth or habit in aqueous solutions, even when the THM was immobilized upon a rigid substrate.

In ultrafiltered urine, THM has been shown to promote the formation of CaOx [Hallson and Rose 1979; Hallson et al. 1981; Rose and Sulaiman 1982; Yoshioka *et al.* 1989]. On the other hand, Ryall *et al.* (1991) found increased inhibition of aggregation and concluded that this inhibition was likely to occur in whole urine *in vivo*.

Doyle *et al.* (1991) have studied the protein content in CaOx crystals precipitated from urines of healthy subjects; they did not detect any THM. They therefore concluded that proteins do not play a direct role in crystal formation. Meanwhile, Hess *et al.* (1991) showed that THM from stone formers with severe nephrolithiasis had lower inhibitory power than normal THM. They suggested that this was due to self aggregation of THM in the stone former.

THM can exist in several different polymeric forms under different ionic conditions [McQueen and Engel 1966; Wiggins 1987; Hess et al. 1989; Hess 1991] which could possibly explain the different results obtained from *in vitro* studies on the role of THM in CaOx crystallisation [Hess *et al.* 1991]. High concentrations of THM itself and cations like calcium, magnesium, sodium and hydrogen ions induce the self aggregation of THM [Hess 1991]. The reversible self aggregation of THM to form a gel leaves fewer monomers in solution, which in turn decreases the effectiveness of THM as an inhibitor.

At low pH and high ionic strength the inhibitory power of THM is lowered. At high pH and low ionic strength, it has been shown to inhibit COM aggregation [Hess 1991]. Fellström *et al.* (1986) found that THM inhibited CaOx

crystallisation at ionic strengths and concentrations usually found in urine. Meanwhile, Scurr and Robertson (1986) have shown that low concentrations of THM produced weak inhibition of CaOx aggregation, while promotion occurred at high concentrations corresponding to polymerization of THM. This dual role of THM was also demonstrated by Hess (1992) who found that THM became a strong promoter of crystal aggregation when the concentration of calcium ions was increased.

I.2.5 THEORIES OF STONE FORMATION

Stone disease is a multifactorial process. No single mechanism has been elucidated which satisfactorily accounts for stone formation. Two main groups of theories have evolved. The first of these describes the formation and retention of the stone nucleus as an intracellular process. Retention is achieved via lesions of the renal papilla (known as Randall's plaque) to which a calculus could remain anchored during its growth period [Randall 1936,1940; Carr 1954].

The second group of theories is based on extracellular processes of stone formation (taking place entirely within the lumen of the urinary tract [Robertson and Nordin 1976]). There are three such theories within this group. The first of these regards the matrix as the cause of stone disease [Boyce *et al.* 1954,1955; Boyce and Garvey 1956]. The primary step is thought to be the condensation of specific organic colloids (mucopolysaccharides and mucoproteins) within the collecting system of the urinary tract [Boyce and King 1963]. The deposition of inorganic salts is then regarded as a rapidly occurring secondary phenomenon.

The second of the extracellular theories postulates that the driving force for precipitation is urine supersaturation [Keyser 1923]. In this theory, CaOx stone formation occurs as a result of persistent oversaturation of Ca^{2+} and/or Ox^{2-} ions. Spontaneous precipitation of the

stone salts occurs when the activity product of the crystal forming ions is greater than the minimum thermodynamic solubility product [Finlayson 1978]. Precipitation therefore occurs independently of a pre-formed matrix.

The inhibitor theory is the third extracellular process in this group. It originated from work by Howard and Thomas (1958), and assumes that there are natural inhibitors in the urine that retard the various processes of stone formation. The presence or absence of these inhibitors can greatly affect the crystallisation kinetics of CaOx [Dent and Sutor 1971]. Typical small molecule inhibitors are thought to be citrate, magnesium and pyrophosphate [Fleisch 1978; Ryall et al. 1981b; Hallson *et al.* 1983; Robertson and Scurr 1986; Scurr and Robertson 1986; Grases *et al.* 1988,1989; Ryall and Marshall 1990; Tiselius 1995] while macromolecules such as THM, chondroitin sulphate and heparan sulphate have been reported as having inhibitor properties [Bowyer *et al.* 1979; Fellström *et al.* 1986; Scurr and Robertson 1986; Kohri *et al.* 1989; Shum and Gohel 1993; Rodgers *et al.* 1994].

The initiation of stone disease involving either the fixation of a free particle or as the origination of a fixed particle at a certain site is still unclear [Finlayson 1977a; Finlayson and Reid 1978b]. The free particle theory addresses the question of whether a free particle could grow large enough to impede urinary flow in a renal tubule. Excessive supersaturation of urine with respect to the stone forming salts may lead to the trapping of an aggregate or large crystal in the urinary tract. Alternatively, the fixed particle theory supposes that blockage of the narrow tubules could result from a high rate of crystallisation. This attachment of the crystal to the wall of the renal system could be aided by a gluing substance. Subsequent growth and aggregation could produce a calculus. Finlayson concluded that the fixation of the particle on the luminal wall was necessary for upper tract urinary stone disease to occur.

Although the basis of the two main theory groups is different, they do have some common features. It is generally believed that stones form from a nidus, and that it is this process that is the most difficult to control. Once nucleation has occurred, growth and aggregation follow. It is the processes of growth and aggregation that have to be overcome so that stone disease can be prevented Grases and Costa-Bauza 1990; Kok *et al.* 1990].

I.3 UROLITHIASIS THERAPY

Surgery, lithotripsy

There are several different types of urinary stone, each one of which requires its own specific method of treatment. This implies that analysis of the stone itself is necessary to help the clinician decide upon the most appropriate therapy. Conservative or non-surgical treatments are based upon the need to decrease the supersaturation of the stone forming salts as this leads to the attainment of a state of undersaturation in which any pre-formed stones should dissolve.

However, in some cases, removal of the stone by surgery or lithotripsy (*in vivo* destruction) is needed [Chaussy and Schmiedt 1983; Chaussy *et al.* 1980,1982; Sofras *et al.* 1988]. The latter method can be achieved in numerous ways with the pulverised stone being eliminated spontaneously in the urine [Wickham 1982; Politis and Griffith 1987; Brecht *et al.* 1988; Chaussy 1988; Tiselius *et al.* 1989; Schulze *et al.* 1989; Constantinides *et al.* 1989]. Litholysis [Sheldon and Smith 1982] refers to dissolution of the stone *in vivo* and has been used successfully in the treatment of uric acid, cystine and struvite stones [Jacobs and Gittes 1976; Smith *et al.* 1979]. However, removal of a renal stone only eliminates the consequences of the disease, but not it's cause.

Diet

Recommendations on dietary management have now become the norm [Baker and Mallinson 1979; Vahlensieck *et al.* 1984; Vahlensieck 1986; Hesse *et al.* 1992,1993; Goldfarb 1994]. Calcium oxalate stone patients are frequently advised to restrict their intake of dietary calcium [Rao *et al.* 1982; Vahlensieck 1986; Rose 1987; Goldfarb 1994] as there exists the widely believed hypothesis that a high calcium intake increases the risk of stone formation. However, in a recent prospective study of the relation between dietary calcium intake and the risk of symptomatic kidney stones in over 45000 male subjects, Curhan *et al.* (1993) demonstrated precisely the converse - namely, a *high* dietary intake of calcium *decreases* the risk. The authors suggest that this might be due to increased binding of oxalate by calcium in the gastrointestinal tract, leading to a decrease in urinary oxalate excretion. Since urinary oxalate is more important than urinary calcium in determining stone formation [Finlayson 1974; Robertson *et al.* 1978; Hesse *et al.* 1993], the risk decreases. Stone formers are therefore advised to restrict their intake of oxalate rich foods such as chocolate and tea.

A high intake of animal protein has been shown to produce an increase in urinary calcium, oxalate and uric acid. It has therefore been suggested that a reduction in dietary animal protein rather than dairy products would be more beneficial for recurrent stone formers [Andersen 1973; Robertson *et al.* 1979]. Indeed, increased stone incidence and recurrence [Leusmann *et al.* 1990] could be a direct consequence of higher animal protein ingestion when compared to population groups from economically poorer regions in which stone incidence is lower.

These findings support the results of other workers who have shown that a low calcium diet increases the excretion of oxalate [Zarembski and Hodgkinson 1969; Marshall *et al.* 1972]. Indeed, the potential danger of dietary calcium restriction has been demonstrated by Bataille *et al.* (1984) who reported an increased probability of forming stones in

patients with certain types of hypercalciuria when following such a diet. Such studies suggest an apparent protective effect of dietary calcium. It therefore seems feasible to advise stone formers to *increase* their dietary intake of calcium.

A high fluid intake is necessary for decreasing the risk of stone formation [Fuss *et al.* 1979; Pak *et al.* 1980; Robertson and Peacock 1983; Vahlensieck 1986]. This advice is given with the knowledge that it will ensure larger volumes passing through the kidney which will ultimately lead to a decrease in residence time of the crystals due to larger flow rates. This in turn, will lead to a decrease in supersaturation.

Medical treatment

Medical treatment of stone formers include careful use of compounds such as cellulosephosphate and thiazides which bind with Ca^{2+} thereby decreasing the supersaturation of the urine with respect to CaOx [Pietrek and Kokot 1973; Williams 1974; Yendt and Cohanim 1978; Ohkawa *et al.* 1992].

Direct inhibition of crystallisation *in vivo* has been accomplished using inhibitors such as disodium ethane-1-hydroxy-1,1 diphosphonate (EDHP) [Bone *et al.* 1979], succinimide for oxalate stones [Thind *et al.* 1980], methylene blue [Boyce *et al.* 1967; Wein *et al.* 1976] and inhibitor stimulating agents such as phosphate (which increase the concentration of the inhibitor pyrophosphate) [Russel *et al.* 1976] for non-obstructive renal calculi.

Role of urate

Allopurinol which has been used for the treatment of uric acid stones [Coe 1977; Fellström *et al.* 1985b; Tiselius *et al.* 1986] has also been used in the treatment of recurrent CaOx stone formers. This arises as a result of its supression of urinary urate [Coe and Raisen 1973; Coe and Kavalach 1974] which has been shown to retard the

inhibitory capacity of naturally occurring UMM's [Finlayson and Dubois 1978; Pak *et al.* 1979; Fellström *et al.* 1982; Ryall *et al.* 1986c; Hesse *et al.* 1987].

There are two theories on the role of urate in the formation of CaOx stones. The first is that sodium urate crystals induce heterogeneous nucleation of CaOx [Coe *et al.* 1975; Pak and Arnold 1975; Finlayson and Dubois 1978; Pak *et al.* 1979; Grases *et al.* 1991]. This theory has arisen due to the good epitaxial fit of Caox and sodium urate (ie CaOx adheres to the sodium urate crystal surface due to a good crystal lattice match) [Lonsdale 1968a,b]. The second theory, as mentioned in the previous paragraph, is that the inhibitory potency of GAGs is reduced by the binding of colloidal urate [Robertson *et al.* 1976b; Fellström *et al.* 1982; Hesse *et al.* 1987].

More recently however, Grover *et al.* (1990b) found that reducing the urate concentration in several urines did not produce any reduction of the metastable limit (MSL), of the volume of crystalline CaOx deposited or of the size of crystals and aggregates. In a later study, they found that the addition of dissolved urate to ultrafiltered urine promoted CaOx crystallisation in a manner similar to that in the presence of GAGs [Grover *et al.* 1992]. In yet another study, they found that the promotion of CaOx crystallisation by urate was not due to its binding of GAGs nor to epitaxial nucleation of CaOx [Grover *et al.* 1993]. They have proposed that a "salting out" mechanism is responsible for the promotion of CaOx crystallisation by urate [Grover *et al.* 1990c,1993].

Alkaline citrate therapy

Prophylactic treatment with alkaline citrate is used to increase the concentration of citrate in urine; this retards the formation of CaOx by decreasing the calcium specific ion activity and the relative supersaturation of CaOx.

Treatment of calcium stone patients using potassium citrate or potassium sodium citrate has been shown to increase urinary pH and citrate excretion both in the short term [Preminger *et al.* 1985; Nicar *et al.* 1984,1986] and the long term [Pak *et al.* 1985; Butz 1986; Barcelo *et al.* 1993]. Decreased levels of calcium have also been reported [Butz and Dulce 1981; Butz 1986; Khazziani *et al.* 1993; Leusmann *et al.* 1993]. The effects of different doses of citrate have been investigated by Berg *et al.* (1990,1992), while Schwille *et al.* (1992) suggest the introduction of medication free intervals for patients on long term oral potassium citrate medication.

Hofbauer *et al.* (1994) however, have been unable to show objectively any evidence to support claims of the therapeutic benefit of oral alkali citrate therapy. They do however justify its use subjectively due to its low incidence of side effects. Their work was carried out using stone formers with regular stone recurrences. They claim that a drawback in other investigations is the absence of randomisation as the time factor of recurrency was not taken into account when selecting stone forming subjects.

Abdulhadi *et al.* (1993) compared patients on long term citrate and hydration therapy with those on hydration alone. They observed an increase in urinary citrate levels but could find no significant difference between the two groups.

The use of alkaline citrate thermodynamically favours the crystallisation of calcium phosphate [Berg and Tiselius 1989]. This is because calcium phosphate precipitates at high pH (>5.5), whereas CaOx precipitation occurs at lower pH levels [Tiselius 1987]. The primary crystallisation of calcium phosphate is capable of inducing heterogeneous crystallisation of CaOx at lower supersaturation levels than those favouring precipitation of pure CaOx. The use of alkaline citrate could therefore increase the risk of heterogeneous crystallisation of CaOx. Despite this

hypothesis, Berg and Tiselius (1989) and Tiselius *et al.* (1993a) found that increasing citrate concentrations inhibited the heterogeneous growth of CaOx on calcium phosphate.

Pak *et al.* (1992) have shown the greater inhibitory action of potassium-magnesium citrate over potassium-citrate (at the same dose of potassium [Koenig *et al.* 1991]) on the crystallisation of calcium oxalate. Recently, therapy using oral sodium-potassium citrate has been investigated by Ogawa (1994) using different regimens and fractional urine collections. He found that an evening dose in addition to a three times a day sodium-potassium citrate regimen significantly reduced the CaOx saturation level in the early morning.

Magnesium therapy

Interest in the beneficial effect of magnesium in the prevention of CaOx stone formation began with animal studies [Cramer 1932; Andrus *et al.* 1960; Lyon *et al.* 1966; MacManus and Heaton 1969; Rushton and Spector 1982] as well as other studies [Takasaki 1972; Robertson *et al.* 1973; Fetner *et al.* 1978; Johansson *et al.* 1980b; Resnick *et al.* 1982]. These studies have since formed the rationale for the treatment of CaOx stone sufferers with magnesium in the form of magnesium oxide [Kohler and Uhle 1966; Melnick *et al.* 1971; Prien and Gershoff 1974; Ahlstrand and Tiselius 1983; Rattan *et al.* 1994] and magnesium hydroxide [Johansson *et al.* 1980a].

Magnesium therapy has been shown to be effective in increasing magnesium excretion and reducing stone incidences [Moore and Bunce 1964; Kohler and Uhle 1966; Melnick *et al.* 1971; Johansson *et al.* 1980a; Danielson 1985]. However, Fetner *et al.* (1978) suggest that magnesium therapy may be successful in reducing the formation product ratio and the growth of CaOx only in the long term. On the other hand, Tiselius *et al.* (1980) found that patients on magnesium oxide therapy increased both

their magnesium and calcium urinary excretion. Since this resulted in the calcium:magnesium ratio being unaffected, they concluded that magnesium therapy is not always effective.

Therapy using magnesium attempts to correct the low magnesium:calcium ratios in stone formers which have been shown by several authors [Tiselius *et al.* 1978; Johansson *et al.* 1980b]. Low values of this ratio can be related to either low magnesium levels [Evans *et al.* 1957] or high calcium levels [Takasaki 1972]. However, Fetner *et al.* (1978) suggest that the physiochemical meaning of this ratio is limited because the action of magnesium would be better described by the urinary concentration of magnesium. They propose that the action of magnesium in decreasing the supersaturation with respect to CaOx by complexing with oxalate or by exerting inhibitor activity is dependent upon the concentration of magnesium rather than the amount of magnesium in relation to calcium. An increase in the magnesium:calcium ratio may therefore not necessarily indicate a lowered supersaturation or an increased inhibitor activity [Fetner *et al.* 1978]. They therefore suggest that the effect of magnesium on CaOx crystallisation should be accounted for in terms of magnesium concentration rather than the magnesium:calcium ratio.

As yet, studies have been unable to demonstrate any difference in the 24 hour magnesium excretion between stone formers and controls [Robertson *et al.* 1968,1978; Welshman and McGeown 1975; Johansson *et al.* 1980b; Bach *et al.* 1981; Resnick *et al.* 1982].

Comment

Once a kidney stone has been formed, there exists the potential for recurrence. Many therapies are based upon prophylactic oral medication, but lifelong management using drug treatments is undesirable. Such drugs should possibly be used when dietary management has been unsuccessful or,

as suggested by Pak *et al.* (1984) as a supplement to dietary management.

I.4 IN VITRO CRYSTALLISATION STUDIES

Methods

Many methods have been used in the *in vitro* study of CaOx crystallisation [Hess and Kok 1996]. As a result, comparison and interpretation of data have been difficult to achieve. Clearly, no single method can model all the processes in stone formation since there are many *in vivo* variables such as urinary flow rates, pH and composition which cannot be mimicked. In addition, factors such as inhibitors of growth and aggregation, matrix, retention and site of crystal formation cannot be reproduced in the crystallisation system.

Although crystalluria cannot be equated with stone formation, much useful information can be deduced from the study of urinary crystallisation processes [Gaur and Nancollas 1984; Baumann 1985; Azoury *et al.* 1987].

The importance of supersaturation as a determining factor in crystallisation theory has already been discussed. The presence of crystalluria in the urine of stone formers and controls [Hallson and Rose 1977] is proof that a state of supersaturation exists or existed somewhere in the urinary tract. On this basis, Kavanagh (1992) has summarised crystallisation experiments into three categories:

(i) initial supersaturation with subsequent supersaturation decay;
(ii) initial undersaturation with concentration to supersaturation;
(iii) constant supersaturation.

Supersaturation decay

Crystallisation experiments using supersaturation decay and substrate depletion are based on a batch principle in which no additions are made to the system once nucleation has begun [Khan *et al.* 1988,1990]. Using this approach, Brecevic *et al.* (1989) found that the order in which feed solutions of calcium chloride ($CaCl_2$) and sodium oxalate (NaOx) were mixed, as well as their initial concentrations affected the abundance of the various CaOx hydrates which were formed. The type of stirring was also found to be a determining factor.

Precipitation can be initiated by use of a seed crystal which does not necessarily have to be a crystal of the nucleating phase [Ryall *et al.* 1981a; Bijvoet *et al.* 1983; Kok *et al.* 1988; Yamaguchi *et al.* 1993]. Baumann *et al.* (1990) measured the inhibition of growth of COM seed crystals in whole urine using an ion-selective calcium electrode. Heterogeneous nucleation of CaOx has been measured by Burns and Finlayson (1980) by examining the CaOx production in the presence of sodium acid urate seed crystals, hydroxyapatite seed crystals and CaOx seed crystals. The inhibition of CaOx monohydrate crystal aggregation by THM and nephrocalcin has also been measured using COM seed crystals [Hess et al. 1989].

Use of seed crystals obviates nucleation so that only growth and aggregation need be considered [Ryall *et al.* 1981a; Will *et al.* 1983; Kok *et al.* 1990]. This does not apply if the seed is heterogeneous, in which case the role of heterogeneous nucleation is then important. The effect of aggregation alone has been measured using seeded systems at saturation [Hess *et al.* 1989]. Although the seeding method does not mimic physiological conditions (because it negates the process of nucleation), it does offer many advantages such as high reproducibility, control over seed variables, and easy interpretation of results [Kavanagh 1992]. However, a disadvantage is that inhibitory activity

is measured at concentrations much lower than those found in urine unlike those of unseeded methods.

A second batch method is that of oxalate tolerance which involves titration of urine with NaOx to determine the MSL [Robertson and Nordin 1976]. This is the minimum amount of oxalate required to induce detectable precipitation [Ryall *et al.* 1985]. After determination of this limit, crystallisation is induced by addition of further oxalate [Edyvane *et al.* 1987a,b; Ryall *et al.* 1991]. Results from this method are more difficult to interpret due to the simultaneous occurrence of several crystallisation mechanisms, but the method can give estimates of a urine's potential for crystallisation.

Consistent results can be obtained for a given set of conditions; however comparisons with other systems are difficult. This is due to results being dependent upon the specific characteristics and conditions of the particular experiment [Hesse *et al.* 1991]. Different conditions existing in different crystallisation systems could induce a "ripple effect" in which numerous inter-related processes involving inhibition and promotion of nucleation, growth and aggregation interact with each other to finally produce a nett, system dependent mechanism [Rodgers and Jappie 1996]. Examples of system dependent factors that would influence crystallisation are: the rate of supersaturation increase above the MSL, agitation [Brecevic *et al.* 1986], and the method of monitoring the crystallisation. This latter factor arises as a result of the limited sensitivity of the individual items of equipment [Hallson and Rose 1990]. This leads to difficulties in measuring the size distribution in the chemically and physiologically most interesting region of 0.1 - 1μm. A review on size measurement techniques has been published by Stresty and Venkateswar (1980).

The progress of the reaction in both seeded and unseeded methods can be monitored continuously or at time intervals using methods such as nephelometry [Grases et al.

1988,1989; Rodgers *et al.* 1993], Coulter Counter [Robertson 1969a; Ryall *et al.* 1981a,b; Tiselius 1985; Grover et al. 1992; Tiselius *et al.* 1993b], calcium electrode [Wolf and Stoller 1994], calcium or oxalate depletion [Gjaldbaek 1982] and flow cytometry [Blomen 1982; Rodgers *et al.* 1995].

Methods for following calcium depletion include atomic absorption spectrophotometry (AAS) [Pak and Holt 1976; Finlayson and Reid 1978a] and the more elaborate methods of colorimetric analysis [Hodgkinson 1980], complexiometric titration [Fleisch and Bisaz 1964], and radioactive ^{45}C tracers [Will *et al.* 1983; Blomen *et al.* 1983; Hesse *et al.* 1987].

Although radioactive ^{14}C-oxalate tracers have been used for the measurement of oxalate depletion [Gill *et al.* 1974; Ligabue *et al.* 1979; Tiselius and Fornander 1981; Rose and Sulaiman 1982; Fellström *et al.* 1982; Grover *et al.* 1990a], there are nevertheless difficulties associated with this method. These arise because of the relatively small change in oxalate concentration which occurs during crystallisation.

In seeded systems, calcium or oxalate depletion methods usually ignore aggregation in interpretation of the results and assume that only growth is occurring. Particle sizing methods can measure the effects of nucleation and growth or nucleation and aggregation by measuring the increases in crystal volume or crystal number respectively [Ryall *et al.* 1981a]. Due to the interdependence of aggregation and growth, it is difficult to measure these processes independently without undertaking complicated mathematical analysis of the data [Blomen *et al.* 1983; Ryall *et al.* 1986a].

Unfortunately, no identification of crystal type is possible using sizing techniques such as the Coulter Counter. Scanning electron microscopy (SEM) is usually used for this purpose [Werness *et al.* 1981; Khan *et al.*

1983; Khan and Hackett 1987b; Kohri *et al.* 1989; Ryall *et al.* 1991; Rodgers *et al.* 1994; Rodgers and Jappie 1996]. Use of particle counters also requires pre-treatment of urine, either by filtration or centrifugation, to remove all non-crystalline material [Robertson 1969a; Ryall 1978]. Such pre-treatment is necessary because sizing instruments cannot distinguish crystals from other particles. Unfortunately filtration removes THM which has been shown to play a role in CaOx crystallisation as discussed earlier [Dawnay *et al.* 1982; Wiggins 1987; Doyle *et al.* 1989,1991; Grover *et al.* 1990b; Ryall *et al.* 1991; Suzuki *et al.* 1994; Rodgers *et al.* 1994]. Despite this, the Coulter Counter has been used successfully in the study of various aspects of CaOx crystallisation [Markovic and Komunjer 1979; Bowyer *et al.* 1979; Ryall *et al.* 1981b,1985,1991; Brecevic and Garside 1981; Scurr and Robertson 1986; Edyvane *et al.* 1987a,b; Grover *et al.* 1992; Doyle *et al.* 1995; Rodgers and Jappie 1996]. Apart from the Coulter Counter, other more sophisticated particle size analysers are now in use that apply Chemical Engineering principles to determine crystal growth processes [Randolph and Larson 1971].

Results obtained by batch methods are usually qualitative due to the changing contributions of nucleation, growth and aggregation on the particle size distribution (PSD) throughout the reaction. A limitation of decreasing supersaturation systems is that different crystal phases may form, making it difficult to relate results to *in vivo* conditions where supersaturation may be maintained at a relatively constant value.

Undersaturation conversion to supersaturation

Supersaturation of an initially undersaturated solution can be achieved by a number of techniques; for example, in semi-batch methods reactants are added to the crystallising medium either continuously or periodically. A second approach involves the process of diffusion. In this approach, no attempt is made to quantify the crystallisation process. The gel crystallisation method

(GCM) [Achilles 1989; Achilles *et al.* 1983,1991] is an example of a diffusion process that has been successfully applied to the crystallisation of CaOx hydrates [Achilles 1985 cited by Baumann 1988b; Achilles 1991; Achilles *et al.* 1994a,b]. The method involves adding the second component of a precipitating binary phase into a gel containing the first component. Crystal growth can be followed by photometric methods.

Concentration by water removal is another method for achieving supersaturation to initiate precipitation. It is postulated that this process is analogous to the concentration of urine in the renal tubules, whereby water is removed and a state of supersaturation is maintained [Hallson and Rose 1978]. The rate of evaporation is slow if it takes place under normal atmospheric conditions, but can be increased using reverse osmosis or evaporation under reduced pressure at 37°C [Azoury *et al.* 1987; Hallson and Rose 1990]. Crystals obtained by these methods can be studied by SEM to give a qualitative estimate of aggregation, or isotopically by the addition of ^{14}C oxalate [Gill *et al.* 1974; Rose and Sulaiman 1982; Grover *et al.* 1990a] before evaporation (which is very expensive), or by chemical analysis [Azoury *et al.* 1987].

Constant supersaturation

Steady state supersaturation systems can be classified as either constant composition crystallisation or mixed suspension mixed product removal (MSMPR) crystallisation techniques. Both have an advantage over those already discussed in that they are more representative of the conditions within the kidney.

The constant composition method [Tomson and Nancollas 1978] involves addition of calcium and oxalate to the reaction vessel to maintain a constant composition. Calcium ion activity is measured by a specific electrode [Sheehan and Nancollas 1980]. Use of a protected calcium electrode has made it possible to study the reaction kinetics in

undiluted urine [Gaur and Nancollas 1984]. Seed crystals have also been used in the constant composition technique [Sheehan and Nancollas 1981; Lanzalaco *et al.* 1988]. Reaction kinetics and inhibitor kinetics are based on the rate of calcium and oxalate depletion. The method can be used at very low supersaturations and is accurate, but may be expensive. An advantage of the method is that it resembles the conditions found in the kidney as crystallisation occurs at a stable supersaturation.

Any chamber continuously receiving a stream of supersaturated liquid and continually ejecting a stream of liquid and suspended particles is a continuous crystalliser [Finlayson 1972]. Finlayson (1972) first described the concept of the urinary tract as a biological analog of a sequence of continuous crystallisers. Further work by Miller *et al.* (1977) led to the development of a continuous MSMPR crystalliser using artificial urine as the crystallising medium.

The MSMPR crystalliser is now widely used for measuring nucleation and growth rates of CaOx [Miller *et al.* 1977; Drach *et al.* 1978,1980,1982; Rodgers and Garside 1981; Li *et al.* 1985; Robertson and Scurr 1986; Kohri *et al.* 1988; Nishio and Kavanagh 1990; Kavanagh *et al.* 1993; Nishio *et al.* 1990,1994] as well as for the determination of inhibitor activities [Drach *et al.* 1978; Li *et al.* 1985]. Drach *et al.* (1980) attempted to distinguish between controls and stone formers by adding 5% urine from each group to 95% synthetic urine. They proposed that uromucoid played a role in producing the differences found between the two groups. Robertson and Scurr (1986) determined the effect of urinary concentrations of various inhibitors using an MSMPR crystalliser with a Coulter Counter. The effects of chondroitin sulphate, hyaluronic acid and heparin on the nucleation and growth rates of CaOx in artificial urine have also been investigated by Kohri *et al.* (1989).

In the past, MSMPR crystallisers have required volumes in excess of 200ml. However, recently an MSMPR crystalliser of volume 20ml has been used successfully to obtain CaOx crystal size distributions (CSD's) using inorganic solutions [Nishio *et al.* 1991].

An advantage of the MSMPR crystalliser system is that simultaneous measurement of nucleation and growth rates can be achieved, at the same level of supersaturation, level of agitation [Brecevic *et al.* 1986], temperature and suspension density. These kinetic measurements are derived easily from the data output of the particle sizers. However, aggregation is more difficult to measure.

An attempt has been made by Springmann *et al.* (1986) to measure aggregation in a flow system using an MSMPR crystalliser with an on-line Couette agglomerator. They compared synthetic urine controls with 5% vol/vol additions of human urine from healthy subjects and stone formers and found that the latter had significantly greater numbers of large particles than those of the healthy subjects. The number of large particles in both the stone formers and healthy subjects was lower than that of the control. They concluded that urine contains an inhibitor of CaOx aggregation and that there is a deficiency of such inhibitors in the urines of stone formers.

Comment

Experiments using synthetic, dilute or fractionated urine have been carried out using many of the techniques mentioned earlier [Gardner and Doremus 1978; Bowyer *et al.* 1979; Kohri *et al.* 1989]. Crystallisation in these artificial solutions requires simple equations for growth calculations [Robertson 1982]. The use however of these solutions in determining inhibitor or promotor activity is questionable [Dhanalekshmy *et al.* 1994]. Extrapolation from such experiments to *in vivo* conditions may not be possible because of an obscuration of the inhibitory power due to dilution [Baumann 1985,1988a]. Most data about

inhibitor activity have been acquired using batch systems [Ryall *et al.* 1985]. Seeded experiments using whole urine have been successful in providing insight into the effects of low molecular weight inhibitors [Baumann 1985, cited by Baumann 1988b], and have allowed the determination of urinary saturation.

Measurements of inhibitor activities should preferably be performed in freshly voided urine, since substances used to preserve the urine (eg HCl) may influence the crystallisation processes. Lanzalaco *et al.* (1982) investigated the effects of freezing and refrigeration on the growth of COM crystals in the absence of bacteriostatic agents. They concluded that the refrigerated samples remained more stable than those that were frozen.

The need for experiments using whole urine is becoming increasingly important. It is therefore necessary to adapt the above systems in order to accommodate such experiments.

The use of whole urine in continuous crystallisation experiments [Springmann *et al.* 1986] has been suggested, but has not been pursued because of the large volumes required. Improvements in the MSMPR crystallisation technique have led to the use of 92% urine [Kavanagh *et al.* 1993]. In addition, two parallel MSMPR crystallisers [Kavanagh 1992] have been used to measure the effect of additives on crystallisation. Baumann *et al.* (1990) have recently adapted the system of Meyer and Smith (1975a) to enable measurements in whole pre-treated urine.

There are however, shortcomings associated with the measurement of crystallisation kinetics in whole urine. In whole urine there is already a high inhibitory presence; this needs to be overcome to enable significant crystallisation to be detected. As a result, the addition of large seed crystal concentrations or the achievement of very high supersaturations which are not found at physiological levels [Baumann 1995] are required.

The point has already been made (Chapter I.4 under "Supersaturation decay") that methods employed in test systems each yield results that could be system specific. In order to compare data, standard methods of experimental procedure and result analysis must be achieved [Kavanagh 1995b]. Grover *et al.* (1990a) have demonstrated this need experimentally by using two methods for determining the effects of THM on crystallisation in ultrafiltered urine. The method of oxalate loading showed that THM had no effect on the amount of CaOx deposited. On the other hand, the evaporation technique using ^{14}C-oxalate showed that THM promoted CaOx crystallisation.

Since the ultimate aims of urinary crystallisation experiments are to understand and thereby attempt to prevent and treat stone disease, such experiments need to be conducted in the same crystallising medium (or as close as possible) as that found *in vivo*, namely whole urine.

I.5 OBJECTIVES

The objectives of the present study are as follows:

(i) to design, construct, develop and test an MSMPR crystalliser that conforms to strict chemical engineering design principles;

(ii) to design, construct, develop and test a thermostatted flow cell which can be accommodated in a Malvern Particle Size Analyser;

(iii) to use a Malvern Particle Size Analyser for the rapid, non-destructive and non-invasive measurement of calcium oxalate crystal size distributions in a series of crystallisation experiments in minimally diluted urine;

(iv) to measure crystal size distributions simultaneously in a parallel series of crystallisation experiments in minimally diluted urine using a Coulter Counter;

(v) to use the data acquired in these experiments to compute calcium oxalate crystallisation kinetics in minimally diluted urine;

(vi) to test the efficacy of the system by investigating the effect of temperature on the kinetics of calcium oxalate crystallisation processes in minimally diluted urine;

(vii) to test the efficacy of the system by investigating the effects of various oxalate feed solution concentrations on the kinetics of calcium oxalate crystallisation processes in minimally diluted urine;

(viii) to test the efficacy of the system by investigating the individual effects of citrate, magnesium and chondroitin sulphate on the kinetics of calcium oxalate crystallisation processes in minimally diluted urine;

(ix) to test the efficacy of the system by investigating the synergistic effect of citrate and magnesium on the kinetics of calcium oxalate crystallisation processes in minimally diluted urine.

REFERENCES: CHAPTER I

Abdulhadi,M.H.; Hall,P.M.; Streem,S.B.
"Can citrate therapy prevent nephrolithiasis?"
Urology (1993), **41** (3), 221-224

Achilles,W.
"Kinetic quantification of crystal growth in gel matrices: an efficient model of urinary stone formation"
in:"Urolithiasis" eds:Walker,V.R.; Sutton,R.A.L.; Cameron,E.C.B.; Pak,C.Y.C.; Robertson,W.G. Plenum Press, New York, London, (1989), 47

Achilles,W.
"Microphotometric quantification of crystal growth in gels for the study of calcium oxalate urolithiasis"
Scann.Micros. (1991), **5**, 1001-1017

Achilles,W.; Dekanic,D.; Burk,M.; Schalk,Ch.; Tucak,A.; Karner,I.
"Crystal growth of calcium oxalate in urine of stone-formers and normal controls"
Urol.Res. (1991), **19**, 159-164

Achilles,W.; Jockel,U.; Schaper,A.; Ulshofer,B.; Riedmiller,H.
"Formation of urinary stones in vitro: growth of calcium oxalate on spherulites of calcium phosphate in gel"
in: "Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis eds: Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.M.; Smith,L.H.; Walker,V.R. Plenum Press, New York, London, (1994a), 161-165

Achilles,W.; Kothe,R.; Jockel,U.; Schalk,C.; Riedmiller,H.
"A new continuous flow microsystem of crystallisation in gels as a model of urinary stone formation"
in: "Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis eds: Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.M.; Smith,L.H.; Walker,V.R.
Plenum Press, New York, London, (1994b), 159-160

Achilles,W.; Mergner,C.H.; Simm,M.
"An optical micromethod of the determination of relative crystallisation rates of calcium oxalate in gels: method and preliminary results"
Urol.Res. (19830, **11**, 87-91

Ackermann,D.; Brown,C.; Dunthorn,M.; Purich,D.L.; Finlayson,B.
"Use of the computer program EQUIL to estimate pH in model solutions and human urine"
Urol.Res. (1989), **17**, 157-161

Ahlstrand,C.; Tiselius,H.G.
"Biochemical effects in patients with calcium oxalate stone disease during combined treatment with bendroflumethiazide and magnesium oxide"
Br.J.Urol. (1983), **56**, 125-130

Andersen,D.A.
"Enviromental factors in the aetiology of urolithiasis"
in:"Urinary calculi:Proceedings of international symposium on Renal stone research" Madrid 1972 eds:Cifuentes Delatte,L.; Rapado,A.; Hodgkinson,A. Karger,Basel (1973), 130-144

Andrews,J.C.; Yarbro,C.L.; Golby,R.L.; Sell,I.T.
"Studies on the mechanism of formation of renal calculi.I."
J.Urol. (1955), **73**, 930-937

Andrus,S.B.; Gershoff,S.N.; Faragalla,F.F.; Prien,E.L.
"Production of calcium oxalate renal calculi in vitamin B_6 deficient rats. Study of the influence of urine pH.
Lab.Invest. (1960), **9**, 7

Atmani,F.; Lacour,B.; Drueke,T.; Daudon,M.
"Isolation and purification of a new glycoprotein from human urine inhibiting calcium oxalate crystallisation"
Urol.Res. (1993), **21**, 61-66

Azoury,R.; Goldwasser,B.; Wax,Y.; Perlberg,S.; Garti,N.; Sarig,S.
"Evaluation of the relative inhibitory potential of fractionated urinary macromolecules"
Urol.Res. (1985), **13**, 199-205

Azoury,R.; Robertson,W.G.; Garside,J.
"Observations on in vitro and in vivo Calcium Oxalate crystalluria in primary calcium stone formers and normal subjects"
Br.J.Urol. (1987), **59**, 211-213

Bach,D.; Hesse,A.; Strenge,A.; Vahlensieck,W.
"Magnesium excretion in urine on condition of individual as well as standard diet in healthy controls and calcium oxalate stone formers"
in:"Urolithiasis: clinical and basic research" eds: Smith,L.H.; Robertson,W.G.; Finlayson,B.
Plenum Press, New York, (1981), 45-49

Baggio,B.; Gambaro,G.; Cicerello,E.; Mastrosimone,S.; Marzaro,G.; Borsatti,A.; Pagano,F.
"Urinary excretion of glycosaminoglycans in urological disease"
Clin.Biochem. (1987), **20**, 449-450

Baggio,B.; Gambaro,G.; Olivia,O,; Favaro,S.; Borsatti,A.
"Calcium oxalate nephrolithiasis: an easy way to detect an imbalance between promoting and inhibitory factors"
Clin.Chim.Acta (1982), **124**, 149

Baker,L.R.I.; Mallinson,W.J.W.
"Dietary treatment of idiopathic hypercalciuria"
Br.J.Urol. (1979), **51**, 181-183

Barcelo,P.; Wuhl,O.; Servitge,E.; Rousaud,A.; Pak,C.Y.C.
"Randomised double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis"
J.Urol. (1993), **150**, 1761-1764

Bataille,P.; Gregoire,J.; Charransol,G.; Pruna,A.; Coevoet,B.; Fournier,A.
"Increased probability of forming stones with a simple calcium restriction in idiopathic hypercalciuria"
Contr.Nephrol. (1984), **37**, 17-21

Baumann,J.M.
"Can the formation of Calcium Oxalate stones be explained by crystallisation processes in urine?"
Urol.Res. (1985), **13**, 267-270

Baumann,J.M.
"How reliable are the measurements of crystallisation conditions in urine?"
Urol.Res. (1988a), **16**, 133-135

Baumann,J.M.
"How to measure crystallisation conditions in urine. A comparison of 7 methods"
Urol.Res. (1988b), **16**, 137-142

Baumann,J.M.
"Methods for crystallisation studies - Introduction"
in: "Urolithiasis: Consensus and Controversies"
eds: Rao,P.N.; Kavanagh,J.P.; Tiselius,H.G. Proc.6th Euro.Symp.Urolithiasis, Manchester, South Manchester, Univ.Hosp. (1995), 57-63

Baumann,J.M.; Ackermann,D.; Affolter,B.
"Rapid method of measuring the inhibition of Calcium-Oxalate monohydrate growth in urine"
Urol.Res. (1990), **18**, 219-222

Bek-Jensen,H.; Tiselius,H.G.
"Inhibition of calcium oxalate crystallisation by urinary macromolecules"
Urol.Res. (1991), **19**, 165-169

Berg,C.; Larsson,L.; Tiselius,H.G.
"Effects of different doses of alkaline citrate on urine composition and crystallisation of calcium oxalate"
Urol.Res. (1990), **18**, 13-16

Berg,C.; Larsson,L.; Tiselius,H.G.
"The effects of a single evening dose of alkaline citrate on urine composition and calcium stone formation"
J.Urol. (1992), **148**, 979-985

Berg,C.; Tiselius,H.G.
"The effects of citrate on hydroxyapatite induced calcium oxalate crystallisation and on the formation of calcium phosphate crystals"
Urol.Res. (1989), **17**, 167-172

Better,O.S.; Melamud,A.; Shabtai,M.; Berenheim,J.; Chaimowitz,C.
"Studies on the pathogenesis of increased incidence of nephrolithiasis in lifeguards in Israel"
Clin.Res. (1978), **26**, 126

Bichler,K.H.; Kirchner,Ch.; Ideler,V.
"Uromucoid excretion of normal individuals and stone formers"
Br.J.Urol. (1976), **47**, 733-738

Bijvoet,O.L.M.; Blomen,L.J.M.J.; Will,E.J.; van der Linden,H.
"Growth kinetics of calcium oxalate monohydrate. III. Variation of solution composition"
J.Cryst.Growth (1983), **64**, 316

Blacklock,N.J.
"The pattern of urolithiasis in the Royal Navy"
in:"Renal Stone Research Symposium"
eds: Hodgkinson,A; Nordin,B.E.C. J&A Churchill Ltd London (1969), 13-47

Blacklock,N.J.
"Epidemiology of urolithiasis"
in:"Scientific foundations of urology" 2nd Edition
eds: Chisholm,G.D.; Williams,D.I. Heineman, London (1982), 235-243

Blomen,L.J.M.J.
"Growth and agglomeration of CaOx monohydrate crystals"
PhD Thesis Univ.Hosp.Leiden, Amsterdam, (1982)

Blomen,L.J.M.J.; Will,E.J.; Bijvoet,O.L.M.; van der Linden,H.
"Growth kinetics of calcium oxalate monohydrate II. The variation of seed concentration"
J.Cryst.G. (1983), **64**, 316-325

Bommmer,J.; Ritz,E.; Tschöpe,W.; Waldherrr,R.; Gebhardt,M.
"Urinary matrix calculi consisting of microfibrillar protein in patients on maintenance hemodialysis"
Kid.Int.(1979), **16**, 722-728

Bone,H.G.; Zerwelch,J.E.; Britton,F.; Pak,C.Y.C.
"Treatment of calcium urolithiasis with diphosphonate:efficacy and hazards"
J.Urology (1979), **121**, 568-571

Bowyer,R.C.; Brockis,J.G.; McCulloch,R.K.
"Glycosaminoglycans as inhibitors of Calcium Oxalate crystal growth and aggregation"
Clin.Chim.Acta (1979), **95**, 23-28

Boyce,W.H.
"Organic matrix of human urinary concretions"
Amer.J.Med. (1968), **45**, 673-683

Boyce,W.H.
"Organic matrix of native human urinary concretions"
in: "Renal Stone Research Symposium"
eds: Hodgkinson,A; Nordin,B.E.C.
London, J&A Churchill Ltd. (1969), 93-102

Boyce,W.H.; Garvey,F.K.
"The amount and nature of the organic matrix in urinary calculi:review"
J.Urol. (1956), **76** (3), 213-227

Boyce,W.H.; Garvey,F.K.; Norfleet,C.M.
"Ion-binding properties of electrophoretically homogeneous mucoproteins of urine in normal subjects and in patients with renal calculus disease"
J.Urol. (1954), **72** (6), 1019-1031

Boyce,W.H.; Garvey,F.K.; Norfleet,C.M. Jr
"The metal chelate compounds of urine"
Am.J.Med. (1955), **19** (1), 87-95

Boyce,W.H.; King,J.S.Jr
"Present concepts concerning the origin of matrix and stones"
Ann.N.Y.Acad.Sci. (1963), **104**, 563-578

Boyce,W.H.; McKinney,W.M.; Long,T.T.; Drach,G.W.
"Oral administration of methylene blue to patients with renal calculi"
J.Urol. (1967), **97**, 783-789

Boyce,W.H.; Swanson,M.
"Biocolloids of urine in health and in calculus disease II. Electrophoretic and biochemical studies of mucoprotein insoluble in molar sodium chloride"
J.Clin.Invest. (1955), **34**, 1581-1589

Brecevic,L.; Garside,J.
"On the measurement of crystal size distributions in the micrometer size range"
Chem.Eng.Sci. (1981), **36**, 867-869

Brecevic,Lj.; Kralj,D.; Garside,J.
"Factors influencing the distribution of hydrates in calcium oxalate precipitation"
J.Cryst.G. (1989), **970**, 460-468

Brecevic,Lj.; Skrtic,D.; Garside,J.
"Transformation of Calcium Oxalate hydrates"
J.Cryst.Growth (1986), **74**, 399-408

Brecht,E.; Moll,V.; Neisus,D.; Ziegler,M.
"Treatment of prevesical ureteral calculi by ESWL"
J.Urol. (1988), **139**, 916-918

Breslau,N.A.; Brinkley,L.; Hill,K.D.; Pak,C.Y.C.
"Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism"
J.Clin.Endocrin.Metab. (1988), **66** (1), 140

Brown,C.M.; Ackermann,D.K.; Purich,D.L.
"EQUIL93: a tool for experimental and clinical urolithiasis"
Urol.Res.(1994), **22**, 119-126

Burns,J.R.; Finlayson,B.
"The effect of seed crystals on calcium oxalate nucleation"
Invest.Urol. (1980), **18** (2), 133-136

Burns,J.R.; Finlayson,B.; Gauthier,J.
"Calcium Oxalate retention in subjects with crystalluria"
in: "Urinary Stone" Proc. of 2nd International urinary stone conference" eds: Ryall.R.; Marshall,V.; Brockis,J.G.; Finlayson,B. Churchill Livingstone, London England (1983), 253-257

Butt,A.J.
"Etiologic factors in renal lithiasis"
Thomas,C.C., Springfield, Illinois.
(1956), 3-47

Butz,M.
"Rational prevention of calcium urolithiasis"
Urol.Int. (1986), **41**, 387-392

Butz,M.; Dulce,H.J.
"Enhancement of urinary citrate in oxalate stone formers by the intake of alkaline salts"
in: "Urolithiasis: Clinical and basic research" eds: Smith,L.H.; Robertson,W.G.; Finlayson,B.
Plenum Press New York (1981), 881-884

Cambell,A.A.; Ebrahimpour,A.; Perez,L.; Smesko,A.; Nancollas,G.H.
"The dual role of polyelectrolytes and proteins as mineralization promoters and inhibitors of calcium oxalate monohydrate"
Calcif.Tissue Int. (1989), **45**, 122-128

Carmona,P.; Rodriguez,M.L.
"Hydrogen bonds between protein side chains and phosphates and their role in biological calcification"
Biophys.Chem. (1987), **28**, 161-167

Carr,J.A.
"The pathology of urinary calculi. Radial Striation"
Br.J.Urol. (1954), **25**, 26-32

Catalina,F.; Cifuentes,L.
"Calcium Oxalate:Crystallographic analysis in solid aggregate in urinary sediment"
Science (1970), **1969**, 183

Caudarella,R.; Stefani,F.; Rizzoli,E.; Malavolta,N.; D'Autuono,G.
"Preliminary results of glycosaminoglycans excretion in normal and stone forming subjects: relationship with uric acid excretion"
J.Urol. (1983), **129**, 665-667

Chaussy,Ch.
"ESWL, past, present and future"
J.Endourol. (1988), **2**, 97-105

Chaussy,C.; Brendel,W.; Schmiedt,E.
"Extracorporeally induced destruction of kidney stones by shock waves"
Lancet (1980), **2**, 1265-1268

Chaussy,C.; Schmiedt,E.
"Shock wave treatment for stones in the upper urinary tract"
Urol.Clin.N.Amer. (1983), **10**, 743-750

Chaussy,C.; Schmiedt,E.; Jocham,D.; Brendel,W.; Forssmann,B.; Walther,V.
"First clinical experience with extracorporeally induced destruction of kidney stones by shock waves"
J.Urol. (1982), **127**, 417-423

Churchill,D.; Bryant,D.; Fodor,G.; Gault,M.H.
"Drinking water hardness and urolithiasis"
Ann.Int.Med. (1978), **88**, 513-514

Clarke,E.
"A history of the stone"
Hosp,.Med. (1968), **2** (June), 1054-1057

Coe,F.L.
"Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder"
Annals of Internal Medicine (1977), **87**, 404-410

Coe,F.L.; Kavalach,A.G.
"Hypercalciuria and hyperuricosuria in patients with calcium nephrolithiasis"
N.Engl.J.Med. (1974), **291**, 1344-1350

Coe,F.L.; Lawton,R.L.; Goldstein,R.B.; Tembe,V.
"Sodium urate accelerates precipitation of calcium oxalate in vitro"
Proc.Soc.Exp.Bio.Med. (1975), **149**, 926

Coe,F.L.; Raisen,L.
"Allopurinol treatment of uric-acid disorders in calcium stone formers"
Lancet (1973), **1**, 129-131

Constantinides,C.; Recker,F.; Jaeger,P.; Hauri,D.
"Extracorporeal shock wave lithotripsy as monotherapy of staghorn renal calculi: 3 years of experience"
J.Urol. (1989), **142**, 1415-1418

Cramer,W.
"Experimental production of kidney lesions by diet"
Lancet (1932), **2**, 174

Crawford,J.E.; Crematy, E.P.; Alexander,A.E.
"The effect of natural and synthetic polyelectrolytes on the crystallisation of Calcium Oxalate"
Aust.J.Chem. (1968), **21**, 1067-1072

Curhan,G.C.; Willet,W.C.; Rimm,E.B.; Stampfer,M.J.
"A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones"
N.Engl.J.Med. (1993), **328**, 833-838

Danielson,B.
"Drugs against kidney stones: effects of magnesium and alkali"
In: "Urolithiasis and related clinical research" eds: Schwille,P.O.; Smith,L.H.; Robertson,W.G.; Vahlensieck,W. Plenum Press, New York, (1985), 525-532

Dawnay,A.B.St.J.; Thornley,C.; Cattell,W.R.
"An improved radio-immunoassay for urinary Tamm-Horfall glycoprotein"
Biochem.J. (1982), **206**, 461-465

Deganello,S.
"The interaction between nephrocalcin and Tamm-Horsfall proteins with calcium oxalate dihydrate"
Scann.Micros. (1993), **7** (3), 1111-1118

Dent,C.E.; Sutor,D.J.
"Presence or absence of inhibitors of Calcium Oxalate crystal growth in urine of normals and stone formers"
Lancet, (1971), **2**, 775-778

Desmars,J.F.; Tawashi,R.
"Dissolution and growth of Calcium Oxalate monohydrate. I. Effect of Magnesium and pH"
Biochim.Biophys.Acta (1973), **313**, 256-267

Dhanalekshmy,T.G.; Aravindakshan,C.; Sindhu,S.; Roshni,S.V.; Vathsala,R.K.; Fazil Marickar,Y.M.
"Natural urine versus synthetic urine for studying inhibitors"
in: "Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis eds: Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.M.; Smith,L.H.; Walker,V.R. Plenum Press, New York, London, (1994), 234

Dirksen,J.A.; Ring,T.A.
"Fundamentals of crystallisation: kinetic effects on particle size distributions and morphology. Review article number 34"
Chem.Eng.Sci. (1991), **46** (10), 2389-2427

Donaldson,D.; Pryce,J.D.; Rose,G.A.; Tovey,J.E.
"Tap water calcium and its relationship to renal calculi and 24h urinary calcium output in Great Britain"
Urol.Res. (1979), **7**, 273-276

Doyle,I.R.; Marshall,V.R.; Dawson,C.J.; Ryall,R.L.
"Calcium oxalate crystal matrix extract: the most potent macromolecular inhibitor of crystal growth and aggregation yet tested in undiluted human urine in vitro"
Urol.Res. (1995), **23**, 53-62

Doyle,I.R.; Ryall,R.L.; Marshall,V.R.
"The effect of low-speed centrifugation and Millipore filtration on the urinary protein content"
in: "Urolithiasis" eds: Walker,V.R.; Sutton,R.A.L.; Cameron,E.C.B.; Pak,C.Y.C.; Robertson,W.G.
Plenum Press, New York, London, (1989), 593

Doyle,I.R.; Ryall,R.L.; Marshall,V.R.
"Inclusion of proteins into calicum oxalate crystals precipitated from human urine: a highly selective phenomenon"
Clin.Chem.(1991), **37** (9), 1589-1594

Drach,G.W.; Kraljevich,Z.; Randolph,A.D.
"Effects of high molecular weight urinary macromolecules on crystallisation of calcium oxalate dihydrate"
J.Urol. (1982), **127**, 805-810

Drach,G.W.; Randolph,A.D.; Miller,J.D.
"Inhibition of calcium oxalate dihydrate crystallisation by chemical modifiers: I. Pyrophosphate and methylene blue"
J.Urol. (1978), **119**, 99-103

Drach,G.W.; Thorson,S.; Randolph,A.
"Effects of urinary organic macromolecules on crystallisation of Calcium Oxalate: enhancement of nucleation"
J.Urol. (1980), **123**, 519-523

Ebisuno,S.; Kohjimoto,Y.; Yoshida,T.; Ohkawa,T.
"Effect of urinary macromolecules on aggregation of calcium oxalate in recurrent calcium stone formers and healthy controls"
Urol.Res. (1993), **21**, 265-268

Edyvane,K.A.; Hibberd,C.M.; Harnett,R.M.; Marshall,V.R.; Ryall,R.L.
"Macromolecules inhibit Calcium Oxalate crystal growth and aggregation in whole human urine"
Clin.Chim.Acta (1987a), **167**, 329-338

Edyvane,K.A.; Ryall,R.L., Mazzachi,R.D.; Marshall,V.R.
"The effect of serum on the crystallisation of Calcium Oxalate in whole human urine:Inhibition disguised as apparent promotion"
Urol.Res. (1987b), **15**, 87-92

Elliot,J.S.; Ribeiro,M.
"Calcium Oxalate solubility in urine: The state of relative saturation"
Invest.Urol. (1967), **5** (3), 239-243

Elwell,D.; Scheel,M.J.
"Crystal growth from high temperature solutions"
Academic Press, London, (1975)

Embon,O.M.; Rose,G.A.; Rosenbaum,T.
"Chronic dehydration stone disease"
Br.J.Urol. (1990), **66**, 357-362

Evans,R.A.; Forbes,M.A.; Sutton,R.A.L.; Watson,L.
"Urinary excretion of calcium and magnesium in patients with calcium containing renal stone"
Lancet (1957), **4**, 958

Felix,R.; Monod,A.l Broge,L.; Hansen,N.M.; Fleisch,H.
"Aggregation of Calcium Oxalate crystals: effect of urine and various inhibitors"
Urol.Res. (1977), **5**, 21-28

Fellström,B.; Backman,U.; Danielson,B.G.; Holmgren,K.; Johansson,G.; Lindsjo,M.; Ljunghall,S.; Wikström,B.
"Allopurinol treatment of renal calcium stone disease"
Br.J.Urol. (1985b), **57**, 357-379

Fellström,B.; Backman,U.; Danielson,B.G.; Holmgren,K.; Ljunghall,S.; Wikström,B.
"Inhibitory activity of human urine on Calcium Oxalate crystal growth: effects of sodium urate and uric acid"
Clin.Sci. (1982), **62**, 509-514

Fellström,B.; Danielson,B.; Ljunhall,S.; Wikström,B.
"The inhibition of calcium oxalate crystal growth by chondroitin sulphate, heparin, pentosan polysulphate and Tamm-Horsfall glycoprotein"
in:"Urolithiasis and related clinical research"
eds: Schwille,P.O.; Smith,L.H.; Robertson,W.G.; Vahlensieck,W. Plenum Press, New York, (1985a), 887

Fellström,B.; Danielson,B.; Ljunhall,S.; Wikström,B.
"Crystal inhibition: The effects of polyanions on Calcium Oxalate crystal growth"
Clin.Chim.Acta (1986), **158**, 229-235

Ferrie,B.G.; Scott,R.
"Occupation and urinary tract stone disease"
Urology (1984), **24**, 443-445

Fetner,C.D.; Barilla,D.E.; Townsend,J.; Pak,C.Y.C.
"Effects of magnesium oxide on the crystallisation of calcium salts in urine in patients with recurrent nephrolithiasis"
J.Urol. (1978), **120**, 399-401

Finlayson,B.
"The concept of a continuous crystalliser"
Invest.Urol. (1972), **9** (4), 258-263

Finlayson,B.
"Renal lithiasis in review"
Symp.Renal Lithiasis Urologic Clinics of North America (1974), **1** (2), 181-211

Finlayson,B.
"Where and how does urinary stone disease start? An essay on the expectation of free and fixed-particle urinary stone disease"
in: "Idiopathic Urinary Bladder Stone Disease"
Fogarty Intern,Center.Proc.no37 U.S.Government printing office Washington D.C. (1977a), 7-31

Finlayson,B.
"Calcium stones: some physical and clinical aspects"
in:"Calcium Metabolism in Renal Failure and
Nephrolithiasis" ed:David,D.S. New York, John Wiley and
Sons (1977b), 337-382

Finlayson,B.
"Physiochemical aspects of urolithiasis"
Kid.Int. (1978), **13**, 344-360

Finlayson,B.; Dubois,L.
"Adsorption of heparin on sodium acid urate"
Clin.Chim.Acta (1978), **84**, 203-206

Finlayson,B.; Khan,S.R.; Hackett,R.L.
"Mechanisms of stone formation - an overview"
Scan.Elec.Micros. (1984), II, 1419-1425

Finlayson,B.; Miller,G.H.
"Urine ion equilibrium - a numerical approach demonstrated
by application to antistone therapy"
Invest.Urol. (1969), **6**, 428

Finlayson,B.; Reid,F.
"The effect of allopurinol on Calcium Oxalate (whewellite)
precipitation"
Invest.Urol. (1978a), **15**, 489-492

Finlayson,B.; Reid,F.
"The expectation of free and fixed particles in urinary
stone disease"
Invest.Urol. (1978b), **15** (6), 442-448

Finlayson,B.; Vermeulen,C.W.; Stewart,E.J.
"Stone matrix and mucoprotein from urine"
J.Urol. (1961), **86** (4), 355-363

Fleisch,H.
"Some new concepts on the pathogenesis and the treatment of
urolithiasis"
Urol.Intern. (1965), **19**, 372-389

Fleisch,H.
"Inhibitors and promoters of stone formation"
Kid.Int. (1978), **13**, 361-371

Fleisch,H.; Bisaz,S.
"The inhibitory effect of Pyrophosphate on Calcium Oxalate
precipitation and its relation to urolithiasis"
Experientia (1964), **20**, 276-277

Fox,M.
"The natural history and significance of stone formation in
the prostrate gland"
J.Urol. (1963), **89**, 716-727

Foye,W.O.; Hong,H.S.; Kim,C.M.; Prien,E.L.
"Degree of sulfation in mucopolysaccharide sulfates in
normal and stone-forming urines"
Invest.Urol. (1976), **14** (1), 33-37

Francis,M.D.
"The inhibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates"
Calcified Tissue Res. (1969), **3**, 151-162

Francois,B.; Cahen,R.; Pascal,B.
"Inhibitors of urinary stone formation in 40 recurrent stone formers"
Br.J.Urol. (1986), **58**, 479-483

Fraser,D.; Russell,R.G.G.; Pohler,O.; Robertson,W.G.; Fleisch,H.
"The influence of disodium ethane-1-hydroxy-1,1-diphosphonate (EDHP) on the development of experimentally induced urinary stones in rats"
Clin.Sci. (1972), **42**, 197-207

Fuss,M.; Simon,J.; Fontinoy,N.; Coussaert,E.
"High flow-low calcium intake: not all renal stone formers adhere to this simple treatment"
Eur.Urol. (1979), **5**, 97-99

Gardner,G.L.; Doremus,R.H.
"Crystal growth inhibitors in human urine. Effect on Calcium Oxalate kinetics"
Invest.Urol. (1978), **15** (6), 478-484

Gardner,R.P.; Verghese,I.C.; Rousseau,R.W.
"On the feasibility of using a radioactive tracer method and a size-discretized mass balance model for the measurement of growth rates in MSMPR crystallisers"
In: "Fine Particles Processing Vol 1" ed: Somasundaran,P. Amer.Inst. Mining, metallurgical and Petroleum Engineers Inc., Proc.Int.Symp.Fine Particles Processing (1980), 51-73

Garside,J.
"Review Article number 15. Industrial crystallisation from solution"
Chem.Eng.Sci. (1985), **40** (1), 3-26

Garside,J.; Davey,R.J.
"Secondary contact nucleation: kinetics, growth and scale-up"
Chem.Engng.Commun. (1980), **4**, 393-424

Garside,J.; Jancic,S.J.
"Growth and dissolution of potash alum crystals in the subsieve size range"
AIChE.J. (1976), **22**, 887-894

Garside,J.; Larson,M.A.
"Direct observation of secondary nuclei production"
J.Cryst.Growth (1978), **43**, 694-704

Garside,J.; Rusli,I.T.; Larson,M.A.
"Origin and size distribution of secondary nuclei"
AIChE.J. (Jan 1979), **25** (1), 57-64

Garside,J.; Shah,M.B.
"Crystallisation kinetics from MSMPR crystallisers"
Ind.Eng.Chem. Process Des.Dev. (1980), **19**, 509-514

Gaur,S.S.; Nancollas,G.H.
"Kinetics of crystal growth in urine"
Kid.Int. (1984), **26**, 767-768

Gianotti,M.; Genestar,C.; Palou,A.; Pons,A.; Conte,A.; Grases,F.
"Investigation of GAGS on 24-hour and 2-hour urines from Calcium Oxalate stone formers and healthy subjects"
Int.Urol.Nephrol. (1989), **21** (3), 281-288

Gill,W.B.; Silvert,M.A.; Roma,M.J.
"Supersaturation levels and crystallisation rates of Calcium Oxalate from urine of normal humans and stone formers determined by a ^{14}C-Oxalate technique"
Invest.Urol, (1974), **12**, 203-209

Gjaldbaek,J Chr.
"Inhibition of Chondroitin Sulphate and Heparin on the growth and agglomeration of Calcium Oxalate monohydrate crystals in vitro"
Clin.Chim.Acta (1982), **120**, 363-365

Gjaldbaek,J.; Robertson,W.G.
"Does urine from stone formers contain macromolecules which promote the crystal growth rate of calcium oxalate crystals in vitro?"
Clin.Chim.Acta (1980), **108**, 75-80

Gohel,M.D.; Shum,D.K.; Li,M.K.
"The dual effect of urinary macromolecules on the crystallisation of calcium oxalate endogenous in urine"
Urol.Res. (1992), **20**, 13-17

Goldberg,J.M.; Cotlier,E.
"Specific isolation and analysis of mucopolysaccharides (glycosaminoglycans) from human urine"
Clin.Chim.Acta (1972), **41**, 19-27

Goldfarb,S.
"Diet and nephrolithiasis"
Annu.Rev.Med. (1994), **45**, 235-243

Grant,A.M.S.; Baker,L.R.I.; Neuberger,A.
"Urinary Tamm-Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi"
Clin.Sci. (1973), **44**, 377-384

Grases,F.; Conte,A.
"Urolithiasis, inhibitors and promoters"
Urol.Res. (1992), **20**, 86-88

Grases,F.; Costa-Bauza,A.
"Study of factors affecting calicum oxalate crystalline aggregation"
Br.J.Urol. (1990), **66**, 240-244

Grases,F.; Costa-Bauza,A.; March,J.G.; Masarova,L.
"Glycosaminoglycans, uric acid and calcium oxalate urolithiasis"
Urol.Res. (1991), **19**, 375-380

Grases,F.; Genestar,C.; Conte,A.; March,P.; Costa-Bauza,A.
"Inhibitory effect of pyrophosphate, citrate, magnesium and chondroitin sulphate in calcium oxalate urolithiasis"
Br.J.Urol. (1989), **64**, 235-237

Grases,F.; Genestar,C.; March,P.; Conte,A.
"Variations in the activity of urinary inhibitors in Calcium Oxalate urolithiasis"
Br.J.Urol. (1988), **62**, 515-520

Grover,P.K.; Marshall,V.R.; Ryall,R.L.
"Tamm-Horsfall mucoprotein reduces promotion of calcium oxalate crystal aggregation induced by urate in human urine in vitro"
Clin.Sci. (1994), **87**, 137-142

Grover,P.K.; Ryall,R.L.; Marshall,V.R.
"Does Tamm-Horsfall mucoprotein inhibit or promote Calcium Oxalate crystallisation in human urine?"
Clin.Chim.Acta (1990a), **190**, 223-238

Grover,P.K.; Ryall,R.L.; Marshall,V.R.
"Effect of urate on calcium oxalate crystallisation in human urine: evidence for a promotory role of hyperuricosuria in urolithiasis"
Clin.Sci. (1990c), **79**, 9-15

Grover,P.K.; Ryall,R.L.; Marshall,V.R.
"Calcium oxalate crystallisation in urine: role of urate and glycosaminoglycans"
Kid.Int. (1992), **41**, 149-154

Grover,P.K.; Ryall,R.L.; Marshall,V.R.
"Dissolved urate promotes calcium oxalate crystallisation: epitaxy is not the cause"
Clin.Sci. (1993), **85**, 303-307

Grover,P.K.; Ryall,R.L.; Potezny,N; Marshall,V.R.
"The effect of decreasing the concentration of urinary urate on the crystallisation of Calcium Oxalate in undiluted human urine"
J.Urol. (1990b), **143**, 1057-1061

Hallson,P.C.; Rose,G.A.
"Seasonal variations in urinary crystals"
Biol.J.Urol. (1977), **49**, 277-284

Hallson,P.C.; Rose,G.A.
"A new urinary test for stone "activity""
Br.J.Urol. (1978), **50**, 442-448

Hallson,P.C.; Rose,G.A.
"Uromucoids and urinary stone formation"
Lancet, (1979), **1**, 1000-1002

Hallson,P.C.; Rose,G.A.
"Chemical measurement of Calcium Oxalate crystalluria: Results in various causes of Calcium urolithiasis"
Urol.Int. (1990), **45**, 332-335

Hallson,P.C.; Rose,G.A.; Samuell,C.T.
"The role of uromucoids in urinary stone formation"
in:"Urinary Calculus" eds:Brockis,J.G.; Finlayson,B.
PSG Publishing, Littleton, Mass. (1981), 285

Hallson,P.C.; Rose,G.A.; Sulaiman,S.
"Raising urinary citrate lowers calcium oxalate and calcium phosphate crystal formation in whole urine"
Urol.Int. (1983), **38**, 179-181

Harrill,J.A.; Stanton-King,J.; Boyce,W.H.
"Structure and composition of salivary calculi"
Laryngoscope (1959), **LXIX**, 481-492

Hastings,B.A.; McLeon,F.C.; Eichelberger,L.; Hall,J.L.; Da Costa,E.
"The ionization of Calcium, Magnesium and Strontium citrates"
J.Biol.Chem. (1934), **107**, 351-370

Hess,B.
"The role of Tamm-Horsfall glycoprotein and nephrocalcin in calcium oxalate monohydrate crystallisation processes"
Scann.Micros. (1991), **5** (3), 689-696

Hess,B.
"Tamm-Horsfall glycoprotein - inhibitor or promotor of Calcium Oxalate monohydrate crystallisation processes?"
Urol.Res. (1992), **20**, 83-86

Hess,B.; Kok,D.J.
"Nucleation, growth and aggregation of stone-forming crystals"
in:"Kidney Stones: Medical and Surgical Management"
eds:Coe,F.L.; Favus,M.J.; Pak,C.Y.C.; Parks,H.; Preminger,G.M.
Lippincott-Raven Publishers, Philadelphia, (1996), 3-32

Hess,B.; Nakagawa,Y.; Coe,F.L.
"Inhibition of Calcium Oxalate monohydrate crystal aggregation by urine proteins"
Am.J.Physiol. (1989), **257**, F99-F106

Hess,B.; Nakagawa,Y.; Parks,J.H.; Coe,F.L.
"Molecular abnormality of Tamm-Horsfall glycoprotein in calcium oxalate nephrolithiasis"
Am.J.Physiol. (1991), **260**, F569-F578

Hesse,A.
"Dietary evaluation and dietary treatment"
in: "Urolithiasis: Consensus and Controversies" eds: Rao,P.N.; Kavanagh,J.P.; Tiselius,H.G. Proc.6th Eur.Symp.Urolithiasis Manchester, South Manchester Univ.Hosp. (1995), 163-168

Hesse,A; Classen,A.; Knoll,M.; Timmermann,F.; Vahlensieck,W.
"Dependence of urine composition on the age and sex of healthy subjects"
Clin.Chim.Acta (1986a), **160**, 79-86

Hesse,A.; Klocke,K.; Nolde,A.; Vahlensieck,W.
"Advances in medical treatment of renal stones"
Urol.Res. (1992), **20**, 90-93

Hesse,A.; Seiner,R.; Heynck,H.; Jahnen,A.
"The influence of dietary factors on the risk of urinary stone formation"
Scann.Micros. (1993), **7** (3), 1119-1128

Hesse,A.; Wuzel,H.; Vahlensieck,W.
"The excretion of glycosaminoglycans in the urine of calcium oxalate stone patients and healthy persons"
Urol.Int. (1986b), **41**, 81-87

Hesse,A.; Wuzel,H.; Vahlensiek,W.
"Significance of glycosaminoglycans for the formation of Calcium Oxalate stones"
Am.J.Kid.Diseases, (1991), **17** (4), 414-419

Hesse,A.; Wurzel,H.; Krampitz,G.; Vahlensieck,W.
"Experimental determination of the kinetics of calcium-binding with Chondroitin Sulphate and the effects of uric acid on this process"
Urol.Res. (1987), **15**, 93-97

Hodgkinson,A.
"Solubility of Calcium Oxalate in human urine, simulated urine, and water"
Invest.Urol. (1980), **18**, 123-126

Hofbauer,J.; Hobarth,K.; Szabo,N.; Margerger,M.
"Alkali citrate prophylaxis in idiopathic recurrent calcium oxalate urolithiasis - a prospective randomized study"
Br.J.Urol. (1994), **73**, 362-365

Howard,J.E.; Thomas,W.C.
"Some observations on rachitic rat cartilage of probable significance to the etiology of renal calculi"
Trans.Am.Clin.Climatol.Assoc. (1958), **70**, 94-102

Hulburt, H.M.
"Perspectives on crystallisation in chemical process technology"
AIChE.Symp.Ser. (1984), **80** (240), 1-14

Hwang,I.S.; Preminger,G.M.; Poindexter,J.; Pak,C.Y.C.
"Urinary glycosaminoglycans in normal subjects and patients with stones"
J.Urol. (1988), **139**, 995

Iwata,H.; Iio,S.; Nishio,S.; Takeuchi,M.
"Architecture of mixed calcium oxalate dihydrate and monohydrate stones"
Scann.Micros. (1992), **6**, 231-238

Jacobs,S.C.; Gittes,R.F.
"Dissolution of residual renal calculi with hemiacidrin"
J.Urol. (1976), **115**, 2-4

Jaeger,P.; Portmani,L.; Jacquet,A.F.; Burckhardt,P.
"Drinking water for stone formers: Is the calcium content relevant?"
Eur.Urol. (1984), **10**, 53-54

Jagannathan,R.; Wey,J.S.
"Diffusion-controlled growth in a crowded environment"
J.Cryst.Growth (1981), **51**, 601-606

Johansson,G.; Backman,U.; Danielson,B.G.; Fellström,B.; Ljunghall,S.; Wikström,B.
"Biochemical and clinical effects of the prophylactic treatment of renal calcium stones with magnesium hydroxide"
J.Urol. (1980a), **124**, 770-774

Johansson,G.; Backman,U.; Danielson,B.G.; Ljunghall,S.; Wikström,B.
"Magnesium metabolism in renal stone disease"
Invest.Urol. (1980b), **18** (2), 93-96

Juuti,M.; Heinonen,O.P.
"Incidence of urolithiasis and composition of household water in southern Finland"
Scand.J.Urol.Nephrol. (1980), **14**, 181-187

Kavanagh,J.P.
"Methods for the study of Calcium Oxalate crystallisation and their application to urolithiasis research"
Scann.Microsc. (1992), **6**, 685-705

Kavanagh,J.P.
"The kidney as a mixed suspension mixed product removal (MSMPR) crystallization chamber"
in: "Urolithiasis: Consensus and Controversies"
Proc.6th Eur.Symp.Urolithiasis. Manchester, South Manchester University Hospitals (1995a), 89-97

Kavanagh,J.P.
"Laboratory methods - are they good enough?"
in: "Urolithiasis: Consensus and Controversies"
Proc.6th Eur.Symp.Urolithiasis eds: Rao,P.N.; Kavanagh,J.P.; Tiselius,H.G. Manchester, South Manchester Univ.Hosp. (1995b), 173-178

Kavanagh,J.P.; Nishio,S.; Garside,J.; Blacklock,N.J.
"Crystallisation kinetics of Calcium Oxalate in fresh, minimally diluted urine: Comparison of recurrent stone formers and healthy controls in a continuous mixed suspension mixed product removal crystalliser"
J.Urol. (1993), **149**, 614

Keyser,L.D.
"The mechanism of the formation of urinary calculi"
Ann.Surg. (1923), **77**, 210-222

Khan,S.R.
"Pathogenesis of oxalate urolithiasis: lessons from experimental studies with rats"
Am.J.Kid.Diseases (1991), **17** (4), 398-401

Khan,S.R.; Finlayson,B.; Hackett,R.L.
"Stone matrix as proteins adsorbed on crystal surfaces: a microscopic study"
in:"Scanning Electron Microscopy" SEM Inc. AMF O'Hare Chicago, (1983), 379-385

Khan,S.R.; Hackett,R.L.
"Crystal-matrix relationships in experimentally induced calcium oxalate monohydrate crystals, an ultrastructural study"
Calcif.Tissue Int. (1987a), **41**, 157-163

Khan,S.R.; Hackett,R.L.
"Role of scanning electron microscopy and x-ray microanalysis in the identification of urinary crystals"
Scann.Micros. (1987b), **1** (3), 1405-1411

Khan,S.R.; Shevock,P.N.; Hackett,R.L.
"In-vitro precipitation of calcium oxalate in the presence of whole matrix or lipid components of urinary stones"
J.Urol. (1988), **139**, 418-422

Khan,S.R.; Shevock,P.N.; Hackett,R.L.
"Membrane-associated crystallisation of calcium oxalate in vitro"
Calcif.Tissue Int. (1990), **40**, 116-120

Khazziani,M.K.; Khanam,A.; Jaffer Naqvi,S.A.; Sheikh,M.A.
"Study of potassium citrate treatment of crystalluric nephrolithiasis"
Biomed.Pharmacother. (1993), **47** (1), 25-28

King,J.S.
"Etiologic factors involved in urolithiasis: a review of recent research"
J.Urol. (1967), **97**, 583-591

Kitamura,T.; Pak,C.
"Tamm and Horsfall glycoprotein does not promote spontaneous precipitation and crystal growth of calcium oxalate in vitro"
J.Urol. (1982), **127**, 1024-1026

Kitamura,T.; Zerwekh,J.E.; Pak,C.Y.
"Partial biochemical and physicochemical characterization of organic macromolecules in urine from patients with renal stones and control subjects"
Kid.Int. (1982), **21**, 379-386

Koenig,K.; Padalino,P.; Alexandrides,G.; Pak,C.Y.C.
"Bioavailability of potassium and magnesium and citraturic response from potassium-magnesium citrate"
J.Urol. (1991), **145**, 330-334

Kohler,F.P.; Uhle,C.A.
"Oral magnesium intake and its effect on various urinary constituents in the prophylaxis of urolithiasis"
J.Urol. (1966), **96**, 812-815

Kohri,K.; Garside,J.; Blacklock,N.J.
"The role of magnesium in calcium oxalate urolithiasis"
Br.J.Urol. (1988), **61**, 107-115

Kohri,K.; Garside,J.; Blacklock,N.J.
"The effect of glycosaminoglycans on the crystallisation of Calcium Oxalate"
Br.J.Urol. (1989), **63**, 584-590

Koide,T.; Takemoto,M.; Itatani,H.; Takaha,M.; Sonoda,T.
"Urinary macromolecular substances as natural inhibitors of calcium oxalate crystal aggregation"
Invest.Urol. (1981), **18**, 382-386

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.M.
"Excessive crystal agglomeration with low citrate excretion in recurrent stone formers"
Lancet (May 1986), 1056-1058

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.M.
"Low inhibition of crystal agglomeration and citrate excretion in recurrent calcium oxalate stone formers"
Contr.Nephrol. (1987), **58**, 73-77

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.M.
"Crystal agglomeration is a major element in Calcium Oxalate urinary stone formation"
Kid.Int. (1990), **37**, 51-56

Kok,D.J.; Papapoulos,S.E.; Blomen,L.J.; Bijvoet,O.L.
"Modulation of Calcium Oxalate monohydrate crystallisation kinetics in vitro"
Kid.Int. (1988), **34**, 346-350

Lanzalaco,A.C.; Sheehan,M.E.; White,D.J.; Nancollas,G.H.
"The mineralization inhibitory potential of urines: a constant composition approach"
J.Urol. (1982), **128**, 845-849

Lanzalaco,A.C.; Singh,R.P.; Smesko.S.A.; Nancollas,G.H.; Sufrin,G.; Binette,M.; Binette,J.P.
"The influence of urinary macromolecules on calcium oxalate monohydrate crystal growth"
J.Urol. (1988), **139**, 190-195

Larson,M.A.; Randolph,A.D.
"Size distribution analysis in continuous crystallisation"
AIChE.Symp.Ser. (1969), **65** (95), 1-13

Leumann,E.; Hoppe,B.; Neuhaus,T.
"Management of primary hyperoxaluria: efficacy of oral citrate administration"
Pediatr.Nephrol. (1993), **7** (3), 207-211

Leusmann,D.B.; Blaschke,R.; Schmandt,W.
"Results of 5035 stone analyses: a contribution to epidemiology of urinary stones"
Scand.J.Urol. (1990), **24**, 205-210

Lewis,A.M.; Thomas,W.C.Jr; Tomita,A.
"Pyrophosphate and the mineralizing potential of urine"
Clin.Sci. (1966), **30**, 389-397

Li,M.K.; Blacklock,N.J.; Garside,J.
"Effects of magnesium on calcium oxalate crystallisation"
J.Urol. (1985), **133**, 123-125

Ligabue,A.; Fini,M.; Robertson,W.G.
"Influence of urine on in vitro crystallisation rate of Calcium Oxalate: determination of inhibitory activity by a ^{14}C-oxalate technique"
Clin.Chem.Acta (1979), **98**, 39-46

Lonsdale,K.
"Human stones"
Science (1968a), **159**, 1199-1208

Lonsdale,K.
"Epitaxy as a growth factor in urinary calculi and gallstones"
Nature, (1968b), **217**, 56

Lynn,K.L.; Shenkin,A.; Marshall,R.D.
"Factors affecting excretion of human urinary Tamm-Horsfall glycoprotein"
Clin.Sci. (1982), **62**, 21-26

Lyon,E.S.; Borden,T.A.; Ellis,J.E.; Vermeulen,C.W.
"Calcium oxalate lithiasis produced by pyridoxine deficiency and inhibition with magnesium diets"
Invest.Urol. (1966), **4**, 133

MacManus,J.; Heaton,F.W.
"The effect of magnesium deficiency on calcium homeostasis in the rat"
Clin.Sci. (1969), **36**, 297

Mall,J.C.; Collins,P.A.; Lyon,E.S.
"Matrix Calculi"
Br.J.Radiol. (1975), **48**, 807-810

Mandel,N.S.; Mandel,G.S.
"Epitaxis between stone forming crystals at the atomic level"
in:"Urolithiasis Clinical and Basic Research"
eds: Smith,L.H.; Robertson,W.G.; Finlayson,B. Plenum Press, New York, (1981), 469

Markovic,M.; Komunjer,L.
"A new method to follow crystal growth by Coulter Counter"
J.Cryst.Growth (1979), **46**, 701-705

Marshall,B.W.; Cochran,M.; Hodgkinson,A.
"Relationship 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**, 91-99

Marshall,R.W.; Robertson, W.G.
"Nomograms for the estimation of the saturation of urine with Calcium Oxalate, Calcium Phosphate, Magnesium Ammonium Phosphate, Uric acid, Sodium acid urate, Ammonium acid urate and Cystine"
Clin.Chim.Acta (1976), **72**, 253-260

Mates,M.J.
"External factors in the genesis of urolithiasis"
in:"Renal Stone Research Symposium"
Churchill, London (1969), 59-64

Mayne,P.D.; Edwards,L.
"What on earth are we drinking?"
Br.J.Urol. (1990), **66**, 123-126

McGeown,M.
"Heredity in renal stone disease"
Clin.Sci. (1960), **19**, 465-471

McQueen,E.G.; Engel,G.B.
"Factors determining the aggregation of urinary mucoprotein"
J.Clin.Pathol. (1966), **19**, 392-396

Melnick,I.; Landes,R.R.; Hoffman,A.A.; Burch,J.F.
"Magnesium therapy for recurring calcium oxalate urinary calculi"
J.Urol. (1971), **105**, 119-122

Meyer,J.L.; Smith,L.H.
"Growth of Calcium Oxalate crystals. I. A model for urinary stone growth"
Invest.Urol. (1975a), **13**, 31-35

Meyer,J.L.; Smith,L.H.
"Growth of Calcium Oxalate crystals. II. Inhibition by natural crystal growth inhibitors"
Invest.Urol. (1975b), **13**, 36-39

Michelacci,Y,; Galshan,R.; Schor,N.
"Urinary excretion of glycosaminoglycans in normal and stone forming subjects"
Kid.Int. (1989), **36**, 1022

Miller,J.D.; Randolph,A.D.; Drach,G.W.
"Observations upon Calcium Oxalate crystallisation kinetics in simulated urine"
J.Urol. (1977), **117**, 342-345

Miller,G.H.; Vermeulen,C.W.; Moore,J.D.
"Calcium Oxalate solubility in urine. Experimental urolithiasis XIV."
J.Urol. (1958), **79**, 607-612

Milvy,P.; Colt,E.; Thornton,J.
"A high incidence of urolithiasis in male marathon runners"
J.Sports.Med.Phys.Fitness (1981), **3**, 295-298

Moore,C.A.; Bunce, G.E.
"Reduction in frequency of renal calculus formation by oral magnesium administration. A preliminary report."
Invest.Urol. (1964), **2**, 7-13

Morse,R.M.; Resnick,M.I.
"Urinary stone matrix"
J.Urol. (1988), **139**, 602-606

Murphy,B.T.; Pyrah,L.N.
"The composition, structure and mechanisms of the formation of urinary calculi"
Br.J.Urol. (1962), **34**, 129-159

Nakagawa,Y.; Abram,V.; Parks,J.H.; Lau,H.S.; Kawooya,J.K.; Coe,F.L.
"Urinary glycoprotein crystal growth inhibitors. Evidence for a molecular abnormality in calcium oxalate nephrolithiasis"
J.Clin.Invest. (1985), **76**, 1455-1452

Nakagawa,Y.; Abram,V.; Kèzdy,F.J.; Kaiser,E.T.; Coe,F.L.
"Purification and characterisation of the principal inhibitor of Calcium Oxalate monohydrate crystal growth in urine"
J.Biological Chemistry (1983), **258** (20), 12594-12600

Nakagawa,Y.; Ahmed,M.A.; Hall,S.L.; Deganello,S.; Coe,F.L.
"Isolation from human calcium oxalate renal stones of nephrocalcin, a glycoprotein inhibitor of calcium oxalate crystal growth"
J.Clin.Invest. (1987), **79**, 1782-1787

Nancollas,G.H.
"The kinetics of growth of seed crystals in solution"
Croatica Chem.Acta (1970), **42**, 311

Nancollas,G.H.; Mohan,M.S.
"The growth of hydroxyapatite crystals"
Arch.Oral.Biol. (1970), **15**, 731-745

Nancollas,G.H.; Smesko,S.A.; Campbell,A.A.; Richardson,C.F.; Johnsson,M.; Ladiccico,R.A.; Binette,J.P.; Binette,M.
"Physical chemical studies of calcium oxalate crystallisation"
Am.J.Kid.Dis. (1991), **17** (4), 392-395

Nesse,A.; Garbossa,G.; Romero,M.R.; Bogado,C.E.; Zanchetta,J.R.
"Glycosaminoglycans in urolithiasis"
Nephron (1992), **62**, 36-39

Nicar,M.J.; Hsu,M.C.; Fetner,C.
"Urinary response to oral potassium citrate therapy for nephrolithiasis in a private practice setting"
Clin.Ther. (1986), **8**, 219-225

Nicar,M.J.; Peterson,R.; Pak,C.Y.C.
"Use of potassium citrate as potassium supplement during thiazide therapy of calcium nephrolithiasis"
J.Urol. (1984), **131**, 430-433

Nielsen,A.E.; Christoffersen,J.
"The mechanisms of chrystal growth and dissolution"
in:"Biological Mineralization and Determination"
ed:Nancollas,G.H. Berlin, Heidelberg, New York, Springer-Verlag (1982), 37-77

Nishio,S.; Abe,Y.; Wakatsuki,A.; Iwata,H., Ochi,K.; Takeuchi,M.; Matsumoto,A.
"Matrix glycosaminoglycan in urinary stones"
J.Urol. (1985), **134**, 503-505

Nishio,S.; Kavanagh,J.P.
"A small scale mixed suspension mixed product removal crystalliser"
Chem.Eng.Sci. (1990), **46**, 709

Nishio,S.; Kavanagh,J.P.; Faragher,E.B.; Garside,J.; Blacklock,N.J.
"Calcium oxalate crystallisation kinetics and the effect of calcium and γ-carboxyglutamic acid"
Br.J.Urol. (1990), **66**, 351-356

Nishio,S.; Kavanagh,J.P.; Garside,J.
"A small scale continuous mixed suspension mixed product removal crystalliser"
Chem.Eng.Sci. (1991), **46** (2), 709-711

Nishio,S.; Terado,T.; Iwata,H.; Takeuchi
"Effects of urinary macromolecules on calcium oxalate crystallisation studied by continuous flow system and fresh undiluted urine"
in:"Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis
eds:Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.; Smith,L.H.; Walker,V.R. Plenum Press, New York, London (1994), 205-297

Norlin,A.; Lindell,B.; Grauberg,P.O.; Lindvall,N.
"Urolithiasis. A study of its frequency"
Scand.J.Nephrol. (1976), **10**, 150-153

Ohkawa,M.; Tokunaga,S.; Nakashima,T.; Orito,M.; Hisazumi,H.
"Thiazide treatment for calcium urolithiasis in patients with idiopathic hypercalciuria"
Br.J.Urol. (1992), **69**, 571-576

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**, 136-141

Pak,C.Y.C.; Arnold,L.H.
"Heterogeneous nucleation of calcium oxalate by seeds of monosodium urate"
Proc.Soc.Exp.Bio.Med. (1975), **149**, 930

Pak,C.Y.C.; Chu,S.
"A simple technique for the determination of urinary state of saturation with respect to brushite"
Invest.Urol. (1973), **11** (3), 211-215

Pak,C.Y.C.; Fuller,C.; Sakhaee,K.; Preminger,G.M.; Britton,F.
"Long-term treatment of calcium nephrolithiasis with potassium citrate"
J.Urol. (1985), **134**, 11-19

Pak,C.Y.C.; Holt,K.
"Nucleation and growth of brushite and Calcium Oxalate in urine of stone formers"
Metabolism (1976), **25**, 665-673

Pak,C.Y.; Holt,K.; Zerwekh,J.E.
"Attenuation by monosodium urate on the inhibitory effect of glycosaminoglycans on calcium oxalate nucleation"
Invest.Urol. (1979), **17** (2), 138-140

Pak,C.Y.C.; Koenig,K.; Khan,R.; Haynes,S.; Padalino,P.
"Physiological action of potassium-magnesium citrate in nephrolithiasis"
J.Bone and Mineral Res. (1992), **7** (3), 281-285

Pak,C.Y.C.; Sakhaee,K.; Crowther,C,; Brinkley,L.
"Evidence justifying a high fluid intake in treatment of nephrolithiasis"
Annals Internal Medicine(1980), **93** (1), 36-39

Pak,C.Y.C.; Smith,L.H.; Resnick,M.J.; Weinerth,J.L.
"Dietary management of idiopathic calcium urolithiasis"
J.Urol. (1984), **131**, 850-852

Pietrek,J.; Kokot,F.
"Treatment of patients with calcium-containing renal stones with cellulose phosphate"
Br.J.Urol. (1973), **45**, 136-143

Politis,G.; Griffith,D.P.
"ESWL: stone-free efficacy based upon stone size and location"
World.J.Urol. (1987), **5**, 255-258

Power,C.; Barker,D.J.P.; Blacklock,N.J.
"Incidence of renal stones in 18 British towns"
Br.J.Urol. (1987), **59**, 105-110

Preminger,G.M.; Sakhaee,K.; Skurla,C.; Pak,C.Y.C.
"Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis"
J.Urol. (1985), **134**, 20-23

Prien,E.L.
"The riddle of urinary stone disease"
JAMA (1971), **216** (3), 503-507

Prien,E.L.; Gershoff,S.F.
"Magnesium oxide-pyridoxine therapy for recurrent calcium oxalate calculi"
J.Urol. (1974), **112**, 509

Ramanarayan,K.A.; Athreya,K.; Larson,M.A.
"Statistical-mathematical modelling of CSD in continuous and batch crystallisers"
AIChE.Symp.Ser. (1984), **80** (240), 76-89

Randall,A.
"Hypothesis for origin of renal calculus"
N.Engl.J.Med. (1936), **214**, 234-242

Randall,A.
"Papillary pathology as a precursor of primary renal calculus"
J.Urol. (1940), **44**, 580-589

Randolph,A.D.; Drach,G.W.
"Some measurements of Calcium Oxalate nucleation and growth rates in urine-like liquors"
J.Cryst.Growth (1981), **53**, 195-201

Randolph,A.D.; Larson,M.A.
"Theory of particulate processes"
Academic Press, New York, (1971), Ch: 2,4,9

Randolph,A.D.; Kraljevich,Z.I.; Drach,G.
"Effect of urinary macromolecules on calcium oxalate dihydrate crystal growth and nucleation rates"
in: "Urolithiasis clinical and basic research"
eds: Smith,L.H.; Robertson,W.G.; Finlayson,B. Plenum Press, New York, (1981), 427-436

Rao,P.N.; Prendiville,V.; Buxton,A.; Moss,D.G.; Blacklock,N.J.
"Dietary management of urinary risk factors in renal stone formers"
Br.J.Urol. (1982), **54**, 578-583

Rattan,V.; Sidhu,H.; Vaidyanathan,S.; Thind,S.K.; Nath,R.
"Effect of combined supplementation of magnesium oxide and pyridoxine in calcium oxalate stone formers"
Urol.Res. (1994), **22**, 161-165

Resnick,M.I.; Munday,D.; Boyce,W.H.
"Magnesium excretion and calcium oxalate urolithiasis"
Urology (1982), **20** (4), 385-389

Resnick,M.I.; Sorrell,M.E.; Bailey,J.A.; Boyce,W.H.
"Inhibitory effects of urinary calcium-binding substances on Calcium Oxalate crystallisation"
J.Urol. (1982), 127, 568-571

Robert,M.; Boularan,A.M.; Colette,C.; Averous,M.; Monnier,L.
"Urinary calcium oxalate saturation in stone formers and normal subjects: an application of the $EQUIL_2$ program"
Br.J.Urol. (1994), **73**, 358-361

Roberts,S.D.; Resnick,M.I.
"Glycosaminoglycans content of stone matrix"
J.Urol. (1986), **135**, 1078-1083

Roberts,S.R.; Resnick,M.I.
"Urinary stone matrix"
in:"Renal Tract Stone Metabolic basis and clinical practice" eds: Wickham,J.E.A.; Colin Buck,A.
Churchill Livingstone. Edinburgh. London Melbourne and New York. (1990), 59-70

Robertson,W.G.
"A method for measuring calcium crystalluria"
Clin.Chim.Acta (1969a), **26**, 105-110
Robertson,W.G.
"Measurement of ionized Calcium in biological fluids"
Clin.Chim.Acta (1969b), **24**, 149-157

Robertson,W.G.
"The solubility concept" in: "Biological mineralisation and determination" ed: Nancollas,G.H.
Springer Verlag Berlin, Heidelberg, New York, (1982), 14

Robertson,W.G.
"Dietary factors important in calcium stone formation"
in: "Urolithiasis and related clinical research"
eds: Schwille,P.O.; Smith,L.H.; Robertson,W.G.; Vahlensieck,W. New York, Plenum Press, (1985), 61-68

Robertson,W.G.; Knowles,F.; Peacock,M.
"Urinary mucopolysaccharide inhibitors of calcium oxalate crystallisation"
in:"Urolithiasis Research" eds:Fleisch,H.; Robertson,W.G.; Smith,L.H.; Vahlensieck,W. New York, Plenum Press (1976b), 331

Robertson,W.G.; Latif,A.B.; Scurr,D.S.; Caswell,A.M.; Drach,G.W.; Randolph,A.D.
"Inhibitors of calcium oxalate crystallisation in urine from stone formers and normals"
in:"Urinary stone" eds: Ryall,R.L.; Brockis,J.G.; Marshall,V.R.; Finlayson,B. Churchill Livingstone, Melbourne (1984), 167-172

Robertson,W.G.; Nordin,B.E.C.
"Physiochemical factors governing stone formation"
in:"Scientific foundations of urology" eds:Innes-Williams,D.; Chisholm,G.D. Year Book Medical Publishers. Chicago (1976), 254-267

Robertson,W.G.; Peacock,M.
"Calcium Oxalate crystalluria and inhibitors of crystallisation in recurrent renal stone formers"
Clin.Sci. (1972), **43**, 499-506

Robertson,W.G.; Peacock,M.
"Review of risk factors in calcium oxalate urolithiasis"
World J.Urol. (1983), **1**, 114-118

Robertson,W.G.; Peacock,M.; Heyburn,P.J.; Marshall,D.H.; Clark,P.B.
"Risk factors in Calcium stone disease of the urinary tract"
Br.J.Urol. (1978), **50**, 449-454

Robertson,W.G.; Peacock,M.; Hodgkinson,A
"Dietary changes and the incidence of urinary calculi in the UK between 1958-1976"
J.Chronic Diseases (1979), **32**, 469-476

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Activity products in stone-forming and non-stone forming urine"
Clin.Sci. (1968), **34**, 597-594

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Calcium crystalluria in recurrent renal stone formers"
Lancet (1969), **2**, 21-24

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Calcium Oxalate crystalluria and urine saturation in recurrent renal stone formers"
Clin.Sci. (1971), **40**, 365-374

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Inhibitors of the growth and aggregation of Calcium Oxalate crystals in vitro"
Clin.Chim.Acta (1973), **43**, 31-37

Robertson,W.G.; Peacock,M.; Marshall,R.W.; Marshall,D.H.; Nordin,B.E.C.
"Saturation inhibition index as a measure of the risk of Calcium Oxalate stone formation in the urinary tract"
N.Engl.J.Med. (1976a), **294**, 249-252

Robertson,W.G.; Scurr,D.S.
"Modifiers of Calcium Oxalate crystallisation found in urine. I. Studies with a continuous crystalliser using an artificial urine"
J.Urol. (1986), **135**, 1322-1326

Robertson,W.G.; Scurr,D.S.; Bridge,C.M.
"Factors influencing the crystallisation of calcium oxalate in urine - critique"
J.Crystal Growth (1981), **53**, 182-194

Rodgers,A.L.; Ball,D.; Harper,W.
"Effect of urinary macromolecules and Chondroitin Sulphate on Calcium Oxalate crystallisation in urine"
Scann.Micros. (1994), **8** (1), 71-77

Rodgers,A.L.; Ball,D.; Harper,W.
"Urinary macromolecules are promoters of calcium oxalate nucleation in human urine: turbidimetric studies"
Clin.Chim.Acta (1993), **220**, 125-134

Rodgers,A.L.; Hibbert,B.; Probyn,T.
"Determination of urinary calcium oxalate crystallisation mechanisms and kinetics using flow cytometry"
Urol.Int. (1995), **55**, 93-100

Rodgers,A.L.; Garside,J.
"The nucleation and growth kinetics of calcium oxalate in the presence of some synthetic urine constituents"
Invest.Urol. (1981), **18** (6), 484-488

Rodgers,A.L.; Jappie,D.
"Studies on the role of urinary macromolecules in urolithiasis: review of methodologies and a proposal for a standard reference crystallisation system"
Scann.Micros. (1996), **10** (1) in press

Rodgers,A.; Spector,M.
"Human stones"
Endeavour, New Series, (1981), **5** (3), 119-126

Rose,G.A.
"Other metabolic causes of stone" in: "Urinary stones. Clinical and laboratory aspects: ed: Rose,G.A.P. Lancaster, MTP Press, (1982), 235

Rose,G.A.
"Current trends in urolithiasis research"
in:"Stone Disease Diagnosis and Management"
ed: Rous,S.N. Orlando, Grune and Stratton, (1987),383-416

Rose,G.A.; Sulaiman,S.
"Tamm-Horsfall mucoproteins promote calcium oxalate crystal formation in urine: quantitative studies"
J.Urol. (1982), **127**, 177-179

Rose,G.A.; Sulaiman,S.
"Effect of different fractions of macromolecules upon triggering of calcium oxalate and calcium phosphate crystal formation in whole urine"
Urol.Int. (1984), **39**, 147-149

Rose,G.A.; Westbury,E.J.
"The influence of calcium content of water, intake of vegetables and fruit and of other food factors upon the incidence of renal calculi"
Urol.Res. (1975), **3**, 61-66

Rushton,H.G.; Spector,M.
"Effects of magnesium deficiency on intratubular calcium oxalate formation and crystalluria in hyperoxaluric rats"
J.Urol. (1982), 127, **598**-604

Russel,R.G.G.; Bisaz,S.; Fleisch,H.
"The influence of orthophosphate on the renal handling of inorganic pyrophosphate in man and dog"
Clin.Sci.Molec.Med. (1976), **51**, 435-443

Ryall,R.L.
"A reappraisal of the Coulter Counter technique for measuring Calcium Oxalate crystal size"
Clin.Chim.Acta (1978), **83**, 99-107

Ryall,R.L.; Bagley,C.J.; Marshall,V.R.
"Independent assessment of the growth and aggregation of Calcium Oxalate crystals using the Coulter Counter"
Invest.Urol. (1981a), **18**, 401

Ryall,R.L.; Harnett,R.M.; Hibberd,C.M.; Edyvane,K.A.; Marshall,V.R.
"Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallisation in undiluted human urine"
Urol.Res. (1991), **19**, 181-188

Ryall,R.L.; Harnett,R.M.; Marshall,V.R.
"The effect of urine, pyrophosphate, citrate, magnesium, and glycosaminoglycans on the growth and aggregation of Calcium Oxalate crystals in vitro"
Clin.Chim.Acta (1981b), **112**, 349-356

Ryall,R.L.; Harnett,R.M.; Marshall,V.R.
"The effect of monosodium urate on the capacity of urine, chondroitin sulphate and heparin to inhibit calcium oxalate crystal growth and aggregation"
J.Urol. (1986c), **135**, 174-177

Ryall,R.L.; Hibberd,C.M.; Marshall,V.R.
"A method for studying inhibitory activity in whole urine"
Urol.Res. (1985), **13**, 285-289

Ryall,R.L.; Hibberd,C.M.; Mazzachi,B.C.; Marshall,V.R.
"Inhibitory activity of whole urine: a comparison of urines from stone formers and healthy subjects"
Clin.Chim.Acta (1986b), **154**, 59

Ryall,R.L.; Marshall,V.R.
"The value of the 24 hour urine analysis in male stone-formers attending a general hospital outpatients clinic"
Br.J.Urol. (1983), **55**, 1-5

Ryall,R.; Marshall,V.R.
"The investigation and management of idiopathic urolithiasis"
in:"Renal Tract Stones: Metabolic Basis and Clinical Practice" eds: Wickham,J.; Buck,A.C. Churchill Livingstone, London, (1990), 307-331

Ryall,R.L.; Ryall.R.G.; Marshall,V.R.
"Interpretation of particle growth and aggregation patterns obtained from the Coulter Counter. A simple theoretical model"
Invest.Urol. (1981c), **18**, 396-400

Ryall,R.G.; Ryall,R.L.; Marshall,V.R.
"A computer model for the determination of extent of growth and aggregation of crystals from changes in their size distribution"
J.Cryst.G. (1986a), **76**, 290-298

Sallis,J.D.; Bichler,K.H.; Korn,S.; Haussmann,A.
"Urinary glycosaminoglycan excretion in patients with urolithiasis"
in: "Urolithiasis:Clinical and basic research"
eds: Smith,L.H.; Robertson,W.G.; Finlayson,R.
New York, Plenum Press, (1981), 619-622

Sallis,J.D.; Lumley,M.F.
"On the possible role of glycosaminoglycans as natural inhibitors of calcium oxalate stones"
Invest.Urol. (1979), **16**, 296-299

Samuell,C.
"Uromucoid excretion in normal subjects, calcium stone formers and in patients with chronic renal failure"
Urol.Res. (1979), **7**, 5-12

Sarles,H.
"Chronic calcifying pancreatitis-chronic alcoholic pancreatitis"
Gastroenterology (1974), **66**, 604-616

Schulze,H.; Hertle,L.; Kutta,A.; Graff,J.; Senge,T.
"Critical evaluation of treatment of staghorn calculi by percutaneous nephrolithotomy and extracorporeal shock wave lithotripsy"
J.Urol. (1989), **141**, 822-825

Schum,D.K.; Gohel,M.D.
"Separate effects of urinary chondroitin sulphate on the crystallisation of urinary calcium oxalate: differences between stone formers and normal subjects"
Clin.Sci. (1993), **85**, 33-39

Schwille,P.O.; Hermann,U.
"Enviromental factors in the pathophysiology of recurrent idiopathic calcium urolithiasis (RCU), with emphasis on nutrition"
Urol.Res. (1992), **20**, 72-83

Schwille,P.O.; Hermann,U.; Wolf,C.; Berger,I.; Meister,R.
"Citrate and recurrent idiopathic calcium urolithiasis"
Urol.Res. (1992), **20**, 145-155

Scurr,D.S.; Robertson,W.G.
"Modifiers of Calcium Oxalate crystallisation found in urine. II. Studies on their mode of action in artificial urine"
J.Urol. (1986), **136**, 128-131

Sheehan,M.E.; Nancollas,G.H.
"Calcium Oxalate crystal growth. A new constant composition method for modelling urinary stone formation"
Invest.Urol. (1980), **17** (6), 446-451

Sheehan,M.E.; Nancollas,G.H.
"A constant composition method for modelling urinary stone formation"
in: "Proc.Fourth Int.Symp.Urol.Res", eds:Smith,L.H.; Robertson,W.G.; Finlayson,B.
New York. Plenum Press, (1981), 391

Sheldon,C.A.; Smith,A.D.
"Chemolysis of calculi"
Urol. Clin. North Am. (1982), **9**, 121-130

Shum,D.K.Y.; Gohel,M.D.I.
"Separate effects of urinary chondroitin sulphate and heparan sulphate on the crystallisation of urinary calcium oxalate: differences between stone formers and normal control subjects"
Clin.Sci. (1993), **85**, 33-39

Shuster,J.; Finlayson,B.; Schaeffer,R.; Sierakowski,R.; Zoltek,J.; Dzegede,S.
"Water hardness and urinary stone disease"
J.Urol. (1982), **128**, 422-425

Siener,R.; Hesse,A.
"Influence of a mixed and a vegetarian diet on urinary magnesium excretion and concentration"
Br.J.Nutrition (1995), **73**, 783-790

Sierakowsky,R.; Hemp,G.; Finlayson,B.
"Water hardness and the incidence of urinary calculi"
in:"Colloquium on renal lithiasis" eds:Finlayson,B.; Thomas,W.C.; Gainesville, Univ.Press of Florida (1976), 213-220

Singh,P.P.; Kiran,R.; Pendse,A.K.; Mathur,H.N.
"Bearing of drinking water quality on the prevalence of urolithaisis"
in:"Urolithiasis" eds:Walker,V.; Sutton,R.A.L.; Cameron,E.C.B.; Pak,C.Y.C.; Robertson,W.G.
New York, Plenum Press, (1989), 771-772

Smith,A.D.; Lange,P.H.; Miller,R.P.; Reinke,D.B.
"Dissolution of cystine calculi by irrigation with acetylcysteine through percutaneous nephrostomy"
Urology (1979), **13**, 422-423

Sofras,F.; Karayannis,A.; Kostakopoulos,A.; Delakas,D.; Kastriotis,J.; Dimopoulos,C.
"Metholodology, results and complications in 2000 extracorporeal shock wave lithotripsy procedures"
Br.J.Urol. (1988), **61**, 9-13

Spector,A.R.; Gray,A.; Prien,E.L.Jr
"Kidney stone matrix, differences in acidic protein composition"
Invest.Urol. (1976), **13** (6), 387-389

Springmann,K.E.; Drach,G.W.; Gottung,B.; Randolph,A.D.
"Effects of human urine on aggregation of Calcium Oxalate crystals"
J.Urol. (1986), **135**, 69-71

Stresty,G.C.; Venkateswar
"Particle size analysis - a review"
In: "Fine Particles Processing Vol 1" ed: Somasundaran,P.
Am.Inst.Mining, Metallurgical and Petroleum Engineers Inc., Proc.Int.Symp.Fine Particles Processing (1980), 329-365

Suzuki,K.; Mayne,K.; Doyle,I.R.; Ryall,R.L.
"Urinary glycosaminoglycans are selectively included into calcium oxalate crystals precipitated from whole human urine"
Scann.Micros. (1994), **8** (3), 523-530

Sutor,D.J.
"Growth studies of Calcium Oxalate in the presence of various ions and compounds"
Br.J.Urol. (1969), **41**, 171-178

Takasaki,E.
"The magnesium:calcium ratio in the concentrated urines of patients with calcium oxalate calculi"
Invest.Urol. (1972), **10**, 147-150

Thind,S.K.; Datta,B.N.; Malik,A.K.; Verma,G.
"Effect of succinic acid administration on urolithiasis"
Indian J.Med.Res. (1980), **71**, 611-616

Thomas,W.C.; Bird,E.D.; Tomita,A.
"Some concepts concerning the genesis of urinary calculi"
J.Urol. (1963), **90**, 521-526

Thomas,W.C.Jr; Howard,J.E.
"Studies on the mineralizing propensity of urine from patients with and without renal calculi"
Trans.Assoc.Am.Physicians (1959), **72**, 181-187

Thorne,I.; Resnick,M.I.
"Urinary macromolecules and renal lithiasis"
World J.Urol. (1983), **1**, 138-145

Thornley,C.; Dawnay,A.; Cattell,W.R.
"Human Tamm-Horsfall glycoprotein: urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay"
Clin.Sci. (1985), **68**, 529-535

Tiselius,H.G.
"Measurement of the risk of calcium oxalate crystallisation in urine"
Urol.Res. (1985), **13**, 297-300

Tiselius,H.G.
"Measurement of the risk of calcium phosphate crystallisation in urine"
Urol.Res. (1987), **15**, 79-81

Tiselius,H.G.; Ahlstrand,C.; Larsson,L.
"Urine composition in patients with urolithiasis during treatment with magnesium oxide"
Urol.Res. (1980), **8**, 197-200

Tiselius,H.G.
"Effects of small molecules on the crystallisation of calcium salts and on the formation of calcium stones"
in: "Urolithiasis: Consensus and controversies"
eds: Rao,P.N.; Kavanagh,J.P.; Tiselius,H.G. Proc.6th Eur.Symp.Urolithiasis. Manchester, South Manchester University Hospitals (1995), 111-141

Tiselius,H.G.; Almgard,L.E.; Larsson,L.; Sorbo,B.
"A biochemical basis for grouping of patients with urolithiasis"
Eur.Urol. (1978), **4**, 241-249

Tiselius,H.G.; Berg,C.; Fornander,A.M.; Nilsson,M.A.
"Effect of citrate on the different phases of calcium oxalate crystallisation"
Scann.Micros. (1993a), **7** (1), 381-390

Tiselius,H.G.; Fornander,A.M.
"Evaluation of a routine method for determination of Calcium Oxalate crystal growth inhibition in diluted urine samples"
Clin.Chem. (1981), **27**, 565-568

Tiselius,H.G.; Fornander,A.M.; Nilsson,M.A.
"The effects of citrate and urine on calcium oxalate crystal aggregation"
Urol.Res. (1993b), **21**, 297-366

Tiselius,H.G.; Larsson,L.; Hellgren,E.
"Clinical results of allopurinol treatment in prevention of calcium oxalate stone formation"
J.Urol. (1986), **136**, 50-53

Tiselius,H.G.; Pettersson,B.; Andersson,A.
"Extracorporeal shock wave lithotripsy of stones in the mid-ureter"
J.Urol. (1989), **141**, 280-282

Tomson,M.B.; Nancollas,G.H.
"Mineralization kinetics: a constant composition approach"
Science (1978), **200**, 1059-1060

Trinchieri,A.; Guarneri,A.; Rovera,F.; Nespoli,R.; Zanetti,G.
"Calcium content of water in the prevention of renal stones" in: "Urolithiasis: Consensus and Controversies" eds: Rao,P.N.; Kavanagh,J.P.; Tiselius,H.G. Proc.6th Eur.Symp.Urolithiasis Manchester, South Manchester Univ.Hosp. (1995), 253-254

Trinchieri,A.; Mandressi,A.; Luongo,P.; Longo,G.; Pisani,E.
"The influence of diet on urinary risk factors for stones in healthy subjects and idiopathic renal calcium stone formers"
Br.J.Urol. (1991), **67**, 230-236

Trump,B.F.; Dees,J.H.; Kim,K.M.
"Some aspects of kidney structure and function with comments on tissue calcification in the kidney"
in: "Urolithiasis" eds: Finlayson,B.; Hench,L.; Smith,L.H. Washington D.C. National Academy of Science (1972), 1

Uhlmann,D.R.; Chalmers,B.
"The energetics of nucleation"
Indust.Eng.Chem. (1965), **57**, 19

Vahlensieck,W.
"Review:The importance of diet in urinary stones"
Urol.Res. (1986), **14**, 283-288

Vahlensieck,W.; Strenge,A.; Hesse,A.
"Nutrition history of recurrent Calcium Oxalate stone formers pre and post dietary"
in: "Urinary Stone" Churchill Livingstone, Melbourne Edinburgh London New York. eds: Ryall,R.; Brockis,J.G.; Marshall,V; Finlayson, B.
(1984), 41-46

Wakatsuki,A.; Nishio,S.; Iwata,H.; Ochi,K.; Takeuchi,M.; Matsumoto,A.
"Possible role of hyaluronate in experimental renal stone formation in rabbits"
J.Urol. (1985), **133**, 319-323

Walton,A.G.
The formation and properties of precipitates
New York. Interscience publishers (1961)

Walton,A.G.
"Nucleation of crystals from solution"
Science, (1965), **148**, 601-607

Wein,A.J.; Benson,G.S.; Raezer,D.M.; Mulholland,S.G.
"Oral methylene blue and the dissolution of renal calculi"
J.Urol. (1976), **116**, 140-141

Welshmann,S.G.; McGeown,M.G.
"The relationship of the urinary cations calcium, magnesium, sodium and potassium in patients with renal calculi"
Br.J.Urol. (1975), **47**, 237-242

Werness,P.G.; Bergert,J.H.; Smith,L.H.
"Crystalluria"
J.Cryst.Growth (1981), **53**, 166-181

Werness,P.G.; Brown,C.M.; Smith,L.H.; Finlayson,B.
"EQUIL2: A basic computer program for the calculation of urinary saturation"
J.Urol. (1985), **134**, 1242-1244

Werness,P.G.; Duckworth,S.C.; Smith,L.H.
"Calcium oxalate dihydrate crystal growth"
Invest.Urol. (1979), **17** (3), 230-233

Wickham,J.E.A.
"The matrix of renal calculi"
in: "Scientific Foundations of Urology" Vol 1
eds: Williams,D.F.; Chisholm,G.D.
Heinemann, London, (1976), 323-329

Wickham,J.E.A.
"Indications for surgical treatment and surgery for renal stones"
in: G.A.Rose:"Urinary Stones", MTP Press Ltd, Lancaster
(1982), 23-66

Wickham,J.E.A.; Buck,A.C.
"Renal Tract Stone: Metabolic Basis and Clinical Practice"
Churchill Livingstone, Edinburgh
(1990), 64-70

Wiggins,R.C.
"Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physiochemical conditions"
Clin.Chim.Acta (1987), **162**, 329-340

Will,E.J.; Bijvoet,O.L.M.; Blomen,L.J.M.J.; Van der Linden,H.
"Growth kinetics of calcium oxalate monohydrate. I. Method and validation"
J.Cryst.Growth (1983), **64**, 297-305

Williams,R.E.
"Long term survey of 538 patients with upper urinary tract stones"
Br.J.Urol. (1963), **35**, 416-437

Williams,H.E.
"Calcium nephrolithiasis and cellulose phosphate"
New Engl.J.Med. (1974), **290**, 224-225

Wilson,J.W.L.; Werness,P.G.; Smith,L.H.
"Inhibitors of crystal growth of hydroxyapatite: A constant composition approach"
J.Urol. (1985), **134**, 1255-1258

Wolf,J.S.; Stoller,M.L.
"Inhibition of calculi fragment growth by metal-bisphosphonate complexes demonstrated with a new assay measuring the surface activity of urolithiasis inhibitors"
J.Urol. (1994), **152**, 1609-1614

Yamaguchi,S.; Yoshioka,T.; Utsunomiya,M.; Koide,T.; Okuyama,A.; Sonoda,T.
"Possible role of stone matrix in calcium oxalate stone formation" in: "Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis eds: Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.M.; Smith,L.H.; Walker,V.R. Plenum Press, New York, London, (1994), 247-252

Yamaguchi,S.; Yoshioka,T.; Utsunomiya,M.; Koide,T.l Osafune,M.; Okuyama,A.; Sonoda,T.
"Heparan sulfate in the stone matrix and its inhibitory effect on calcium oxalate crystallisation"
Urol.Res. (1993), **21**, 187

Yendt,E.R.; Cohanim,M.
"Thiazides and calcium urolithiasis"
Can.Med.Assoc.J. (1978), **18**, 755-757

Yoshioka,T.; Koide,T.; Utsunomiya,M.; Itatani,H.; Oka,T.; Sonoda,T.
"Possible role of Tamm-Horsfall glycoprotein in Calcium Oxalate crystallisation"
Br.J.Urol. (1989), **64**, 463-467

Zarembski,P.M.; Hodgkinson,A.
"Some factors influencing the urinary excretion of oxalic acid in man"
Clin.Chim.Acta (1969), **25**, 1-10

CHAPTER II
PARTICLE SIZING

Particle size analysis can be traced back to antiquity with evidence of the use of sieving by the Egyptians in 4000BC [Groves 1974]. The behaviour of particulate solids has gained increasing importance since then and is now used in modern technologies such as pharmaceuticals, pesticides, polymers, emulsions and biological materials.

Development of particle sizing techniques [Laws *et al.* 1963] has led to sophisticated instrumentation that require mathematical equations to manipulate the data. As a result of the wide range of methods that are available today, theoretical and practical factors must be considered when deciding which method is to be implemented.

This chapter describes the theory and principles of two well known particle sizing techniques, viz Malvern and Coulter, both of which were used in the present study.

II.1 MALVERN THEORY

II.1.1 THEORIES OF LIGHT SCATTERING

The scattering of light is fundamental to the Malvern particle sizing technique and as such, a discussion thereof is therefore included in the following section.

Historical background

At the beginning of the 19th century Young and Fresnel completed decisive studies which contributed towards understanding the nature of light [van de Hulst *et al.* 1964]. Their work was followed in the latter part of the century by Maxwell's electromagnetic theory of light [Chu 1974; Bohren and Huffman 1983; Haken 1984] in which electric and optical phenomena were linked. A problem of the era was the scattering of light by a homogeneous sphere

and though many special cases were solved, its full solution was eventually formulated by Mie in 1908.

Classically, light can be expressed as an oscillating electric field. The electrons from particles upon which the light impinges, vibrate due to this electric field and radiate light in all directions causing scattering. The vibrations of the electrons depend on their natural frequency in a particular atomic configuration and on the frequency of the electric field oscillations [Haken 1984; Narducci and Abraham 1988].

When light falls on a small particle, a fraction of the light is absorbed into the particle while the rest of the incident light is scattered. The angle of the scattered light relative to the incident beam is known as the scattering angle.

Light scattering can be divided into models dependent upon particle size relative to the wavelength of the light and also dependent upon the refractive index (RI) value of the particle relative to that of the surrounding medium.

The RI of the medium characterises the potential of an electron in a particular atomic configuration to follow the oscillations of the electric field of light [Jonasz 1991]. The RI depends on the chemical composition of the particle and its density. A sphere with a high RI will scatter light at large scattering angles in comparison to the low scattering angle of a low RI particle. The relative refractive index is the ratio of the RI of the particle to that of water. Therefore the higher the water content of a particle, the closer to 1 is the RI of the particle. Measurement of the RI is explained by Lightfoot and Watson (1990).

Raleigh theory

Raleigh partially solved the problem of electromagnetic wave scattering from small homogeneous particles

[Rayleigh,Lord 1871,1881; van de Hulst *et al.* 1964; Bohren and Huffman 1983]. He assumed the homogeneity of the electric field of the incident wave within the particle. His theory required firstly that the particle be very much smaller than the wavelength of the incident light, and secondly that the velocity of light does not decrease appreciably inside the particle [van de Hulst *et al.* 1964].

Raleigh-Debye-Gans theory

Expanding on the first model led to descriptions for small particles with RI's close to that of the suspending medium [van de Hulst *et al.* 1964; Bohren and Huffman 1983]. To account for the inhomogeneity of the electric field in a large particle, the latter was considered as being made up of many small fragments of Raleigh's model. The light scattering from a particle fragment depends on the field of the incident wave and on the waves radiated by other fragments. The latter waves depend on the light scattered by the fragment in question. If the RI of the particle is only slightly different to that of the medium, then the particle interacts weakly with the incident light. The scattered waves from the fragment are too weak to affect neighbouring fragments which "feel" mostly the incident wave. The model also requires that relative to a common origin, the phase differences between waves scattered by the fragments depend on the RI of the particle.

Fraunhofer and Anomalous theories

For spherical particles with a diameter greater than the wavelength of the illuminating radiation, the diameter of the diffraction pattern is inversely proportional to the particle diameter. In other words, the scattering angle for particles of sizes greater than the wavelength of light is independent of the optical properties of the particles or suspension. Therefore, for particles bigger than this size, no knowledge of the material or of the medium's optical properties is necessary. However, in the range 0.5-1μm (measurement over 50° from forward direction) the

light coupled into the particle is not completely absorbed as in the case above 1μm, but can emerge as a refracted ray [Lightfoot and Watson 1990; Kusters *et al.* 1990].

The Fraunhofer theory assumes that particles scatter light like discs of the same diameter. The assumptions are that:

(i) the particle is much larger than the wavelength of the light employed (typically 5λ) [de Boer *et al.* 1987];

(ii) all particles scatter with equal efficiencies;

(iii) the particle is opaque and does not transmit any light.

The anomalous theory proposed by van der Hulst *et al.* (1964) is used for a particle in a liquid and is similar to the Fraunhofer theory. Light passes through transparent particles and interferes with the diffracted light producing a modified scattering pattern. The anomalous theory invokes equations that take into account the contribution from light passing through particles. If the condition that the RI of the particle is close to that of the medium is fulfilled, the particle merely shifts the phase of the ray. The shift is the product of the ray's path length inside the particle and of the difference between the RI's of the particle and the medium. Thus the electric field immediately behind the particle can be calculated.

The Fraunhofer and anomalous assumptions are not completely valid especially when the relative refractive index of the particle and medium are close to 1 [Lightfoot and Watson 1990; Kusters *et al.* 1990]. When the particle size approaches the wavelength of light, the scattering becomes a complex function with maxima and minima. Mie theory fully describes light scattered from spherical particles.

Mie theory

Gustav Mie (1908) (cited by Jaggard *et al.* 1981) completely solved the equations for the interaction of light with matter [van de Hulst *et al.* 1964; Kerker 1969; Bohren and Huffman 1983]. The model is a solution of the Maxwell equations [Born and Wolf 1975] describing the propogation of the electromagnetic wave of light in space. Although the theory is valid for any particle diameter and particle RI, it is only fully applicable to spherical, optically homogenous particles. Mie theory often requires a knowledge of the shape and orientation of the non-spherical particles. However, an equivalent sphere diameter can be obtained due to the thousands of randomly orientated non-spherical particles involved in a single measurement. Greenberg (1980) gives a brief review of the uses of Mie theory and the difficulties associated with non-spheres.

The Mie theory assumes a volume for the particle as opposed to the Fraunhofer theory which assumes a projected area for the particle. It also requires a knowledge of the ratio of the RI of the particle to that of the suspending medium. The relative refractive index has both a real and imaginary contribution. The real contribution controls how the light is deflected by the particle and the imaginary contribution determines how much light is absorbed by the particle. In practice it is found that non-spherical particles or particles with a high degree of surface roughness behave as if they had a higher than expected imaginary RI compared with the properties of the bulk material [Bohren and Huffman 1983].

The theory assumes that the dispersant is transparent and therefore has a zero imaginary contribution. For crystals in a saturated solution, the relative refractive index is close to 1. For systems with relative RI's close to 1, refraction of light through the particle contributes to the forward scattering. Quantification of the errors in this respect using a Malvern 2600 particle sizer (as used in the

present study) has been carried out by Kusters *et al.* (1990).

Thus, Mie theory describes exactly how particles of different sizes and optical properties interact with light [Jonasz 1991]. However, for some materials in which the particles have sizes smaller than the wavelength of light, a simplification of Mie theory (Raleigh-Debye-Gans theory) can be used. For particles less than 1/10th the wavelength of the illuminating beam, all materials act as Raleigh scatterers. In other words, the scattering is isotropic (equal energy is scattered at all angles), allowing for further simplification.

Limitations of the Mie theory for various particles (eg latex spheres, air molecules) have been examined by Jaggard *et al.* (1981). They validated the use of this theory for latex spheres, but noted an underestimation of the phase function at large scattering angles. This has also been reported by other workers [Zerull *et al.* 1977; Perry *et al.* 1978], and suggests that the difference between experimental data and theoretical Mie values will increase for larger or more irregular shaped particles.

The Equivalent Sphere theory

A problem in particle size analysis is the calculation of volume distributions for non-spherical particles, since the shape of such particles is unknown. However, if the mass of the particle is known, it can be converted into the weight of an equivalent sphere ($w = 4\pi r^3 \rho$) from which the diameter can be calculated.

For example, to calculate the spherical equivalent diameter D_2 of a cylinder of diameter $D_1 = 20\mu m$ and height $100\mu m$ the following calculations are necessary (where X is the radius of the equivalent sphere):

Volume of cylinder $= \pi r^2 h = 10000\pi(\mu m^3)$ 1

Volume of sphere $= (4/3)\pi X^3$ 2

$\therefore \quad X = (3V/4\pi)^{1/3} = 19.5\mu m$

$\therefore \quad D_2 = 39.1\mu m$

The equivalent spherical diameter for the cylinder is therefore approximately 40μm.

II.1.2 MALVERN PARTICLE SIZER: THEORY

Research into particle size measurements using non-imaging light scattering techniques [Cornillault 1972; Weiss and Frock 1976] led to the development of the first commercial laser diffraction size analyser. One such device which is now marketed by Malvern Instruments was invented in the Department of Chemical Engineering and Fuel Technology at Sheffield University, England [Swithenbank *et al.* 1977; Felton 1978]. Early research into crystal growth rates of potassium iodide, formed using seed crystals, were conducted using this machine [Felton and Brown 1980].

The first particle size analysers used Fraunhofer theory to account for the extra scattering of light by small particles. Newer machines (eg Malvern Mastersizer) use a combination of the Fraunhofer and full Mie scattering theories [Agrawal *et al.* 1991]. The Malvern 2600 used in the present study uses the Fraunhofer theory for measurements of liquid droplet sprays and metal in liquids. The anomalous theory is used for particles in air and particles in liquid (the latter was used in this study).

The Malvern particle sizer uses Low Angle Laser Light Scattering (LALLS). The method is rapidly becoming the preferred standard for measurements in industry due to its accuracy and wide range of applications [Mohamed *et al.* 1981; Burkholz and Polke 1984].

A diagram of the 2600 Malvern optical unit is shown in Figure 1 [Malvern Instruments User manual].

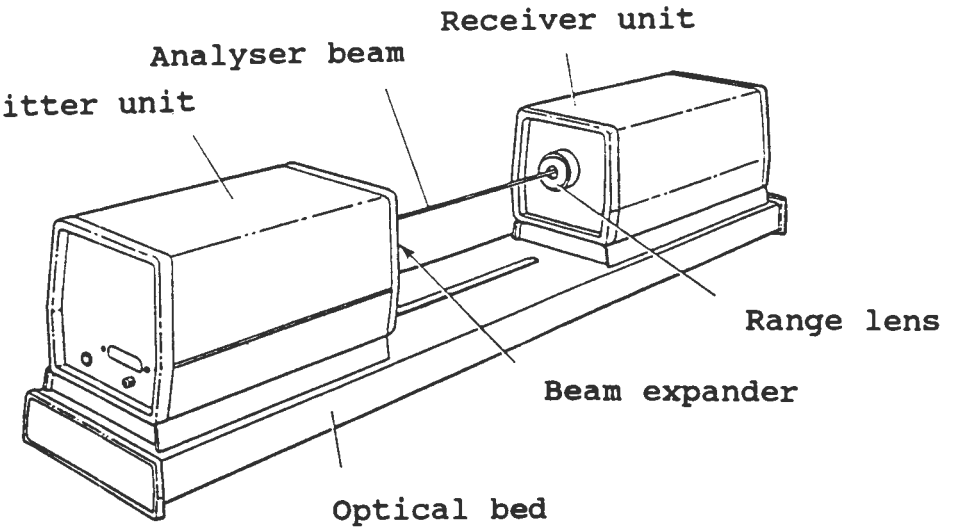


Figure 1: 2600 Malvern particle sizer optical unit used in the present study.

The optical measurement unit consists of an optical transmitter, a receiver and an optical bench. The transmitter unit consists of a He/Ne laser, its power supplies and the beam expanding optics that create the laser beam. The receiver unit consists of the range lens, the detector and the associated electronics and computer interfaces. The optical bench maintains the alignment of the transmitter and receiver units relative to each other. Mounted on this bench is a moveable holder for the flow cell. The optical configuration is shown in Figure 2 [Malvern Instruments User Manual].

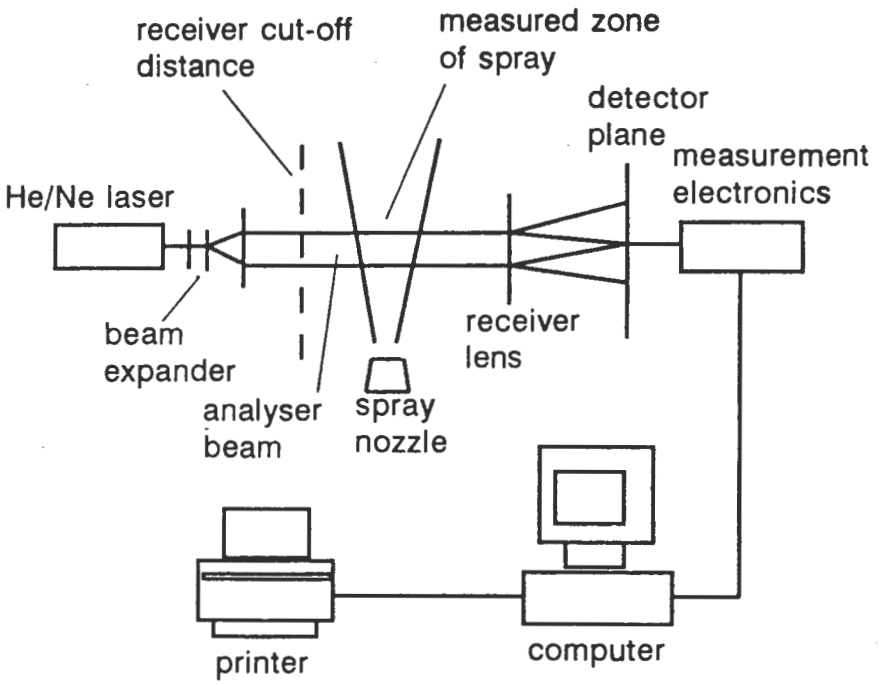


Figure 2: The Malvern optical configuration.

Diffraction

Light from the low power He/Ne laser is used to form the analyser beam. This beam is collimated and is monochromatic; it is typically about 18mm in diameter. The light scattered by the particles in the sample cell and the unscattered light are incident on the receiver lens (range lens). The unscattered light is focussed to a point on the axis. The Fourier transform lens forms the far field Fraunhofer diffraction pattern of rings of the scattered light around the central spot [Thyagaragjan and Ghatak 1981].

The scattered light over a range of solid angles of scatter is then gathered by a detector which is usually a thin wafer of photosensitive silicon. This detector, which is placed at the focal plane of the lens, is in the form of 31 concentric annular sectors. The dimensions of the detector are listed by Dodge (1984) and Hirleman *et al.* (1984). The diffracted light is concentrated concentrically at a distance from the point of focus. This distance is a function of the particle diameter. The remaining light which is not diffracted is focussed on the detector and passes straight through a small aperture and out of the

optical system. A sample volume concentration can thus be determined by monitoring the total laser power passing out of the system in this way.

The position of individual particles and their state of motion have little effect on the diffraction pattern which is created. The range lens configuration has the property that whenever the particle is in the laser beam, it's diffraction pattern is stationary and centered on the range lens optical axis. This means that no matter where the particle is in the beam or even if it is moving through the beam, the light diffracted at an angle θ will result in the same radial displacement in the focal plane. The lens transformation is optical and fast. This property is shown in Figure 3 [Malvern Instruments User Manual].

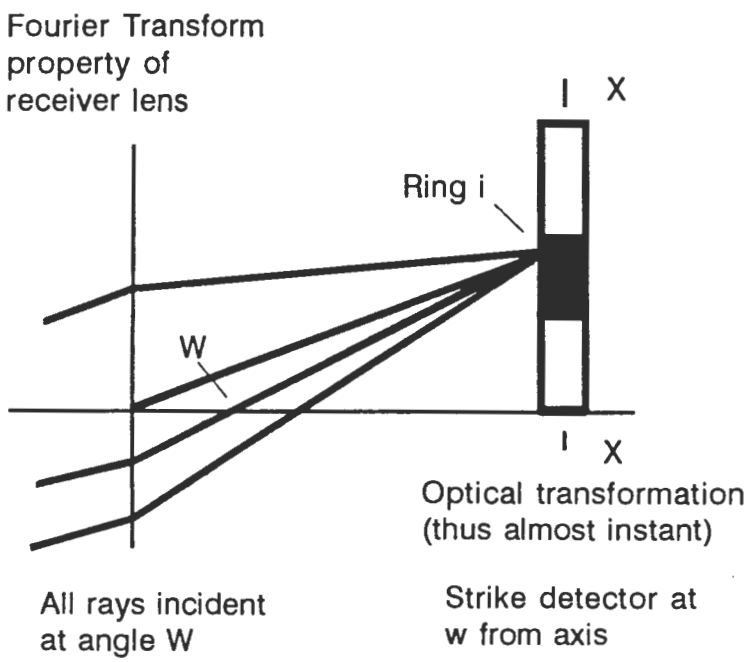


Figure 3: Properties of the range lens.

In practice many particles of different diameters are illuminated by the beam at the same time. A series of concentric light rings are generated at various radii, each being a function of a particular size. The rings of light intensity are superimposed to form a combined pattern. The amount of light intensity at any point on the matrix of detectors allows the number of particles present to be calculated and the particle size is given by the distance

from the center of the diffraction pattern. The smaller the particle is, the further is the diffraction response from the center as shown in Figure 4 [Lines 1990].

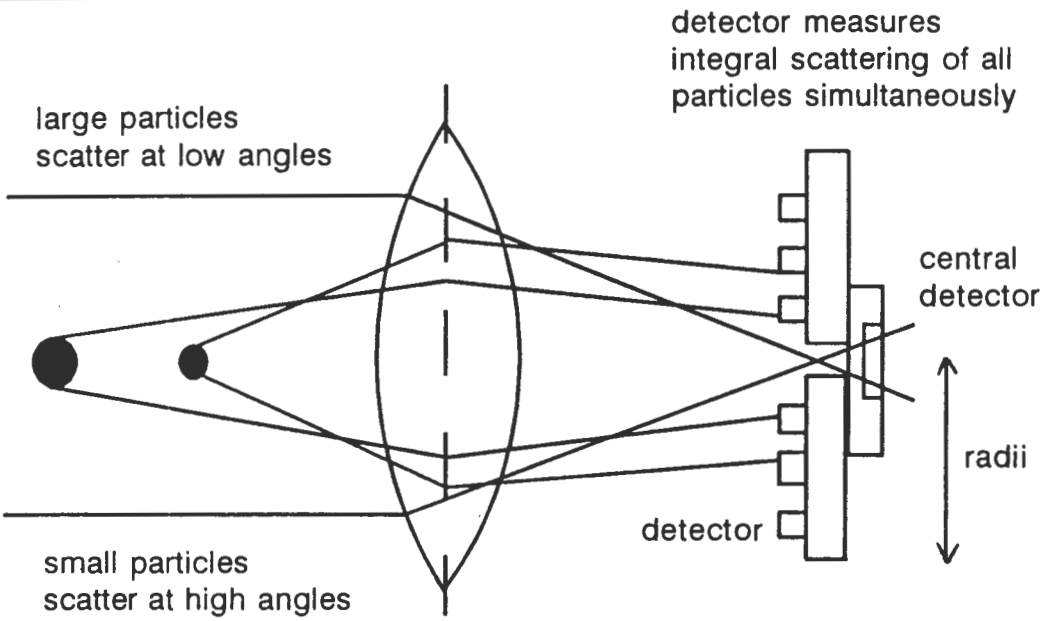


Figure 4: Properties of the scattered light.

To avoid unrepresentative sampling of the bulk, a time averaged observation of the scattering as the material continuously flows through the analyser beam, is used. This time averaging occurs over any period in the range between 5 seconds and 2 minutes.

During this time, the measured light scattering is continuously changing, forming the instantaneous integral of the material illuminated by the beam. By averaging the measurements of the detector readings (sweeps) it is possible to build up an integral light scattering matrix based on millions of individual particles.

Range lens

For particles of size greater than 1μm, the scattering angle is independent of the optical properties of the medium or the suspended particles. The scattering is therefore due to the diffraction of light around the particle. No knowledge of the optical properties of the medium or the suspended particles is necessary. This is

due to the fact that any absorption of light into the particle is ignored by the Fraunhofer and Anomalous theories.

For particles of size less than 1μm, the coupled light is not completely attenuated and can therefore emerge as a refracted ray. Knowledge of the refractive indices then becomes necessary and light scattering theory is used.

Photon correlation spectroscopy (PCS) [Berne and Pecora 1976; McCave and Syvitski 1991] can be used in the analysis of the particle range where the diffraction theory breaks down. The method is able to size particles in a state of Brownian motion suspended in a liquid. The technique can measure particles in the size range 1nm-1μm at scattered light intensities so low that a photomultiplier tube with a signal correlator is needed. Measurement of movement enables calculation of particle size due to the inverse proportionality relationship.

Alignment of the optical system

Alignment of the laser beam is achieved using the align display mode with subsequent movement of the detector using X and Y adjusters on the transmitter unit. The detector is moved until the focussed laser spot coincides with the central detector which is located behind a small hole in the main detector. Maximising the power of the beam on the central detector achieves alignment of the optical system. This procedure is followed in the absence of any sample. Diagramatically the change from misalignment to alignment is shown in Figure 5 [Malvern Instruments User Manual].

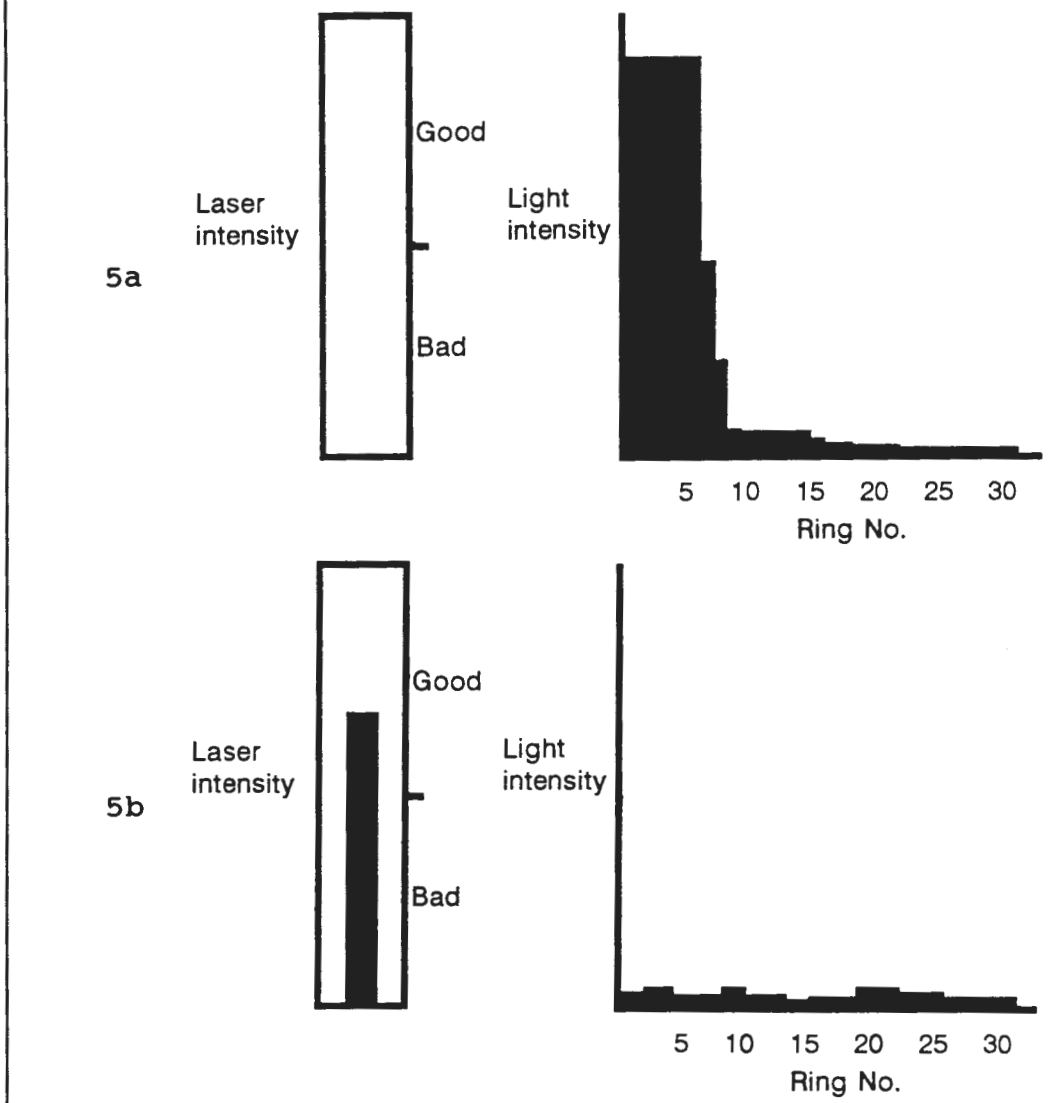


Figure 5: Misalignment (a) and alignment (b) of the laser.

Vignetting

Lens cut off (vignetting) occurs when the sample to be measured is too distant from the range lens. As a result, the range lens, with its limited aperture, is unable to capture the maximum scattering angle of the material.

Vignetting is noticeable in the alignment procedure of the Malvern instrument. A display of the beam alignment shows the light energy falling on each detector. Rapidly decreasing energy at large angles is characteristic of vignetting and this is illustrated in Figure 6 [Malvern Instruments User Manual].

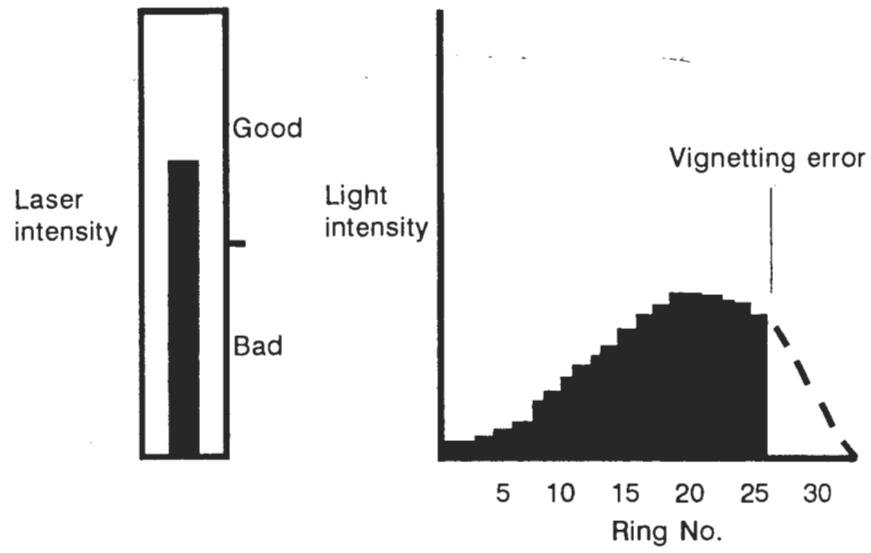


Figure 6: Align display showing vignetting error.

Background measurement

The range lenses have a wide angle of light acceptance which makes the subtraction of extra contributions from the sample measurement necessary. This background measurement takes into account both the "blank" sample and the stray light contributions. Background light should be measured under the same conditions as sample measurement. It is also necessary to measure the background as close as possible to the time of sample measurement to ensure that there are no time variations in the stray light. After the blank measurement, the sample cell should not be moved as the cell windows are part of the measurement zone [Chu 1974]. An internal electronic background is automatically subtracted from the blank and sample measurements.

Refractive index

Laser light scattering is a flexible technique with one qualification that each phase must be optically distinct from the other and the medium must be transparent to the laser wavelength. In other words, the RI of the material and the supporting medium must be different.

Measurement of the RI of the particle is achieved by suspending the sample particles in a mixture of two

liquids, the RI of each being known. The mixture of these two liquids is constituted in such a way that minimum scattering is obtained. At this point, the RI's of the sample and the medium are the same. The RI of the sample is then obtained from measurement of the RI of the suspension. Choice of the suspension liquids is limited as the RI of the sample must be between those of the two suspending liquids [Phahla 1991].

Since calcium oxalate is the material of interest in the present study, calculation of its RI is accomplished using the Lorentz-Lorentz formula which was obtained from the Clausius-Mossotti relation (Equation 3) [Atkins 1982; Guenther 1990]:

$$\alpha(n) = (3/4\pi N)[(n_r - 1)/(n_r + 2)] \quad \ldots\ldots\ldots\ldots 3$$

where $\alpha(N)$ = the molar polarisation of the suspending medium
N = Avogadros number
n_r = refractive index of the medium.

Molar refractivity, R_m, is given by Equation 4:

$$R_m = [(n_r - 1)/(n_r + 2)]Mm/\rho \quad \ldots\ldots\ldots\ldots 4$$

Where Mm = molar mass and ρ = density.

Equation 5 is used for a mixture of liquids:

$$\frac{[n^2 - 1]}{[n^2 + 2]} w/\rho = \sum_j [(n_j^2 - 1)/(n_j^2 + 2)](w_j/\rho_j) \quad \ldots\ldots 5$$

where j = 1 to N (where N is the number of liquids)
w = weight of mixture
w_j = weight of jth component of the mixture.

Thus:

$$R_m = \sum_j [(n_j^2 - 1)/(n_j^2 + 2)](w_j/\rho_j)$$
$$= \sum_j [(n_j^2 - 1)/(n_j^2 + 2)]V_j \quad \text{because } V_j = w_j/\rho_j$$

where j = 1 to N.

The refractive index of the suspension is thus given by Equation 6:

$$n_r = [(V_m + 2R_m)/(V_m - 2R_m)]^{1/2} \quad .....6$$

where V_m = total volume of the medium.

Substituting for solid CaOx:

$$R_m = (Ca^{2+}) + (C-C) + 2(C=O) + 2(C-O)$$
$$= 1.19 + 1.2 + 2(3.34) + 2(1.41)$$
$$= 11.89$$

Mm = 146.111g/mol

therefore $V_m = 146.11/2.25 = 64.94$

and $n_r = [(64.94 + 23.78)/(64.94 - 11.89)]^{1/2}$

$= 1.29$

This value is very close to that of water ie 1.33. Assuming a value of 1.33 for urine, a large log difference value can be expected because of the closeness of the refractive indices. The log difference value gives an indication of the fit of the data to the chosen model. The larger the difference, the worse the fit. Smaller log difference values might be possible if an approximate scattering matrix is used (ie one that is determined from the calculated refractive index).

Obscuration and sample measurement

Under measurement conditions, the suspending medium must have a stable refractive index. Variations in the index arising from inconsistencies in the suspension may lead to extra scattering signals during sample measurement.

Air bubbles in the suspension, turbulence and beam steering all cause scattering and result in unstable alignment. Beam steering is caused when the magnitude of the variation in the RI is large relative to the magnitude of the beam diameter. Such variations arise from slight changes in the temperature (temperature gradients) between different

regions in the sample. On the other hand, turbulence gives rise to small scale index variations that diffract light in the same way as particles do. These variations appear as apparent scattering contributions at low angles and appear to come from very large size particles. This is represented in Figure 7 [Malvern Instruments User Manual].

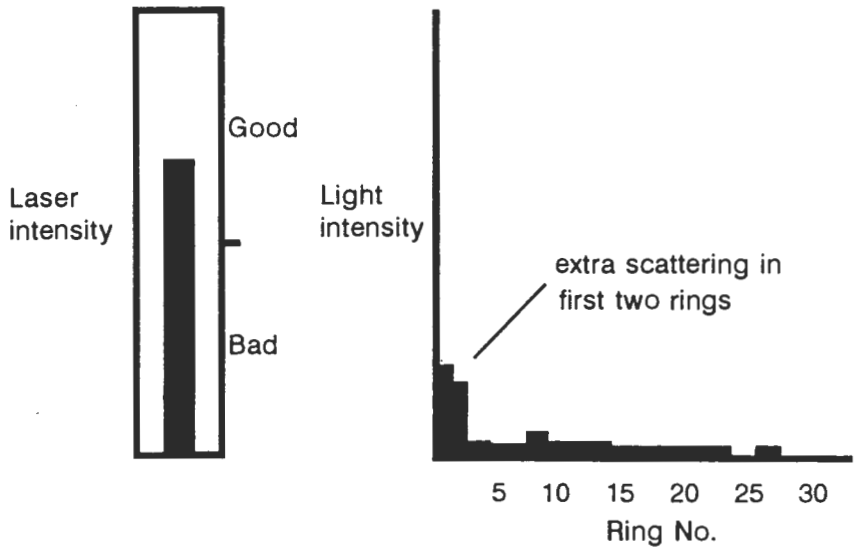


Figure 7: Alignment display showing extra scattering in the first two inner rings.

Figure 7 represents the alignment screen display of the Malvern in the presence of the suspending fluid only. The fluid flows through the beam under identical conditions as those when the sample particles are also present. The extra scattering appears in the first 2 rings of the bar graph at the right of the figure. These lower rings represent scattering by small particles. In the case shown in Figure 7, the extra scattering would be erroneously measured as small particles. On the other hand, second order scattering would show fluctuations in the outer, high angle detectors [Chigier 1984]. This scattering does not occur in the sample shown in Figure 7; however, if it were present it would appear in rings 30 and 31 in a similar fashion to that shown in rings 1 and 2.

These index variations also manifest themselves in the changing obscuration values in the Malvern data. A stable obscuration shows a stable size distribution. If the

obscuration decreases constantly from one reading to the next, then the crystal size could be increasing or the material could be precipitating because of inadequate pumping speeds. Another possibility is that the sample could be dissolving into the suspending fluid. On the other hand, if the obscuration values increase rapidly it could be indicative of the particles preferentially attaching themselves to the glass cell windows due to surface charges. The particles would thus be in the beam continuously, causing increased concentration values.

Over-concentration of the particles in the sample can also lead to multiple scattering [Felton and Brown 1980]. This widens the angle of light scatter. This in turn, results in a broader size distribution with a smaller mean size because the scattering angle is inversely proportional to the particle diameter. Corrections for multiple scattering using the independent model have been developed by Hamidi and Swithenbank (1986).

The Malvern instrument has an option to view the sample concentration before measurements to allow dilution to be effected if necessary to obtain acceptable obscuration values (dimensionless) within 0.1 - 0.5.

II.1.3 MALVERN PARTICLE SIZER: DATA ANALYSIS

The scattering matrix

Both the Mie and diffraction theories may be used to calculate scattered light intensity as a function of scattering angle and particle size. The total light energy distribution is the sum of the products of the energy distribution for each size range and the weight fraction in that range. Because only relative distributions are produced, the weight and volume are synonymous.

The scattering pattern for a single sphere is shown in Figure 8 [Felton 1990].

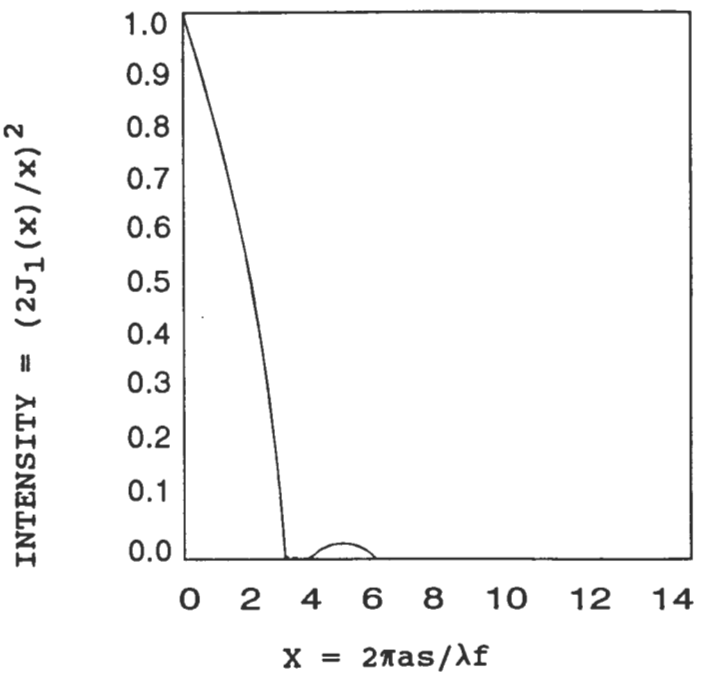


Figure 8: A single sphere scattering pattern showing an intensity distribution of scattered light energy (symbols are explained below in equation 7).

The centre of the diffraction pattern gives maximum light intensity which oscillates with decreasing amplitude as the distance from the axis increases. The diameter of the diffraction pattern is inversely proportional to the particle diameter [Riley and Agrawal 1990]. The particle size distribution cannot be determined from the intensity distribution, but it can be determined from the light energy distribution. The light energy within a circle of radius s in the focal plane is given by Equation 7 [Brown and Felton 1985; Felton 1990]:

$$L = 1 - J_0^2(2\pi as/\lambda f) - J_1^2(2\pi as/\lambda f) \quad .....7$$

where L = light energy
f = focal length of the lens
a = radius of the particle
x = diffracted light energy (Figure 8)
s = radius of circle in which the light energy is contained
λ = wavelength
J_0, J_1 = Bessel functions [Felton 1990]

Felton (1990) and also Gouesbet and Grehan (1988) have shown that the light energy within a ring between radii s_1 and s_2 from a particle of radius a is given by Equation 8:

$$L_{s1,s2} = E\{J_0^2(kas_1/f) + J_1^2(kas_1/f) - J_0^2(kas_2/f) - J_1^2(kas_2/f)\} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 8$$

where $k = 2\pi/\lambda$
E = energy falling on particle.

This is proportional to the cross-sectional area of the particle for a uniform beam:

$$E = C'\pi a^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 9$$

With more than one particle, the sum of contributions from the size classes leads to Equation 10:

$$E = C''V_i/a_i \quad \ldots\ldots\ldots\ldots\ldots\ldots 10$$

V_i is the volume fraction in the i^{th} size class.

Substitution for a particle of radius a, gives the first maximum at:

$$2\pi as/\lambda f = 1.357 \quad \ldots\ldots\ldots\ldots\ldots\ldots 11$$

The total light energy distribution is equal to the sum of products of the energy distribution for each size range and the volume fraction in that size range.

The derivation of the volume size distribution V(j) can be related to the measured data L(i) by the following matrix equation [Malvern Instruments User Manual]:

$$L(i) = V(j)T(i,j) \quad \ldots\ldots\ldots\ldots\ldots\ldots 12$$

where i = the index of size bands
j = the index of detector elements
T(i,j) describes how particles in size band i scatter light to detector element j.

Figure 9 shows the matrix calculated from the Fraunhofer diffraction method [Lightfoot and Watson 1990].

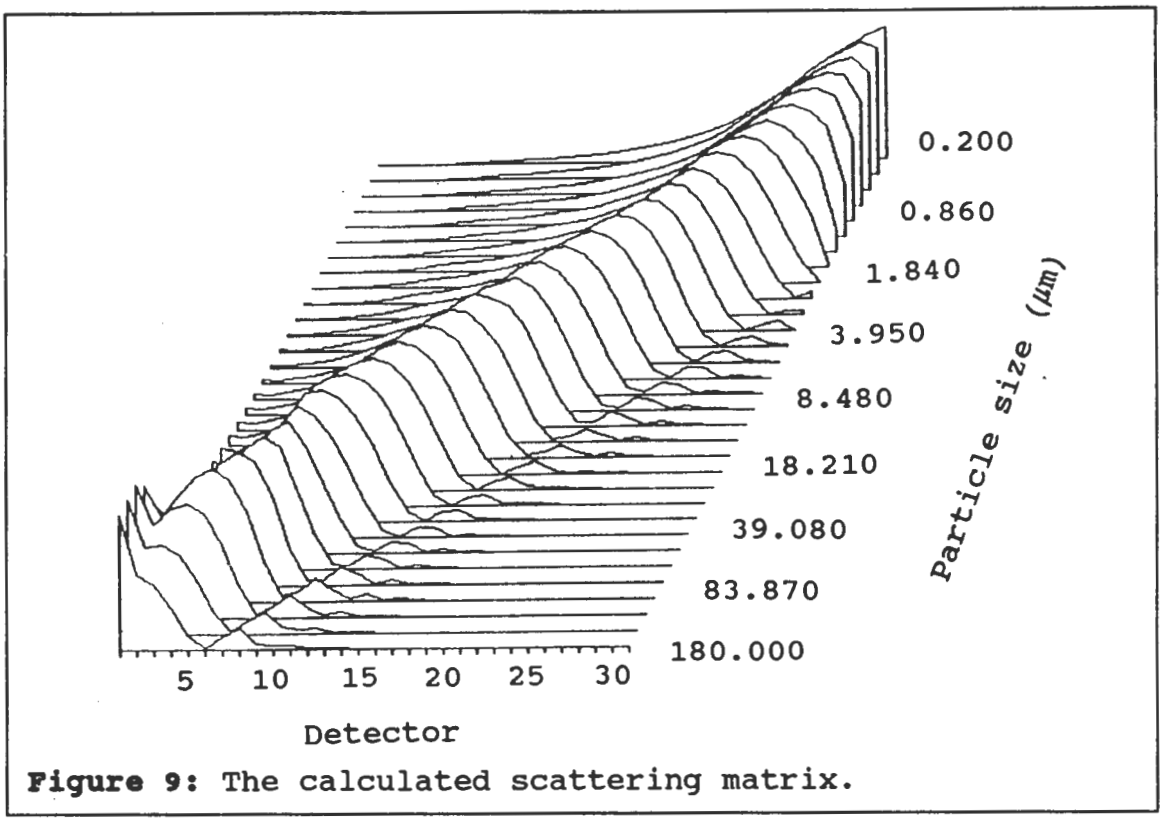


Figure 9: The calculated scattering matrix.

The main peak arises from the interaction of the forward scattering lobe and the detector. As the particles get smaller, the forward scattering lobe gets broader and therefore the matrix peak moves to larger angles. The forward scattering lobe is the major component of the Bessel function which describes the diffracted light distribution. The smaller peaks to the right of the main peaks are the 2nd and 3rd Bessel function peaks. The detector dimensions and size bands are optimised so that the peaks in the matrix fall close to the diagonal. The scattering functions have been normalised so that the peaks all have the same height [Hirleman *et al.* 1984].

Light passing through the particle would be indicated by higher secondary peaks which, if not analysed properly, could be mis-interpreted as the main peaks generated by smaller particles. Only one matrix is calculated for each lens corresponding to a particular refractive index ratio. Kusters *et al.* (1990) computed that the Malvern matrix corresponds to a refractive index ratio of approximately

1.2. They proved that serious errors can occur when the systems to be sized have a refractive index ratio close to 1.

The distribution functions available for use with the Malvern instrument are the Normal, Log normal, Rosin-Rammler and the Model Independent (Histogram) [Cadle 1955].

Analysis model

In the present study, no mathematical description relating the volume in one sizeband to that in any other was assumed, viz the Model Independent Analysis [Randolph and Larson 1971] model was used. This model performs a deconvolution analysis of the measured intensity to extract a size distribution without relying on a specified size distribution function. Inversion methods are not possible because of the constraint that $V(j) \geq 0$ (Equation 12). Therefore a volume distribution is estimated based on the measured light energy data and a new light energy distribution is calculated using Equation 12. The parameters are then iteratively adjusted until the sum of the squared errors in L(i) are minimised [Malvern Instruments User Manual].

The residual (log difference) is calculated as

$$\text{Residual} = \log_{10} \left(\Sigma(L(i) - V(j)T(i,j)\right)^2 \quad ....13$$

where L(i) is the data calculated from the estimated volume distribution. The parameters of the volume distribution above are then iteratively adjusted until the residual is a minimum. A log difference of less than 5 shows a good fit of the data to the model.

Various intrinsic sample factors and the experiment protocol can contribute to poor fitting of the data. Most of these have been described in detail in this Chapter II.1.2. Briefly, sample errors include contamination from particles too large to measure, and use of an inappropriate model for the sample distribution. Errors arising from the

experiment include incorrect optical alignment, incorrect background measurement, incorrect choice of lens and dirt on the optics. Sufficient signal accumulation is also necessary for representative measurements.

Sample concentration

As previously mentioned, the undiffracted light which is focussed onto the center spot allows for the calculation of the sample concentration. This was calculated directly without "in-situ" calibration for the first time by Brown and Felton (1985).

The ratio of the light intensity at the center spot before and after sample measurement gives the fraction of light obscured by the particles [Felton 1990]. Using the Beer Lambert Law (Equation 14):

$$\ln (I/I_0) = -\tau l \quad \text{.....14}$$

where $\tau_i = 2\ \Sigma N_i A_i \quad (i = 1 \text{ to } m)$

I = light intensity with sample
I_0= light intensity without sample
l = optical path length
N_i= number of particles of size class i per unit volume
A_i= mean particle projected area of i^{th} size class.

The measured obscuration can be related to the total projected cross-sectional area of the particles. The concentration is also dependent on the beam length.

The concentration is expressed as a % of the volume of dispersant in Equation 15 [Malvern Instruments User Manual]:

$$C = \frac{100 \log_e(1-Ob)}{-(3/2)b\sum_i (V_i Q_i / d_i)} \quad \text{.....15}$$

where b = beam length
Ob = obscuration
Q_i = extinction coefficient of sizeband i
V_i = volume in sizeband i
d_i = mean diameter in sizeband i.

Measured and derived diameters

The relative volume corresponding to the 32 size bands is obtained from the output of the Malvern and is presented in table form. The entire volume of the particles present is assumed to lie within this range. Since the weight of a sphere = $4/3\pi r^3\rho$, the mean diameter is calculated by Equation 16:

$$\text{Mean diameter} = (\Sigma d^3/n)^{1/3} \quad \text{.....16}$$

This is a number mean (number volume or number weight) because the number of particles (n) is taken into account in Equation 16. Mathematically this is represented by D[3,0]. D[4,3] and is known as the volume mean diameter (VMD) or De Broukere diameter. In terms of an arbitrary shaped particle, D[4,3] is the sphere of equivalent volume to the particle and is calculated in Equation 17:

$$D[4,3] = \Sigma d^4/\Sigma d^3 \quad \text{.....17}$$

The sphere of equivalent surface area is D[3,2] also known as the Sauter Mean diameter (SMD) and is given by Equation 18:

$$D[3,2] = \Sigma d^3/\Sigma d^2 \quad \text{.....18}$$

The number of particles does not appear in Equations 17 and 18. Laser diffraction does not require the number of particles to obtain a size distribution.

In a similar way, the number mean (number length mean) and number-surface mean are defined in Equations 19 and 20 respectively:

$$D[1,0] = \Sigma d/n \quad \text{.....19} \qquad D[2,0] = (\Sigma d^2/n)^{1/2} \quad \text{.....20}$$

A D[1,0] would be generated using an electron microscope for example and a method using image analysis would generate D[2,0].

The natural result of the Malvern measurement is a volume distribution. This may be converted to a number, surface area or length distribution, but care must be taken when doing so to ensure that the reproducibility error in one distribution is not too large in another. The different means can be converted to each other using the Hatch-Choate transformation [Hatch and Choate 1929].

The transformation of the distribution is achieved by Equation 21:

$$X_i = V_i d_i^{T-3} \quad \text{.....21}$$

where X_i = transformed distribution
V_i = volume in sizeband i
d_i = mean diameter of sizeband i
(geometric mean, square root of the product of size boundaries)
T = 3:volume, 2:surface area, 1:length, 0:number

The distributions can be described using statistical parameters. The first 3 moments, mean, standard deviation and skewness are best described in terms of the derived diameters D[m,n] as shown in Equation 22:

$$D[m,n] = [(\Sigma V_i d_i^{m-3})/(\Sigma V_i d_i^{n-3})]^{1/(m-n)} \quad \text{.....22}$$

where V_i = volume in sizeband i
d_i = mean diameter of sizeband i.

Equation 23 sums the relative volume in each sizeband multiplied by a power of the mean diameter of that sizeband:

$$D[m,n]^{(m-n)} = \frac{D|m,o|^m}{D|n,o|^n} \quad \text{.....23}$$

The span is calculated from the percentiles in Equation 24 [Malvern Instruments User Manual]:

$$\text{Span} = \frac{D[v,0.9] - D[v,0.1]}{D[v,0.5]} \quad \text{.....24}$$

D[v,0.9] and D[v,0.1] show the diameters at 90% and 10% of the distribution respectively.

The span gives a measure of the width of the volume distribution relative to the median diameter D[v,0.5].

The uniformity (volume median diameter) D[v,0.5] is defined in Equation 25:

$$U = \frac{\Sigma V_i \left| d[v,0.5] - d_i \right|}{d[v,0.5] \Sigma V_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots 25$$

and is a measure of absolute deviations from the median diameter.

The specific surface area is the surface area of a unit volume of particles expressed in Equations 26 and 27:

$$SSA = \frac{\text{Total area}}{\text{Total volume}} = \frac{6\Sigma V_i / d_i}{\Sigma V_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 26$$

V_i = volume in size band i
d_i = mean diameter of size band i.

$$SSA = 6/D[3,2] \quad \ldots\ldots\ldots\ldots\ldots 27$$

All of these measured and derived diameters are produced in the output from the Malvern, the software of which allows for the easy transformation into the other distributions.

II.2 COULTER MULTISIZER: THEORY

Single particle stream scanning methods involve a stream of particles passing through an optical or electrical sensing zone.

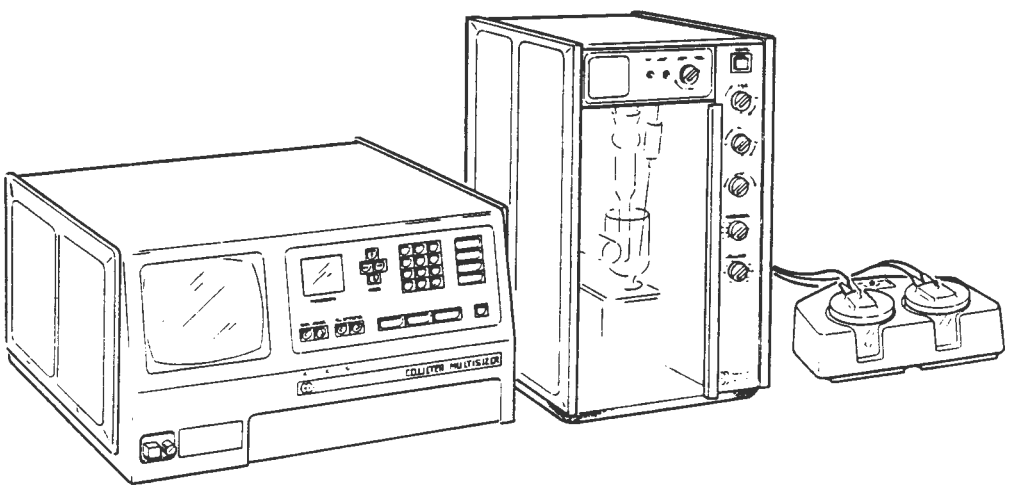
The Coulter Counter is an example of the electrical sensing zone method. It was developed by Coulter in 1956 [Coulter Multisizer Manual Issue C] and was originally applied to blood cell counting and later to bacterial cell counting [Coulter 1957]. Use of the method for cell-volume

distribution measurements was demonstrated later by Kubitschek (cited by [Allen 1981]). Further improvements by Coulter Electronics have led to the use of the Coulter Counter in particle sizing.

The counting and sizing of CaOx crystals in urine using the Coulter Counter was reported first by Robertson (1969). Since then, the technique has been applied extensively in the analysis of CaOx crystallisation processes in urine [Markovic and Komunjer 1979; Ryall *et al.* 1981a; Tiselius 1985; Azoury *et al.* 1987; Grover *et al.* 1990b] with much emphasis being placed upon inhibitor activities [Robertson and Peacock 1972; Robertson *et al.* 1973; Bowyer *et al.* 1979; Ryall *et al.* 1985,1991; Edyvane *et al.* 1987; Berg and Tiselius 1989; Grover *et al.* 1990a]. Computer modelling programs to aid in the calculation of growth in supersaturated solutions using the changes in size distributions have been developed by Ryall *et al.* (1981b,1984,1986). Calculation of growth in the presence of simultaneous aggregation is made possible by these computer crystallisation models.

Operating principles

A diagram of the Coulter Multisizer equipment [Allen 1981] used in the present study is shown in Figure 10.



A B C

Figure 10: The Coulter Multisizer.

The instrument comprises a sampling stand (B), a vacuum control unit (C) and the main electronics unit with printer (A).

The Coulter method involves determining the number and volume of singular particles suspended in an electrically conductive liquid. The stirred suspension is forced through a small aperture by means of a vacuum. Two electrodes are placed in the conductive liquid on either side of the aperture and a constant current is maintained between them.

As each particle, moving along a randomly distributed path, passes through the aperture, it changes the impedence between the two electrodes. The first electrode is inside the aperture tube and holds that part of the electrolyte at ground potential. The second electrode is connected to a constant current supply and is immersed in the solution in the sample cup. The change in resistance between the two electrodes generates a voltage pulse, the amplitude of which is approximately proportional to the displaced volume of the particle. The principle limiting resistance to the current is the electrolyte within the aperture itself. The Coulter is able to maintain a positive voltage pulse due to internal corrections of positive and negative current polarity.

The number of particles in a unit volume of suspension can also be determined using the built-in manometer syphon system. This allows for the determination of particle concentration. Particle shape cannot be measured by the method, so results are expressed in spherical equivalents. This proves to be valid, as sufficiently high numbers of particles are counted to ensure an averaging of orientations of particles traversing the aperture [Fine Particle Application Coulter Issue D].

Measurement of crystal growth is achieved by monitoring the increase in the crystal volumes [Markovic and Komunjer 1979; Ryall *et al.* 1981a] whereas the percentage change in

total crystal numbers gives an indication of crystal aggregation [Ryall *et al.* 1981a].

After coincidence corrections, the measured pulses are electronically amplified, sized, counted and accumulated. This accumulation occurs in equivalent spherical diameter size channels which are pre-calibrated. The size distribution of the suspended phase can then be determined from derived data.

The analysis of liquid and electrical current flow through the aperture is not simple. Assumptions are therefore necessary in the derivation of the electrical response from particle passage. These assumptions are as follows [Fine Particle Application Coulter Issue D]:

1. The aperture contents form a cylindrical resistor in which current density is uniform.

2. Multiplying the aperture length by an appropriate factor incorporates the electrically effective zones outside the aperture.

3. The passages of individual particles occur at random and are evenly distributed through the aperture cross-section.

4. The electrically effective volume of a particle in the aperture may be expressed as a cylinder having the same resistivity as the particle.

Notwithstanding that the electrical pulse is correlated with the particle volume, the two variables cannot be related simply unless the particle is a smooth sphere. This is due to the dependency of the signal on the shape of the particle. Calibration is effected by smooth latex spheres and is based upon the assumption that the pulse height is proportional to the particle volume. The data from the Coulter are therefore equated for spheres and as such are not absolute for irregularly shaped particles.

Figure 11 illustrates the Coulter principle (adapted from [Allen 1981]).

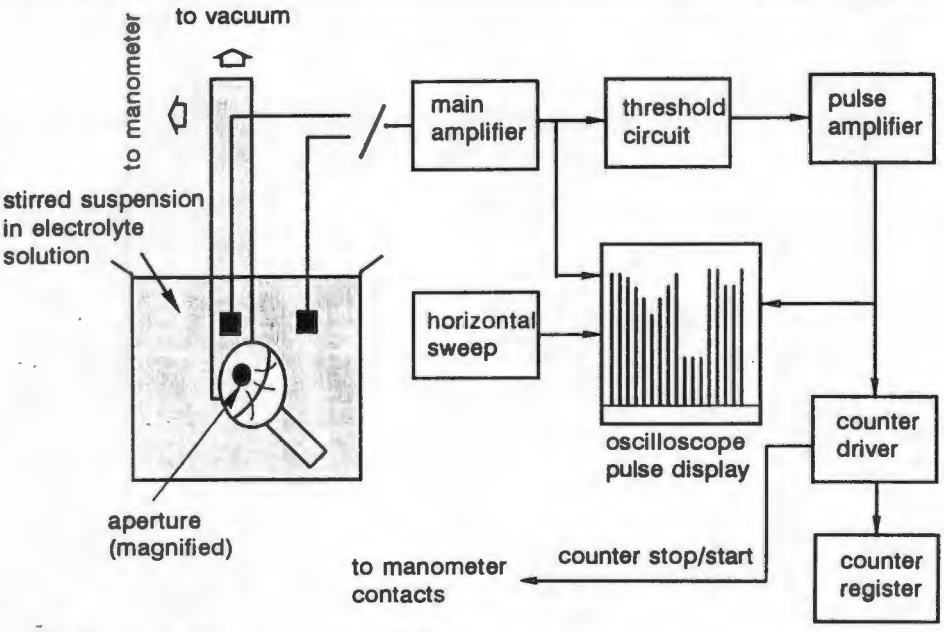


Figure 11: The Coulter principle.

A controlled external vacuum unbalances the mercury siphon and initiates sample flow from the standard sample cup, through the aperture and up into the aperture tube. The system is then isolated from the external vacuum and sample flow continues due to the siphoning action of the rebalancing mercury. The count procedure begins when the mercury column contacts the start probe. The sample suspension continues to flow through the aperture, up through the aperture tube and into the waste flask. Counting stops when the mercury column reaches the stop probe. The distance between the start and stop probes is fixed and is directly related to sample volume.

The immersed electrodes measure the change in resistence resulting from particle passage through the orifice. The size distribution of the suspended phase is calculated from the derived data generated from the voltage pulses. All amplified voltage pulses that are above the adjustable threshold level are counted. These counts represent the number of particles larger than a determinable volume proportional to the appropriate threshold setting [Allen 1981]. The determination of number frequency as a function

of volume can be achieved by analysing counts at various amplifications and threshold settings.

A modification for urinary crystal size measurement with the Coulter Counter was described by Ryall and Marshall (1978). The method ensured that there was no skewing of the results by large particles. This was accomplished by ensuring that the counting was taken at equal size increments instead of the constant threshold settings previously used.

There is a wide range of operating modes available to obtain optimum results from the sample analysis [Operating Manual Coulter Multisizer Issue C]. Methods of sampling include manual start/stop, and pre-selection of time, volume and total count. Various levels of resolutions of the size distribution are also available ranging from division into 16 to 256 channels [Operating Manual Coulter Multisizer Issue C].

Interfacing of the Coulter with a computer has allowed the manipulation of raw data [Muerdter *et al.* 1981]. Distributions of number, volume and surface area are available in both differential and cumulative modes.

Calibration

Calibration of the Coulter Counter is effected by the count integration procedure using narrow distribution (monosized) smooth particles of known density.

The scaling of current to voltage conversion is set during the calibration so that specific particle sizes can be recognised. Effectively the calibration establishes the particle size (volume) that is represented at one channel edge. (Explanation of the calculation of channel sizes is described later in this Chapter II.2 under "Channel size calculation"). There is a regular geometric size progression between all the channels in use so that as soon as a particle size is represented by one channel, the

remaining channel sizes are automatically computed. The Coulter is equipped with automated calibration procedures for standard orifice tube sizes to make calibration easier [Coulter Multisizer manual Issue C].

Electrolyte

In general, water insoluble particles can be dispersed using appropriate surfactants in aqueous electrolytes such as sodium chloride (NaCl). When choosing an electrolyte, it is necessary to ensure that it is compatible with the material being analysed.

Orifice tube

The percentage current change as each particle passes through the aperture is proportional to the ratio of particle volume to volumetric aperture size (cross section multiplied by length through wafer). Although there is a wide range of aperture diameters available, the upper particle volume diameter which can be measured by each aperture is limited. This is due to the previously mentioned proportionality being linear to only 40% of the aperture diameter because of aperture blockage when larger particles are present in the suspension. For particle diameters below about 2% of the aperture diameter, the current change is too small to detect reliably.

Coincidence correction

The Coulter Counter automatically corrects the particle counts for coincident particle passages [Wales and Wilson 1961,1962]. There are two types of coincident particle passage, namely primary and secondary [Allen 1981]. Primary coincidence, also known as horizontal interaction, occurs when two particles give rise to pulses at the same instant. The result is an overlapping of the two pulses. Secondary coincidence, or vertical interaction, occurs when the individual pulses from two particles are additively large enough to be sensed. The result is the measurement of a single large pulse. This occurs when the two

particles are of similar size and are close to each other. Coincident particle passage can affect the measured size distribution due to the errors of primary count loss, secondary count gain, and shadow loss [Kaye 1981]. The latter occurs when a particle is not sensed due to its proximity to another particle that is being measured. The measured particle can desensitize the measurement zone around itself for a short period thus enabling closely following particles to go undetected.

Overall coincidence can be avoided by observing allowable limits in counts per second for the aperture in use. Calculation of these limits from first principles is given by the formula described by McCave and Jarvis (1973).

Dilution of the sample is necessary if the coincidence limit is exceeded. This is because loss of data in the small size classes will occur, leading to a narrowing of the distribution. As long as the acceptable coincidence levels are maintained, the number of particles in the large size classes will be low. This is due to the logarithmic decrease in the population distribution as size increases. To avoid poor analytical resolution in these size ranges, sufficient crystals must be counted leading to long counting times.

It is necessary to filter the electrolyte and blank sample solution so that the lowest possible background count is obtained. Attempts have been made to analyse unfiltered and undiluted urine with the Coulter Counter [Hennequin *et al*. 1991]. However, their coincidence levels were found to exceed the limit of 20%. This was due to the large number of particles in the unfiltered sample. Blocking of the aperture was found to be due to gross particulate matter which is present in all unfiltered urines as opposed to it being caused by large crystals per se.

In practice it is necessary to keep the coincidence correction at a level of about 10% while ensuring the standard deviations are kept below 0.5% by reading particle

numbers in the tens of thousands. This will lead to an overall accuracy of counting better than 1%.

Channel size calculation

A simplified diagrammatic representation of the full range analysis is shown in Figure 12 [Coulter Multisizer manual Issue C] where LC and RC are the left and right edges respectively of any channel other than channel X.

With reference to Figure 12, the size of the right edge (RE) of any channel (X) is given by Equation 28:

$$d_{\mu m} = (K_C * X)/(J(I*G)^{1/3} \quad \ldots\ldots\ldots\ldots\ldots 28$$

where K_C is the overall calibration constant, J is the number of channels in use, and I and G are the current and gain respectively.

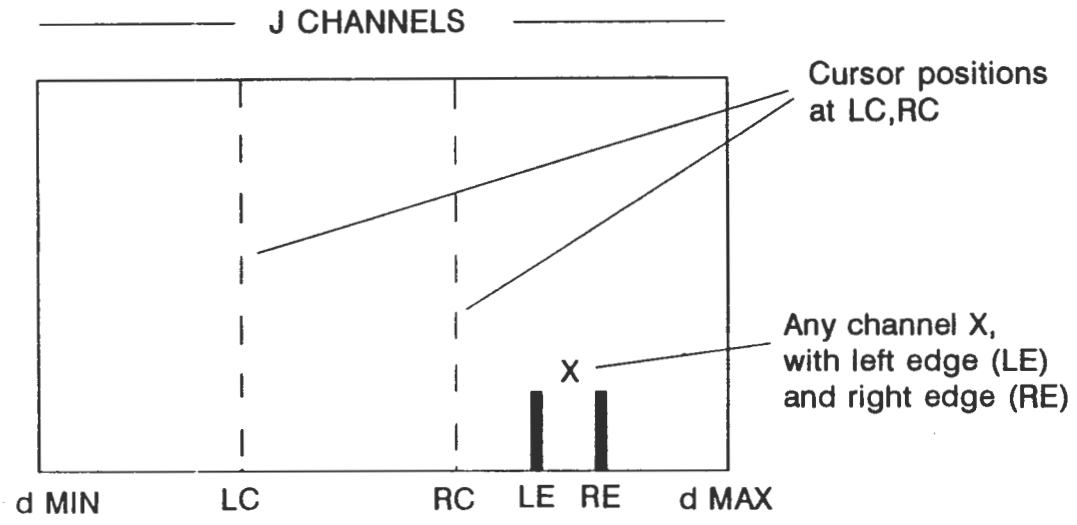


Figure 12: Full range analysis of the Coulter counter.

The calculation of the diameter of any channel X is given by Equation 29:

$$D_{\mu m} = d_{256} * i^{U} \quad \ldots\ldots\ldots\ldots\ldots\ldots 29$$

where $U = 256(X/J - 1)$.

Equations 30 and 31 allow for the conversion into area and volume respectively:

$$\text{area } (\mu m^2) = \pi d^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots 30$$

$$\text{volume } (\mu m^3) = \pi d^3/6 \quad \ldots\ldots\ldots\ldots 31$$

The true midpoint (d_m) of each channel (Equation 32) is used in the weighting procedure:

$$d_m = (d_{(x-1)} + d_x)/2 \quad \ldots\ldots\ldots\ldots 32$$

Diameter weighting is then given by Equation 33:

$$\bar{X} = \Sigma[f(d_m)]/\Sigma f \quad \ldots\ldots\ldots\ldots\ldots 33$$

where m = (X-0.5) and f represents the channel contents.

A similar procedure to that for the full range shown above, is followed for the narrow and window ranges. These ranges are used when greater detail of a specific size range is required after an accumulation in full range has given some knowledge of the sample distribution.

REFERENCES: CHAPTER II

Agrawal,Y.C.; McCave,I.N.; Riley,J.B.
"Laser diffraction size analysis"
in:"Principles, methods and applications of particle size analysis" ed:Syvitski,J.P.M. Cambridge Univ.Press (1991), 119-128

Allen,T.
"The electrical sensing zone method of particle size distribution determination (the Coulter principle)"
in: "Particle Size Distribution. Powder Technology Series", 3rd Edition, ed: Scarlett,B., Chapman and Hall, London, New York, (1981), 393-401

Atkins,P.W.
"Physical Chemistry"
Oxford Univ.Press, Oxford 2nd Ed (1982)

Azoury,R.; Robertson,H.G.; Garside,J.
"Observations on in vitro and in vivo calcium oxalate crystalluria in primary calcium stone formers and normal subjects"
Br.J.Urol. (1987), **59**, 211-213

Berg,C.; Tiselius,H.G.
"The effects of citrate on hydroxyapatite induced Calcium Oxalate crystallisation and on the formation of Calcium Phosphate crystals"
Urol.Res. (1989), **17**, 167-172

Berne,B.J.; Pecora,R.
"Dynamic light scattering"
New York, J.Wiley, (1976)

Bohren,C.F.; Huffman,D.R.
"Absorption and scattering of light from small particles"
John Wiley, New York, (1983), 279

Born,M.; Wolf,E.
"Principles of optics" 5th edition, Pergamon Press, Oxford, (1975), 635-664

Bowyer,R.C.; Brockis,J.G.; McCulloch,R.K.
"Glycosaminoglycans as inhibitors of calcium oxalate crystal growth and aggregation"
Clin.Chim.Acta (1979), **95**, 23-28

Brown,D.J.; Felton,P.G.
"Direct measurement of concentration and size for particles of different shapes using laser light diffraction"
Chem.Eng.Res.Des. (1985), **63**, 125-132

Burkholz,A.; Polke,R.
"Laser diffraction spectrometers - experience in particle size analysis"
Part.Charact. (1984), **1**, 153-160

Cadle,R.D.
"Particle size determination"
Interscience Publishers Inc. New York (1955), 92-101

Chigier,N.
"Comparative measurements using different particle size instruments"
in"Liquid particle size techniques" vol 1, eds: Tishkoff,J.M.; Ingebo,R.D.; Kennedy,J.B.
Am.Soc.Testing and Materials (1984), 169-186

Chu,B.
"Laser light scattering. Quantum Electronics, Principles and applications"
Academic Press, New York, (1974), Ch 2,7

Cornillault,J.
"Particle size analyser"
Appl.Opt. (1972), **11**, 265-268

Coulter,W,H.
"High speed automatic blood cell counter and cell size analyser"
Proceeds of the National Electronics Conference (1957), 1034-1042

"Coulter Multisizer manual (Software level 2.1 and 3.1) Issue C"
Coulter Electronics Ltd, England, (May 1987)

Davies,J.A.; Collins,D.L.
"Comparison of the size distribution of Boron powders as measured by Malvern Diffractometer and Coulter counter"
Part.Part.Syst.Charact. (1988), **5**, 116-121

de Boer,G.B.J.; de Weerd,C.; Thoenes,D.; Goossens,H.W.J.
"Laser diffraction spectrometry: Fraunhofer versus MIE scattering"
Part.Charact. (1987), **4**, 14-19

Dodge,L.G.
"Calibration of the Malvern particle sizer"
Appl.Opt. (1984), **23**, 2415-2419

Edyvane,K.A.; Ryall,R.L.; Mazzachi,R.D.; Marshall,V.R.
"The effect of serum on the crystallization of Calcium Oxalate in whole human urine: Inhibition disguised as apparent promotion"
Urol.Res. (1987), **15**, 87-92

Felton,P.G.
"In-stream measurement of particle size distribution"
in: "Proceedings, the international Symposium on in-stream measurements of particle solid properties" Bergen Norway (Aug 1978)

Felton,P.G.
"A review of the Fraunhofer diffractions particle sizing technique" in: "Liquid particle size measurement techniques" 2nd Vol. eds: Hirleman,E.D.; Bachelo,W.D.; Felton,P.G.; American society for testing and materials. Philadelphia (1990), 47-59

Felton,P.G.; Brown,D.J.
"Measurement of crystal growth rates by laser diffraction"
Solids Separation Processes Dublin, IChemE. Symposium Series (1980), 59-61

"Fine particle application notes for Coulter counters Issue D" Coulter Electronics Ltd, England, (July 1987)

Gowesbet,G.; Grehan,G.
"Optical particle sizing"
Plenum Press, New York (1988)

Greenberg,J.M.
"Focussing in on particle shape"
in: "Light scattering by irregularly shaped particles" ed: Schuerman,D.W., Plenum Press, (1980), 7-23

Grover,P.K.; Ryall,R.L.; Marshall,V.R.
"Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallisation in human urine?"
Clin.Chim.Acta (1990a), **190**, 223-238

Grover,P.K.; Ryall,R.L.; Potezny,N.; Marshall,V.R.
"The effect of decreasing the concentration of urinary urate on the crystallisation of calcium oxalate in undiluted human urine"
J.Urol. (1990b), **143**, 1057-1061

Groves,M.J.
"Particle size analysis: Past, Present and Future"
Analyst (Dec 1974), **99**, 959-972

Guenther,R.
"Modern optics"
John Wiley and Sons Inc, Canada, (1990), 278-279

Hamidi,A.A.; Swithenbank,J.
"Treatment of multiple scattering of light in Laser diffraction measurement techniques in dense sprays and particle fields"
J.Institute of Energy (1986), **59**, 101-105

Haken,H.
"Laser theory"
Springer-Verlag, Berlin, Heidelberg, New York (1984), Ch3

Hatch,T.; Choate,S.P.
"Statistical description of the size properties of non-uniform particulate substances"
J.Franklin Inst. (1929), **207**, 369-387

Hennequin,C.; Gaubil,J.; Bader,C.; Daudon,M.; Drueke,T.; Lacour,B.
"Impossibility to study crystalluria directly with Coulter counter"
Clin.Chim.Acta (1991), **200**, 227-230

Hirleman,E.D.; Oeahsle,V.; Chigler,N.A.
"Response characteristics of laser diffraction particle size analysers. Optical sample volume extent and lens effect"
Opt.Eng. (1984), **23**, 610-619

Jaggard,D.L.; Hill,C.; Shorthill,R.W.; Stuart,D.; Glantz,M.; Rosswog,F.; Taggart,B.; Hammond,S.
"Light scattering from particles of regular and irregular shape"
Atmos.Enviro. (1981), **15** (12), 2511-2519

Jonasz,M.
"Size, shape, composition and structure of microparticles from light scattering"
in: "Principles, methods and application of particle size analysis" ed:Syvitski,J.P.M. Cambridge Univ. Press (1991), 143-162

Kaye,B.H.
"Direct characterisation of fineparticles"
in: "Chemical analysis. A series of monographs on analytical chemistry and its application" Vol 61,
eds: Elving,P.J.; Winefordner,J.D.; Kolthoff,I.M.
John Wiley & Sons, New York (1981), 271-297

Kerker,M.
"The scattering of light (and other electromagnetic radiation)"
Academic Press. New York. (1969)

Kusters,K.A.; Wijers,J.G.; Thoenes,D.
"Determination of particle size distributions of ice crystals in aqueous solutions using laser diffraction spectrometry"
in: "Proc.2nd Int.Conf.Optical Particle Sizing" (March 1990) Arizona State Univ. Press ASU 585-592

Laws,E.Q.; Ashworth,R de B.; Beech,D.G.; Furmidge,C.G.L.; Heywood,H.; Hibbott,H.W.; Hinsley,J.F.; Howes,R.; Jackson,R.; Kayle,B.H.
(Particle size analysis sub-committee)
"Classification of methods for determining particle size. A review"
Analyst (1963), **88**, 156-187

Lightfoot,N.S.; Watson,D.J.
"The effect of optical properties of particles and dispersing media on particle size analysis by laser diffraction" Proc.2nd Int.Cong.Opt.Part.Sizing
Mech.Aerospace Eng. Arizona State Univ. March 5-8 (1990), 511-519

Lines,R.
"Sizing up the options for particle characterisation"
Reprinted from Process Engineering, Coulter Electronics Ltd, England (Nov 1990)

Malvern Instruments 2600 Series User Manual
Issue 2 Malvern Instruments, Malvern (1989)

Markovic,M.; Komunjer,L.
"A new method to follow crystal growth by Coulter counter"
J.Cryst.Growth (1979), **46**, 701-705

McCave,I.N.; Jarvis,J.
"Use of the model T Coulter Counter in size analysis of fine to coarse sand"
Sedimentology (1973), **20**, 305-315

McCave,I.N.; Syvitski,J.P.M.
"Principles, and methods of geological particle size analysis"
in: "Principles, methods and application of particle size analysis" ed: Syvitski,J.P.M., Cambridge Univ. Press, Cambridge (1991) 1-21

Mohamed,N.; Fry,R.C.; Wetzel,D.L.
"Laser Fraunhofer diffraction studies of aerosol droplet size in atomic spectrochemical analysis"
Anal.Chem. (1981), **53**, 639-644

Muerdter,D.R.; Dauphin,J.P.; Steele,G.
"An interactive computerised system for grain size analysis of silt using electroresistance"
J.Sedimentary Petrology (1981), **51**, 647-650

Narducci,L.M.; Abraham,N.B.
"Laser physics and laser instabilities"
World Scientific, Singapore, (1988), Ch1

"Operating manual for the Coulter Multisizer (Software level 2.1 and 3.1) Issue C" Coulter Electronics Ltd, England, (May 1987)

Perry,R.; Hunt,A.; Huffman,D.
"Experimental determination of Mueller scattering matrices for non-spherical particles"
Appl.Opt. (1978), **17**, 2700-2710

Phahla,L.
"The determination of refractive index of the particles"
Honours project, Dept Physics (1991)

Randolph,A.D.; Larson,M.A.
"Theory of particulate processes"
Academic Press, New York, London (1971), 15

Rayleigh,Lord J.W.S.
"On the transmission of light through an atmosphere containing small particles in suspension, and on the origin of the blue of the sky"
Phil.Mag. 5th Series, (1871), **47**, 375-384

Rayleigh,Lord J.W.S.
"On the electromagnetic theory of light"
Phil.Mag. 5th series, (1881), **12**, 81-101

Riley,J.B.; Agrawal,Y.C.
"Sampling and inversion of data in diffraction particle sizing"
Appl.Opt. (1990)

Robertson,W.G.
"A method for measuring calcium crystalluria"
Clin.Chim.Acta (1969), **26**, 105-110

Robertson,W.G.; Peacock,M.
"Calcium Oxalate crystalluria and inhibitors of crystallisation in recurrent renal stone formers"
Clin.Sci. (1972), **43**, 499-506

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Inhibitors of the growth and aggregation of Calcium Oxalate crystals in vitro"
Clin.Chim.Acta (1973), **43**, 31-37

Ryall,R.L.; Bagley,C.J.; Marshall,V.R.
"Independent assessment of the growth and aggregation of Calcium Oxalate crystals using the Coulter counter"
Invest.Urol. (1981a), **18** (5), 401-405

Ryall,R.L.; Harnett,R.M.; Hibberd,C.M.; Edyvane,K.A.; Marshall,V.R.
"Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallisation in undiluted human urine"
Urol.Res. (1991), **19**, 181-188

Ryall,R.L.; Hibberd,C.M.; Marshall,V.R.
"A method for studying inhibitory activity in whole urine"
Urol.Res. (1985), **13**, 285-289

Ryall,R.L.; Marshall,V.R.
"A reappraisal of the Coulter counter technique for measuring Calcium Oxalate crystal size"
Clin.Chim.Acta (1978), **83**, 99-107

Ryall,R.L.; Ryall,R.G.; Marshall,V.R.
"Interpretation of particle growth and aggregation patterns obtained from the Coulter counter: a simple theoretical model"
Invest.Urol. (1981b), **18**, 396-400

Ryall,R.L.; Ryall,R.G.; Marshall,V.R.
"A computer model for the determination of extents of growth and aggregation of crystals from changes in their size distribution"
J.Cryst.G. (1986), **76**, 290

Ryall,R.G.; Ryall,R.L.; Villis,R.; Marshall,V.R.
"Calculation of crystal growth and crystal aggregation when both processes occur simultaneously"
in: "Urinary Stone" eds: Ryall,R.L.; Brockis,J.G.; Marshall,V.R.; Finlayson,B. Churchill Livingstone (1984), 372-377

Swithenbank,J.; Beer,J.M.; Taylor,D.S.; Abbott,D.; McCreath,G.C.
"A laser diagnostic technique for the measurement of droplet and particle size distribution"
in: "Experimental diagnostics in gas phase combustion systems" ed: Zinn,B.T. Prog.Astronaut.Aeronaut. (1977), **53**, 421-447

Thyagaragjan,K.; Ghatak,A.K.
"Lasers. Theory and application"
Plenum Press, New York, London (1981), 418

Tiselius,H.G.
"Measurement of the risk of calcium oxalate crystalluria in urine"
Urol.Res. (1985), **13**, 297-300

van de Hulst,H.C.; Moore,A.F.; DeJager,C.
"Light scattering by small particles"
John Wiley & Sons Inc. New York (1964), pg7,12,75,119

Wales,M.; Wilson,J.N.
"Theory of coincidence in Coulter Particle Counters"
Review of Scientific Instrumentation (1961), **32**, 1132-1136

Wales,M.; Wilson,J.N.
"Coincidence in Coulter Counters"
Review of Scientific Instrumentation (1962), **33**, 575-576

Weiss,E.L.; Frock,H.N.
"Rapid analysis of particle-size distributions by laser light scattering"
Powder Technol. (1976), **14**, 287-293

Zerull,R.; Giese,R.; Weiss,K.
"Scattering measurements of irregular particles vs Mie theory"
Opt.Polar. 12 (instrumentation and applications)
Pro.Soc.Photo-Optical Instrum.Engrs. (1977), 191-199

CHAPTER III

DESIGN AND DEVELOPMENT OF THE MSMPR CRYSTALLISATION SYSTEM

III.1 INTRODUCTION

The mixed suspension mixed product removal (MSMPR) crystalliser system is an example of a steady state continuous crystalliser. It can be used to generate particles by evaporation, cooling, or chemical reaction [Randolph and Larson 1971; Youngquist and Randolph 1972; Jancic and Garside 1975; Garside and Jancic 1976; Brecevic and Garside 1981; Grootscholten *et al.* 1982; Bennett 1984; Nishio *et al.* 1990,1991; Kavanagh *et al.* 1993]. Evaporation is normally achieved under vacuum to remove the solvent which causes deposition of the solute [Grootscholten *et al.* 1982]. Crystallisation by cooling involves lowering the temperature of the crystalliser which contains a concentrated pre-heated solution. The cooling results in deposition of the solute due to a decrease in solubility at lower temperatures [Garside and Jancic 1978].

Precipitation systems are the most common for laboratory use. In this type of crystalliser, a third component (in which the solute is insoluble) is added to the system, or a chemical reaction is initiated in which the product is insoluble in the crystallising medium [Brecevic and Garside 1981]. An advantage of the precipitation crystalliser is that it can be used in isothermal conditions and at atmospheric pressure [Randolph and Larson 1971].

The MSMPR crystalliser which generates supersaturation by chemical reaction is the type used in the present study. Supersaturation is generated by the continual feeding of reactants into the crystalliser; constant supersaturation is achieved after 10 volumes have passed through the chamber [Randolph and Larson 1971; Brecevic and Garside 1981; Rodgers and Garside 1981; Li *et al.* 1985]. Constraints of the steady state operation are that the feed rate, composition and

temperature are constant as well as crystalliser volume and temperature [Randolph and Larson 1971].

III.2 CRYSTALLISER

Several design requirements were necessary in order to satisfy fundamental MSMPR crystalliser constraints.

Firstly, the design must allow perfect mixing to be achieved in the crystalliser such that the liquor composition and crystal size distribution are uniform throughout.

Secondly, there must be no size classification (ie the mean retention probability of the particles must not vary over the particle size range to be measured) at the offtake, ensuring that the discharge suspension density, size distribution and liquor composition are the same as in the crystalliser.

Thirdly, the size of the crystalliser should be such that a minimum volume of feed is needed and minimum disturbance is caused by sampling [Randolph and Larson 1971].

Crystal breakage is assumed to be negligible and shape is assumed to be uniform.

The design features of the crystalliser used in the present study were modelled on guidelines by Brodkey and Hershey (1988) and are shown in Figure 3.1.

The following definitions are used to describe the physical dimensions of the agitation vessel:

B : Width of each baffle
C : Distance from tank bottom to impeller center line
D : Impeller diameter
T : Inside diameter of tank
Z : Depth of fluid in the tank

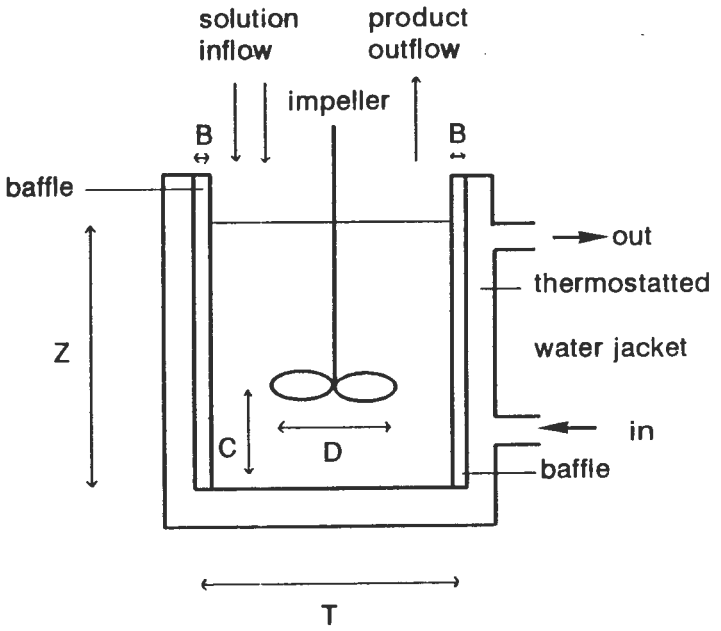


Figure 3.1: The physical dimensions of the crystalliser.

An impeller is a device that provides agitation and mixing of a liquid. Impellers are classified into both axial and radial flow with the three main types being propellers, paddles and turbines.

In designing the crystallisation unit, the following ratios had to be satisfied [Brodkey and Hershey 1988]:

D/T = 1/3 (For turbulent flow regimes)
B/T = 1/18 (For propellers)

(The ratio B/T applies for center-mounted impellers and is usually equal to 1/12 for turbine or paddle agitation. Propellers are high speed, axial-flow impellers for low viscosity liquids, and as such, do not require baffles as large as those for radial-flow impellers).

C/T = 1/3 (Bottom clearance of any impeller)
Z/T = 1 (Fluid level in the tank)

These ratios have been derived from many laboratory scale experiments and are the principle dimensions used during the scale-up to plant size systems. The first three ratios ensure a maximum level of mixing while at the same time keeping the power input and operating costs to a minimum. The fourth

ratio ensures that the most distant regions of the crystalliser are mixed.

The physical dimensions that satisfied these conditions and which were used in the present study for a vessel of volume $200cm^3$ (as quoted by other workers in the literature) are:

T = 63.5mm
Z = 63.5mm
D = 21.17mm
C = 21.17mm
B = 3.52mm

The crystalliser consisted of a rounded flat bottomed $200cm^3$ glass vessel. It was surrounded by a water jacket to allow for thermostatting and was closed at the top by a tight fitting lid. The lid incorporated holes to allow for an overhead stirrer and for input and output feed lines [Bennett 1988]. The overhead stirrer was centrally mounted and the input and output feed lines were on opposite sides to each other. This was done to ensure that the solutions were not withdrawn from the crystalliser prior to their being mixed. The feed lines and offtake were also positioned midway between adjacent baffles. Introduction and withdrawal of the solutions in this position ensured better mixing than if they were introduced along the baffle positions.

III.3 IMPELLER DESIGN

Mixing

The overall process of mixing consists of macro-, meso- and micromixing. Macromixing is concerned with mixing on the scale of the whole vessel (ie. bulk blending) [Baldyga and Pohrecki 1995] and is considered in more detail later in the following four pages. Mesomixing is the turbulent dispersion of the inlet feed just after it has entered the reaction vessel [Baldyga *et al.* 1993]. Micromixing then occurs in the turbulent concentration field created by the mesomixing process. Baldyga and Pohorecki (1995) have reviewed turbulent micromixing while Bourne and Dell'ava (1987) have considered the different types of mixing on scale-up.

Garside and Tavare (1984, 1985) and Tavare (1986,1989) have shown that micromixing can significantly influence the overall performance of the crystalliser. Tavare (1994) has also shown that micromixing effects are more important when reactants enter the MSMPR crystalliser separately rather than when they are pre-mixed. Baldyga and Bourne (1992), showed experimentally that competition between micromixing and the reaction determines the product distribution at low feed rates. Using a semi-batch reactor, they considered the importance of micro- and mesomixing as process controlling rates.

Bourne and Yu (1994) integrated their theoretical model of micromixing with an experimental flow model. Using two and three parallel reactions they investigated the effect on micromixing of feed positions, dished and flat bottomed vessels, vessel size, stirrer speeds and impeller diameter. Using a Rushton turbine to develop and maintain fully turbulent flow, Bourne *et al.* (1995) have shown that fluid viscosity is a relevant variable for micromixing. Also, using a computational fluid mixing model, Bakker and Fasano [1994] theoretically calculated blend time and chemical product distributions in turbulent, agitated vessels and considered the effect of inlet feed positions.

In precipitation processes, the crystal size distribution (CSD) is dependent upon all the three processes of mixing [Pohorecki and Baldyga 1988]; however, since chemical reactions, nucleation and growth occur on a molecular level, only molecular level mixing should affect their course.

Laminar and turbulent flow

As already mentioned, the flow patterns of a liquid in a vessel depend upon various factors such as the size of the vessel, presence of baffles, the characteristics of the fluid, and the mode of stirring [Skrtic *et al.* 1987]. The design of the impeller has a strong impact on the type of flow and the energy requirements [Bates *et al.* 1963]. The degree of suspension required also determines the amount of power used

with each specific impeller. Although there is a large range of impellers available for mixing, the choice is restricted to whether laminar or turbulent flow is required [Brodkey and Hershey 1988]. Laminar flow occurs when the flow patterns produced by the impeller are radial and tangential only. Turbulent flow causes axial flow patterns which incorporate the radial, tangential and longitudinal components of flow as discussed later in the next section "Velocity components of flow".

If laminar flow is required an anchor or a helix impeller can be used. Typically laminar flow impellers are large and operate at low speeds, providing poor mixing. For turbulent flow there is a range of turbines with pitched blades and also a range of pitched blade mixing propellers.

The performance of various impellers and the effect of the D/T ratio has been examined by Shaw (1992). More specifically, three and six bladed axial flow propellers have been considered by Musil *et al.* (1984). To ensure thorough mixing for a uniform size distribution, turbulent flow was necessary in the system used in the present study.

Velocity components of flow

The overall flow pattern in the vessel depends upon variations of the three velocity components of fluid flow from point to point. The radial component acts perpendicular to the shaft of the impeller. The longitudinal component acts parallel to the shaft, and the tangential or rotational component acts in a direction tangent to a circular path around the shaft [McCabe and Smith 1976].

For mixing, tangential flow is undesirable. Using an impeller located vertically and at the center of an unbaffled vessel, the tangential component poses two problems. The first is laminar flow of the liquid around the impeller. This flow causes rotation of the fluid in distinct levels which do not intermix, leading to the absence of longitudinal flow necessary for thorough mixing. Centrifugal forces from these

circulatory currents then force the particles to the vessel walls. This eventually leads to a concentration of the particles at the center of the vessel bottom. This entire process is due to the same angular velocities of the impeller and fluid.

Vortices

The second problem of tangential flow is the formation of a surface vortex, which, if deep enough, can entrain air into the fluid. The presence of a vortex can also raise the level of the outer fluid which could lead to spillage.

Vortex systems also occur in the region around the impeller blades [Calabrese and Stoots 1989]. These are different for each impeller design, and an understanding of this region is necessary for various agitation applications [Kumar *et al.* 1991a,b].

To counteract the tangential velocity component, four equi-spaced baffles were incorporated into the crystalliser design for the present study. Mounting the impeller off-center [Garside and Jancic 1976], or in the side of the vessel [McCabe and Smith 1976] can also prevent the circulatory flow.

The effect of mixing intensity on the particle size obtained from both baffled and unbaffled vessels in a semi-continuous and batch process has been investigated by Pohorecki and Baldyga (1983) both theoretically and experimentally. They developed a new model of micromixing to determine the CSD from precipitation reactions. Their predicted results agreed with experimental results for the precipitation of $BaSO_4$ in a stirred crystalliser and showed that the rate of crystallisation and the CSD are directly influenced by the mixing intensity.

Propeller

The flow currents leaving a propeller continue through the fluid until they are deflected by the wall or the bottom of

the vessel. If there is no slip between propeller and the fluid, then one full revolution of the propeller would move the liquid longitudinally through a fixed distance [McCabe and Smith 1976]. This distance is dependent upon the angle of inclination of the propeller blades. The ratio of this distance to the propeller diameter is known as the pitch of the propeller.

To provide the necessary turbulent flow, a vertical, center mounted two blade propeller with a pitch of 45° was chosen for the present study. The steel propeller and baffles were coated with teflon [Bourne and Zabelka 1980] as soft-coated impellers were found to produce fewer nuclei than steel impellers in studies of secondary nucleation of K_2SO_4 [Shah *et al.* 1973; Randolph and Sidkar 1974,1976; Evans *et al.* 1974]. In the present system, the propeller was driven by a Heidolph RZR50 motor. A commercially available heavy base stand was required to support the motor as a consequence of its weight and strong vibrational motion [Fasano *et al.* 1995].

A schematic diagram of the liquid flow in a baffled vessel is shown in Figure 3.2. The propeller drives the liquid straight down to the bottom of the vessel. The flow currents then spread radially outwards toward the wall, upwards along the wall, and then finally return to the suction of the propeller from the top.

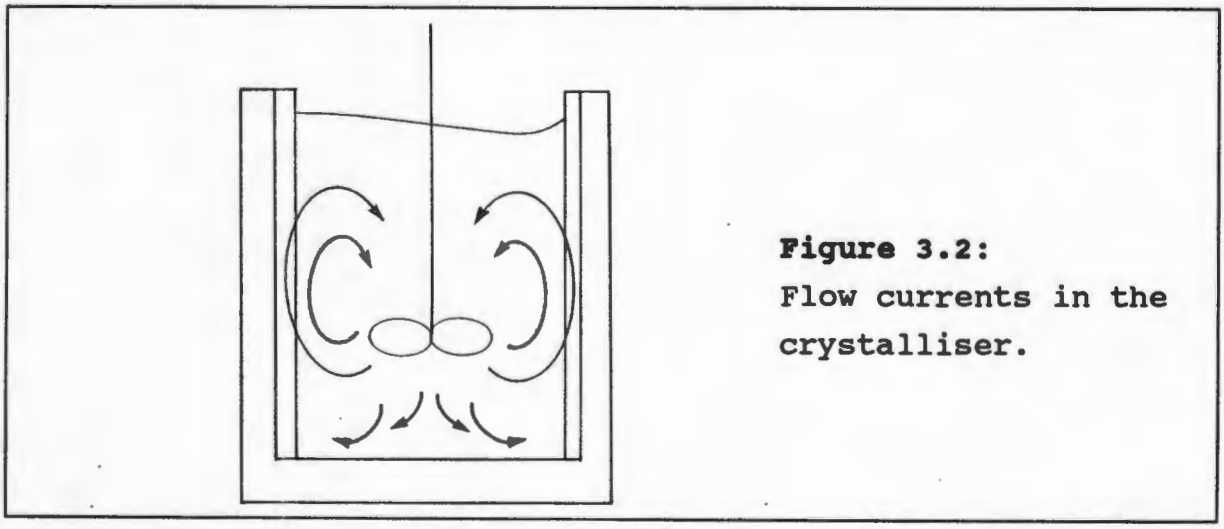


Figure 3.2:
Flow currents in the crystalliser.

If control over the direction and velocity of flow to the suction of the impeller is required then a draft tube can be

used [Garside and Jancic 1978]. Oldshue (1969b) has discussed the general principles of suspending solids and gases in liquids and has considered variables such as D/T, the height of suspension and settling velocities. He has also reviewed general and specific mixing [Oldshue 1969a,1989].

III.4 THE OFFTAKE TUBE

The validity of the kinetic measurements depend upon achieving the conditions which were assumed when the size distribution equations were derived. However, even if the crystalliser conditions are achieved, the sampling procedures and subsequent sample treatment can destroy the validity of the results. Therefore, much time was spent on achieving representative sampling in the present study.

A pipette was used by Rodgers and Garside (1981) for the intermittent removal of the product from the crystalliser. In the present study, it was decided to withdraw the crystalliser product using a vertical tube immersed into the sample rather than a built-in offtake at the side of the crystalliser [Zacek *et al.* 1982]. This ensured that the offtake direction was the same as the direction of flow in the crystalliser. In this way it was hoped to mimic sampling by a pipette. The offtake was positioned midway between two adjacent baffles and also on the opposite side to the input feeds [Zacek *et al.* 1982].

The first design was a simple glass offtake tube. The tube was made as short as possible to minimize any changes which might occur in CSD as the mixed product flowed from the crystalliser to the cell in which the CSD was to be measured (Figure 3.16 page 99). The glass was not connected directly to the cell because of the difficulty of glueing it to teflon. Therefore a short connecting section of tygon tubing was used. Due to restricted laboratory space for the crystalliser and cell, the offtake tube incorporated a u-bend. The bend angle was made as small as possible to minimize any laminar flow effects.

With this design, it was not possible for the entire offtake tube to be jacketed for thermostatting due to constraints on the physical space available.

During this early stage of system development, an attempt was made to use the offtake tube not only for sampling, but as a levelling device as well. The offtake design and configuration are represented in Figure 3.3. During blank determinations, the sample tube was immersed to a depth equal to that of the midpoint of the propeller blades [Zacek *et al.* 1982] for sample withdrawal (Figure 3.3a). During unsteady state conditions, when the product from the crystalliser was withdrawn to waste and not to the cell, it was positioned at the top of the fluid in the crystalliser (Figure 3.3b). When steady state was finally attained, the offtake tube was immersed to the level shown in Figure 3.3a.

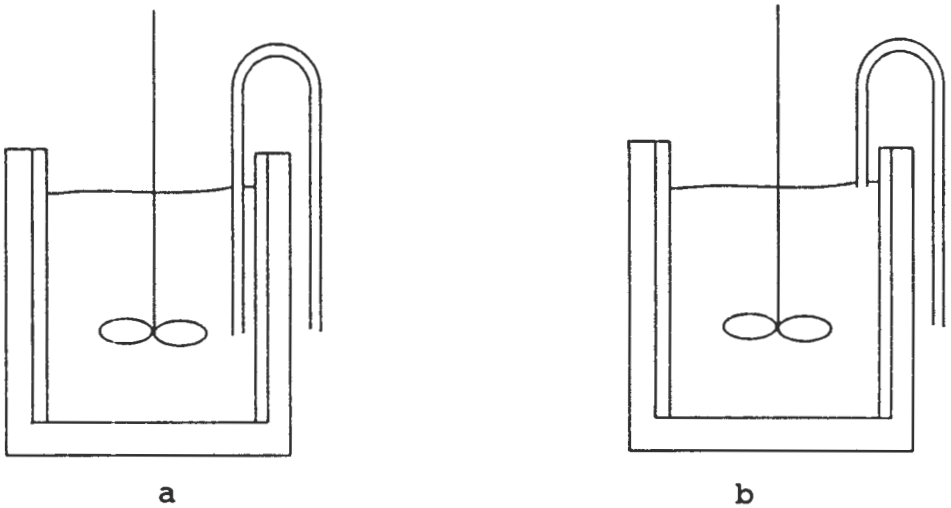


Figure 3.3: Sampling positions inside the crystalliser.

A problem arose with this first design. While the tube was being used as a levelling device (Figure 3.3b), air bubbles became entrapped inside it. These bubbles remained in the offtake tube until they grew large enough to be swept away. Often, these same bubbles were pulled down into the cell during sampling (Figure 3.3a) and were erroneously counted as very large particles $\geq 180\mu m$.

It was therefore decided to keep the sampling tube immersed in the crystallising medium (Figure 3.3a) during unsteady state

in an attempt to stop air being entrained into the offtake flow.

This led to a further problem of deposition of CaOx crystals on the outside of the immersed section of the tube prior to steady state being attained. This effect occurred as a result of the (unheated) glass tube being immersed in the crystallising medium while steady state was being approached.

These shortcomings led to the following modifications of the offtake tube.

Elimination of the bubbles was tackled first. The propeller speed was increased to 1100 rpm and the flow rate of hot filtered water was increased to 60 ml/min. This was done to exaggerate the number and size of the bubbles that might be expected under normal experimental conditions (950rpm, 40ml/min), thereby allowing for easy observation.

Due to the suction action of the pump, it was impossible to prevent minute bubbles (originating from the crystalliser), from entering the glass offtake. Therefore, it was decided to attempt to entrap the bubbles somewhere in the tube before they reached the cell. The idea was to create a small reservoir at the top of the u-bend (Figure 3.4).

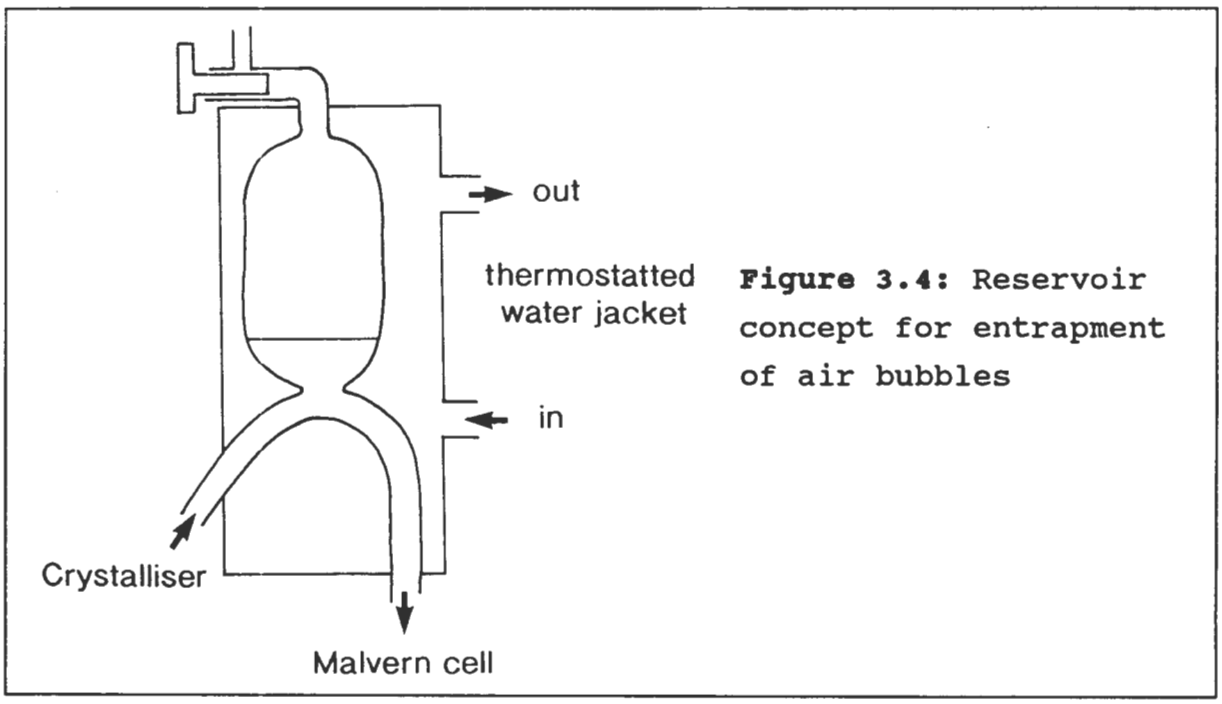


Figure 3.4: Reservoir concept for entrapment of air bubbles

To maintain the constant temperature, the whole of the reservoir was surrounded by a waterjacket.

To allow entry of a small quantity of air into the reservoir after it had been filled with untreated urine, a tap was fitted. In this way it was hoped that bubbles entering the system would rise to the top of the reservoir and hence would not be sucked down the outlet to the Malvern cell.

It was appreciated that the reservoir at the top of the u-bend should be as small as possible to ensure minimal effect on the steady state CSD. However, in the early stages of design development, the reservoir had a relatively large volume of 3ml to observe the bubbles more easily.

Unfortunately, due to the action of the pump, a few tiny bubbles moving along the lower section of the u-bend in the glass tube still managed to reach the cell. A minor modification to the above design was therefore required. This comprised a small lip at the position where some of the bubbles had been concentrated (Figure 3.5).

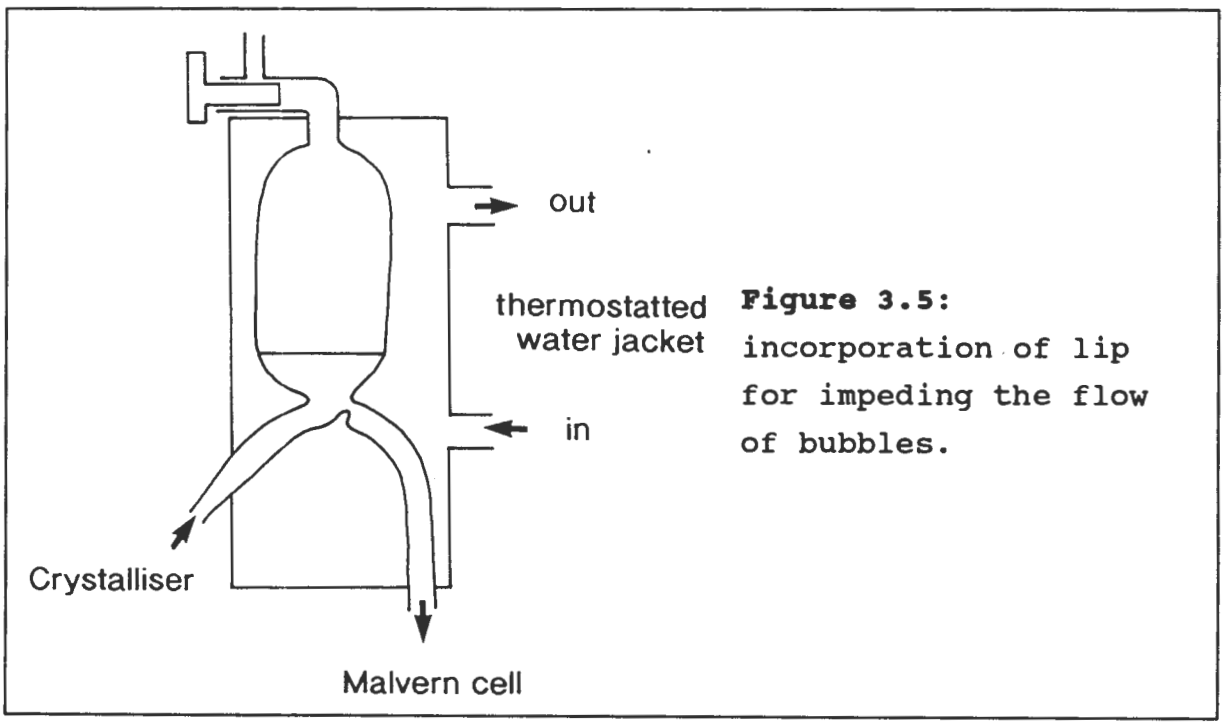


Figure 3.5: incorporation of lip for impeding the flow of bubbles.

The lip had the desired effect of impeding the flow of tiny bubbles until they concentrated and grew into a larger bubble. Once the bubble was big enough it managed to escape from the

glass lip and rose up to the air trap. In this way the lip managed to prevent bubbles that were travelling along the bottom of the u-bend from being pulled into the Malvern cell. However, despite these design modifications, close inspection of the cell windows and the section of tubing between the reservoir and cell still showed the presence of bubbles.

Further improvements in the design were therefore necessary and the next modification is shown in Figure 3.6. In the design shown in Figure 3.5, the close proximity of the inlet (from the crystalliser) and outlet (to the Malvern cell) had permitted the easy passage of bubbles to the Malvern cell. This had been obviated by the introduction of the lip.

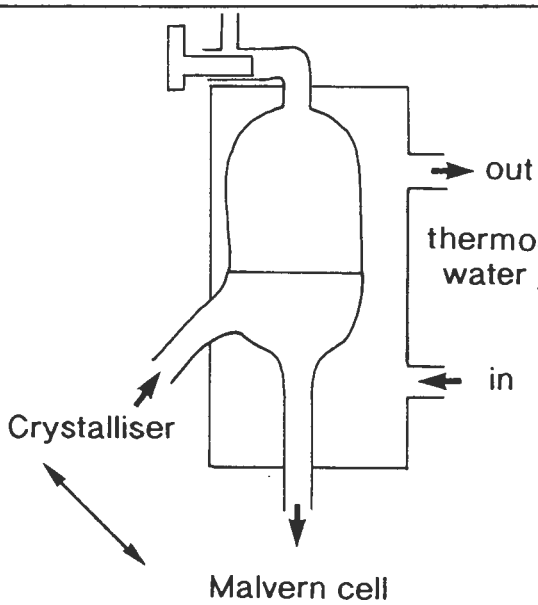


Figure 3.6: Raised inlet (from the crystalliser) in relation to the outlet (to the Malvern cell).

separation has been increased relative to Figure 3.5

In the design shown in Figure 3.6, the distance between the inlet and outlet was increased in an attempt to reduce the force with which the bubbles were pulled down into the cell. It can be seen that relative to the designs shown in Figures 3.4 and 3.5, the inlet to the reservoir is raised in relation to its outlet. It was therefore anticipated that the lip would no longer be necessary and hence it was removed.

Unfortunately, this modification was not successful. Due to the high flow rate of 60ml/min, the force exerted on the bubbles was too strong for them to become trapped in the

reservoir. Instead, they were immediately pulled down into the outlet tube (to the Malvern cell) and never entered the air trap. To overcome this, the inlet and outlet were positioned still further apart as shown in Figure 3.7. This reduced, but did not eliminate, the downward movement of small bubbles.

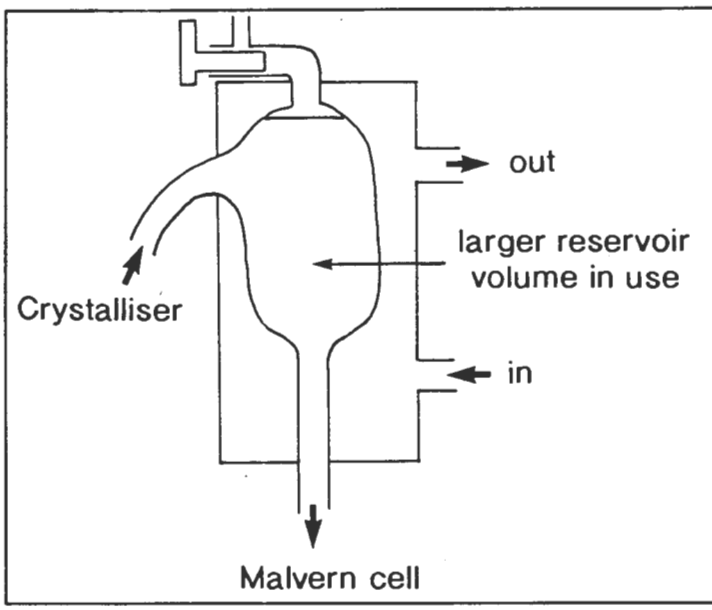


Figure 3.7: Inlet and outlet positioning at opposite sides of the reservoir to maximise the distance between them.

It was realised that an obstruction of some kind was needed to counteract the strong pumping action. Four glass indents were incorporated into the design (Figure 3.8). These proved to be successful and completely stopped the downward movement of the bubbles.

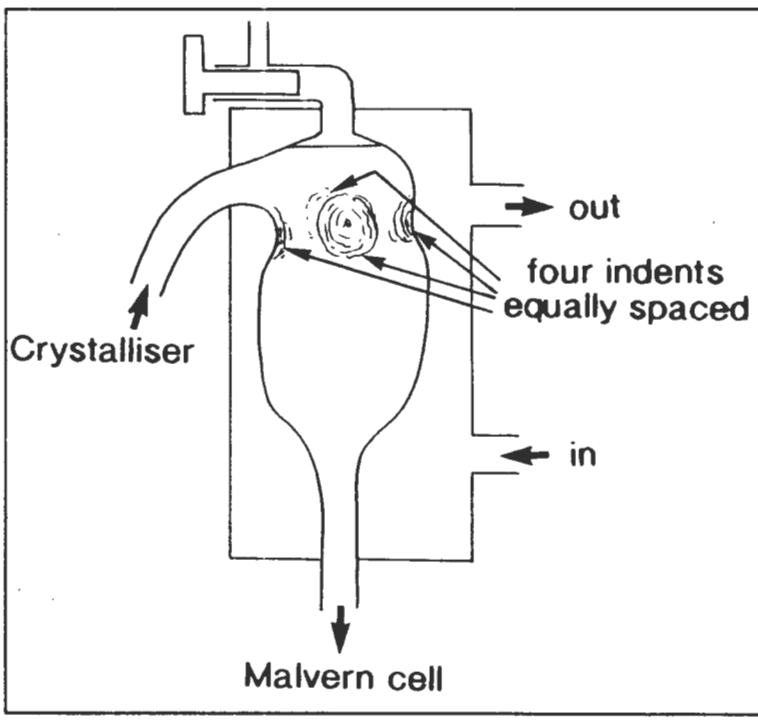


Figure 3.8: Incorporation of glass indents.

Having solved the problems of air bubbles, a new problem was identified. Initial experiments demonstrated that the reservoir produced a concentrating effect on the solution causing crystal deposition on the glass close to the outlet tube, well before steady state was achieved. Thus the sample being measured (in the Malvern cell) was not representative of the steady state distribution in the crystalliser.

This deposition was caused by the presence of a large volume of urine in Figures 3.7 and 3.8 relative to the preceding designs.

In the next design (Figure 3.9), the reservoir was retained but, to alleviate the problem of deposition, it was made as small as possible. In doing so, the inlet and outlet were placed closer to each other as in Figure 3.4. The connecting volume of the reservoir and u-tube was once again made as small as possible, but spirals were introduced to impede the downward flow of bubbles into the cell. The spiral was constructed in a stretched configuration to maintain a downward flow and therefore reduce the likelihood of crystal deposition within the loops (Figure 3.9).

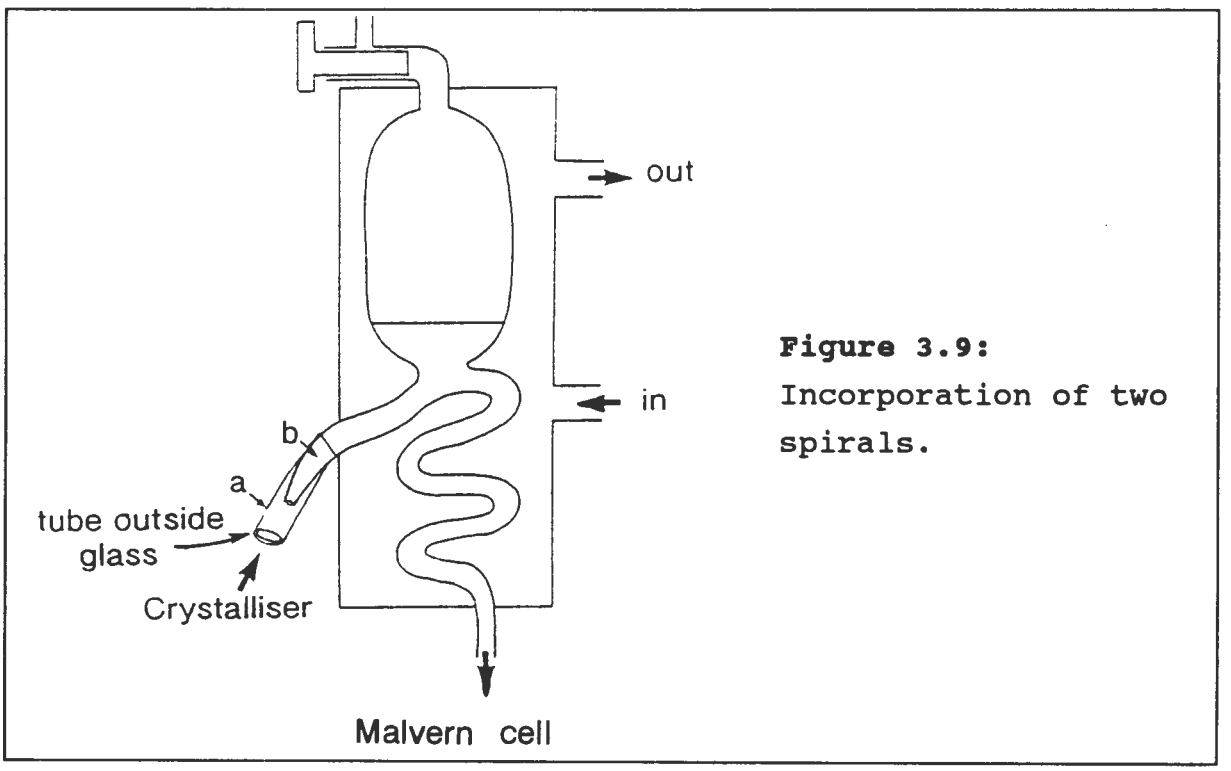


Figure 3.9: Incorporation of two spirals.

This design was successful in preventing the bubbles from entering the Malvern cell. The bubbles either rose to the top of the reservoir or were trapped inside the spirals.

To minimise the distance through which the sample had to travel between the crystalliser and the cell a spiral of only one loop was finally used (Figure 3.10). The volume of the reservoir was also kept to a minimum of $1cm^3$.

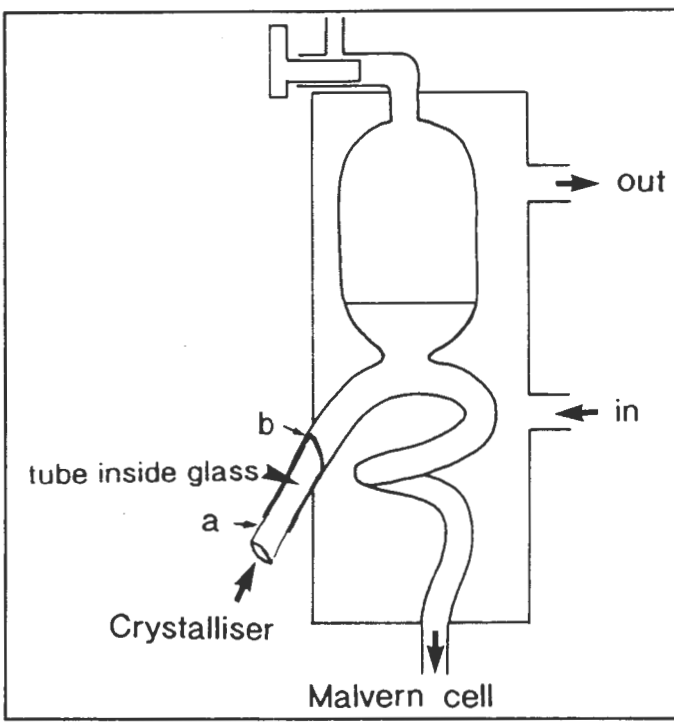


Figure 3.10: Final offtake design with a single spiral.

Another problem with all of the designs (Figures 3.6-3.9) arose as a result of the distance between the sampling point (inside the crystalliser) and the joint of the unheated and heated sections of the glass offtake ("a" to "b" in Figure 3.9). In the initial designs (Figures 3.6-3.9), a tiny deposit was sometimes seen at the connection point because as mentioned previously, thermostatting of the entire offtake was not possible.

In an attempt to overcome this problem the glass section "a-b" was replaced by tygon tubing to determine whether deposition would occur on the tubing (implying that deposition occurred on the glass only). The glass was tapered at point "b" (Figure 3.9) and tygon tubing was fitted over the glass. However, this proved to be unsuccessful as deposition occurred at the tip of the glass taper possibly due to the narrowing of

the glass hole. Noting this, a short section of glass near point "b" was widened and tygon tubing was inserted *inside* the glass up to point "b" (Figure 3.10). To ensure a complete seal, the tubing was covered lightly with high vacuum grease before being inserted into the glass section. Thereafter, deposition was not noted at point "b" nor in the entire offtake.

The design shown in Figure 3.10 successfully eliminated the bubbles and the deposition on the immersed section of tubing inside the crystalliser. (Indeed, deposition was noted on the immersed tygon tubing in only a few experiments; these were then discarded and a lower concentration of the feed NaOx solution was used).

III.5 THE CONNECTION BETWEEN THE OFFTAKE TUBE AND THE SAMPLING (MALVERN) CELL

In initial experiments using urine, the total flow from the MSMPR crystalliser was directed into the Malvern cell during both the unsteady and steady state periods. This protocol involved measurement of a background count using an untreated sample, followed by a period during which there occurred the flow of unsteady and steady state product through the cell. Finally the steady state sample was measured.

As described in Chapter III.4, these initial experiments were plagued with the problem of bubbles in the cell. As a result, bubbles appearing in the cell during unsteady state operation could not be removed before a measurement.

Emptying the cell to remove the bubbles would involve reversing the flow of the sample through the cell. As the crystalliser is directly connected to the cell, the reversal of sample flow would imply the re-introduction of the unsteady state product into the crystalliser. This would therefore cause contamination of the sample within the crystalliser.

It was therefore necessary to divert the flow of the unsteady state product from the Malvern cell to avoid this problem.

In order to change the direction of flow from the crystalliser to waste (during unsteady state) and to the cell (during steady state), a 4-port flow switching device was used. Figure 3.11a indicates the operation of the switching device allowing sample flow from the crystalliser to the Malvern cell (Figure 3.11a(i)) or to waste (Figure 3.11a(ii)).

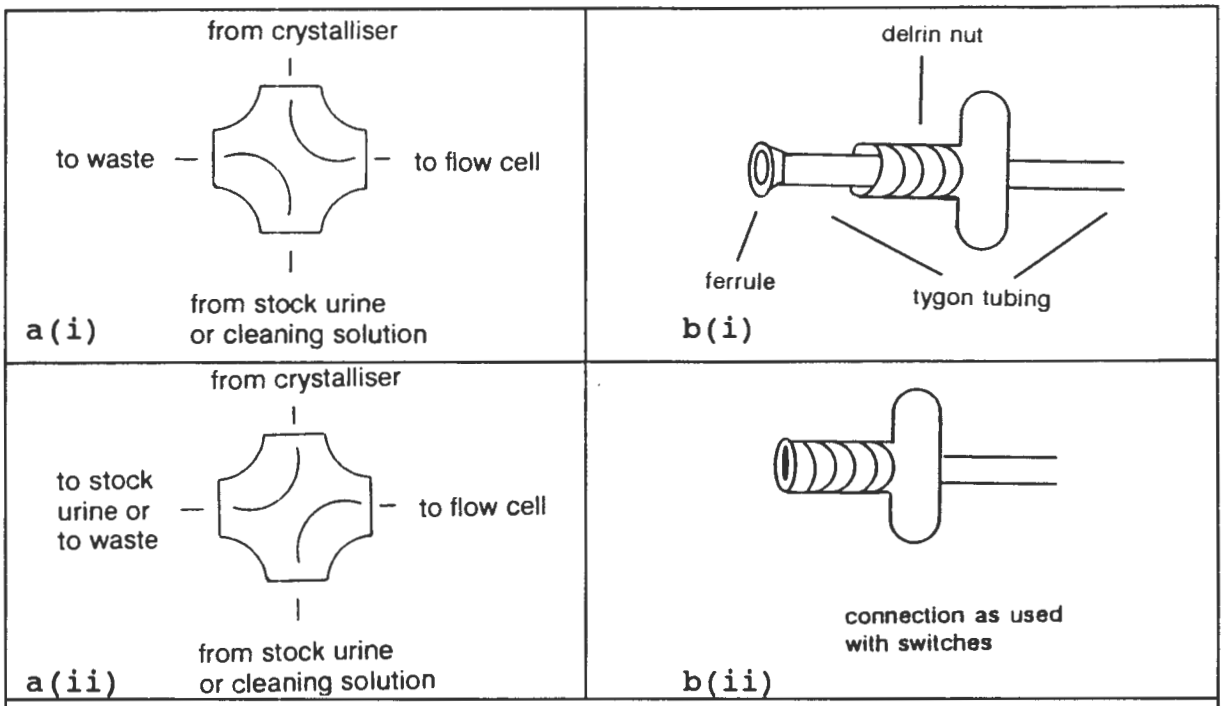


Figure 3.11: 4 port flow switching device and connector.

In Figure 3.11a(i) the switch allowed flow from the crystalliser to the flow cell. The switch was used in this position for both blank and sample determinations. At the same time, the other port of the switch served a dual purpose for allowing the recycling of stock untreated urine or the flow of a cleaning solution to waste after sample determination.

In Figure 3.11a(ii) the switch is reversed to that shown in Figure 3.11a(i). In this position, the switch allowed the flow from the crystalliser to be diverted to waste. Simultaneously, the urine from the stock solution flowed to the cell through the other port and was cycled back to the stock volume. If multiple determinations were necessary, this port was used to pass a cleaning solution through the flow

cell and then to waste. The switch was used in this position during the unsteady state operation of the MSMPR crystalliser.

A plastic connector that screwed into the switching device is shown in Figure 3.11b. Tygon tubing was inserted into a tight fitting, threaded delrin nut which was later screwed into the switch. To ensure that the tubing was flanged against the inner section of the switch (so that there were no leaks from a gap between the hole in the switch and the hole in the tubing), a ferrule was inserted into the end of the tubing (Figure 3.11b(i)). The delrin nut was then pushed to the end of the tubing so that it formed a tight fit onto the ferrule (Figure 3.11b(ii)). Four of these connectors were attached to the four ports of the switch.

Unfortunately, proper sealing of the tygon tubing to the switch was not possible. This caused small leakages of the sample as well as incorporation of air bubbles into the flowing stream. Another problem arose as a result of the holes in the switch being of much smaller diameter than that of the tygon tubing. This caused blockage of the holes in the switch by deposited CaOx. When blockage occurred, the experiment was stopped due to overflow of the MSMPR crystalliser. The problems of sample leakages and air bubbles were overcome, using high pressure vacuum sealant around the connections.

The problem of blockage of the holes in the switch could not be solved and it was therefore decided to remove the switch from the system.

With the removal of the switch, the problem arose again as to how to divert the unsteady state product from flowing through the Malvern cell. An alternative approach was to use a 2-way valve in place of the switch. Two variations are shown in Figure 3.12.

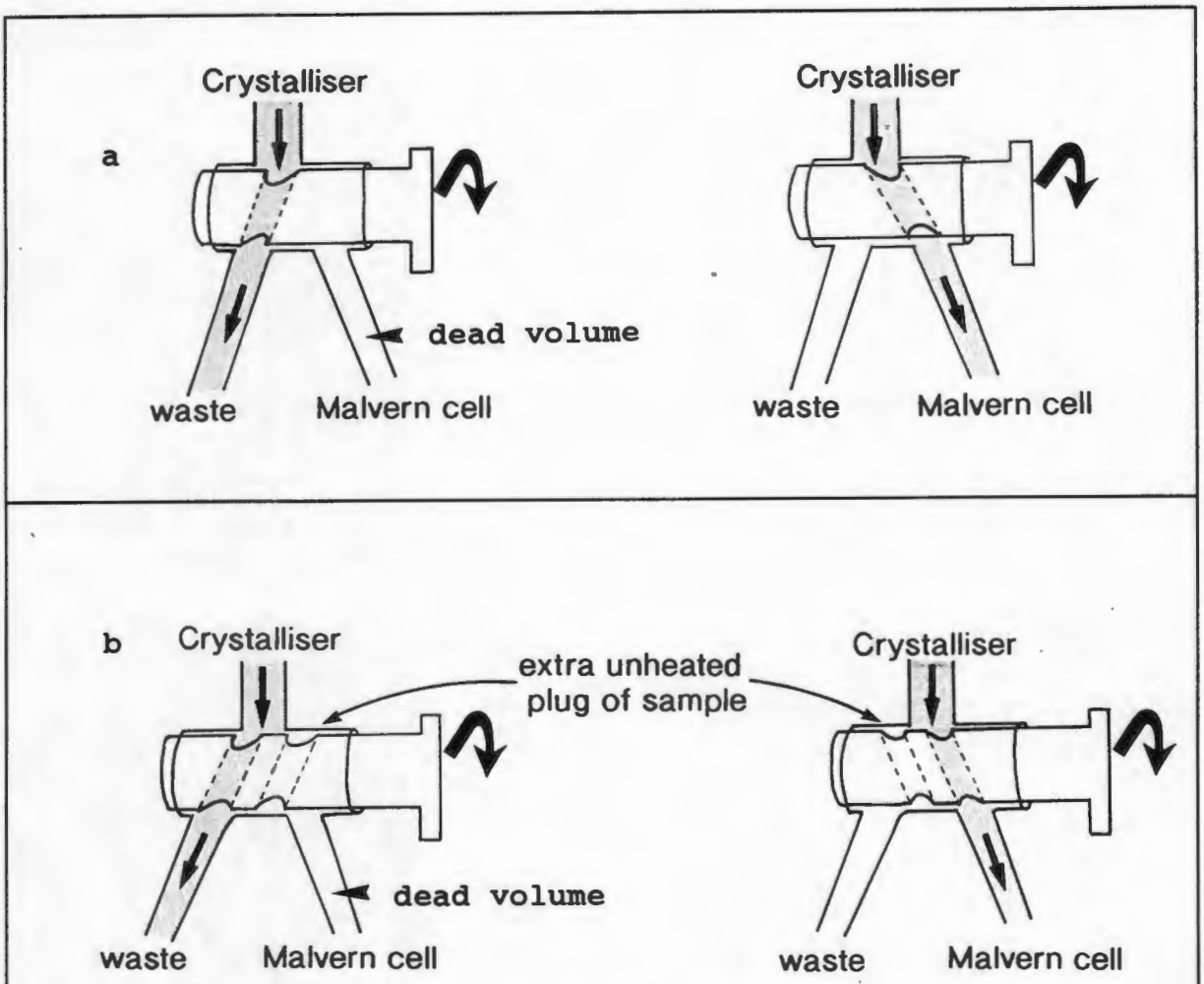


Figure 3.12: Two types of 2 way valves used. (a): single channel; (b): double channel.

In both of the 2 way valve designs, the glass offtake tube and the glass valves were tapered and were connected by tygon tubing. This involved warming the glass taper and simply pushing on the tygon tubing as a tight fitting sleeve. Once the glass cooled, the seal was complete.

There were several drawbacks associated with these designs. Firstly, the valves and the immediate area around them could not be thermostatted at the temperature of the experiment because of physical constraints with respect to manipulating the taps. As a result, CaOx deposition occurred near the valves before steady state was achieved. Secondly, in both of the two way valves shown in Figure 3.12 there exists a small dead volume that is not heated during unsteady state operation because it is isolated from the sample flow. In addition, in Figure 3.12b there is an additional unheated sample plug

trapped in the unused channel of the valve during unsteady state operation. Since mixing of the heated steady state sample with these unheated sections of sample would have had an effect on the CSD, it was necessary to either avoid or at least minimize such regions. Thirdly, even though the fittings were tight, both valves leaked despite the application of high vacuum grease. Administration of more grease led to blocking of the valve.

Use of these valves meant that it was not possible to have flow through the cell during the unsteady state period as had been done in using the switch. This meant that the flow cell would either have to be empty or be filled with untreated stock urine during this stage. The presence of static urine in the cell was preferred as this would allow the cell windows to be thermostatted at the correct temperature since the untreated urine in the cell was maintained at the same temperature as the sample in the crystalliser. There would therefore be no temperature gradient when the steady state product was diverted into the cell.

The problem of leakage from the valves was not solved so it was decided to remove them from the system and replace them with screws (Figure 3.13).

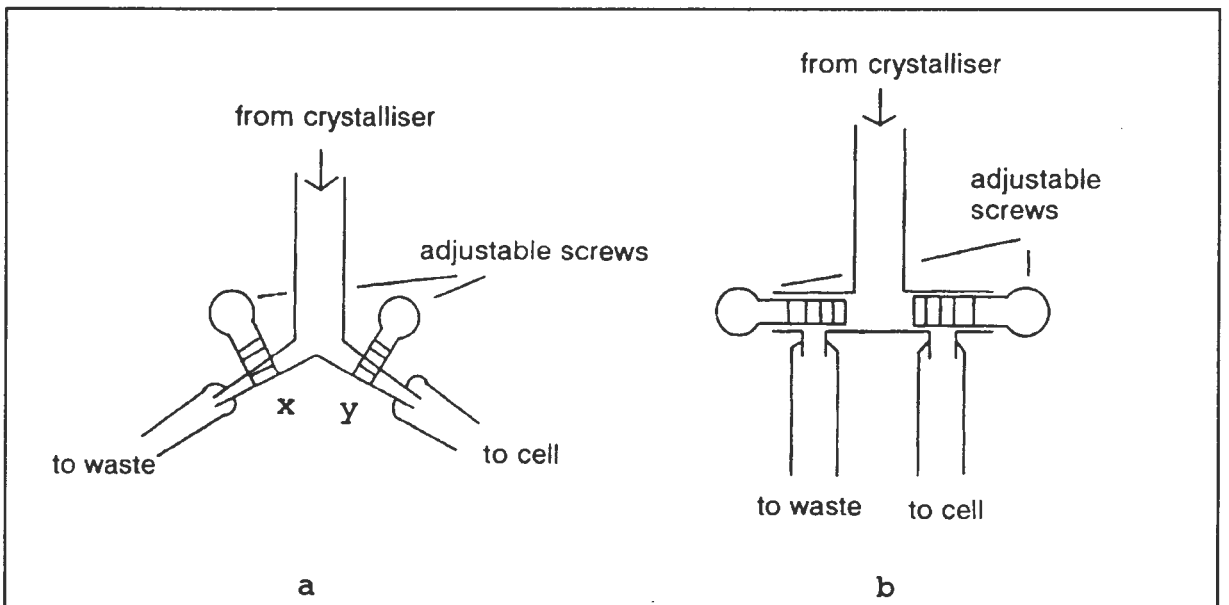


Figure 3.13: Two types of 2 way valves used with screws.

In Figure 3.13a the glass junction was angled into a Y shape. During unsteady state operation, screw "x" was open and "y" was closed. (This was reversed for steady state operation). However, a consequence of this configuration was that a small volume of sample was left static in branch "y" leading to a small quantity of crystal deposition before steady state was attained. Therefore, the design was modified (Figure 3.13b) to a T junction but, due to the presence of the horizontal connecting section, even more deposition occurred along the entire length near the adjustable screws.

This design concept was therefore abandoned. Instead, the glass tube was split into two angled branches (as in Figure 3.13a) and is shown in Figure 3.14. Clamps were used to control the flow of sample.

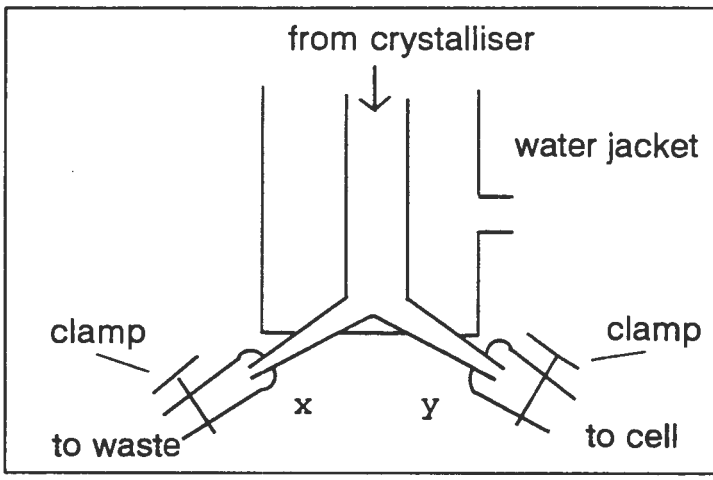


Figure 3.14: Final connection used with the introduction of clamps to restrict flow.

During unsteady state operation, clamp "x" was open and clamp "y" was closed. Although there still existed a tiny volume of liquid in branch "y" that would not become mixed with the rest of the sample, no observable deposition occurred here, possibly due to the unheated volume of branch "y" in Figure 3.14 being much smaller than that in Figure 3.13a. When steady state was attained, clamp "x" was closed and clamp "y" was opened. In this mode, no apparent temperature effect on the Malvern readings was noted as a stable Malvern obscuration value was obtained.

With this design, the entire glass offtake (with the exception of the two small sections of the tapered glass) could be thermostatted. The dead volume that was not heated (between

the glass tubing and the cell) was kept to a minimum of less than $0.25cm^3$.

The final offtake configuration and its connection to the Malvern cell are shown in Figure 3.15.

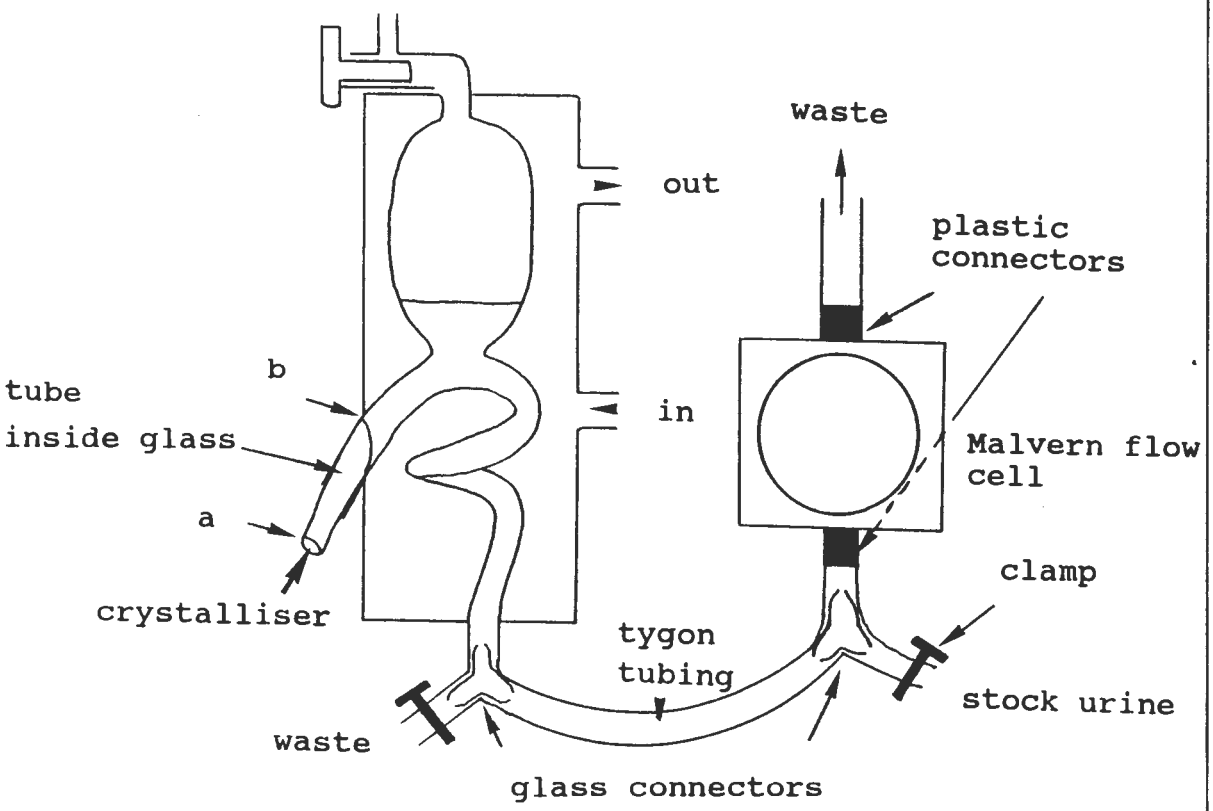


Figure 3.15: The complete offtake design and connections from the crystalliser to the flow cell.

III.6 MALVERN FLOW CELL

A flow cell was designed for use in the present study (Figure 3.16) since the cell supplied by the Malvern manufacturers was unsuitable for the specific requirements of the project. For example, the volume of the original flow cell was too small, the connections would have required larger inner diameter tygon tubing than that needed for the pre-determined flow rates, and the cell was constructed from metal onto which CaOx might deposit.

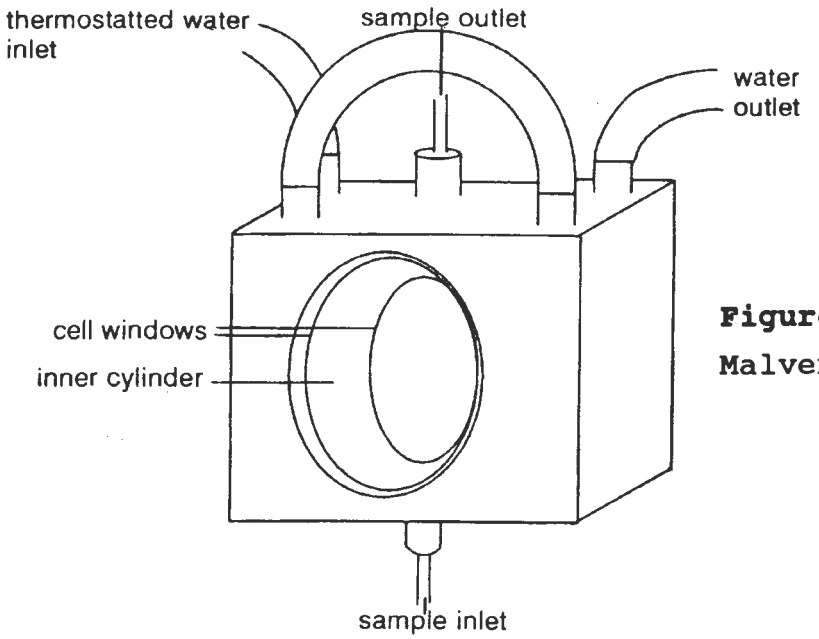


Figure 3.16:
Malvern flow cell.

The newly designed flow cell was constructed from a teflon block through the centre of which a cylinder was cut to ultimately form the sample chamber. Teflon was selected as no deposition of CaOx occurs on this material (Mueller private communication). The diameter of the cylinder was equal to the diameter of the receiver lens. This ensured that the laser beam would not be obstructed by any part of the cell.

The cylinder was bored in such a way that the windows on either side were flanged against an inner ridge which ultimately defined the sample cylinder diameter as 10mm. The inner rim of the cylinder facing the laser beam was milled with increasing diameter to ensure maximum capture of the laser beam. The side facing the transmitter unit was slightly tapered. The path length of the laser beam (10mm) was small enough to ensure that the scattered light would be measured and that it would not be lost due to scattering at too wide an angle within the cell. The total sample volume within the cell was 7.07ml.

III.6.1 FLOW CONNECTORS

Threaded holes were drilled in the top and the bottom of the cell. These threaded holes were not drilled to facilitate

flow per se, but were drilled to accomodate two screws. Tygon sample tubing was pushed into the center of these two custom-made plastic screws to form a tight seal. The tubing-to-screw connections were sealed with epoxy sealant. High vacuum grease was also applied to the connection.

Initially, these flow connectors were screwed in as far as possible so that they were aligned with the circumference of the inner sample cylinder. However this proved unsuitable for two reasons. Firstly, the flow of the sample into the cell formed a fast stream in the path of the laser beam causing fluctuations in the sample measurement. This effect occurred irrespective of whether the flow direction through the cell was from top to bottom or visa versa. Secondly, when the flow direction was downwards, obscuration values increased significantly. This was attributed to poor withdrawal of sample particles leading to their accumulation in the lower curved regions of the cell. As this section is in the laser path, these particles were continually measured thereby causing a large error in the CSD.

Improvements in the cell involved boring the input and output holes into a funnel shape which was tapered to the size of the connectors at the outer sections. In addition, the flow connectors were screwed in half way only. This resulted in a gentle flow of sample along the sides of the cell thereby diminishing the effect of the flow patterns. When the particles exited the cell through the funnelled offtake they were no longer in the path of the laser beam and hence no concentration effect occurred.

III.6.2 CELL WINDOWS

The cell windows were positioned on either side of the cylindrical sample chamber (Figure 3.16). On the inner side, the cell windows were held in place by the ridge of the inner cylinder of the teflon cell. On the outer side they were held in position by an O-ring and a perspex retaining wall. The retaining wall was itself held in position by screws that

passed through the cell and were bolted in at the opposite end. This configuration is shown in Figure 3.17.

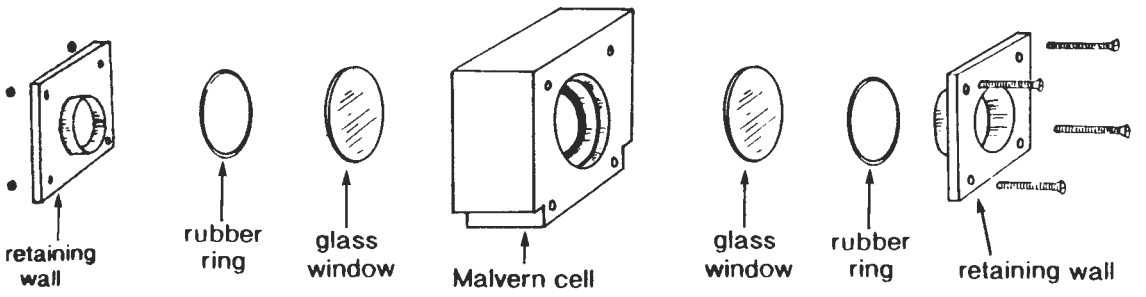


Figure 3.17: Placement of cell windows.

Initial testing of this cell was accomplished using warm, filtered, glass-distilled water which was cycled through it. While attempting to align the cell, poor laser intensity values were obtained due to spurious light energy readings in the middle rings as shown in Figure 3.18.

Source B'ground Record no.0 256
Focus 63

Ring	Data	Ring	Data
0	524.97	16	17.85
1	50.47	17	17.76
2	28.16	18	18.77
3	17.29	19	20.06
4	15.99	20	20.20
5	14.03	21	20.40
6	14.84	22	21.30
7	13.78	23	22.15
8	14.22	24	23.18
9	13.76	25	24.10
10	14.27	26	25.84
11	14.92	27	27.45
12	15.16	28	28.71
13	151.20	29	26.74
14	16.76	30	24.38
15	16.83		

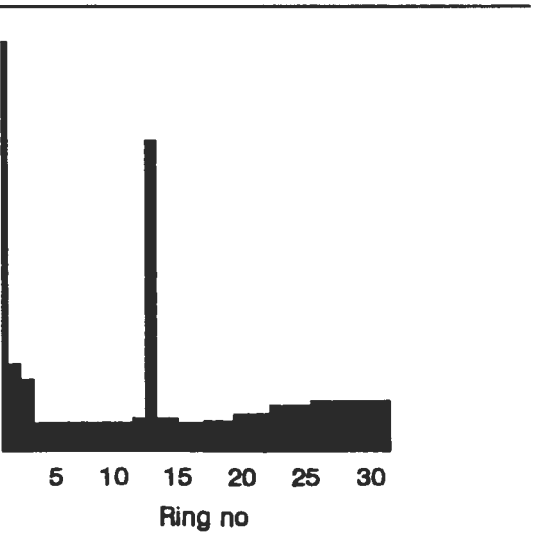


Figure 3.18: Background reading showing error in ring 13.

When the laser beam impinges on the windows of the cell, part of the light is reflected back towards the transmitter. Usually this reflection appears as a well defined circle however, due to the error in alignment, this circle was poorly defined.

The table and corresponding bar graph in Figure 3.18 indicate the light energy in each of 31 concentric rings. Ring 1 is associated with the smallest particles and ring 30 is associated with the largest measureable particles for a particluar lens. Ring 0 indicates the light energy impinging on the central detector; it should be made as high as possible when aligning the laser beam. Rings 1-30 should indicate values below 30 otherwise either the alignment is incorrect, or the cell windows are not clean. In the case shown in Figure 3.18, ring 13 indicates an irregular and unacceptably high reading during alignment.

The cell was repositioned by adjusting 2 screws on the cell holder to ensure that the laser beam was not obscured by any part of the cell. Since repositioning of the cell did not totally eliminate the spurious peak, it was concluded that it was due to the windows not being perfectly parallel. Accordingly, the inner cylinder of the cell was remilled. Care was also taken to ensure that the screws were tightened equally when securing the windows. These modifications successfully eliminated the spurious peak.

A second method to ensure that no obscuration of the laser beam occurs due to the cell, involves the correct positioning of the reflection of the beam from the cell windows. The reflection from the parallel windows was therefore maintained above the beam expander for each experiment by adjusting the screws in the cell holder and this is shown in Figure 3.19. (The beam expander is also known as the transmitter lens and its function is to expand the diameter of the laser beam, which was created in the transmitter unit, to 10mm).

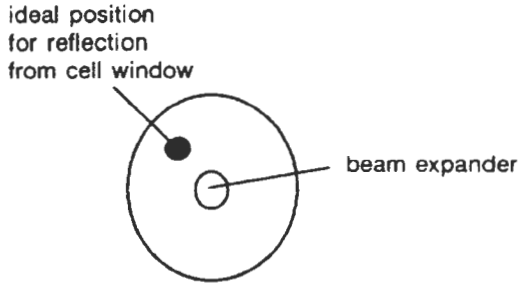


Figure 3.19: Alignment of cell.

It was also necessary to ensure that the glass windows were of the same thickness. Differences in the thickness can cause large errors in the first and second rings giving rise to the observation of two laser spots from the cell window reflection rather than one.

III.6.3 THERMOSTATTING

The cell was thermostatted by circulating heated water as shown in Figure 3.20.

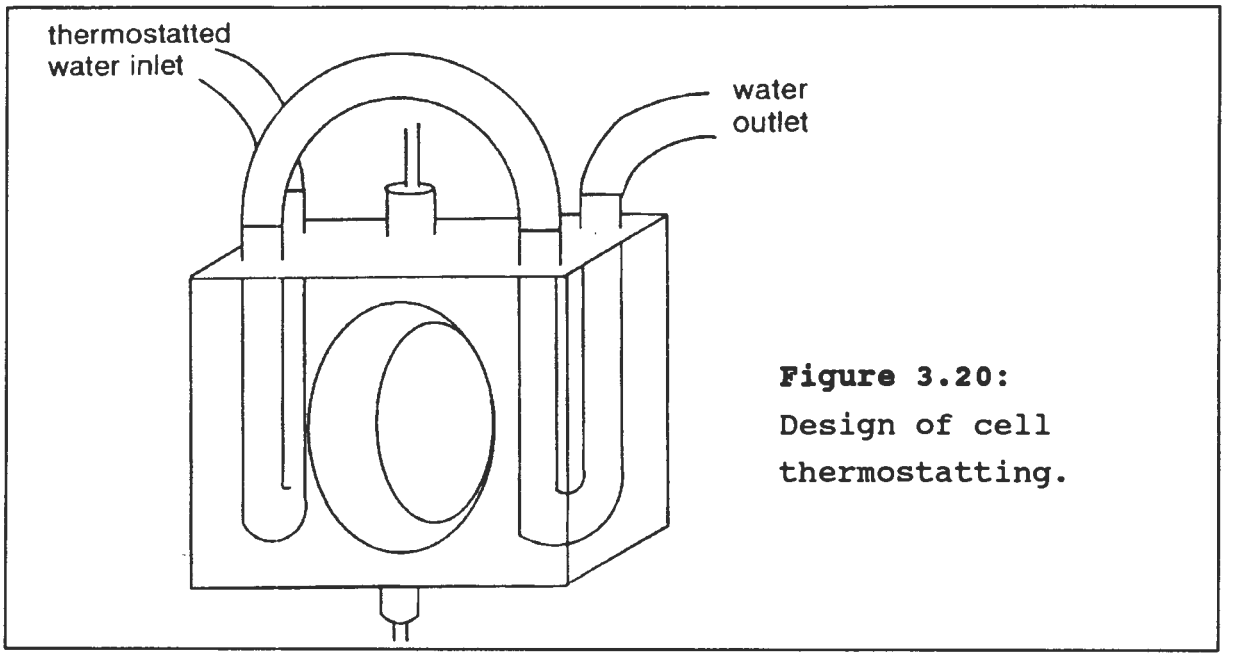


Figure 3.20: Design of cell thermostatting.

After the water had reached the required temperature, 30 minutes were allowed for temperature equilibration to be achieved.

III.7 SYSTEM DEVELOPMENT

DESIGN no.1

A flow diagram of the first design is shown in Figure 3.21. It is noted that a simple glass tube offtake was used to pump sample from the crystalliser into the cell. Thus, unsteady state products flowed through the cell prior to the attainment of steady state, causing deposition of CaOx on the glass windows. This preferential attachment of the crystals to the glass cell windows is attributed to surface charges which attract the crystals to attach themselves to the charge sites on the window. The crystals were therefore continuously in

the path of the laser beam thereby causing inflated obscuration readings.

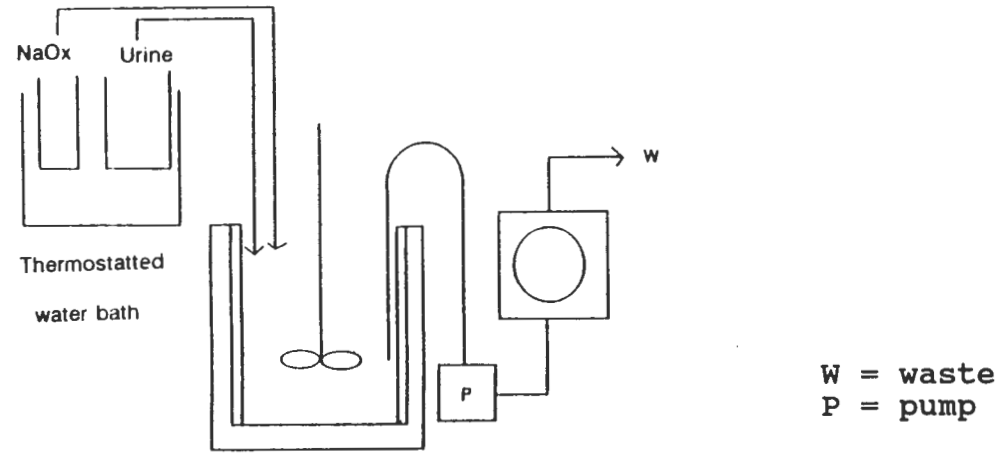


Figure 3.21: Design no.1

In an attempt to counteract this effect, a surfactant, Triton X100 was added to the bulk untreated urine in varying concentrations. Volumes ranging from 1 drop per liter to $1cm^3$ per liter were used. Care was taken to avoid adding too much surfactant which might cause frothing. The increasing volumes of Triton used, led to increasing concentrations of NaOx being required to produce measurable crystallisation. Shor and Larson (1971) have described the effects of various ionic and surface-active agents on nucleation and growth rates in their experiments involving crystallisation of KNO_3 from aqueous solution. They concluded that the inorganic ionic additives used decreased the nucleation rate and resulted in larger crystal sizes, and that the surfactants increased the nucleation rate to produce a smaller sized product. This latter finding confirms the findings of the present study that higher concentrations of NaOx are required to produce measurable precipitation in the presence of increasing surfactant concentrations.

After several trial experiments in the present study, it was decided to abandon the use of Triton and rather to restrict modifications of the urine to a minimum. Instead, a simple procedure was implemented: after thorough cleaning, the windows were carefully wiped to remove dust particles and a thin film of untreated filtered urine was applied on one side

of each window using a chamois leather. This prevented preferential crystal deposition.

DESIGN no.2

The next modification was to allow the sample through the cell only after steady state was reached.

To achieve this, it was necessary to divert the flow of unsteady state product (once a blank determination of untreated sample had been made) to waste until steady state had been reached after 10 residence times.

A 4 port flow switching valve (described in Chapter III.5) was therefore incorporated into the system as shown in Figure 3.22.

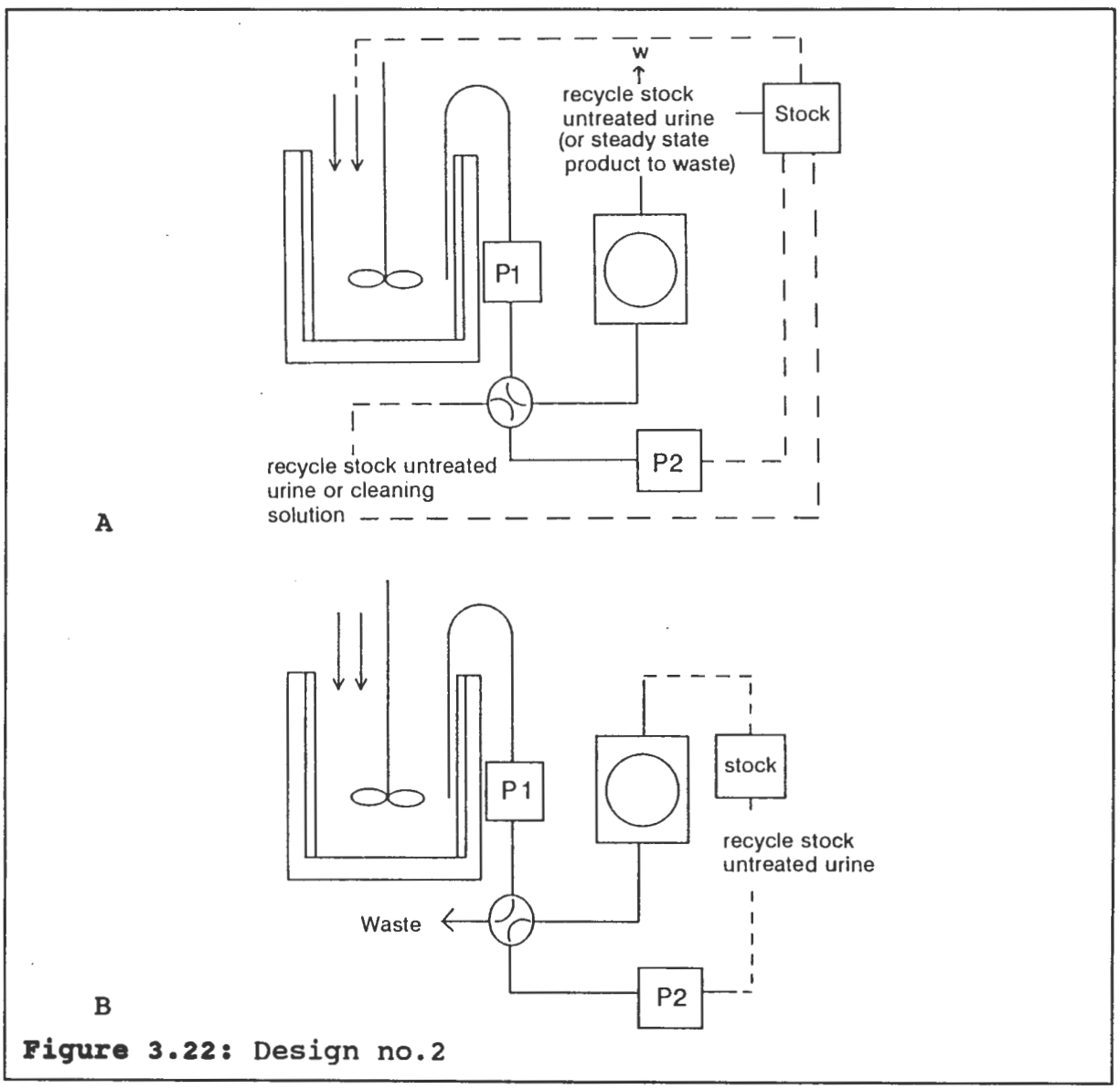


Figure 3.22: Design no.2

With the valve in position "A", untreated urine from the stock sample was cycled by pump P1 through the crystalliser and the cell so that a representative blank measurement could be taken. Pump P2 was used to cycle the stock untreated urine to warm the tubing and to achieve thorough mixing.

Once a blank reading had been taken, the switch was turned to position "B" (Figure 3.22), and the unsteady state product from the crystalliser was pumped out (P1) to waste until steady state was reached. Meanwhile, heated, untreated urine was cycled through the cell by pump P2 to allow the windows of the cell to be maintained at a constant temperature.

Once steady state was reached, the switch was reversed back to position "A". Upon entering the cell, the steady state product therefore mixed with the untreated urine already present and the mixture was then pumped out to waste. In the meantime, the tubing connected to P2 was not needed for the experiment and was therefore cleaned.

DESIGN no.3

In the early stages of the work, it was necessary to determine during the unsteady state operation of the crystalliser whether the crystal concentration was within the optimal range of the Malvern and Coulter instruments. At the same time, attempts were being made to obtain a range of NaOx concentrations that would create measureable particle concentrations for these two instruments. Using Design no.2, it was possible to measure a sample from the crystalliser during the unsteady state as well as during steady state.

The procedure that was followed involved reversing the switch (from "A" to "B" in Figure 3.22) after sample measurement so that a cleaning solution of warm dilute HCl followed by warm filtered water could be pumped (P2) through the cell. Thereafter, it was rinsed with untreated urine which was eventually recycled back into the stock supply. In order to facilitate this process of multiple sample determinations at

various times before steady state, Design no.2 was altered as shown in Figure 3.23.

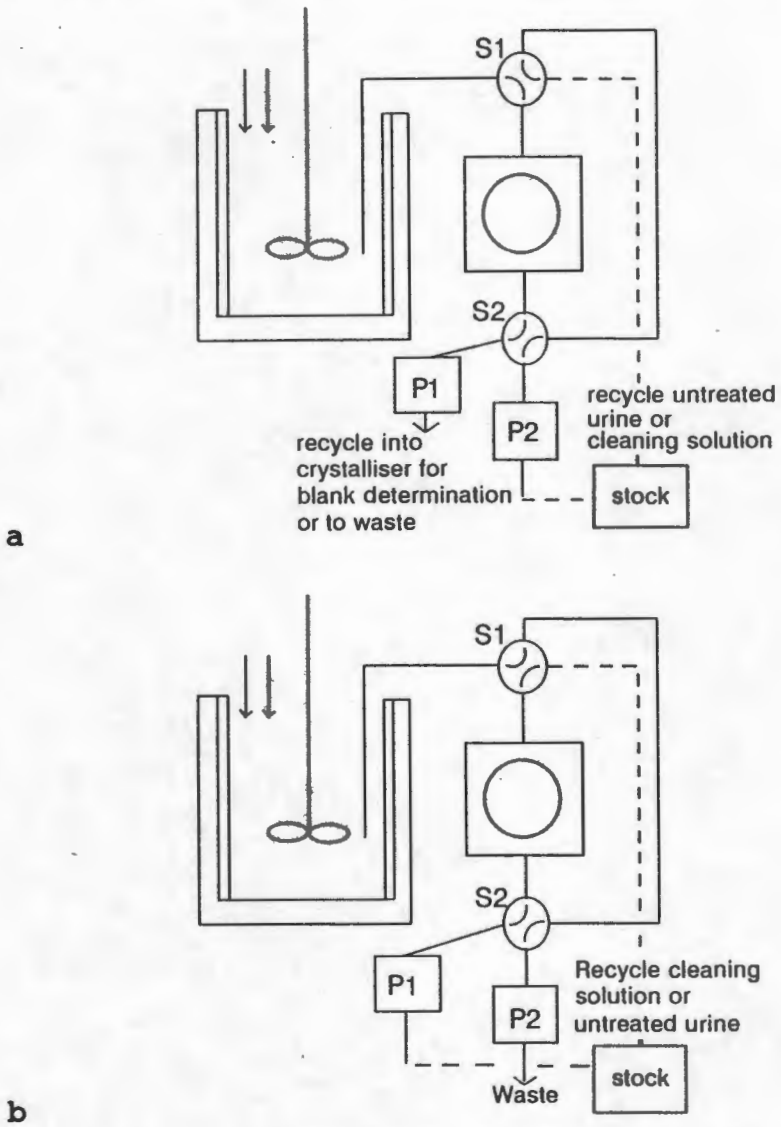


Figure 3.23: Design no.3

In this design, pump (P1) was placed on the opposite side of the cell to that of the crystalliser and a second switch (S2) was incorporated into the system on the same side of the cell as the pump. Switch S2 was not used to change the direction of the flow, but was used rather as a connection for the various tubes. Note that in Figure 3.23, both "a" and "b" have switch S2 in the same position while switch S1 is reversed.

Changing the position of S1 changed the operation of pumps P1 and P2. With the switch S1 in positon "a", urine from the

crystalliser was pumped (P1) through the cell to enable the measurement of a blank sample. Simultaneously, P2 pumped untreated urine through the other section of tubing. After the blank determination, S1 was reversed as shown in position "b". This positioning of S1 represents the unsteady state operation where the product from the crystalliser flowed to waste (P2) while the cell contained untreated stock urine pumped by P1.

When a sample determination was necessary during the unsteady state operation, S1 was reversed to position "a" so that sample from the crystalliser flowed through the cell and then out to waste (P1). A cleaning solution was simultaneously pumped (P2) through the other section of tubing. After an unsteady state determination, S1 was reversed to position "b" and the product from the crystalliser was diverted to waste (P2). While this mode was operating, P1 was used to pump a cleaning solution followed by untreated stock urine through the flow cell to prime it for another determination.

The re-positioning of pump P1 resolved another problem that had been encountered in Design no.2. Using this latter configuration resulted in crystals suffering a milling effect caused by their passage through P1 prior to reaching the cell. As a result, crystals flowing through the cell after steady state was attained, were not representative of the sample in the crystalliser. The re-positioning of P1 on the opposite side of the cell to that of the crystalliser (Figure 3.23) caused the sample to exit the cell instead of entering it.

DESIGN no.4

Although placing the pump on the opposite side of the cell (Design no.3) negated all milling effects, the required flow rate of 40ml/min could not be attained because the path length of the sample (2 switches and the cell) had increased. Also, the diameter of the holes in the switches was smaller than that of the tygon tubing thereby making it more difficult for the pump to pull the sample through 2 switches rather than just one.

Attempts to achieve this flow rate sometimes resulted in air being pulled into the cell through the sides of the glass windows and therefore overflow of the crystalliser volume above the required 200cm^3 resulted.

At this stage of system development, the concentrations of NaOx required to produce measureable crystallisation had been identified. Determinations during the unsteady state operation were therefore no longer necessary.

Design no.3 was therefore modified by removing the second switch (to allow the required flow rate) as shown in Figure 3.24.

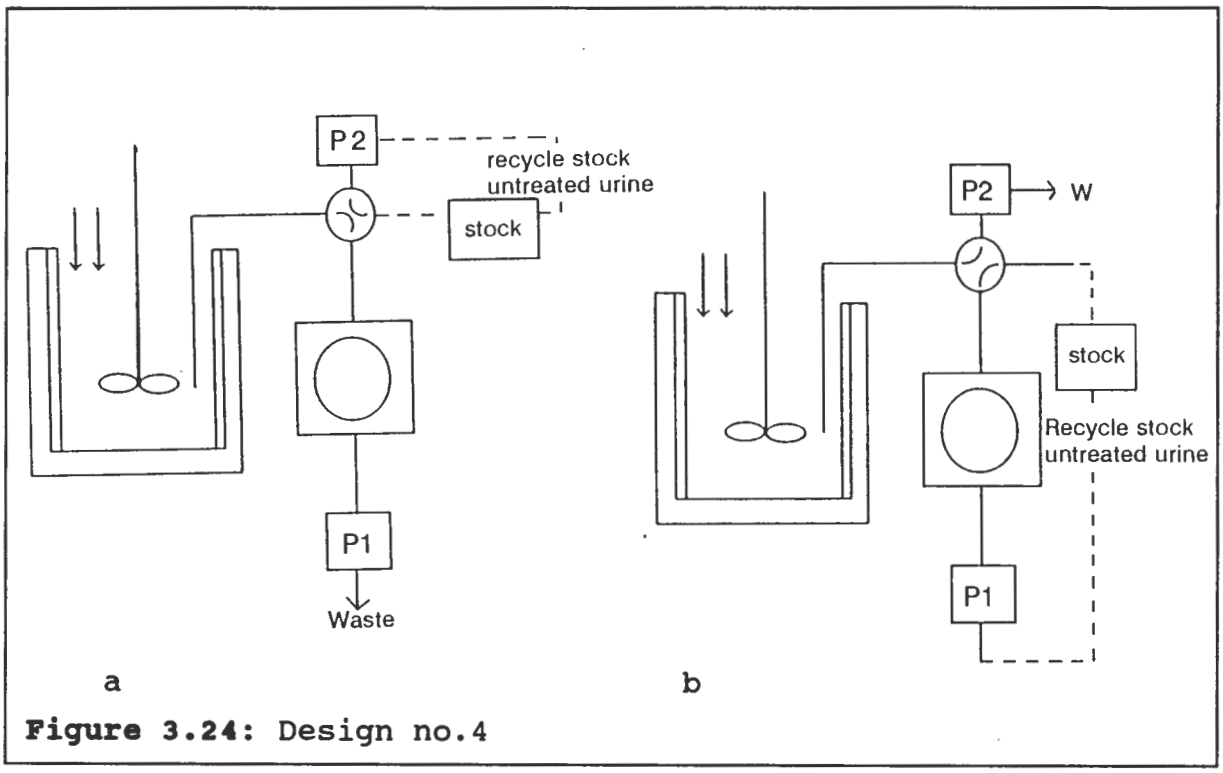


Figure 3.24: Design no.4

This design was similar to design no.2 except for the positioning of the pump (P1) after the cell in the flow system. The switch was positioned as in "a" for blank and steady state determinations, and was in position "b" during unsteady state operation when the product from the crystalliser was pumped (P2) to waste while untreated urine was cycled through the cell.

DESIGN no.5

Tygon tubing had to be replaced frequently because of wear. As a result, it became necessary to incorporate small glass connectors between certain lengths of tubing. These are shown in Figure 3.25. The tygon tubing was fitted onto these connectors by heating the glass and pushing the former onto the tapered sections of the connections.

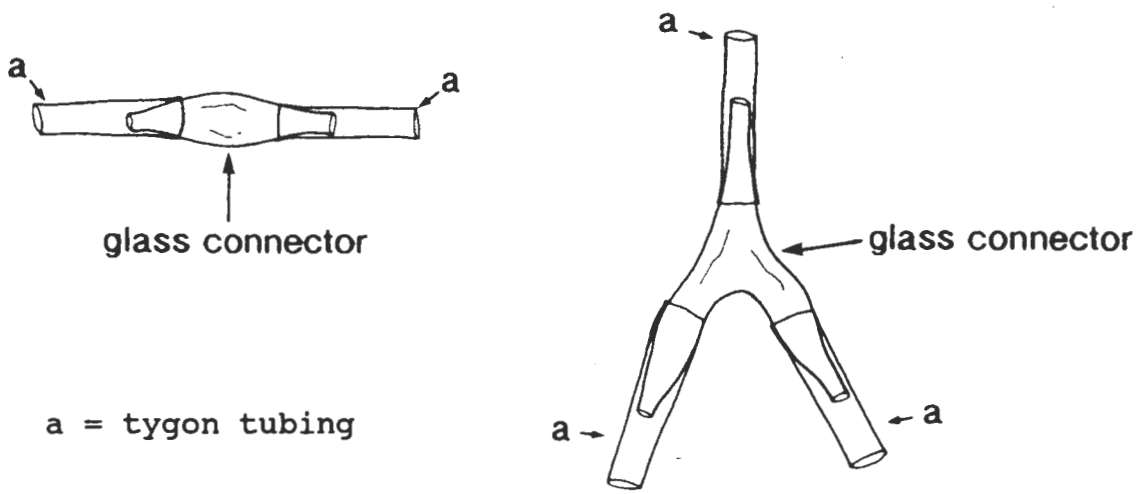


Figure 3.25: Glass connections between tubing.

The use of these connectors allowed sections of tubing that were worn by the pumps to be replaced without disturbing the other sections that were fixed into position (eg. connectors on the flow cell).

It was during this experimental stage that it was decided to remove the switching device. Reasons for the removal have been discussed in Chapter III.5. Various two way valves were used in place of the switch but these were later replaced by the use of clamps (as discussed in Chapter III.5). Figure 3.26 shows the fifth system design indicating the clamps between the crystalliser and waste (C1) and the crystalliser and cell (C2). Figure 3.26a shows downward flow into the cell while Figure 3.26b shows upward flow into the cell.

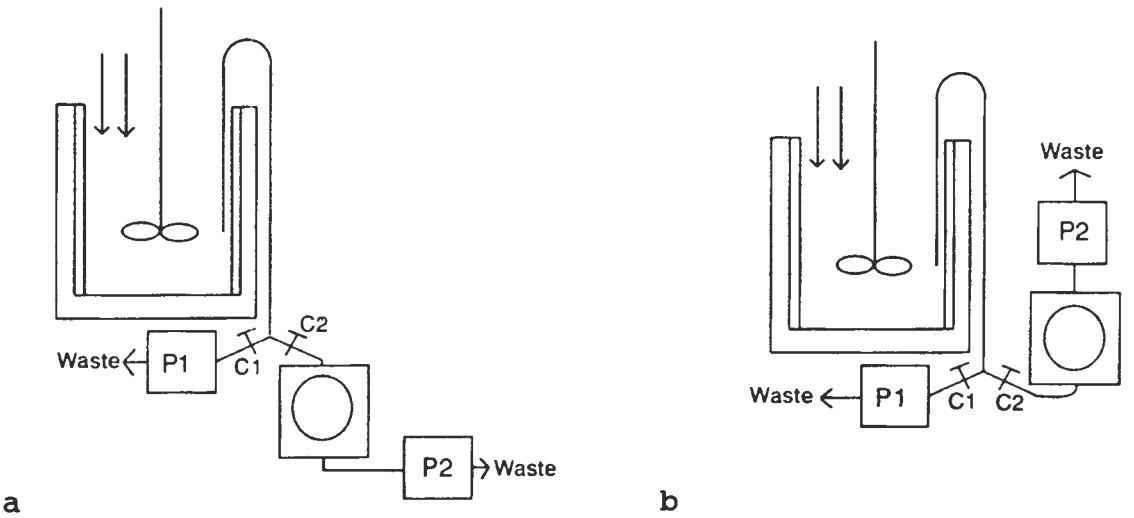


Figure 3.26: Design no.5 showing both downward (a) and upward (b) flow in the cell.

As explained in Chapter III.5, the single offtake from the crystalliser was branched with one branch to waste and the other to the Malvern cell. Use of the clamps on these two branches meant that only one branch (ie one clamp) was open at a time.

Figure 3.26a indicates downward flow of sample from the crystalliser to the cell. In this mode, it was necessary to invert the cell as sample entering from the top would immediately be pulled out at the bottom by P2 and the cell would not become filled.

Therefore, with C2 open (C1 closed), the cell was inverted and initially was filled (P2) with untreated urine from the stock via the crystalliser. Once filled, the cell was turned upright and untreated urine was cycled through it (P2) to enable a blank determination to be recorded.

After measuring the blank, two different protocols were possible. The first of these would involve emptying the cell and keeping it as such until steady state in the crystalliser had been achieved. Thereafter, the steady state product from the crystalliser would flow downwards into the empty cell but, as explained, would exit immediately and inversion of the cell would be required. However, since a blank determination had

already been recorded, physical movement of the cell would cause gross errors in the measured CSD.

The second possible protocol was to close C2 after the blank determination and to open C1, leaving the untreated urine in the cell. After attainment of steady state, C1 would be closed and C2 would be opened, thereby allowing flow into the untreated urine in the cell. Mixing of the steady state product and untreated urine in this way would therefore not be representative of the CSD in the crystalliser. Thus, both possible protocols for downward flow into the cell were non-ideal. It became obvious that upward flow would have to be considered.

It was appreciated that there were two potential disadvantages of upward flow. Firstly, since upward flow necessitated the crystalliser offtake tubing to be connected to the bottom of the cell, a further bend in the tubing would be required. It was realised that this might cause laminar flow in this bend resulting in crystal precipitation. Secondly, the possibility existed that under the effect of gravity, large crystals would not be pumped out of the top of the cell (ie, the flow rate might not be large enough to counteract the effect of gravity). This would mean that large particles would circulate in the cell and would eventually precipitate thereby giving distorted CSD's.

In order to facilitate the decision of whether to utilize downward or upward flow, trial runs using both configurations were conducted. No significant differences in the CSD's were observed. However, since upward flow had fewer inherent difficulties this configuration was deemed to be the more suitable.

Design no.6

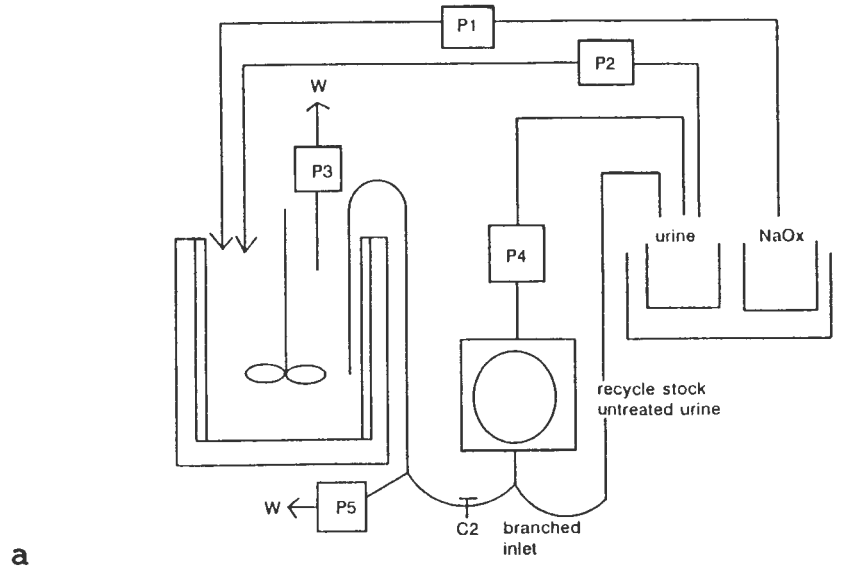
After measurement of the blank in the system depicted by Design no.5 (Figure 3.26), untreated urine was retained in the cell during unsteady state operation. When steady state was attained, the flow of the steady state product was diverted to

the cell. With untreated urine already present it was "bubble free". However, errors in the CSD arose as a result of the mixing which occurred.

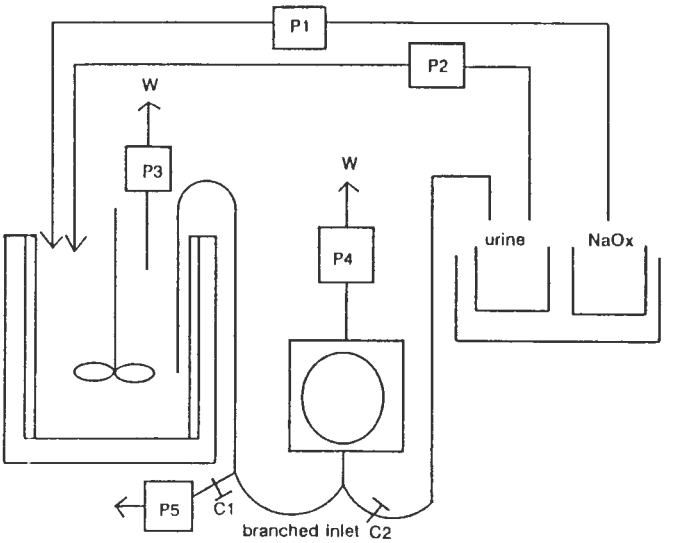
Experiments were accordingly conducted in which the cell was emptied after a blank determination, and left as such for 10 residence times. However, due to a combination of the high flow rate (40 ml/min) of the urine from the crystalliser into the empty cell and the cooling of the glass windows (due to the cell being empty for 10 residence times), bubbles inevitably formed in the cell when the steady state product was introduced. These bubbles were erronously measured by the Malvern as very large particles.

Since both approaches for introduction of the steady state product into the Malvern cell were problematic, a further modification involving the incorporation of three additional pumps P3, P4 and P5 was necessary (Figure 3.27).

The inclusion of P4 allowed a third approach to be implemented: untreated urine was cycled *through* the cell during unsteady state operation "a". In order to achieve this the inlet to the cell at the bottom was branched (see Figure 3.27) with pump P4 positioned on the opposite side of the cell relative to the crystalliser.



a



b

Figure 3.27: The final system design.

P1,P4: Watson Marlow single channel 502 model pumps
P3 : Minipuls 2 Gilson double channel pump
P2 : Perimax 12 spectec six channel pump
P5 : Cole Palmer high flow rate pump with solid state masterflex speed controller
P2 and P3 both used PVC calibrated flow tubing 1.42mm ID

Unsteady state operation is shown in Figure 3.27a. The product from the crystalliser flowed to waste via pump P5; simultaneously, untreated urine from the stock sample was continually cycled through the flow cell via pump P4 as described in the previous paragraph. A few minutes prior to steady state being attained, air bubbles in the cell were

removed by emptying the latter and refilling it with untreated urine from the stock supply.

Emptying the cell involved reversing the direction of flow through it (ie reversing P4 to allow downward flow through the cell). The sampling tube was removed from the stock untreated urine and the cell was emptied as a result of P4 pumping air instead of urine. Once the cell was empty, the flow through the cell was changed (ie P4 was reversed) to upward flow once again. The cell was refilled with the stock untreated urine without bubbles appearing on the glass windows. A blank measurement was then made by cycling the untreated urine from the stock through the cell by P4.

After the blank measurement, the cell was emptied once again, by reversing P4 which was subsequently switched off. Since urine had flowed through the cell minutes before, the cell windows were still warm. After steady state had been achieved, the product from the crystalliser was diverted into the flow cell as shown in Figure 3.27b. This involved using C1 to clamp the tube that had been used for the removal to waste of unsteady state product. In addition, clamp C2 to the cell from the crystalliser was moved to the other inlet of the cell which had previously been used to cycle untreated stock urine.

Thus the main features of the final design shown in Figure 3.27 are as follows:

(i) the offtake from the crystalliser is maintained at a depth equal to the midpoint of the propeller blades (Chapter III.4 and Designs 1-5);

(ii) clamps are used to divert the sample flow to waste or the crystalliser (Chapter III.5 and Design 5);

(iii) the flow through the cell is upwards (Design 5);

(iv) pump P4 is placed on the opposite side of the cell relative to that of the crystalliser to avoid milling effects;

(v) untreated sample is cycled through the cell during unsteady state operation to maintain the cell windows at the correct temperature and to ensure mixing of the stock untreated sample.

III.8 OPERATING CONDITIONS

Residence time

The particle size distribution (PSD) obtained by manipulating kinetic data is only applicable to the MSMPR crystalliser provided that the holding time τ is the mean residence time of the crystals in suspension [Randolph and Larson 1971], ie: provided that

$$\tau = V/Q \quad \text{.....1}$$

where V = volume of crystalliser
Q = total flow rate (in or out) of the crystalliser

To operate the various pumps within their optimum ranges using the available tubing, a residence time of 3.6 minutes was initially tested. However, the high flow rate of 55ml/min necessary to maintain this residence time caused excessive wear and tear on the tubing; as a consequence the residence time was changed to 5 minutes. This reduced the required flow rate to 40ml/min.

Flow rate

The flow system consisted of two running feeds: one of filtered, untreated urine and a second of aqueous NaOx. To ensure minimal dilution of the urine in the crystalliser, the flow rate of the urine was arbitrarily set at 90% of the total flow rate (ie 36ml/min), while that of the aqueous NaOx was set at 4ml/min.

Propeller speed

Circulation rate is defined as the volume of fluid moved by an impeller in a unit time (ie pumping capacity of the impeller) [McCabe and Smith 1976]. The volumetric flow rate must be sufficient to sweep out the entire volume of the vessel in a reasonable time. The impeller must also be rotating fast enough to ensure that the flow currents it produces reach the remotest regions in the crystalliser. The stream leaving the propeller carries a fixed amount of kinetic energy (E_k) which is released by the shear friction as the current flows through the fluid. If the stream does not have sufficient E_k to reach the furthest regions of the tank, dead zones are created. Therefore, the fluid velocity must be sufficient to provide at least a minimum amount of E_k. The flow rate for a propeller is proportional to agitator speed in revolutions per second (rps) multiplied by the square diameter of the impeller [McCabe and Smith 1976] (Equation 2).

$$\text{Flow rate} \propto nDa^2 \quad \text{.....2}$$

where n = the agitator speed in rps
Da = the diameter of the propeller

The propeller speed must also be such that a turbulent regime exists inside the crystalliser providing the means of entraining material from the bulk of the liquid in the tank and incorporating it in the flowing system.

Bourne and Zabelka (1980) conducted experiments with glass beads and potash alum in a gradual classification continuous crystalliser. Classification occurs when the mean residence time $\tau(L)$, of a crystal of size L, is different to the mean residence time τ of the slurry inside the crystalliser. Small, medium and large sized crystals can each have different mean residence times relative to τ. Gradual classification occurs when $\tau(L)$ is a smooth continuous function of L. Using a contoured base tank, Bourne and Zabelka concluded that the limiting step in homogenising the suspensions was the pick-up of the particles from the base of the tank. The potash alum

system is an example of a size-dependent growth process [Garside and Jancic 1978].

Measurement of suspension speeds is achieved by direct and indirect methods. Direct methods include visual, contact, radiation and observation of transparent liquid and sediment interface. Indirect methods are based upon the height of the interface between suspension and clear liquid, measurement of solid phase concentration, or measurement of mass transfer rates [Rieger and Ditl 1994]. Recently, Godfrey and Zhu (1994) used a matched refractive index technique to measure axial concentration profiles. Using tracer particles, they were able to visualise flow patterns by streak photography.

Zweitering (1958) was the first to use visual methods to determine the conditions for complete suspension of all solid particles. Using a baffled vessel, he determined the required impeller speed as a function of varying vessel dimensions, type of stirrer, dimensions and position of stirrer and amounts of solids and densities of solids and liquids.

Using dimensional analysis, Zweitering was able to determine suitable dimensionless groups for his experimental data. Expansion of the groupings led to Equation 3 to calculate the stirrer speed N_{js} required to just suspend the crystals [Zacek *et al.* 1982]:

$$N_{js} = \frac{S\nu^{0.1}(g\Delta\rho/\rho)^{0.45}d^{0.2}X^{0.13}}{D^{0.85}} \qquad \text{.....3}$$

where S = constant, dimensionless
ν = kinematic viscosity, cm^2/s
g = gravitational acceleration, cm/s^2
$\Delta\rho$ = difference between solid and solution density, g/cm^3
ρ = solution density, g/cm^3
d = crystal size, cm
X = weight percent of solids in suspension
D = vessel diameter, cm

Suspension is defined as having zero particles at rest at the bottom of the tank for periods in excess of 1 or 2 seconds. This equation applies to a flat bottomed tank with impeller agitation and has been used by several investigators [Weisman and Efferding 1960; Zacek *et al.* 1982]. It has been supported by Nienow (1968) who also established the importance of impeller clearance.

Oldshue and Sharma (1992) visually calculated N_{js} in tanks of differing diameter in which impeller heights above the bottom of the tank (measured from centerline of impeller) were varied. They also considered shape of the tank bottom, varying D/T ratios (defined in Chapter III.2) and effect of percentage solids (ie the percentage of total solids compared to the total weight of liquid and corresponding solid) on N_{js}. They found that there are three regions in the crystalliser where C/T (defined in Chapter III.2) has an effect on N_{js} for both the axial flow pitched blade turbine and the radial flow flat blade turbine tested.

Calculations of minimum stirrer speed for complete suspension have been proposed by numerous authors all of whom accepted Zweitering's definition of suspension. Dietl and Rieger (1980) and Musil and Vlk (1980), have critically reviewed these proposals.

The rate of nucleation depends strongly upon the stirring rate [Youngquist and Randolph 1972]. Micro-attrition and secondary nucleation are a direct consequence of an increased rate of stirring and particle concentration [Conti 1980]. Qualitatively, induction of nucleation by crystal-solid impacts has been demonstrated by Lal *et al.* (1969) and Clontz and McCabe (1971). Bennett *et al.* (1973) studied the effect of crystal suspension circulation on nucleation and reported a correlation between agitator tip speed and nucleation rate.

To ensure that the possibility of these effects was minimized in the present study, the stirring rate was set as low as possible while still allowing complete mixing. Scanning

electron microscopy (SEM) micrographs were used to check that there was no visible attrition of the crystals.

Using plots of S vs T/D (where T and D are the vessel and impeller diameters respectively) [Zweitering 1958] the value of the dimensionless constant S for the present system was calculated to be 7. (The tank diameter is 6.35cm and the maximum crystal size d, was assumed to be 45μm).

Using Zweitering's formula, the minimum stirrer speed to suspend a particle of size 5μm was found to be 387 rpm, while that for a particle of size 45μm was 601 rpm. To ensure complete homogeneity the stirrer speed was maintained above 601rpm.

Reynolds number inside the crystalliser

There are at least ten dimensionless numbers associated with agitation involving heat and mass transfer [Brodkey and Hershey 1988]. Dimensional analysis is based on dimensional homogeneity existing between variables describing the given system. Using a minimum number of dimensions, all other dimensions are related to these.

Reynolds (1883) [cited by Brodkey and Hershey 1988] illustrated the differences between laminar and turbulent flow. He found from his experiments on laminar - turbulent transition that a dimensionless group of numbers, N_{Re} (Equation 4), could be used to predict empirically the transition point. In defining N_{Re}, the characteristic velocity is the tip speed, defined as $U_t = \pi ND$ with rotational speed N in units of s^{-1}.

$$N_{Re} = D^2 N\rho/\mu \quad \text{.....4}$$

where D = impeller diameter, cm
N = impeller rotational speed, s^{-1}
ρ = density of liquid, g/cm^3
μ = viscosity of liquid, g/cm.s

When N is slowly increased from zero, a laminar flow regime exists initially. A large transitional region extends from N_{Re} = 10 to N_{Re} = 10^3. In this range, vortex systems near the impeller begin to form. When N_{Re} > 10^3 turbulent flow exists [Perry and Chilton 1973; Brodkey and Hershey 1988].

To ensure that the solution flow inside the crystalliser would be fully turbulent, the impeller tip speed N was calculated using a Reynolds number of 10^4 and was found to be 914.5 rpm. The impeller was therefore set to 950 rpm with a corresponding Reynolds number of 10388. (These figures were calculated using values of density and viscosity for pure water).

Reynolds number inside tubing

To ensure fully turbulent flow inside the offtake tubing between crystalliser and flow cell, the required Reynolds number was calculated. The critical value for turbulent flow inside a tube is N_{Re} > 2100 [Brodkey and Hershey 1988].

Viscous sheer stress is defined as $\tau_v = \mu V/d$.
Inertial sheer stress $\tau_i = \rho V^2$

$$N_{Re} = \text{inertial / viscous}$$
$$= \rho V d_0/\mu \quad \text{.....5}$$

where ρ = density, g/cm^3
μ = viscosity, g/cm.s
d_o = pipe diameter, cm
V = average velocity accross the pipe (ie flow rate/area of a circle: $V = Q/\pi r^2$)

A small Reynolds number (<2100) means large viscous forces damping out turbulence thereby causing laminar flow. In laminar flow, the fluid moves in straight lines along the tube axis (z) with no fluid groups moving in the r or ϕ directions (Figure 3.28). A large Reynolds number (>10000) means large inertial forces leading to turbulent flow. (The region 2100 < N_{Re} < 10000 is the transitional region from laminar to fully turbulent flow).

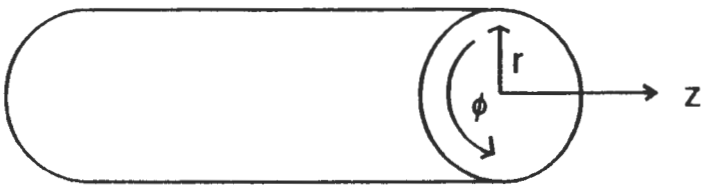


Figure 3.28: Flow directions in a tube.

Using the total flow rate (Q) = 40ml/min and the diameter of the offtake tube as 1.6mm, the value of N_{Re} for the system designed for the present study was calculated to be 780,5.

Since this value is less than the critical value of 2100, laminar flow would exist in the tubing. To increase the value of N_{Re}, a larger flow rate Q would be needed. However, since residence time (= V/Q) must be held constant, a larger flow rate Q would need to be offset by a larger crystalliser volume. This in turn would cause the required volume of bulk urine to increase also. Thus it was decided to accept the N_{Re} value, recognising that a consequence thereof was that the solution would not be fully turbulent in the tubing. In the earlier offtake designs of the present study (designs no.2-4), this effect caused crystallisation in the tubing and switches. To minimize the effect of laminar flow in the tubing (in the final design no.6), the offtake tube was made as short as possible and was positioned such that the flow of sample from the crystalliser was directed downwards, ie in the same direction as gravity.

Settling velocity of crystals inside Malvern cell

For upward flow of the sample through the flow cell, it was necessary to determine the maximum size of crystal that would be kept in suspension. Newton's second law of motion states:

$$\Sigma F = \dot{P} = d(mU)/dt \quad \text{.....6}$$

where $\dot{P}$ = rate of change of momentum
ΣF = sum of the forces exerted
$d(mU)/dt$ = change of momentum at an instant.

A force balance using this law enables the formulation of the equation for the terminal velocity (U_t) (Equation 7) of a small particle at steady state [Clift *et al.* 1978; Brodkey and Hershey 1988].

$$U_t = (2gr_p^2)(\rho_p - \rho)/(9\mu)$$

$$= d^2(\rho_p - \rho)g/(18\mu) \quad \text{.....7}$$

where g = gravitational acceleration, cm/s^2
ρ_p = particle density, g/cm^3
ρ = fluid density, g/cm^3
μ = viscosity of fluid, g/cm.s
r_p = radius of particle, cm
d = diameter of particle, cm

flow rate through cell = 40ml/min; volume of cell = 7.07ml; d = 45μm:
Time for 1ml to flow through = $40/7.07 = 5.658min^{-1}$
Speed of particles = 942.95μm/sec
[private communication Dr Hounslow]

U_t = 1963,86μm/s (from Equation 7)

Therefore $d_{max}/45 = (942,95/1963,80)^{0.5}$
[private communication Dr Hounslow]

and d_{max} = 31,18μm

For a crystal of this size, the force of gravity is more influential than Brownian motion collisions. As a result, the larger crystals would be prevented from escaping the cell at the top, thus causing them to remain in the path of the laser beam. In the present system however, crystals of this size were uncommon and no error was reflected in the obscuration value during measurement (ie no rapid increase in obscuration).

Reynolds number inside Malvern cell

Stokes (1851) [cited by Brodkey and Hershey 1988], solved the problem of a sphere moving very slowly through a stationary fluid. Stokes' Law relates the force exerted by the fluid on the sphere to viscosity, particle radius r_p, and free stream velocity U_∞. The equation for terminal velocity is restricted to the region where Stokes' Law applies. The Law is found to be accurate up to $N_{Re} = 0.5$ [Brodkey and Hershey 1988].

Assuming that the maximum crystal diameter is 45μm, the particle Reynolds number $N_{Re,p}$ is calculated by Equation 8:

$$N_{Re,p} = d_p U_\infty \rho / \mu \quad \text{.....8}$$
$$= 0.129$$

where U_∞ = the free stream velocity if the particle is at rest (ie the difference in velocity between the fluid and the particle)

$U_\infty = U_t$ (calculated in Equation 7)

The flow in the cell is therefore in the Stokes' Law region.

III.9 REPRODUCIBILITY AND SAMPLE TESTING

Obscuration values

Initial tests were performed by removing samples directly from the crystalliser to ensure that the crystalliser was perfectly mixed. Initial testing was accomplished using a suspension of 14,4μm average diameter PDVB latex beads (Coulter Electronics Ltd. Luton, England) in 0,2μm filtered isoton [AIChE testing committee 1980]. A blank measurement of this stationary isoton was taken using the 63mm lens of the Malvern Multisizer instrument. Thereafter, the bead suspension was introduced into the crystalliser and the stirrer speed of the impeller was set to 950rpm. Three samples of this suspension were then pipetted from the crystalliser into the empty cell and were measured. The suspension was then pumped through the flow system to obtain 10 readings for comparison with the static

measurements. Average volume mean diameters and obscuration values are shown in Table 3.1.

	D[4,3] (μm)	Obscuration
Static system	15.18 ± 0.01	0.2476 ± 0.0050
Flow system	15.19 ± 0.05	0.2274 ± 0.0134

Table 3.1: Comparative readings of flow and static system using Coulter calibration beads in isoton.

The D[4,3] values are in good agreement. The stationary system showed more stable obscuration values than those for the flow system. Ideally, the standard deviation of the obscuration values should be < 0.01.

Metastable limits

Using a residence time of 3,6 minutes, urine was introduced into the flow system. Single urine samples were used initially for each experiment. The test involved the determination of the metastable limit (MSL) of the urine with respect to NaOx. The MSL is the minimum amount of oxalate required to induce detectable precipitation [Ryall *et al.* 1985].

Ten aliquots (10ml) of 0.45μm filtered urine were pipetted into particle free Coulter sampling cups. The aliquots were incubated at 38°C and each fraction was then titrated with 0.1ml of successively increasing concentrations of NaOx in the concentration range 0.01 to 0.2 mol/dm^3. The presence of crystals after 30 minutes was investigated using a Coulter Counter.

Thereafter, precipitation was induced in the crystalliser of the flow system by dosing each urine with NaOx corresponding to the MSL concentration. The NaOx concentrations were usually between 0.08 to 0.12 mol/dm^3. However, in most cases, the dosing concentration proved to be too high for the Malvern measurements as the crystalliser suspension became very

cloudy, sometimes within the first few minutes of the experiment. For optimum results, the concentration bar on the Malvern screen should be within the ideal range (Figure 3.29) as indicated in the loading bar on the right of the figure. The center graph (Figure 3.29) indicates the light energy in each of the 31 rings, while the graph on the left gives an approximate indication of the sizes of the measured particles. The obscuration value is indicated at the bottom left of the figure and correlates with the loading bar on the right.

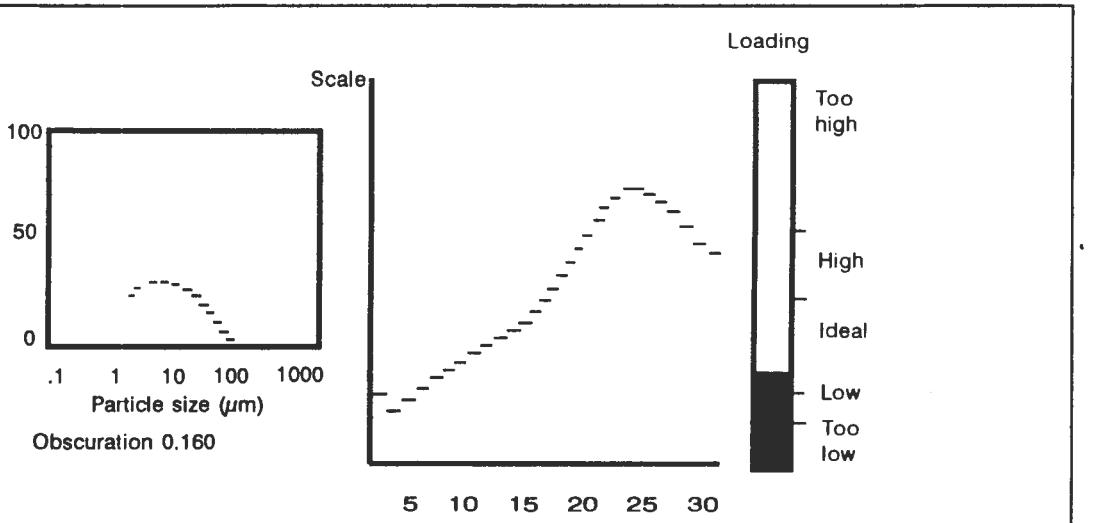


Figure 3.29: Diagram of screen during sample concentration inspect.

Pooled urines were used to obtain a set of comparative experiments on the same sample. At this stage, specimens were arbitrarily pooled to provide sufficient volume. The MSL for each of five pools was determined each day over the experimental period of a week, and were all found to remain stable with respect to the MSL determined on the first day. Using pooled urines, various dosing concentrations of NaOx based upon the measured MSL, were tested. In this way, it was possible to identify a range of NaOx concentrations that would produce measurable crystallisation (in the ideal range of the Malvern) in almost every pool. Concentrations of 0.02 to 0.03 mol/dm^3 usually achieved this.

Particle size distributions

Initially, there was a poor fit between experimental data (from urine samples innoculated with aqueous NaOx) and the

proposed model (Chapter II.1.3 under "Analysis model"). The goodness of fit depends upon the log difference values between experimental data and the proposed model: the bigger the difference, the poorer the fit. Attempts to improve the fit included using a program written for the 63mm lens, but which had been modified for the 100mm lens and which implemented the more correct MIE theory.

Figure 3.30 shows the results obtained from pooled samples used in this study with both the Malvern program (a) and the MIE theory program (b).

(3.30a)

High Size	Und %	High Size	Und %	High Size	Und %	High Size	Und %	High Size	Und %	High Size	Und %
188	100	84.5	100	38.0	95.3	17.1	64.6	7.69	41.1	3.46	16.3
175	100	78.6	99.9	35.4	94.0	15.9	62.8	7.15	37.9	3.21	14.9
163	100	73.1	99.9	32.9	92.4	14.8	61.4	6.65	35.5	2.99	13.2
151	100	68.8	99.8	30.6	90.8	13.7	60.3	6.18	33.6	2.78	11.7
141	100	63.2	99.6	28.4	88.9	12.8	59.4	5.75	31.6	2.59	10.3
131	100	58.8	99.4	26.4	86.9	11.9	58.6	5.35	29.6	2.40	9.0
122	100	54.7	99.1	24.6	84.6	11.1	57.8	4.97	27.5	2.24	7.9
113	100	50.8	98.6	22.9	81.5	10.3	56.8	4.62	25.3	2.00	6.7
105	100	47.3	98.1	21.3	77.5	9.56	55.0	4.30	23.1	1.33	5.7
97.8	100	44.0	97.3	19.8	72.4	8.39	50.6	4.00	21.0		
90.9	100	40.9	96.4	18.4	67.4	8.27	45.5	3.72	18.8		

Source = Sample Focal length = 100mm Presentation = pil Volume conc = 0.0201%	Beam length = 10mm Log diff = 4.187 Obscuration = 0.2921 Sp.S.A. = 1.1163m^2/cc.	Model indp Shape off

Span	D[4,3]	D[3,2]	D[v,0.9]	D[v,0.1]	D[v,0.5]
3.88	13.73um	5.37um	29.66um	2.54um	8.81um

(3.30b)

Log Diff = 4.483 Max = 32.1 at band centered at 1.78μm
obscuration = 0.2921 lens = 100

High Size	V%	HSize	V%	HSize	V%	HSize	V%
183.99	0.06	27.33	5.41	9.95	4.74	3.36	2.99
81.93	0.93	21.34	17.15	7.76	1.98	2.98	6.11
51.26	2.59	16.38	3.11	6.87	1.28	2.38	13.31
36.38	4.31	12.76	1.98	4.76	1.76	1.92	32.10

Figure 3.30: Comparison of the fit between experimental data and a proposed model using two dfferent programs: (a) original Malvern program using the anomalous theory and (b) the MIE theory.

Figure 3.30a shows the tabulated equivalent Malvern data which is divided into 64 size classes giving a more detailed PSD. In comparison, Figure 3.30b indicates a PSD having 16 size divisions.

For the data analysed by the the original Malvern program (a), the log difference value is 4.187 while that from the MIE theory (b), is 4.483. It is thus apparent that a better fit is achieved with the Malvern program.

Calibration using latex beads

Final reproducibility testing was accomplished using a mixture of calibration beads suspended in 0.45μm filtered isoton. Three bead sizes were used namely, 3.2, 8.5 and 14.0μm. Five samples were taken from the waste line after flowing through the offtake system, and five samples were pipetted directly from the crystalliser during flow experiments. The median particle sizes are shown in Table 3.2.

	Median diameters (μm)		
	Bead size		
	no.1	no.2	no.3
Flow through			
1	3.44	8.31	13.98
2	3.40	8.35	13.83
3	3.56	8.32	14.15
4	3.62	8.42	14.00
5	3.66	8.44	13.80
Pipette			
1	3.32	8.32	13.90
2	3.48	8.32	13.83
3	3.48	8.48	13.95
4	3.57	8.29	13.90
5	3.71	8.45	14.05

Table 3.2: Median particle diameters for a mixture of 3 different sized calibration beads.

Comparison of the means of the median diameters for each bead size using the Students t-test showed no significant difference at a 99% confidence level [Miller and Miller 1993].

CaOx precipitation

In addition to beads, reproducibility tests were also performed on the kinetics of CaOx crystallisation from solutions of 10^{-2}M $CaCl_2$ and NaOx. The residence time used was 5 minutes. Measurements were taken using the 100mm lens of the Malvern instrument. To mimic the anticipated experimental conditions in the present study, the NaOx flowed at 90% and the $CaCl_2$ at 10% of the total flow rate of 40ml/min. Flow rates were monitored by collecting outflow from the crystalliser in a measuring cylinder as a function of time. Two sets of five experiments were performed using the flow system. Readings were taken between 8 and 11 residence times. The best fit straight lines of plots of log(population density) vs size [Randolph and Larson 1971; Jancic and Garside 1975; Rodgers and Garside 1981] were calculated for the size range 7.15 - 24.6μm [Brecevic and Garside 1981]. The values of growth rate (G), nucleation rate (B_o) and mean particle diameters (D[4,3]) are shown in Table 3.3, and are each calculated from an average of 8 measurements. Table 3.4 shows the average growth and nucleation rates of the two sets of experiments in Table 3.3.

Set One				
Exp	G (μm/min)	B_o (μm/min.ml)	D[4,3] (μm)	Obscuration
1	0.686	2373	16.43	0.3306
2	0.691	2358	16.15	0.3232
3	0.698	2268	15.94	0.3296
4	0.706	2188	15.49	0.3320
5	0.691	2344	16.45	0.3352
Set Two				
Exp	G (μm/min)	B_o (μm/min.ml)	D[4,3] (μm)	Obscuration
1	0.735	1954	16.80	0.3395
2	0.724	2051	16.80	0.3392
3	0.718	2129	16.66	0.3386
4	0.713	2168	16.61	0.3321
5	0.712	2146	16.95	0.3416

Table 3.3: Kinetic data from reproducibility experiments using 10^{-2}M $CaCl_2$ and NaOx. G = growth rate; B_o = nucleation rate; D[4,3] = mean particle diameter. Note that the range of the obscuration values is < 0.01 for each set.

	G (μm/min)	B_o (μm/min.ml)
Set 1	0.694 ± 0.050	2306 ± 482
Set 2	0.720 ± 0.056	2090 ± 545

Table 3.4: 95% Average growth and nucleation rates of the data from the two sets of experiments shown in Table 3.3.

Reproducibility of the experiments was good ($p < 0.05$) with Table 3.4 indicating the 95% confidence levels for the growth and nucleation rates.

REFERENCES: CHAPTER III

Aeschbach,S.; Bourne,J.R.
"The attainment of homogeneous suspension in a continuous stirred tank"
Chem.Eng.J. (1972), **4**, 234-242

American Institute of Chemical Engineers. Equipment testing procedures committee
"A.I.Ch.E. testing procedure for particle size classifiers" in:"Particle size classifiers: a guide to performance evaluation"
New York, A.I.Ch.E. (1980), 1-16

Andrigo,P.; Bagatin,R.; Cavalieri d'Oro.P.; Perego,C.; Raimondi,L.
"Micromixing in a chemical quenching apparatus for fast kinetics"
Chem.Eng.Sci. (1988), **43** (8), 1923-1928

Bakker,A.; Fasano,J.B.
"Time dependent, turbulent mixing and chemical reaction in stirred tank reactors"
AIChE.Symp.Ser. (1994), **90**, (299), 71-77

Baldyga,J.; Bourne,J.R.
"Interactions between mixing on various scales in stirred tank reactors"
Chem.Eng.Sci. (1992), **47** (8), 1839-1848

Baldyga,J.; Bourne,J.R.; Yang,Y.
"Influence of feed pipe diameter on mesomixing in stirred tank reactors"
Chem.Eng.Sci. (1993), **48** (19), 3383-3390

Baldyga,J.; Podgorska,W.; Pohorecki,R.
"Mixing-precipitation model with application to double feed semi-batch precipitation"
Chem.Eng.Sci. (1995), **50** (8), 1281-1300

Baldyga,J.; Pohorecki,R.
"Turbulent micromixing in chemical reactors - a review"
Chem.Eng.J. (1995), **58**, 183-195

Bates,R.L.; Fondy,P.L.; Corpstein,R.R.
"An examination of some geometric parameters of impeller power"
I&EC Process Des.Dev. (1963), **2**, 310-314

Bennett,R.C.
"Matching crystalliser to material"
Chem.Eng. (1988), **95** (8), 119-127

Bennett,R.C.
"Advances in industrial crystallisation techniques"
AIChE.Symp.Ser. (1984), **80** (240), 45-55

Bennett,R.C.; Fiedelman,H.; Randolph,A.D.
"Crystalliser influenced nucleation"
Chem.Eng.Prog.Symp.Ser. (1973), **69** (7), 86-93

Bourne,J.R.; Dell'ava,P.
"Micro and macromixing in stirred reactors of different sizes"
Chem.Eng.Res.Des. (1987 March), **65**, 180-186

Bourne,J.R.; Gholap,R.V.; Rewatkar,V.B.
"The influence of viscosity on the product distribution of fast parallel reactions"
Chem.Eng.J. (1995), **58**, 15-20

Bourne,J.R.; Yu,S.
"Investigation of micromixing in stirred tank reactors using parallel reactions"
Ind.Eng.Chem.Res. (1994), **33**, 41-55

Bourne,J.R.; Zabelka,M.
"The influence of gradual classification on continuous crystallisation"
Chem.Eng.Sci. (1980), **35**, 533-542

Brecevic,L.; Garside,J.
"On the measurement of crystal size distributions in the micrometer size range"
Chem.Eng.Sci. (1981), **26**, 867-869

Brodkey,R.S.; Hershey,H.C.
"Transport Phenomena. A unified approach"
McGraw-Hill Inc. (1988), 359, 198, 113

Calabrese,R.V.; Stoots,C.M.
"Flow in the impeller region of a stirred tank"
Chem.Eng.Prog. (1989 May), 43-50

Clift,R.; Grace,J.R.; Weber,M.E
"Bubbles, drops and particles"
Academic Press Inc. (1978), ch9

Clontz,N.A.; McCabe,W.L.
"Contact nucleation of magnesium sulfate heptahydrate"
Chem.Eng.Progr.Symp.Ser. (1971), **67** (110), 6-17

Conti,R.
"Particle abrasion at high solids concentration in stirred vessels II"
Chem.Eng.Sci. (1980), **35**, 543-547

Ditl,P.; Reiger,F.
"Suspension of solid particles"
Chem.Eng.Sci. (1980), **35**, 764-765

Evans,T.W.; Margolis,G.; Sarofim,A.F.
"Mechanism of secondary nucleation in agitated crystallisers"
AIChE.J. (1974), **20**, 950

Fasano,J.B.; Miller,J.L.; Pasley,S.A.
"Consider mechanical design of agitators"
Chem.Eng.Progr. (1995 August), 60-71

Garside,J.; Jancic,S.J.
"Measurement and scale-up of secondary nucleation kinetics for the potash alum-water system"
AIChE.J. (1979), **25** (6), 948-958

Garside,J.; Jancic,S.J.
"Prediction and measurement of crystal size distributions for size dependent growth"
Chem.Eng.Sci. (1978), **33**, 1623-1630

Garside,J.; Jancic,S.J.
"Growth and dissolution of potash alum crystals in the subsieve size range"
AIChE.J. (1976), **22** (5), 887-894

Garside,J.; Tavare,N.S.
"Mixing, reaction and precipitation in an MSMPR crystalliser: effects of reaction kinetics on the limits of micromixing"
in:"Industrial Crystallisation 84"
eds:Jancic,S.J.; de Jong,E.J. (1984), 131-136

Garside,J.; Tavare,N.S.
"Mixing, reaction and precipitation: limits of micromixing in an MSMPR crystalliser"
Chem.Eng.Sci. (1985), **40** (8), 1485-1493

Gates,L.; Morton,J.; Fondy,P.
"Selecting agitator systems to suspend solids in liquids"
Chem.Eng. (May 1976)

Godfrey,J.C.; Zhu,Z.M.
"Measurement of particle-liquid profiles in agitated tanks"
AIChE.Symp.Ser. (1994), **90** (299), 180-185

Grootscholten,P.A.M.; De Leer,B.G.M.; De Long,E.J.
"Factors affecting secondary nucleation rate of sodium chloride in an evaporative crystalliser"
AIChE.J. (1982), **28** (5), 728-737

Hounslow,M.J.
Dept.Chemical Engineering, Univ.Cambridge, Penbroke Street, Cambridge CB23RA, United Kingdom
Private communication, May 1994

Jancic,S.; Garside,J.
"On the determination of crystallisation kinetics from crystal size distribution data"
Chem.Eng.Sci. (1975), **30**, 1299-1301

Kavanagh,J.P.; Nishio,S.; Garside,J.; Blacklock,N.J.
"Crystallisation kinetics of calcium oxalate in fresh minimally diluted urine: comparison of recurrent stone formers and healthy controls in a continuous mixed suspension mixed product removal crystalliser"
J.Urol. (1993), **149**, 614-617

Kumar,S.; Kumar,R.; Gandhi,K.S.
"Alternative mechanisms of drop breakage in stirred vessels"
Chem.Eng.Sci. (1991a), **46** (10), 2483-2489

Kumar,S.; Kumar,R.; Gandhi,K.S.
"Influence of the wetting characteristics of the impeller on phase inversion"
Chem.Eng.Sci. (1991b), **46** (9), 2365-2367

Li,M.K.; Blacklock,N.J.; Garside,J.
"Effects of magnesium on calcium oxalate crystallisation"
J.Urol. (1985 January), **133**, 123-125

Lal,D.P.; Mason,R.E.A.; Strickland-Constable,R.F.
"Collision breeding of crystal nuclei"
J.Cryst.G. (1969), **5** (1), 1-8

McCabe,W.L.; Smith,J.C.
"Unit Operations of Chemical Engineering"
2nd Edition, McGraw-Hill Inc. (1976), 241-276

Miller,J.C.; Miller,J.N.
"Statistics for analytical chemistry"
Ellis Horwood, PTR Prentice Hall, (1993), 3rd Edition, ch3

Mueller,I.; Linder,P.W.; Rodgers,A.L.; Ball,D.; Knight,J.
"Studies of calcium oxalate stone formation: introducing a multi-faceted crystalliser"
Paper presented at the International symposium on metal ions in solution, Transvaal, South Africa, 20-23 April 1993.
(Private communication, July 1993)

Musil,L.; Vlk,J.; Jiroudkova,H.
"Suspending solid particles in an agitated tank with axial-type impeller"
Chem.Eng.Sci. (1984), **29** 621-628

Musil,L.; Vlk,J.
"Suspending solid particles"
Chem.Eng.Sci. (1980), **36**, 789

Nienow,A.W.
"Suspension of solid particles in turbine agitated baffled vessels"
Chem.Eng.Sci. (1968), **23**, 1453-1459

Nishio,S.; Kavanagh,J.P.; Faragher,E.B.; Garside,J.; Blacklock,N.J.
"Calcium oxalate crystallisation kinetics and the effects of calcium and γ-carboxyglutamic acid"
Br.J.Urol. (1990), **66**, 351-356

Nishio,S.; Kavanagh,J.P.; Garside,J.
"A small-scale continuous mixed suspension mixed product removal crystalliser"
Chem.Eng.Sci. (1991), **46** (2), 709-711

Oldshue,J.Y.
"Suspending solids and dispersing gases in mixing vessel"
Ind.Eng.Chem. (1969a), **61** (9), 79-89

Oldshue,J.Y.
"Fluid mixing in 1989"
Chem.Eng.Prog. (1989 May), 33-41

Oldshue,J.Y.
"Mixing. Annual review"
Ind.Eng.Chem. (1969b), **61** (11), 121-127

Oldshue,J.Y.; Sharma,R.N.
"The effect of off-bottom distance of an impeller for the just suspended speed Njs"
AIChE.Symp.Ser. (1992), **88**, (286), 72-76

Perry,R.H.; Chilton,C.H.
"Chemical Engineers Handbook"
5th Edition, McGraw-Hill Inc. (1973), ch19

Pohorecki,R.; Baldyga,J.
"The use of a new model of micromixing for determination of crystal size in precipitation"
Chem.Eng.Sci. (1983), **38** (1), 79-83

Pohorecki,R.; Baldyga,J.
"The effects of micromixing and the manner of reactor feeding on precipitation in stirred tank reactors"
Chem.Eng.Sci. (1988), **43**, 1949-1954

Randolph,A.D.
"Effect of crystal breakage on crystal size distribution in a mixed suspension crystalliser"
I&EC Fundamentals (1969), **8** (1), 58-63

Randolph,A.D.; Larson,M.A.
"Theory of particulate processes"
Acad.Press. New York, London, (1971), 162, 65

Randolph,A.D.; Sidkar,S.K.
"Effect of a soft impeller coating on the net formation of secondary nuclei"
AIChE.J. (1974), **20** (2), 410-412

Randolph,A.D.; Sidkar,S.K.
"Creation and survival of secondary crystal nuclei. The potassium sulfate-water system"
Ind.Eng.Chem.Fundam. (1976), **15** (1), 64-71

Rieger,F.; Ditl,P.
"Suspension of solid particles"
Chem.Eng.Sci. (1994), **49** (14), 2219-2227

Roberts,R.M.; Gray,M.R.; Thompson,B.; Kresta,S.M.
"The effect of impeller and tank geometry on circulation time distributions in stirred tanks"
Trans.IChem. (1995), **73** (A), 78-87

Rodgers,A.L.; Garside,J.
"The nucleation and growth kinetics of calcium oxalate in the presence of some synthetic urine constituents"
Invest.Urol. (1981), **18** (6), 484-488

Rosen,H.N.; Hulbert,H.M.
"Continuous vacuum crystallisation of potassium sulfate"
Chem.Eng.Progr.Symp.Ser. (1971), **67**, (110), 18-27

Ryall,R.L.; Hibberd,C.M.; Marshall,V.R.
"A method for studying inhibitory activity in whole urine"
Urol.Res. (1985), **13**, 285-289

Shah,B.S.; McCabe,W.L.; Rousseau,R.W.
"Polyethylene vs. stainless steel impellers for crystallisation processes"
AIChE.J. (1973), **19** (1), 194

Shaw,J.A.
"Succeed at solids suspension"
Chem.Eng.Progr. (1992 May), 34-41

Shor,M.; Larson,M.A.
"Effect of additives on crystallisation kinetics"
Chem.Eng.Progr.Symp.Ser. (1971), **67** (110), 32-42

Skrtic,D.; Furedi-Milhofer,H.; Markovic,M.
"Precipitation of calcium oxalate from high ionic strength solutions. V. The influence of precipitation conditions and some additives on the nucleating phase"
J.Cryst.G. (1987), **80**, 113-120

Tavare,N.S.
"Mixing in continuous crystallisers"
AIChE.J. (1986), **32** (4), 705-732

Tavare,N.S.
"Micromixing limits in an MSMPR crystalliser"
Chem.Engng.Technol. (1989), **12**, 1-10

Tavare,N.S.
"Mixing, reaction and precipitation: an interplay in continuous crystallisers"
Chem.Eng.Sci. (1994), **49**, (24B), 5193-5201

Weisman,J.; Efferding,L.E.
"Suspension of slurries by mechanical mixers"
AIChE.J. (1960), **6** (3), 419-426

Youngquist,G.R.; Randolph,A.D.
"Secondary nucleation in a class II system: ammonium sulphate-water"
AIChE.J. (1972), **18** (2), 421-429

Zacek,S.; Nyvlt,J.; Garside,J.; Nienow,A.W.
"A stirred tank for continuous crystallisation studies"
Chem.Eng.J. (1982), **23**, 111-113

Zweitering,Th.N.
"Suspending of solid particles in liquid by agitators"
Chem.Eng.Sci. (1958), **8**, 244-253

CHAPTER IV
IN VITRO EXPERIMENTS

IV.1 INSTRUMENT SETTINGS

IV.1.1 MALVERN

Malvern measurements are susceptible to stray light as discussed in Chapter II.1.2 under "Background measurement". Thus, care was taken to locate the instrument in a windowless, dark and dust-free room.

Range lens

Initially the 63mm lens with a particle detection capability in the size range 1.2 - 118μm was used. However, this was subsequently changed to the 100mm lens (range 1.9 - 188μm) to be more compatible with the Coulter settings in which the lower limit of measurements was 2.8μm.

Despite a lower limit determined by the particular lens selected, it is nevertheless possible to measure particles below this limit as the instrument has a sub-size measurement compensation. However, reduced accuracy of measurement will occur if more than 20% of the material is in this sub-size class [McCave *et al.* 1986]. The lower detection limit of measurement with the 63, 100 and 300mm lenses is approximately 0.5μm. Fraunhofer theory breaks down in this range because the particles do not diffract light in the manner required for valid application of the theory [de Boer *et al.* 1987; Bayvel and Jones 1981].

Vignetting

Each range lens has a limited aperture and a maximum scattering angle through which it is able to collect scattered light. If the sample is too distant from the range lens, it is possible that the maximum scattering angle for all the particles will not be captured. This error is called

"vignetting". It is therefore necessary to ensure that all the particles in the sample pass through the beam within the working distance of the range lens. This working distance is known as the lens cut-off distance x, and is calculated as follows [Felton 1990]:

$$x = f(R_l - R_b)/R_d \quad \text{.....1}$$

Where f = focal length
R_l = lens radius
R_b = laser beam radius
R_d = detector radius

For the 100mm lens, x was calculated to be 133mm.

Refractive index instability

Instability in the optical alignment was noticed in the early stages of the present study when the blank sample was passing through the flow cell. Investigation showed that this refractive index instability was due to both temperature gradients and to the fast flowing stream of sample into and out of the cell. The problems of temperature differences was eliminated easily as it was noted that the instability occurred during initial flow of the sample through the cell but that it later disappeared after the glassware, tubing and flow cell had warmed up.

The contribution from sample flow was noticed only when very high flow rates (60 ml/min) were used. When instability occurred at the high flow rate (60 ml/min during early testing for the presence of bubbles in the cell), the Malvern was programmed to selectively ignore the scattering in the lowest angle (ie rings 1 and 2). However, this procedure should be avoided as it leads to a limitation of the dynamic range of the lens.

The instability was totally absent in the presence of very low flow rates; there was no instability at the pre-selected flow rate for experimentation (40ml/min).

Analysis time

In this work, a maximum analysis time of 2 minutes was used, during which 32760 measurement sweeps were collected. During this time, a few cell volumes passed through the cell ensuring that the largest possible volume of sample was analysed. The two minute time averaged measurement was therefore the most representative reading possible.

Scattering matrix

As discussed in Chapter II.1.2 under "Refractive index", more acceptable log difference values can be obtained if necessary from an approximate scattering matrix (ie the scattering matrix calculated by the Malvern but manipulated using the Mie theory). Log difference values from the Malvern were acceptable, however the more correct Mie theory was used to determine whether lower log difference values could be obtained.

In the case when the difference between the refractive index of the particle and of the suspending medium is small, analysis of size distributions should be done using the Mie theory (Chapter II.1.1 under "Fraunhofer and Anomalous theories" and "Mie theory"). In an attempt to determine whether smaller log difference values could be obtained from the system, a program using Mie theory written in the Chemistry Department at UCT [Seymore PhD Thesis, UCT 1996] was used to analyse Malvern data. The results from this program are shown in Chapter III.9 under "Reproducibility and sample testing".

Log difference values from this program were not better than those from the Malvern program. Since the values obtained from the latter were well within the acceptable limit, the data from the Malvern was used directly without any further treatment.

IV.1.2 COULTER

Orifice tube

Since the experimental protocol involved comparison of Malvern and Coulter data, the dynamic ranges of the two machines had to be matched as closely as possible. Initially the 63mm lens of the Malvern was selected, the particle size range of which is 1.2 - 118μm. Accordingly, the 70μm orifice tube for the Coulter was chosen with a nominal particle size range of 1.4-42μm. Other Coulter orifice tubes that were available (with their nominal particle size ranges indicated in brackets) were the 140μm (2.8 - 84μm), 200μm (4.0 - 120μm) and the 560μm (11.2 - 336μm).

However, air bubbles on the 70μm orifice wafer were encountered. These expanded under the vacuum during counting and caused electrical signal noise, leading to reduced accuracy of the counts. Cleaning of the orifice tube overnight did not improve the situation. Although it was possible to remove the air by releasing the vacuum, draining and careful refilling of the orifice tube, it was very time consuming and impractical when readings had to be taken immediately.

It was therefore decided to use the 140μm orifice tube (nominal particle size range 2.8 - 84μm) which was found to be free of air on the wafer, possibly because a gentler flow of sample occurs through it. The aperture current setting was 1600μA.

The Malvern lens was accordingly changed; the 100mm lens was selected as it provided the closest match to the lower limit size range of the 140μm Coulter orifice tube.

Since the crystalliser was removed from the immediate location of the Coulter Counter, no noise transmission was encountered.

Calibration

Latex calibration beads (Coulter Electronics Ltd. England) of diameter 14.4μm were used to calibrate the 140μm aperture. For optimum results the calibration particles should be 20% of the

aperture diameter [Operating Manual Coulter Multisizer Issue C]. The beads were suspended in 0.22μm filtered Isoton II (a bacteriostatted phosphate-buffered saline solution supplied by Coulter Electronics) in a measurable concentration such that the coincidence was less than 2%. The resulting calibration constant K_d was calculated at 1331,1.

Electrolyte and signal noise

It is standard practice when using the Coulter Counter to have the same electrolyte on each side of the aperture. If this is not the case, inconsistencies can occur when the electrolyte inside the orifice tube (usually Isoton II) mixes with the different electrolyte in which the sample is suspended. In the present study, CaOx crystals in urine were to be measured. Therefore, a sample was withdrawn from the stock untreated 0.45μm filtered urine and was used to fill the Coulter sample jar and orifice tube. (The total volume of raw urine required from each participant was therefore of necessity, large).

Signal noise can also arise as a result of a change in temperature; therefore, it was necessary to maintain the urine in the Coulter reservoir and the orifice tube at the same temperature as that of the sample from the crystalliser. This was achieved by introducing the untreated urine into the Coulter reservoir and orifice tube a few minutes before sampling the steady state product from the crystalliser. To ensure that the orifice tube had equilibrated to 37°C, it was immersed in thermostatted 0.45μm filtered untreated urine which was flushed through the aperture for a minute, after which three blank readings were recorded.

Stirring

When recording measurements of the suspension solution, adequate mixing is necessary to minimize agglomeration or flocculated particle passage, either of which would lead to an incorrect size distribution. Stirring of the sample also ensures that there is no sedimentation which could lead to size classification. This latter situation is especially

important since the sample was originally withdrawn from the crystalliser where great care was taken in mixing to avoid such effects. Careful placement of the overhead glass stirrer was necessary to negate possible electrical disturbances from itself and the Coulter cup.

Sampling

To prevent errors from too small sampling volumes, a minimum volume of 500μl for the 140μm aperture was used. For optimum results a volume of 2000μl is recommended but this volume was not suitable in the present study as each reading requires 25 seconds. Since crystallisation occurs inside the Coulter sampling cup, initial and final readings would differ substantially. On the other hand, sampling of the 500μl volume required an average of 7 seconds and therefore allowed an initial and final reading to be recorded. The advantages of taking an initial reading were that it introduced suspension sample into the orifice tube and that it provided a value for comparison with the final reading.

Careful cleaning of the orifice tube and aperture was necessary because of deposits of CaOx after many readings. This was due to the high concentration of CaOx in the samples taken from the crystalliser. Details of the cleaning procedures are described in this Chapter IV.4.3 under "Cleaning".

Fortunately, blocking of the aperture did not occur. This is attributed to careful filtering of the blank sample prior to commencement of the experiments and to the fact that none of the crystals grew or aggregated to sufficient size.

A problem that was encountered with the Coulter Counter was breakage of the mercury column. This seriously affected the reading time during sampling since the breaks in the mercury caused start/stop probes to react at the wrong times. If breakage occurred while sampling, the reading was abandoned. In general, breakage is due to either dirt in the mercury, or operating faults. Apart from changing the mercury, the

breakage was usually corrected by applying the vacuum a few times. When the mercury breakage was corrected quickly, the experimental run was continued with minimum interruption.

Data analysis

The size range capability in this work (as determined by the use of the 140μm orifice tube) was 2.8 - 84μm and was divided into 256 channels. Each channel can be designated as being of size (X) which represents either diameter, area, or volume. X is defined as the right edge of that particluar channel. For convenience, the left edge of the channel is designated (X-1), and the centre (X-0.5) (Chapter II.2 under "Channel size calculation").

In all the determinations, the full range option was used and was sometimes supplemented with narrow or window range readings. All readings were taken in the linear diameter mode. Explanation of these options is discussed in Chapter II.2 under "Channel size calculation". The theory of area and volume scales with both linear and log laws is discussed in the Coulter Multisizer manual Issue C.

Further manipulations concerning specific areas of the distribution in narrow and window selections were applied only when time allowed, as the facility of an on-line computer for storage of the data was not available.

IV.1.3 SCANNING ELECTRON MICROSCOPY

To validate the trends in growth and nucleation rates of CaOx in the MSMPR crystallisation experiments, precipitated crystals were examined in a scanning electron microscope (SEM). This instrument provides information on morphology and allows qualitative and semi-quantitative comparisons to be made of crystal number, size and extent of aggregation.

Using a pasteur pipette, a sample of crystals was aspirated from the MSMPR crystalliser after kinetic measurements had been completed. This sample was filtered through a 0.2μm

Nucleopore filter (13mm diameter) supported in a Sartorious membrane filter clamp (G.m.b.H. Göttingen). The filter paper, with the deposited crystals, was pasted onto an aluminum stub for SEM analysis. The sample was coated with approximately 20nm of Au/Pd in a Polaron 5100 Sputter Coater.

Specimens tilted at 35° to the collector were examined using a Cambridge S200 scanning electron microscope operating at a nominal beam potential of 10kV and beam current of 100μA. The secondary electron images were recorded on Ilford FP4 roll film at 60s frame period and 800 lines per frame.

IV.2 CRYSTALLISATION KINETICS

By making a number of simplifying assumptions as outlined in Chapter II.2 under "Crystalliser" [Randolph and Larson 1971], the steady state crystal size distribution in an MSMPR crystalliser is represented by Equation 2.

$$n = n^{o}\exp(-L/G\tau) \quad \ldots\ldots\ldots\ldots\ldots\ldots 2$$

where

$$n^{o} = B_{o}/G \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

$$\tau = V/Q \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

L = crystal size, μm
τ = crystalliser residence time, mins
G = crystal growth measured in μm/min
n^{o} = the nuclei number density which is related to the nucleation rate B_{o} (no/min.ml) as shown in Equation 3.

By taking logarithms of Equation 2, the following linear expression arises.

$$\log n = \log n^{o} - L/(2.303G\tau) \quad \ldots\ldots\ldots\ldots 5$$

A plot of log(n) vs L will therefore yield a straight line of slope $1/(2.303G\tau)$ and intercept $\log(n^{o})$.

By using Equations 3 and 5, the values of G and B_o may be deduced from the steady state crystal size distribution. The values of G and B_o given in this chapter are all deduced from such plots.

Hounslow [Hounslow *et al.* 1988; Hounslow 1990a,b] has described another technique to determine the CSD (hence nucleation and growth) from an MSMPR crystalliser. The technique involves transforming the population balance into a series of algebraic equations in terms of moments [Randolph and Larson 1971].

The first four moments (m_o - m_3) have a physical meaning and are proportional to total number, length, area and volume of solid per unit volume of suspension. Once the moments of the distribution have been determined, nucleation and growth rates can be calculated from Equations 6 and 7 respectively:

$$(B_o/2)m_o^2 + m_o/t - B_o = 0 \quad \text{.....6}$$

$$3Gm_2 - m_3/t = 0 \quad \text{.....7}$$

Hounslow has further taken into account the process of aggregation and has described an index of aggregation which allows the determination of nucleation, growth and aggregation when these processes occur simultaneously in the MSMPR crystalliser. This technique was however not used in the present study as the method of Randolph and Larson (1971) (Equations 3 and 5) was preferred.

The Coulter Counter automatically measures the total number of particles in the distribution and therefore results can be used directly with Equation 5. On the other hand, the Malvern Particle Sizer is not based on a number measurement but gives a percentage volume distribution. However, using the following Equation (8), values proportional to the population density can be evaluated [Brecevic and Garside 1981].

$$\bar{n} = w/L^3\Delta L \quad \text{.....8}$$

where $\Delta L = L_2 - L_1$

To compare results from the Coulter and Malvern, the procedure of Brecevic and Garside (1981) was followed. They calculated the best-fit straight lines of plots of log(population density) versus crystal size in the size range 7.3 - 23.2μm. In the present study, the size range for the best-fit straight line was taken to be 7 - 24μm. The values for n obtained with the Malvern Particle Sizer were then multiplied by a constant factor in order that the lines obtained from the Malvern and the Coulter gave the same value of population density at L = 10μm [Brecevic and Garside 1981]. All experiments performed in the present study were repeated on three different urine pools.

Figure 1 shows an example of a population density plot obtained from experiments using both the Coulter and Malvern particle sizer. The two size distributions demonstrate good aggreement. It appears that the population density plots from the Malvern and the Coulter form a curve that can be fitted by two straight lines: one below about 7μm and the other between 7μm and 25μm. The plot therefore indicates an increase in population density below 7μm. This increase is greater than would be expected from the theoretical exponential function of Equation 2.

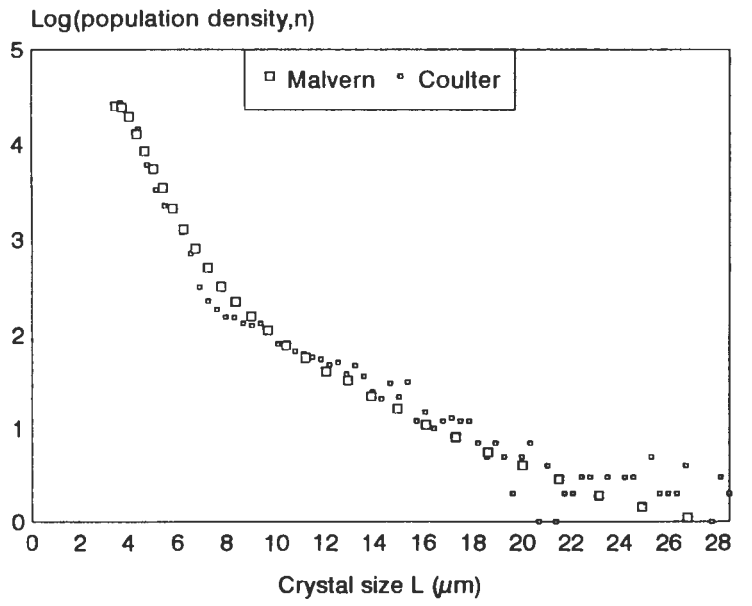


Figure 1: Steady state crystal size distribution (τ = 5 minutes) showing measurements by the Coulter Counter and Malvern Particle Sizer.

Possible explanations for this deviation include that on average smaller crystals grow at a slower rate than larger crystals, and that secondary nuclei are born directly into the micrometer size range [Brecevic and Garside 1981]. These two effects would lead to a greater increase in the number of large particles relative to the increase in the number of small particles. A plot of log(population density) versus crystal size will therefore lead to curvature of the graph.

Each distribution was well represented ($R^2 > 0.95$) by a straight line in the size range 7μm - 24μm.

Multiple regression analyses (Statgraphics version 6.1, Statistical Graphics Corp. 1992) were performed to determine significant differences ($p < 0.05$) in the intercept and slope (from which nucleation and growth rates of CaOx are calculated) between the various experiments within each pool. Results from the multiple regression analyses are shown in Appendix 1.

The suspension density M_T (mmol/dm^3) represents the total crystal mass produced and was calculated in the present study using the following equation published by Randolph and Larson (1971):

$$M_T = 6 f_V \rho B G^3 \tau^4 \quad \text{.....9}$$

where f_V is the volume shape factor (taken to be $\pi/6$ [Kohri *et al.* 1988]) and ρ is the density of the CaOx crystals (2.22g/cm^3).

The total calcium and oxalate concentrations in solution in the crystalliser were calculated using the following equations [Kohri *et al.* 1988]:

$$[Ca]_{sol} = Ca - M_T \quad \text{.....10}$$

$$[Ox]_{sol} = Ox - M_T \quad \text{.....11}$$

where Ca and Ox represent the calcium and oxalate concentrations (mmol/dm^3) respectively in the feed solutions. Dilution factors were taken into account when calculating these values. The endogeneous oxalate (diluted to 90% of its

original concentration) and the exogenous oxalate concentration were summed. This exogenous oxalate was diluted to 10% of its original concentration in experiments investigating the effect of oxalate concentration (Chapter IV.4.1 under "Effect of NaOx") and temperature (Chapter IV.4.2 under "Effect of temperature") while it was diluted to 5% in experiments using inhibitors (Chapter IV.4.3 under "Effect of various inhibitors").

The supersaturation ratio S was calculated using the computer program Equil developed by Werness *et al.* (1985). The total concentrations of citrate and magnesium in solution inside the crystalliser were calculated by summing their respective endogeneous and exogenous concentrations after dilution factors had been taken into account. Dilution of all other urinary constituents to 90% of their original concentration were also taken into account when calculating the supersaturation. Values of suspension density, calcium and oxalate concentrations and supersaturation are tabulated in Appendix 2.

IV.3 EXPERIMENTAL PROCEDURE

Urine collection and pre-treatment

Participants were all healthy males without any prior history of stone disease. All were in the age group 20-30 years. Each participant was required to collect a 24-hour urine sample in a 2.5 dm^3 glass bottle while remaining on their normal diets.

The collection bottles were thoroughly cleaned overnight by soaking in a 5% Contrad solution (Merck KGa, Darmstadt, Germany) and rinsing with deionised water. To eliminate effects which the detergent might have on the urine, the bottles were then soaked in a 5% HNO_3 solution overnight and rinsed with deionised water. To preserve the urine samples, 1ml/dm^3 of a 20% w/v sodium azide solution was added per 2 dm^3 urine.

Individual urine samples were tested for infection using Boehringer Mannheim dipsticks (Combur-10-test). 1.5 dm^3 of urine from each of 8 individuals was then pooled and thoroughly mixed in a 25 dm^3 vat. The minimum number of samples required for statistical pooling was determined in a separate experiment using ANOVA (Appendix 3). This experiment showed that a minimum of 5 samples were necessary to ensure that each pool was statistically different [Jappie PhD Thesis, UCT 1996].

The pooled urine sample was then filtered through Sartorious cellulose acetate filters (prefilter and 0.45μm) to remove all crystalline and non-crystalline matter. The filtration apparatus was cleaned using the method described previously for the collection bottles. The bulk urine was used at its native pH.

Representative samples were then withdrawn from the pool and analysed for the following constituents, and these values are tabulated in Appendix 4.

1. Chloride, phosphate, urate and creatinine were determined by private pathologists using the methods described by Tietz (1976).

(1a). The determination of chloride in urine involves the use of a two phase Ag/AgCl type ion-selective electrode (Beckman CX3 instrument). The equilibrium developed at the surface of the electrode is dependent on the solubility product Ksp of silver and chloride ions in solution. Introduction of chloride ions into the system disrupts the Ksp with the resultant movement of Ag^+ ions from the AgCl phase of the electrode to the sample solution which is mixed with a buffered solution. The Ag^+ metal phase of the chloride electrode responds indirectly to the chloride ion activity in the sample. The potential at the chloride electrode is then referenced to a sodium reference chamber.

(1b). The determination of phosphate in urine is achieved by a 10 to 20 fold dilution and acidification of the sample to

pH 3. The phosphate reacts with ammonium molybdate in sulphuric acid solution to form the coloured complex ammonium phosphomolybdate. Absorbance is measured at 340nm using a Boehringer Mannheim Hitachi 717 spectrophotometer.

(1c). An enzymatic colorimetric test using uricase is applied for the determination of uric acid.

$$\text{uric acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{3,5-dichloro-2-hydroxybenzenesulphonic acid} + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{N-(4-antipyryl)-3-chloro-5-sulphonate-p-benzoquinonemonoimine} + HCl + 4H_2O$$

The hydrogen peroxide (H_2O_2) which is formed reacts (under catalysis of peroxidase) with the compounds shown to give a red-violet quinoneimine. Absorbances are measured at 750 and 546nm using a Boehringer Mannheim Hitachi 717 spectrophotometer.

(1d). Creatinine concentration in urine is determined by the colour change occurring in the following reaction:

$$\text{creatinine} + \text{picric acid} \xrightarrow{(OH)^-} \text{1:1 creatinine-alkaline picrate complex (red)}$$

The rate of formation of the creatinine picrate complex is proportional to the creatinine concentration in the sample. Measurements are made at 520 and 560nm using a Beckman CX3 instrument.

2. Calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) were determined using a Varian Techtron AA-5 flame atomic absorption spectrophotometer, (Techtron Appliances, South Melbourne, Australia, 1970).

An air-acetylene flame was used with a 10cm burner head (serial no.5639). Settings of the AAS for each element are shown on page 145.

Element	λ (nm)	slit width (nm)	lamp current (mA)
Ca	422.7	50	5
K	766.5	300	5
Mg	285.2	50	5
Na	589.0	200	5

Lanthanum chloride was added to samples for the determination of Ca, K and Mg. The lanthanum acts as a complex inhibitor to bind with phosphate thereby eliminating any interferences due to this ion. The lanthanum chloride was added to the sample in a concentration to give 10,000 ppm in the final solution [Willis 1961]. A drop of concentrated HCl was added to clear cloudiness that developed on addition of the lanthanum solution.

For the determination of Na, potassium chloride was added to the sample to suppress its ionization [Bassett *et al.* 1978]. It achieves this because it has a lower ionization potential than Na and therefore creates an excess of electrons when the solution is nebulized into the flame; this results in the suppression of Na ionization.

3. Oxalate was determined by enzymatic assay (Sigma kit, procedure no. 591).

The method is based upon the oxidation of oxalate by oxalate oxidase followed by the measurement of hydrogen peroxide (H_2O_2) produced during the reaction by a peroxidase-catalyzed reaction as shown below:

$$\text{oxalate} + O_2 \xrightarrow{\text{oxalate oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine dye} + H_2O$$

The hydrogen peroxide reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-

(dimethylamino)benzoic acid (DMAB) in the presence of peroxidase. Indamine dye, having an absorbance maximum at 590nm, is produced in this reaction. The intensity of the colour produced is measured by a spectrophotometer (LKB Biochrom Novaspec 4049 spectrophotometer) and is directly proportional to the concentration of the oxalate in the sample.

4. Citrate was determined by enzymatic assay (Boehringer Mannheim UV method, cat.no. 139076).

Citrate is converted to oxaloacetate and acetate in a reaction catalyzed by the enzyme citrate lyase (CL). Oxaloacetate and its decarboxylation product pyruvate are then reduced to L-malate and L-lactate respectively by reduced nicotinamide-adenine dinucleotide (NADH) in the presence of the respective enzymes malate dehydrogenase (MDH) and lactate dehydrogenase (LDH). The amounts of NADH oxidised in the last two reactions are stoichiometric with the amount of citrate present in the sample, and is determined by measuring the NADH using a spectrophotometer set at a wavelength of 340nm.

$$\text{citrate} \xrightarrow{\text{CL}} \text{oxaloacetate} + \text{acetate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

Storage

After aliquots of urine had been withdrawn from the bulk, filtered urine for the above analyses, the latter was refrigerated overnight at 6°C. Before each experiment, it was warmed to room temperature. A representative sample of $3dm^3$ was then withdrawn from the bulk sample and refiltered through the 0.45μm Sartorious cellulose acetate filters to remove any crystalline matter that had deposited overnight. This second filtration step was found to be necessary as background

measurements on the unfiltered overnight sample were sometimes unacceptably high.

Sometimes massive overnight precipitation in the bulk sample occurred. After filtration, further heavy deposition of crystals occurred when the sample was warmed to 37°C. These samples were discarded. Analysis of the deposits using xray powder diffraction (XRD, Philips PW1130/90/96, CuK_2 wavelength = 1.542Å) showed uric acid crystals. By testing each individual urine prior to pooling, it was possible to identify two particular participants whose samples were those which consistently induced crystal deposition. These individuals were consequently excluded from the experimental population group.

Cleaning

Prior to the experimental runs, all the glassware (ie crystalliser and adjoining glassware, conical and volumetric flasks) was cleaned thoroughly for 12 hours in a 5% contrad solution and for a further 12 hours in a 5% HNO_3 solution followed finally by rinsing with 0.45μm filtered glass distilled water. The baffles and stirrer were also cleaned in this way.

In addition, the supply tubing and the Malvern cell were flushed firstly with deionised water and then with a 10% HNO_3 solution. They were then rinsed with 0.45μm filtered glass distilled water.

The flow-cell windows were soaked in a 5% HNO_3 solution overnight and thoroughly rinsed with deionised water. They were wiped with lens tissue to remove dust particles and a thin layer of urine was applied to the inside face of each window to ensure that surface charges which might potentially attract and attach air bubbles or crystals were minimized.

Thermostatting

Heated water from a water bath was used to thermostat the crystalliser, offtake tube and flow cell at 37°C. All solutions were incubated at 37°C for 90 minutes prior to commencement of the crystallisation experiments. The crystalliser, offtake tube, flow cell and adjoining tubing were all rinsed with the bulk thermostatted urine. The supply tubing for the NaOx and inhibitor solutions were also rinsed with their respective solutions to avoid minute dilution from the deionised water used for cleaning. The thermostatted urine was then cycled through the crystalliser, offtake tube and cell and finally back to the stock solution for a further 15 minutes. This was done to ensure mixing of the stock urine and also to warm the connecting tygon tubing.

Experimental method

At this stage, the propeller was set in motion to agitate the urine in the crystalliser. In order to achieve the pre-selected residence time of 5 minutes, the total flow rate into and out of the crystalliser was required to be 40ml/min (Chapter III.8 under "Residence time"). Therefore the flow rate of the urine into the crystalliser was set at 36ml/min and that of the NaOx solution at 4ml/min.

The connecting tygon tubing between the offtake from the crystalliser and the Malvern cell was clamped tightly so that no product from the former could enter the cell during unsteady state operation. After crystallisation had been initiated, all the product from the crystalliser was diverted to waste prior to the attainment of steady state. Meanwhile, untreated urine from the stock supply was cycled through the Malvern cell ensuring that it became thoroughly mixed and that the cell itself and connecting tygon tubing was maintained at the temperature of the experiment.

After 8 residence times, the flow cell was emptied and refilled with the untreated urine by reversing the direction of flow into the cell (Chapter III.7 under "Design no.6").

This was done to remove any air bubbles that might have developed on the cell windows. The laser was then aligned with the flow cell and blank measurements were recorded until a stable reading was registered. Usually, three such readings were necessary.

The flow cell was then emptied and the clamp from the crystalliser offtake to the flow cell was released (Chapter III.7 under "Design no.6"). At the same time, the clamp from the crystalliser to waste was tightened. The offtake from the crystalliser was therefore diverted into the flow cell after 10 residence times. After allowing the flow of several volumes of the cell, several measurements were recorded. Steady state was indicated by a stable obscuration value.

IV.4 IN VITRO EXPERIMENTS

IV.4.1 EFFECT OF NaOx

The first set of experiments were performed to investigate the effect of varying the concentration of NaOx on the crystallisation of calcium oxalate within the same urine pool. As described previously, the first task was to identify a range of exogenous NaOx concentrations that produced measureable precipitation. This range was found to be 20-30 $mmol/dm^3$ and was independent of the endogenous oxalate in the urine. Using analytical grade reagents, an aqueous solution of NaOx within this range was prepared and was used as the first concentration in the sequence of experiments on a particular urine pool. As explained earlier, urine flowed into the crystalliser at 36ml/min and a separate feed was used for NaOx at 4ml/min.

The extent of crystallisation produced from this first NaOx concentration (as indicated by the obscuration value on the Malvern), was used to identify two further suitable concentrations for the in-flowing NaOx solution. These three NaOx concentrations were then used to initiate crystallisation in three urine pools. Nucleation and growth rates in the three pools are shown in Table 1.

		GROWTH μm/min		NUCLEATION no/min.ml (*10^3)	
Sample	[NaOx] mmol/dm^3	Coulter	Malvern	Coulter	Malvern
5	2	0.6066	0.6568	5.303	5.056
	2.5	0.7191 *	0.6532	3.662 *	3.514
	3	0.8186	0.7532	3.322	3.264
6	2	0.6596	0.7345	4.018	2.920
	2.5	0.8183 *	0.7915	4.053	4.059 *
	3	0.8102	0.8106	5.449	5.106
7	2	0.7856	0.6623	2.827	3.217
	2.5	0.8204 *	0.7085	1.890	3.419 *
	3	0.9743	0.7736	2.710	3.723

Table 1: Growth and nucleation rates for varying concentrations of exogneous NaOx after being admitted to the crystalliser. (* denotes significant difference $p < 0.05$)

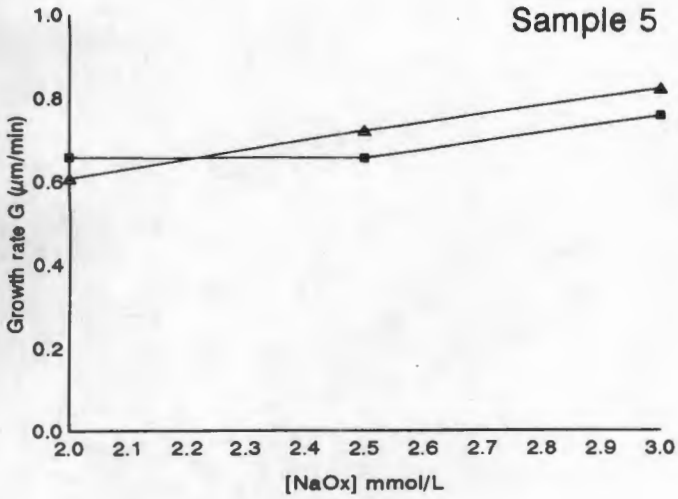
The concentration of NaOx shown in the table corresponds to the concentration of exogenous oxalate after being admitted to the crystalliser.

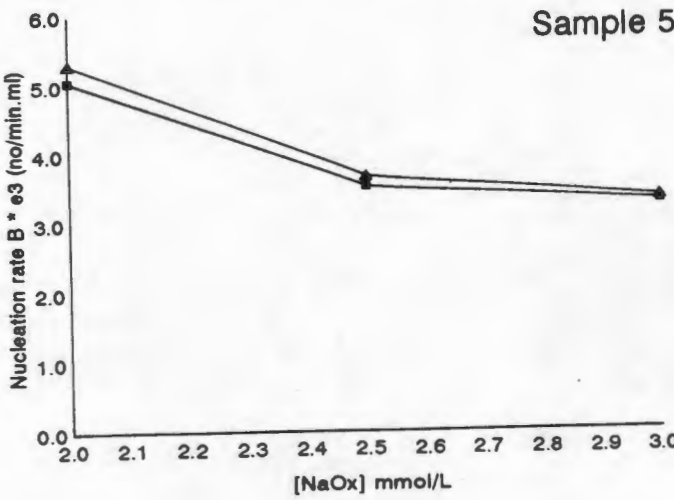
Figure 2 is a graphical representation of the data given in Table 1 and shows growth and nucleation rates of CaOx crystals as a function of increasing NaOx concentrations. These rates were calculated as described in Chapter IV.2. under "Crystallisation kinetics". Although in general, values for the Coulter are higher than those for the Malvern, the trends in the nucleation and growth rates for the two instruments are in good aggreement. The higher Coulter values can be explained in terms of the time delay in getting the sample to the Coulter for analysis. The graphs indicate an increase in growth rates with increasing NaOx for all the samples. However, trends in the nucleation rate are not consistent.

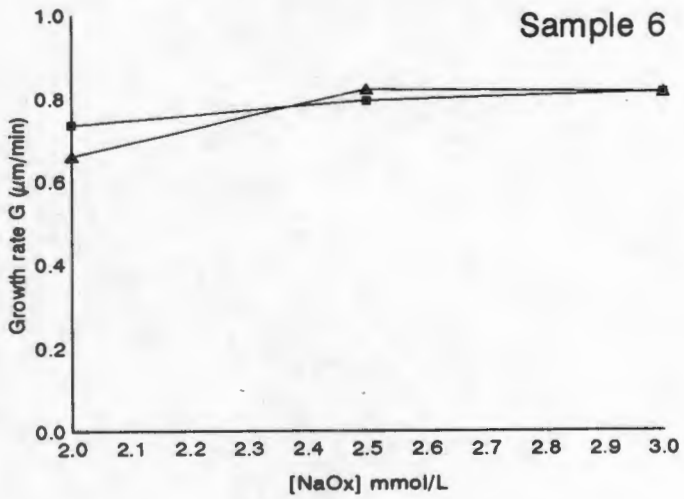
Growth and nucleation rates are plotted as a function of the supersaturation ratio S in Figure 3. The growth rate increases with increasing supersaturation for all samples while trends for the nucleation rate are not apparent. In general, the trends for the Coulter and Malvern agree with the exception of the nucleation variation in sample 7.

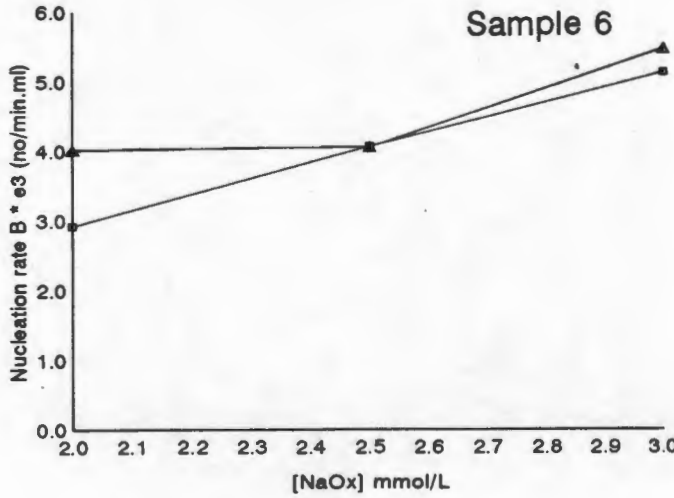
Figure 4 indicates the relationship between nucleation and growth rates for each sample. No trend is observed for the Malvern data; however samples 6 and 7 indicate an increase in nucleation rates with increasing growth rates for the Coulter.

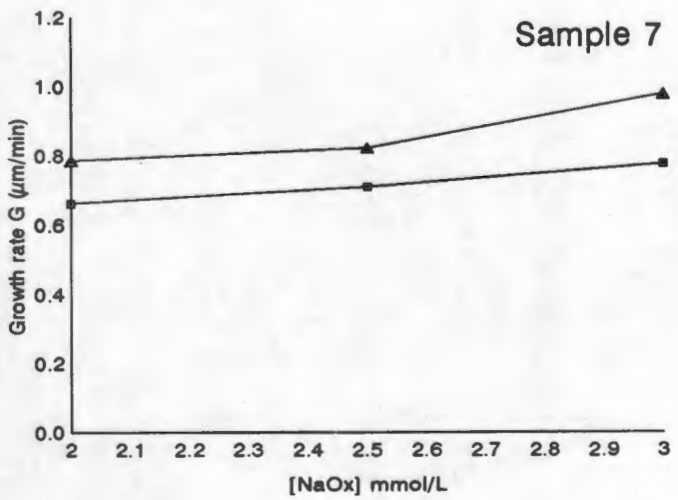
The increase in the growth rates as more concentrated NaOx was added to the crystalliser (shown in Figure 2) is supported by SEM (Figure 5). The most common morphology observed was that of COD crystals; their size increased from 15-28μm as the concentration of NaOx increased. COM crystals were observed at sizes below 10μm. Aggregation of COD crystals was present in some samples with the largest aggregate size being 10μm. Semi-quantitatively, the number of crystals increased with increasing concentrations; samples 6 and 7 showed increases in the number of small crystals below 5μm (this is shown in the micrographs of sample 6 in Figures 5A,B).











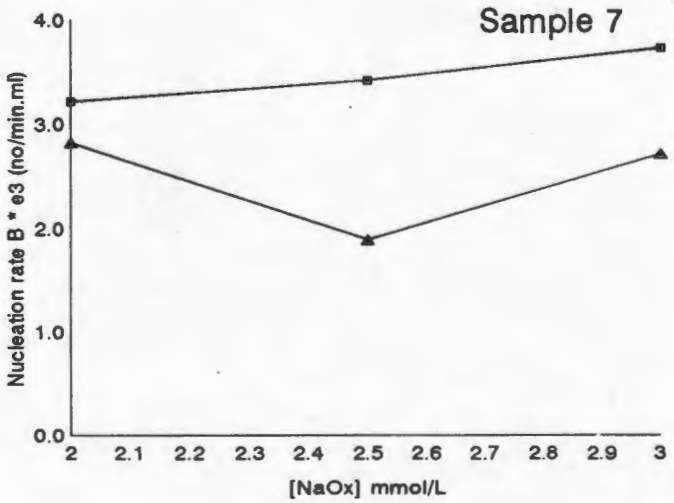
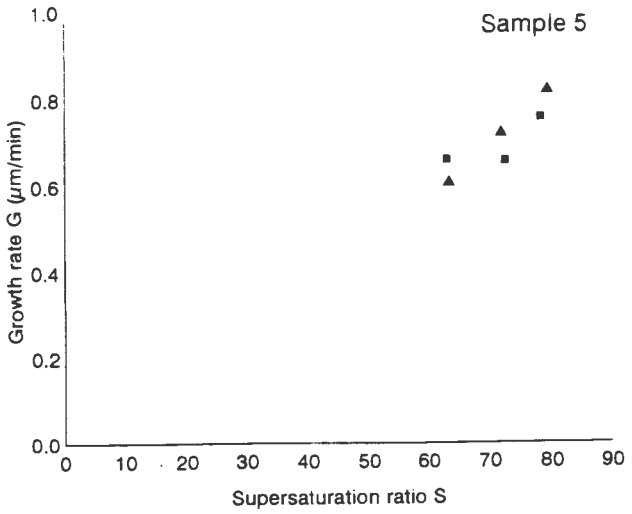
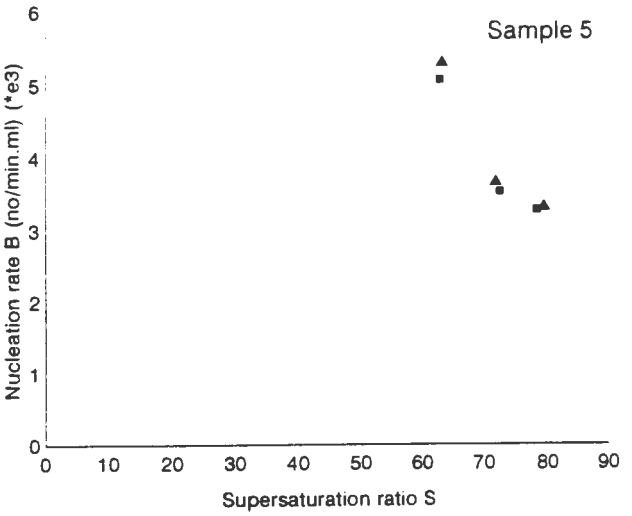
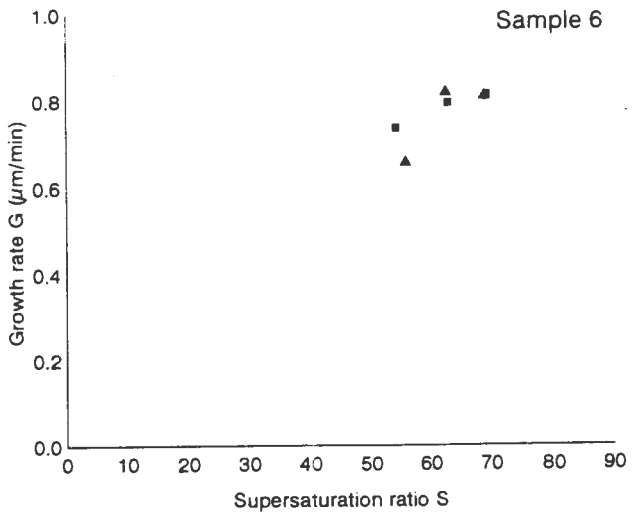
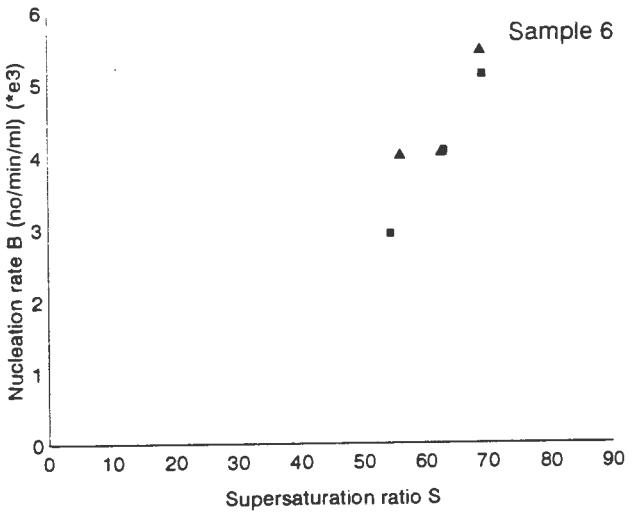


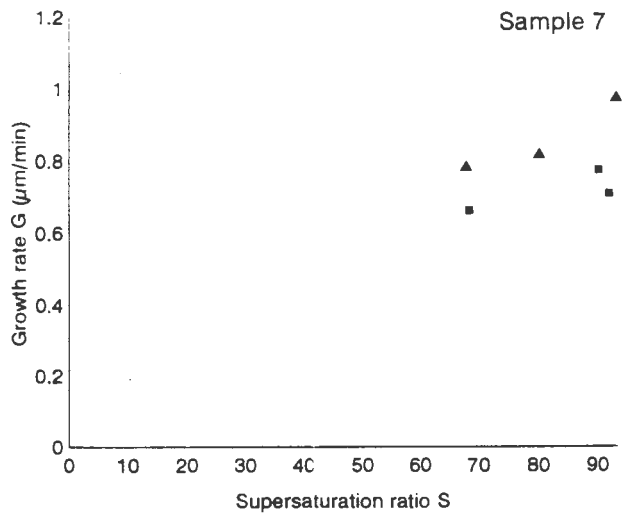
Figure 2: Growth and nucleation rates calculated in the presence of varying concentrations of NaOx.











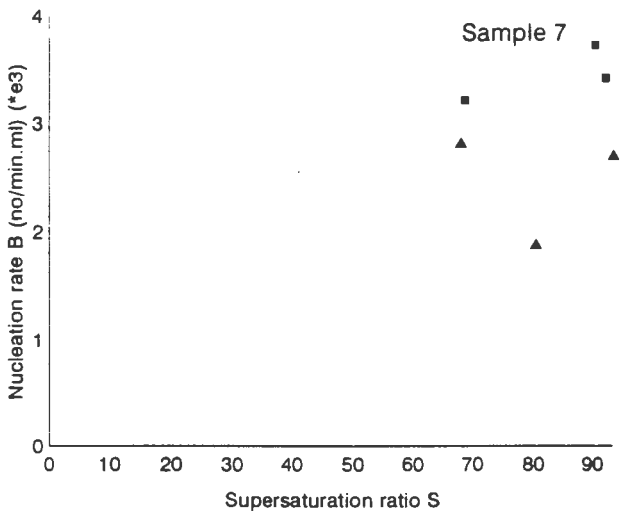


Figure 3: Growth and nucleation rates as a function of supersaturation ratio for varying concentrations of NaOx.

▲ Coulter
■ Malvern

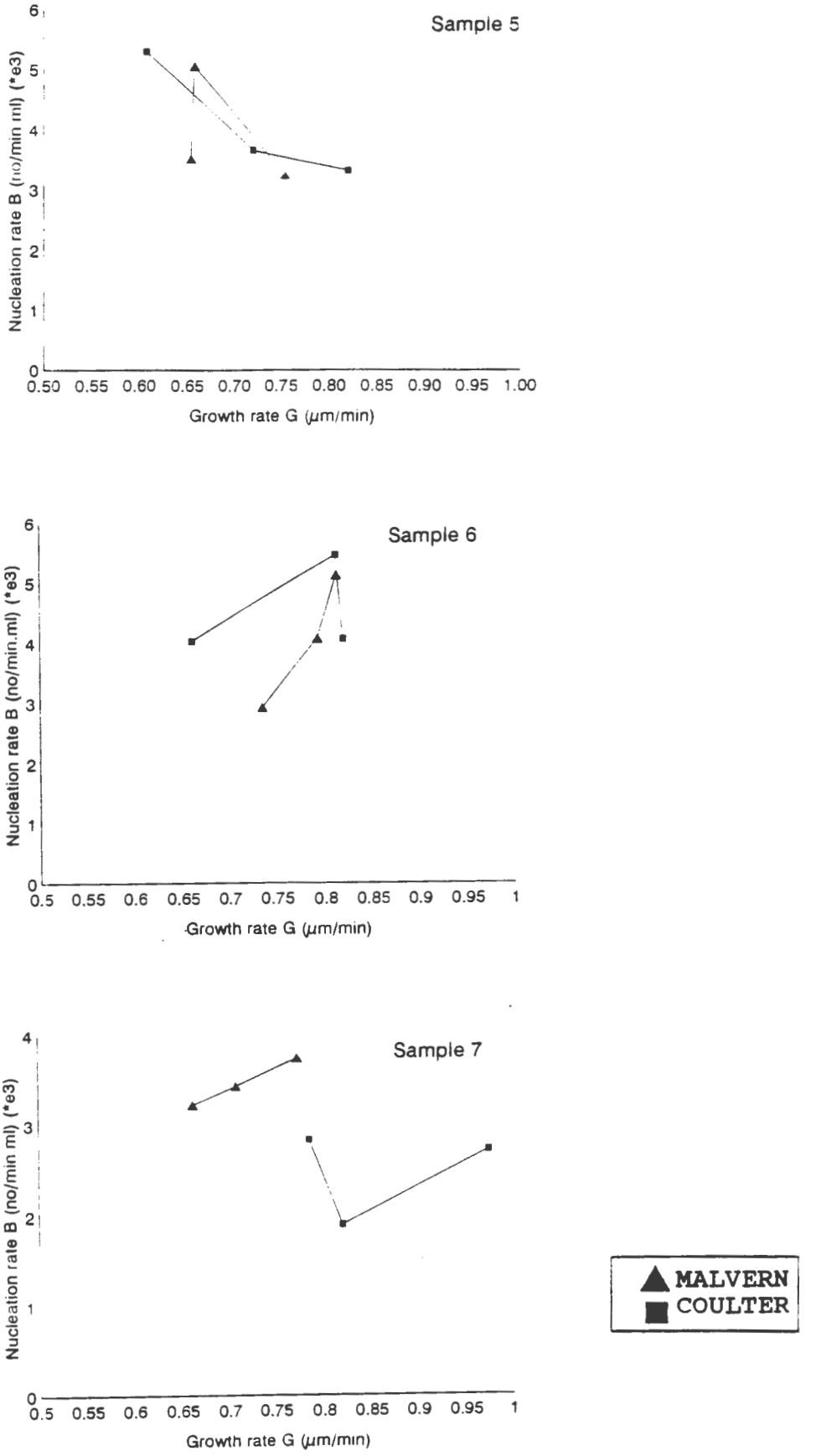
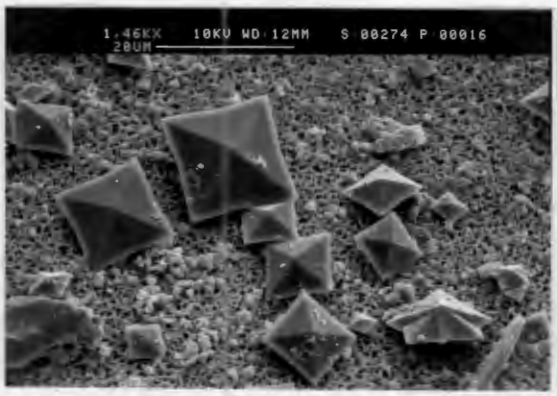
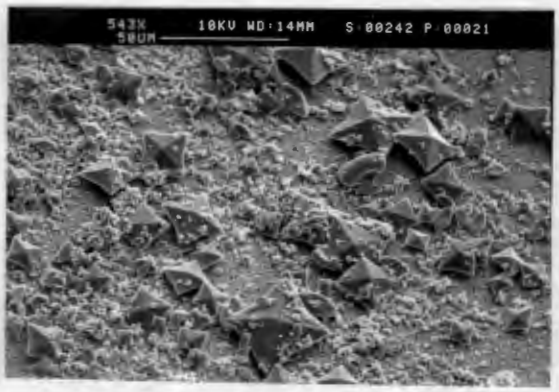


Figure 4: Nucleation rate plotted against growth rate for varying concentrations of NaOx.





5A: [NaOx] = 2mmol/dm^3
mag: 1.46KX
maximum crystal size = 15μm

5B: [NaOx] = 3mmol/dm^3
mag: 543x
maximum crystal size = 25μm

Figure 5: SEM micrographs of samples innoculated with 2 and 3mmol/dm^{-3} NaOx.

IV.4.2 EFFECT OF TEMPERATURE

The effect of three temperatures (18, 38 and 58°C) on the crystallisation of CaOx was investigated using an arbitrary concentration of NaOx = 3mmol/dm^3 throughout. Nucleation and growth rates for these experiments are given in Table 2.

		GROWTH μm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	Temp(°C)	Coulter	Malvern	Coulter	Malvern
8	18	0.5108	0.4640	5.267	10.30
	38	0.8811 *	0.4624 *	2.524 *	5.395
	58	0.9461	0.7452	1.160	3.587
9	18	0.6989	0.5942	4.125	5.781
	38	1.111 *	0.8012 *	1.405 *	2.268 *
	58	1.504	1.227	0.8814	0.6255
10	18	0.6346	0.1946	7.014	10.15
	38	0.6237	0.6069 *	3.669 *	8.644 *
	58	1.037	0.9182	2.142	2.351

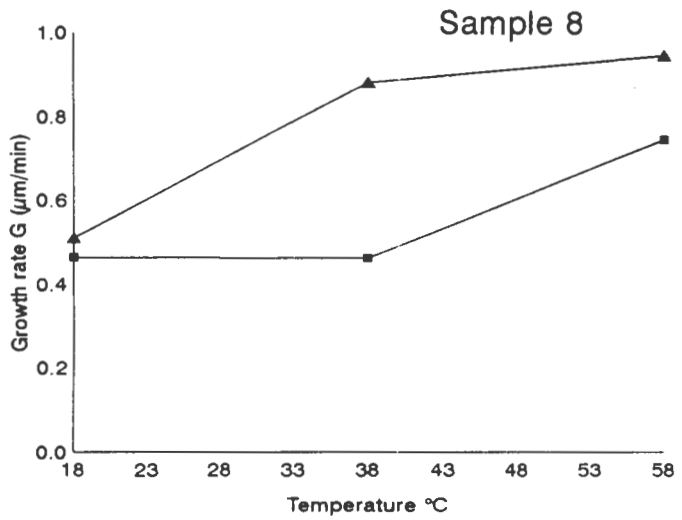
Table 2: Growth and nucleation rates for various temperatures at a constant exogenous NaOx concentration of 3mmol/dm^3 inside the crystalliser. (* denotes significant difference $p < 0.05$).

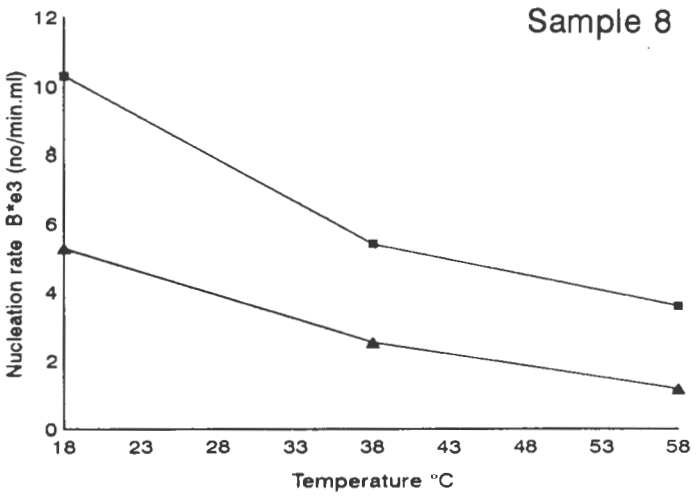
Graphical representations of these data are given in Figure 6. Within each sample, general trends for the Coulter and Malvern showed good agreement in that growth rates increased with increasing temperature for each sample while nucleation rates decreased. With regard to the individual rates at each temperature, growth rates are lower and nucleation rates are higher for the Malvern data relative to the Coulter data.

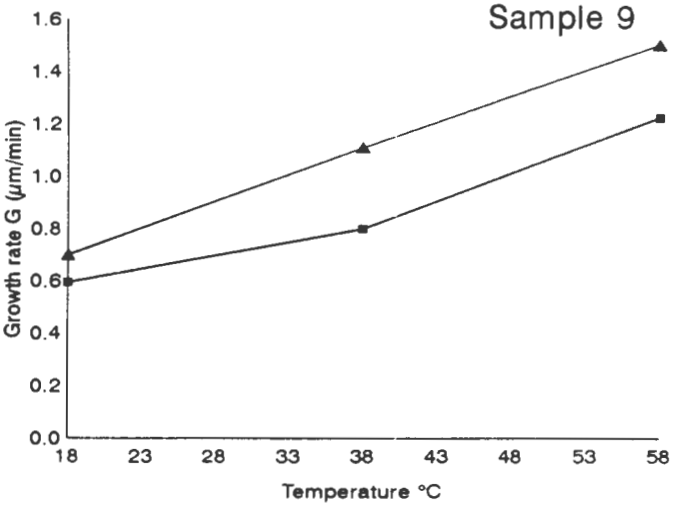
Figure 7 illustrates growth and nucleation rates at different temperatures plotted against the supersaturation ratio. Inspection of Figure 7 in conjunction with Table 2 shows that the growth rates increase sharply with increasing temperature, while supersaturation values show little variation. On the other hand, nucleation rates show the opposite trend. The small variation in supersaturation is not surprising since a constant NaOx concentration was used to initiate crystallisation.

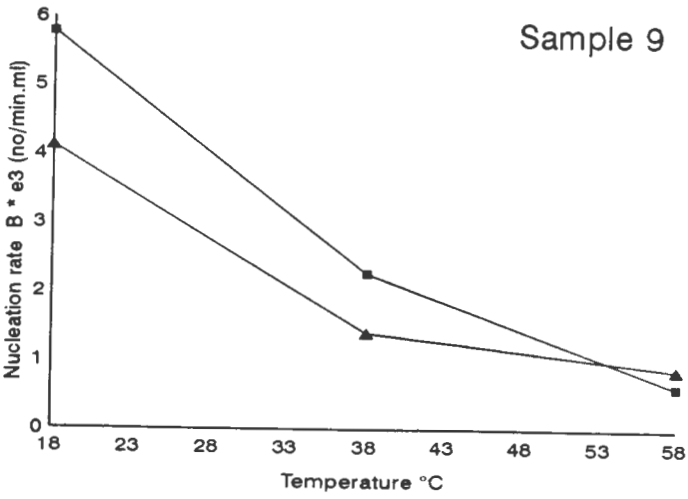
Figure 8 shows the rate of nucleation as a function of the growth rate. All three samples indicate an increase in nucleation rate as the growth rate increased. This observation was substantiated by both the Coulter and Malvern data.

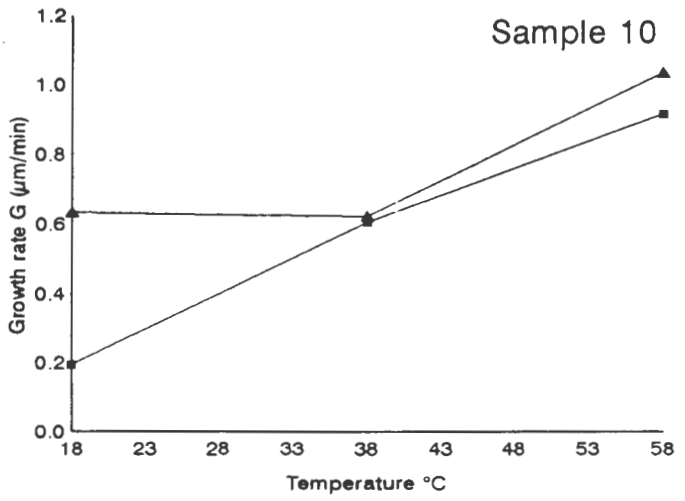
SEM showed little deposition at 18°C. However, COT (in the size range 25-30μm) (Figure 9A) was the predominant phase in all samples; in addition some COD crystals of size 20μm were also present. COM were observed occasionally. An increase in temperature produced a decrease in the size of COT crystals to 20μm, an increase in the number of COD crystals and the formation of COM (Figure 9B). At 58°C a mixture of COM and COD of sizes 10-15μm were observed (Figure 9C). Although an aggregate is shown in Figure 9B, it was not a commonly observed feature.











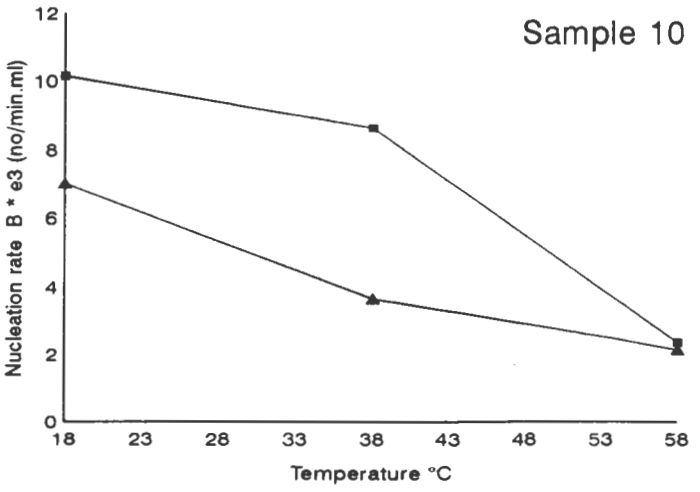
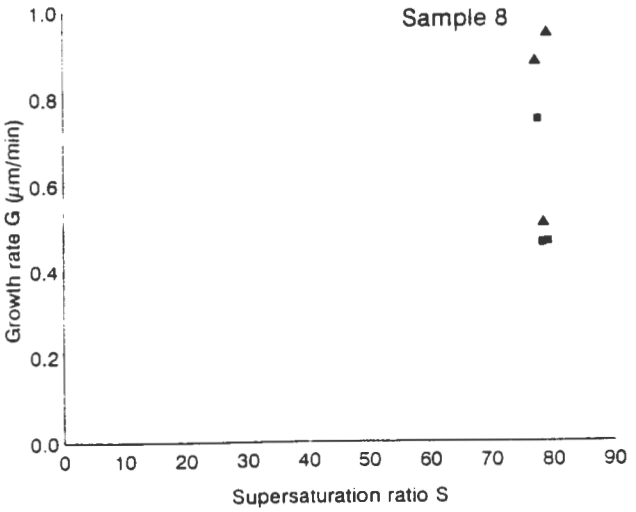
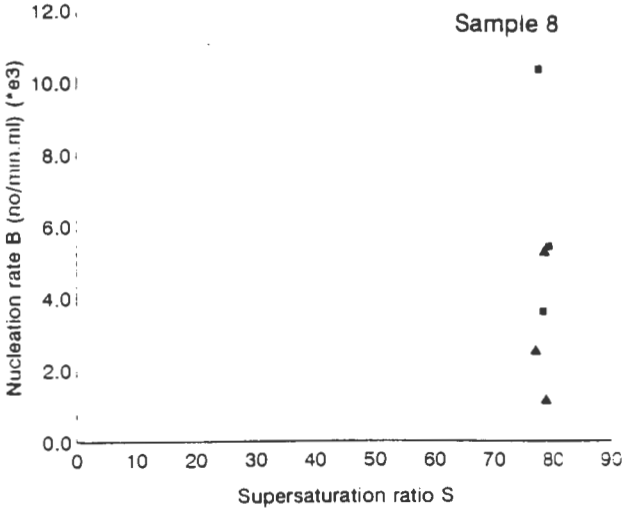
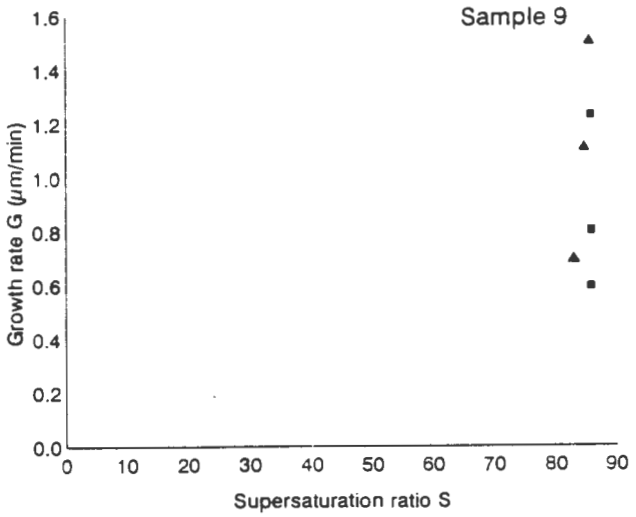


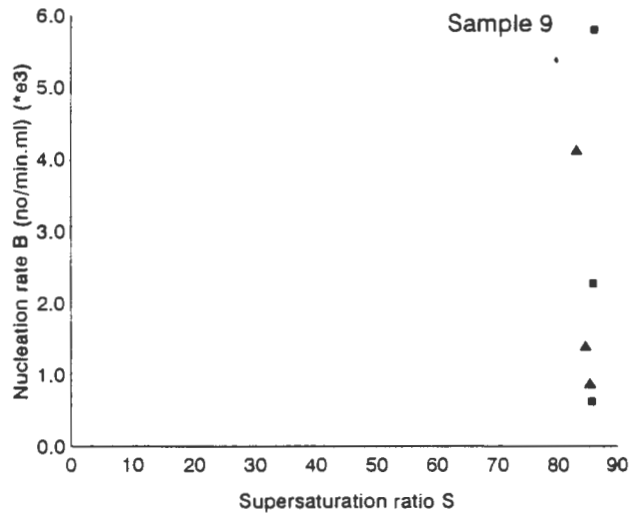
Figure 6: Growth and nucleation rates as a function of temperature.

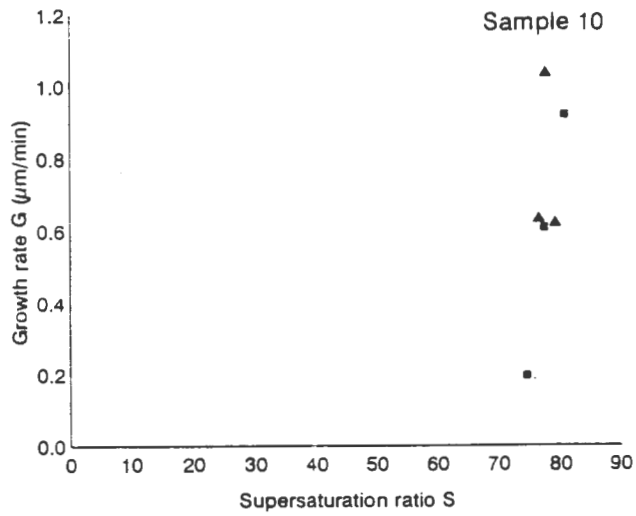
▲ Coulter
■ Malvern











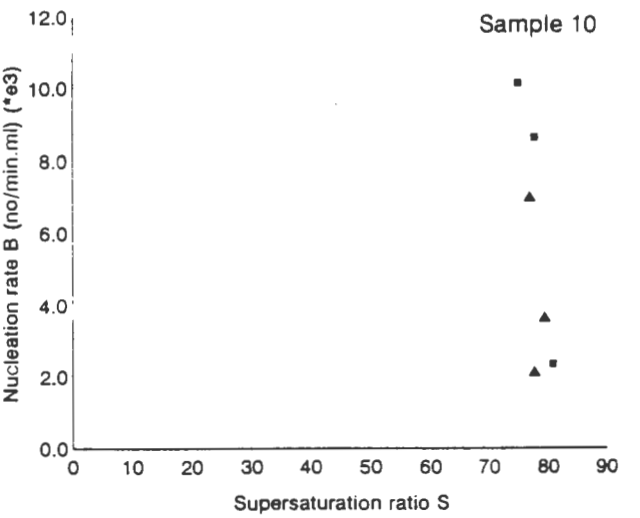


Figure 7: Nucleation rates and growth rate as a function of supersaturation ratio for varying temperatures.

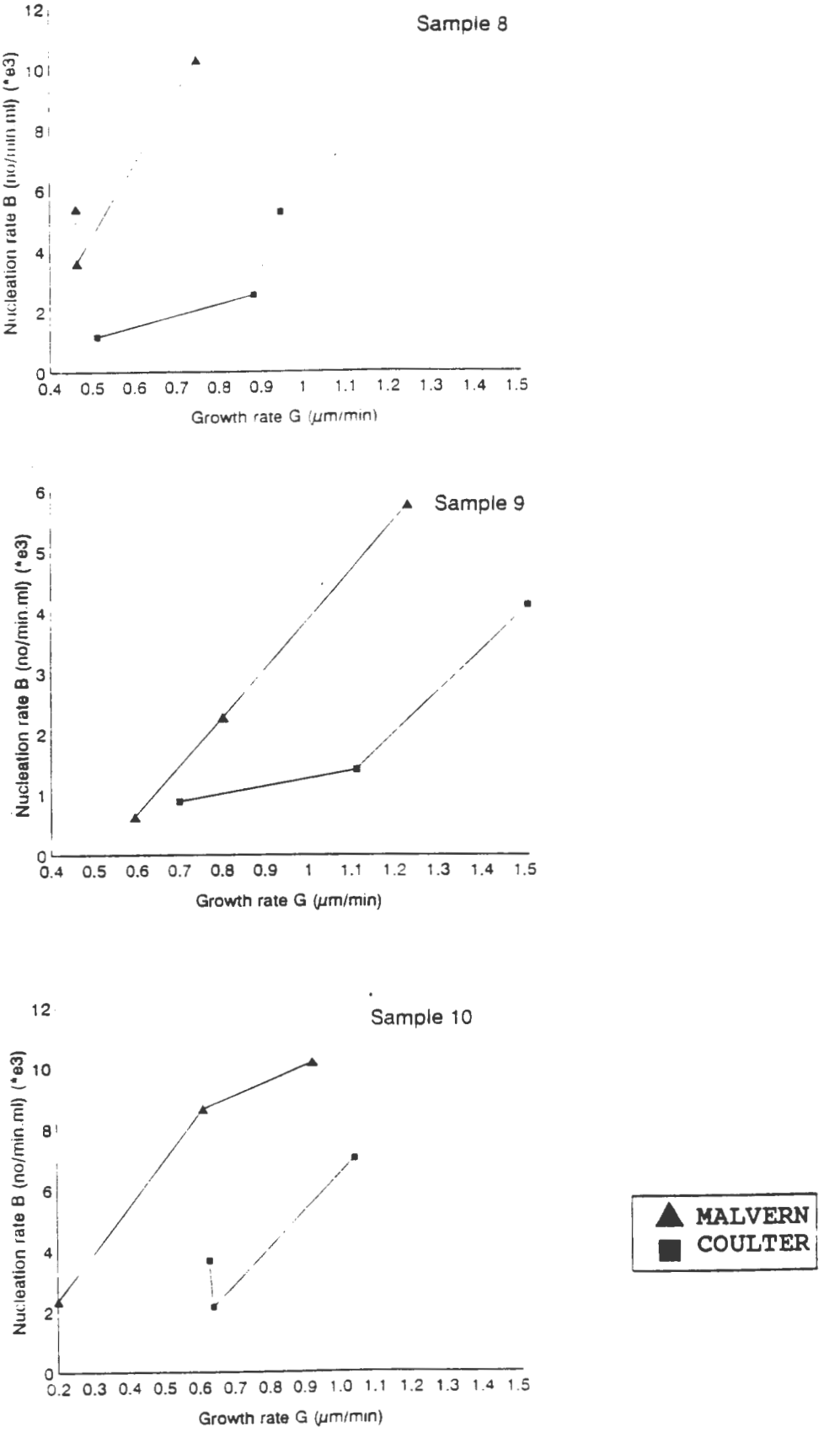
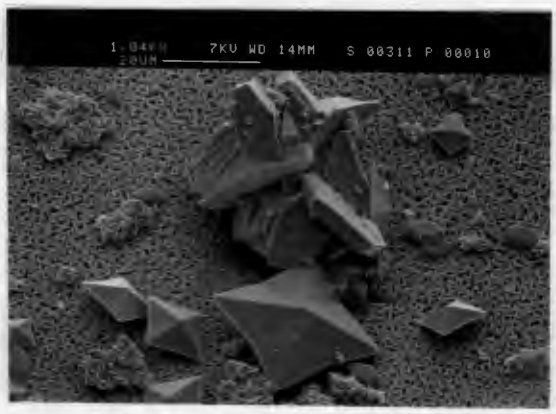


Figure 8: Variation in nucleation rate with growth rate for varying temperatures.

9A: COT and COD and some isolated COM
18°C, mag: 982x



9B: Aggregate of COT with COD and COM's
38°C, mag: 1.04Kx

9C: COM and COD at 58°C
mag: 2.16KX

Figure 9: SEM micrographs showing varying CaOx morphologies at varying temperatures.

Comparison of results obtained in the temperature and oxalate experiments

A comparison of the temperature and oxalate experiments can be made. Temperature experiments were performed using a constant exogenous oxalate concentration of 3mmol/dm^3; therefore the experiment at 38°C can be compared to the oxalate experiment at 37°C in which the NaOx feed concentration was 3mmol/dm^3. From Tables 1 and 2, it is seen that growth and nucleation rates for the two experiments are similar.

A comparison of the change in the growth and nucleation rates as a function of temperature and oxalate concentration can also be made. From Tables 1 and 2, it is apparent that an increase in temperature from 18°C to 58°C (Table 2) has a much greater effect on both the nucleation and growth rates than

that which occurs when the exogenous oxalate concentration inside the crystalliser is increased from 2 to 3mmol/dm^3 (Table 1). The change in the nucleation and growth rates with increasing temperature is more than double that of the change with increasing oxalate concentration.

This difference in rates is also reflected in the large change in both the nucleation and growth rates at the different temperatures which were accompanied by little change in the supersaturation (Table 2 and Appendix 2). In comparison, results for the varying oxalate concentrations show smaller changes in nucleation and growth rates with an accompanying larger change in supersaturation (Table 1 and Appendix 2).

The large difference in rates is also indicated by connsideration of the graphs of nucleation rate vs growth rate (Figures 4 and 8 respectively).

IV.4.3 EFFECT OF VARIOUS INHIBITORS

To further test the efficacy of the entire crystalliser system, a series of experiments involving three common urinary substances regarded as inhibitors, was undertaken. For this purpose, the concentration of NaOx that was used to initiate crystallisation was that which produced the highest measureable precipitation in the untreated sample. The crystallisation response of the urine to this NaOx challenge alone was used as the control for comparison with experiments incorporating the inhibitor. Inhibitors that were tested were citrate, magnesium and chondroitin sulphate A. Separate experiments were also conducted using combinations of citrate and magnesium to investigate their synergistic relationship.

In addition, the effect of introducing these inhibitors into the crystalliser via different routes was investigated. In the first set of such experiments, the pooled urine was dosed with the inhibitor prior to being introduced into the crystalliser. 140ml of various concentrations of an aqueous solution of the inhibitor was added to the stock urine of volume 2800ml. In this method, the flow of the treated urine

was kept at 36ml/min while an aqueous solution of NaOx was introduced at 4ml/min through a different inlet.

In the second set of these experiments, a solution of the inhibitor was introduced into the crystalliser via an independent feed flowing at 2ml/min; the flow rate of the NaOx was adjusted to 2ml/min while that of the urine (via a third inlet) was maintained at 36ml/min. In order that the final concentration of oxalate inside the crystalliser be equivalent to that used in the first set of experiments, the concentration of the NaOx feed solution was doubled.

Effect of citrate

Inhibitor dosing of pooled urine prior to introduction into the crystalliser

Urines were dosed with citrate concentrations usually occurring in normal urine. These were in the range 1-2 mmol/dm^3 [Berg and Tiselius 1989]. The ensuing growth and nucleation rates are given in Table 3.

		GROWTH μm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	[Citrate] mmol/dm^3	Coulter	Malvern	Coulter	Malvern
11	0	0.9517	0.7682	4.291	3.248
	1.020	0.9010	0.6887	1.216 *	2.226
	1.275	0.8664	0.6613	2.264 *	3.564
	1.530	0.5529	0.7250	8.332	5.863
12	0	0.7306	0.6836	3.602	5.797
	1.020	0.5882 *	0.6350 *	3.685	3.255
	1.275	0.5973 *	0.6051 *	2.081	2.083
	1.530	0.5473	0.5841	3.158	2.997
13	0	0.8446	0.7207	5.403	8.736
	1.020	0.7921 *	0.6886	3.596 *	4.991
	1.275	0.7116 *	0.6793	2.517 *	3.241
	1.530	0.6949	0.6650	5.130	5.656

Table 3: CaOx growth and nucleation rates in the presence of various exogenous concentrations of citrate. The concentration of the feed NaOx was kept constant at 3mmol/dm^3 inside the crystalliser. (* denotes significant difference $p < 0.05$).

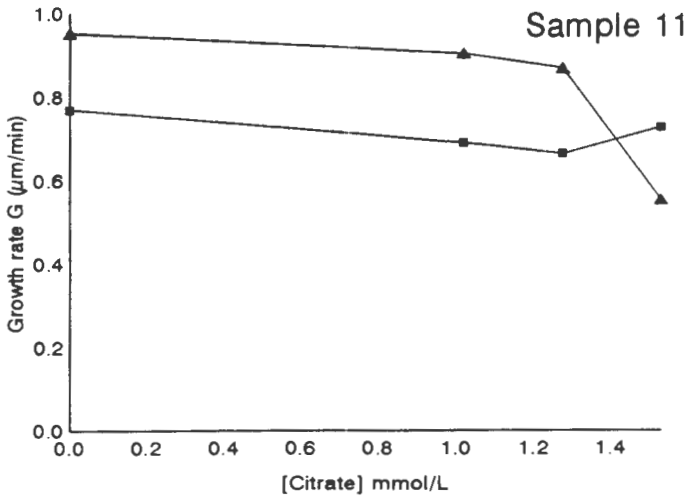
Concentrations shown in Table 3 indicate the final concentration of exogenous citrate in the crystalliser.

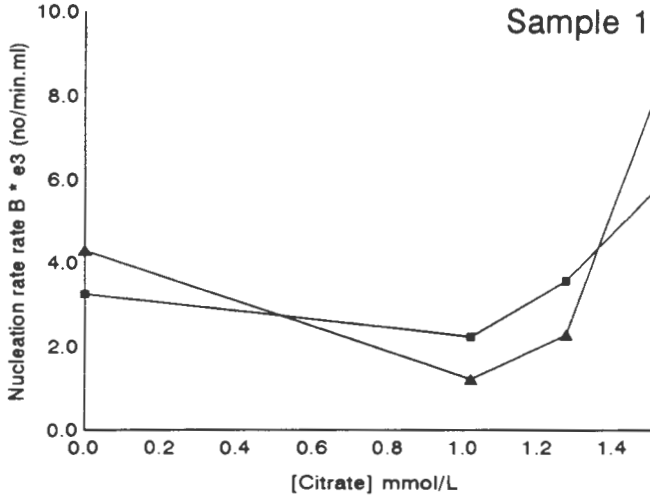
Growth and nucleation rates are plotted as a function of citrate concentration in Figure 10. Results obtained from both the Malvern and Coulter instruments show that with increasing concentrations of citrate, growth decreases. On the other hand, nucleation rates show a general decrease up to a citrate concentration of 1 to 1.2mmol/dm^3 after which nucleation increases.

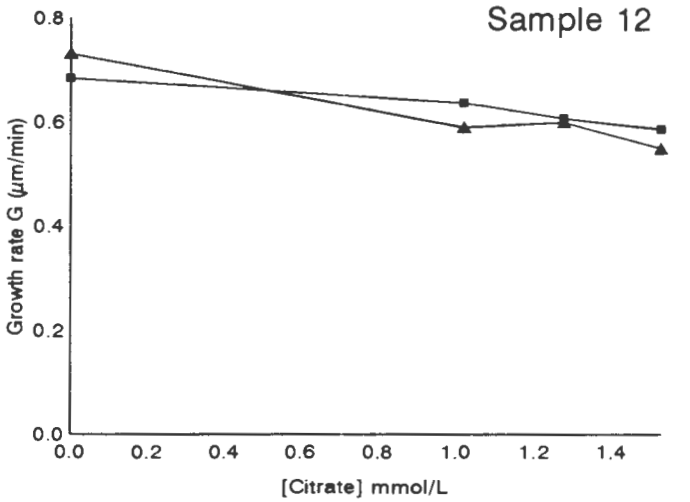
Growth rates were found to increase with increasing supersaturation ratio as indicated in Figure 11. However, the trend in nucleation rates is not clear.

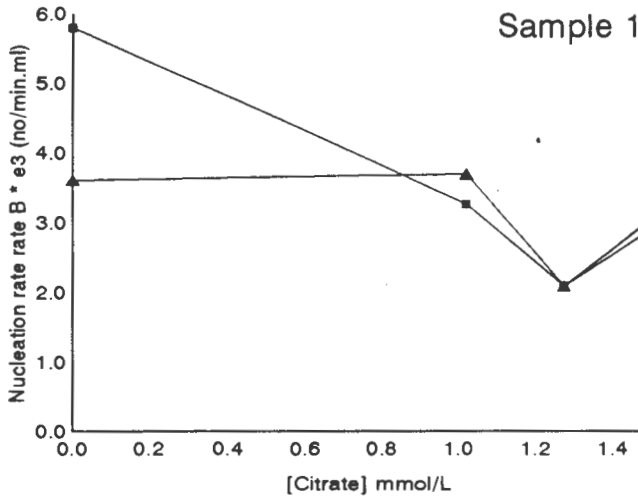
The relationship between nucleation and growth rates is shown in Figure 12. No trend for the Coulter or the Malvern is observable for the three samples.

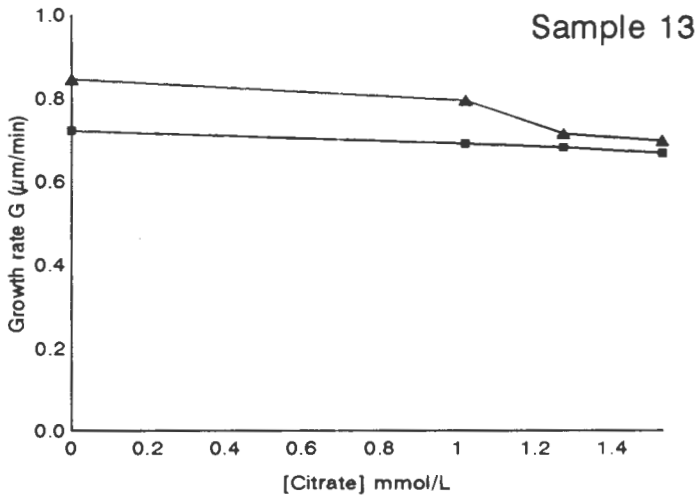
SEM confirmed the trends in growth rates as a function of citrate concentration. This manifested itself by a decrease in the average size of the COD crystals (most common morphology) from 20-10μm in all samples (Figure 13). A few large COT were noted in some samples, but they were not typical. Increasing the citrate concentration led to an increase in the number and size (up to 10μm) of COM crystals (Figure 13B). In addition, the total number of crystals appeared to decrease with increasing citrate concentration (Figure 13). Aggregation was not typical in any of the samples analysed.











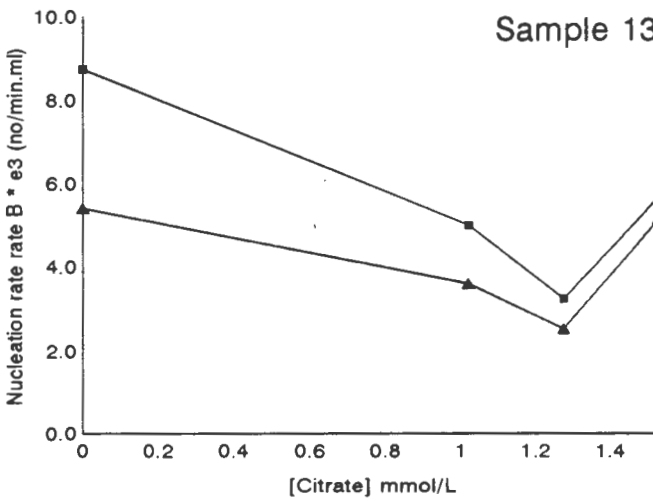
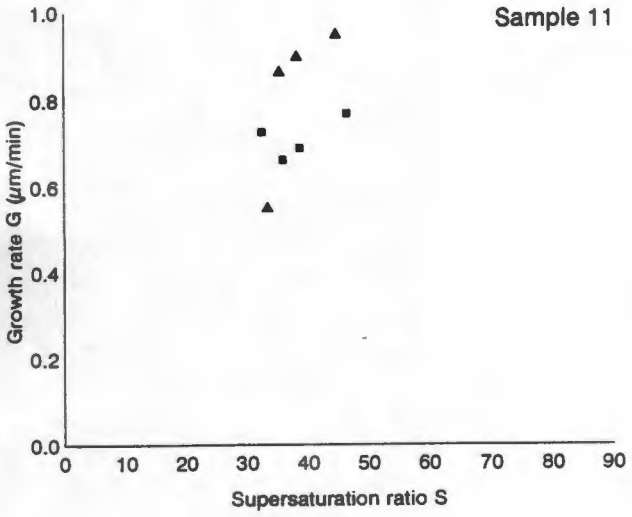
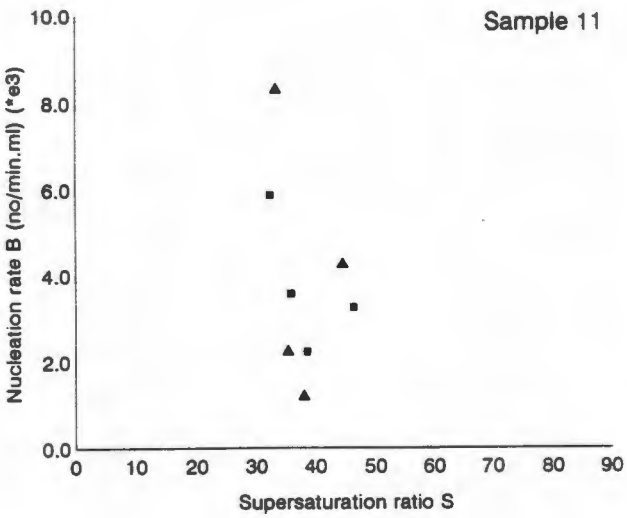
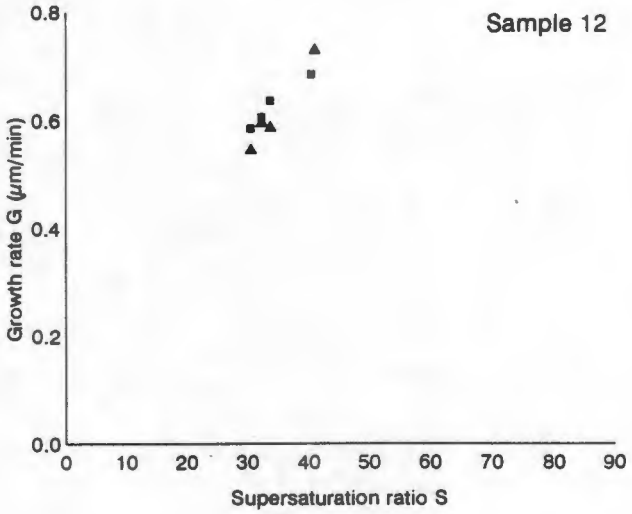


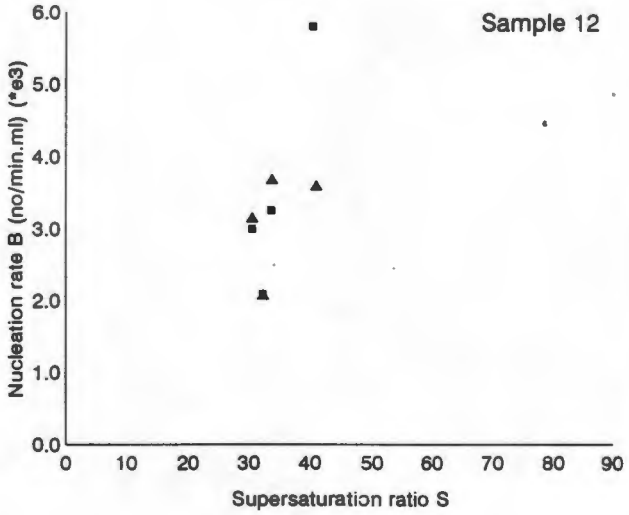
Figure 10: Growth and nucleation rates as a function of citrate concentration in the urine pool.

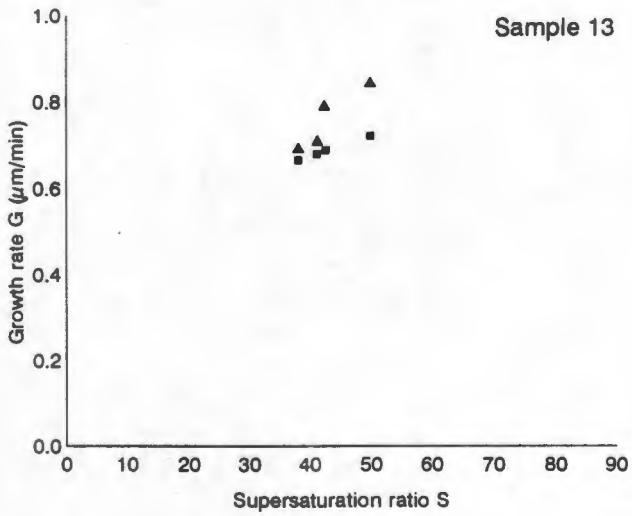
▲ Coulter
■ Malvern











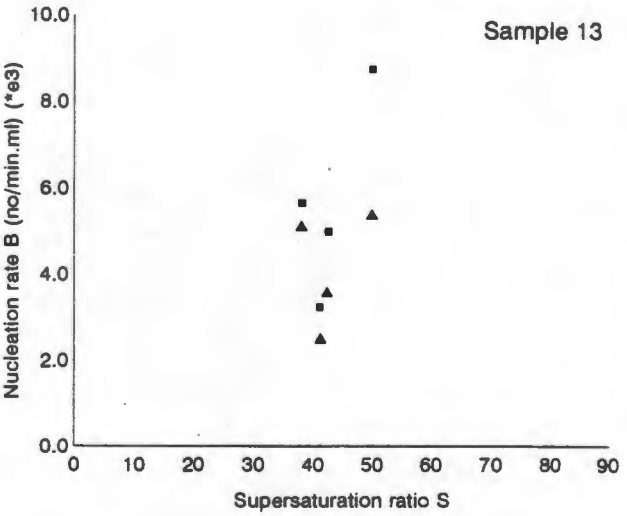
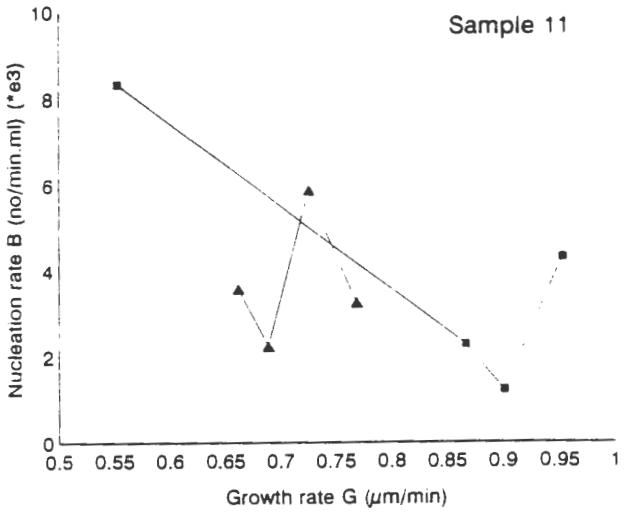
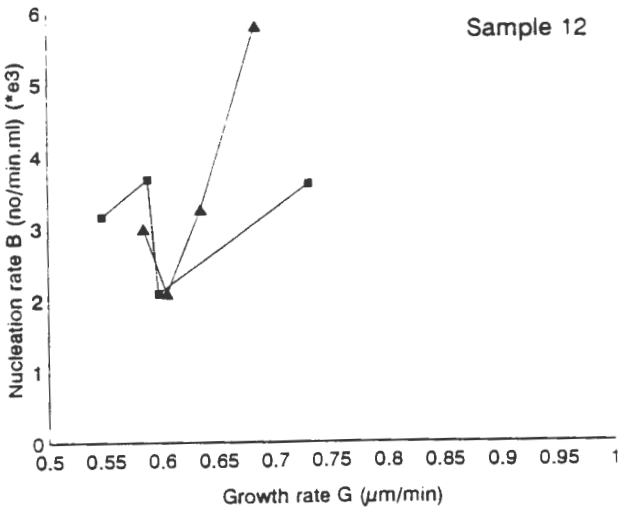


Figure 11: Growth and nucleation rates as a function of supersaturation ratio S for varying concentrations of citrate added to the urine pool.

▲ Coulter
■ Malvern





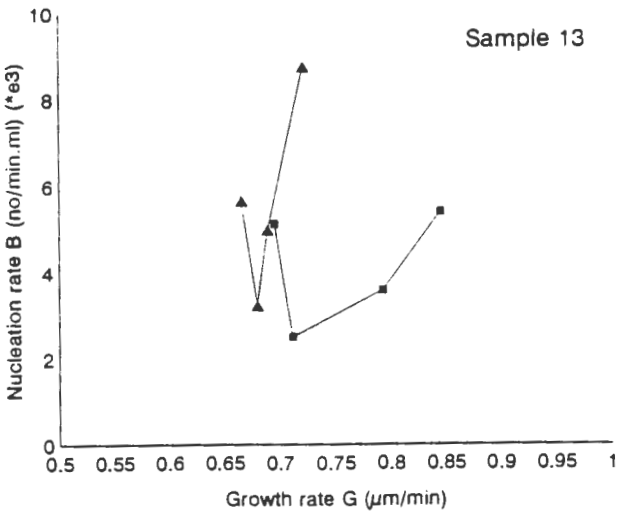
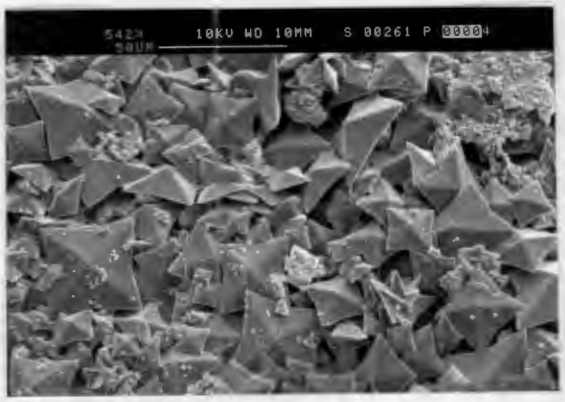
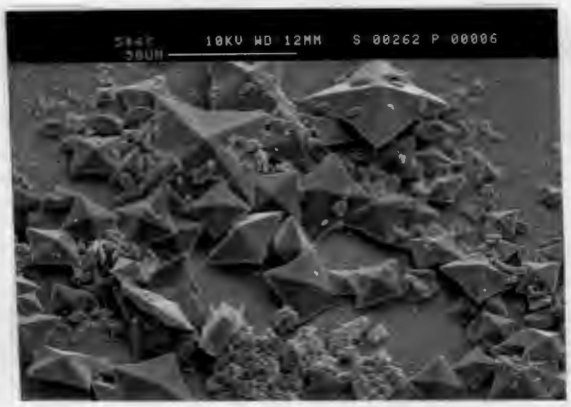


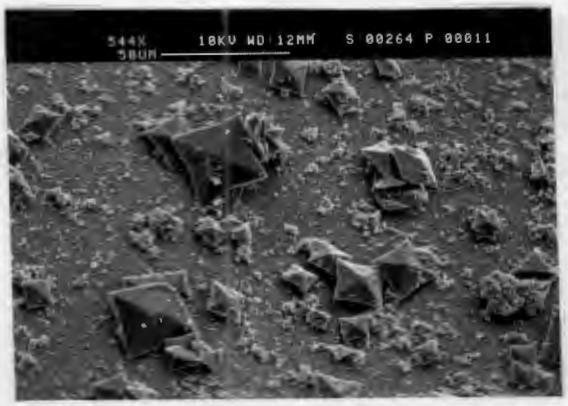
Figure 12: Nucleation rate as a function of growth rate for varying concentrations of citrate added to the urine pool.



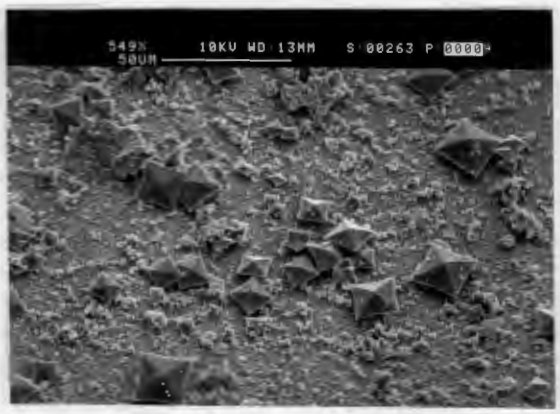
25A: [Mg] = 1.927mmol/dm^3
mag: 542x
max crystal size = 35μm



25B: [Mg] = 3.850mmol/dm^3
mag: 544x
large COD at 43μm

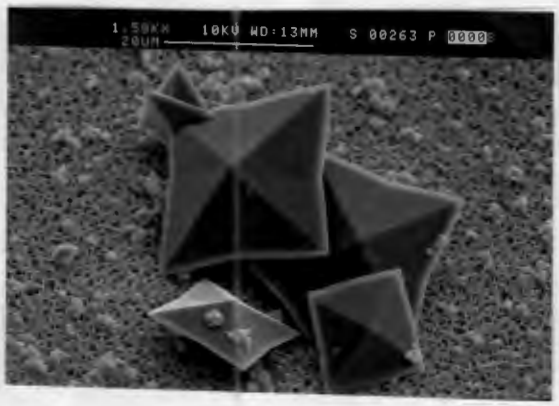


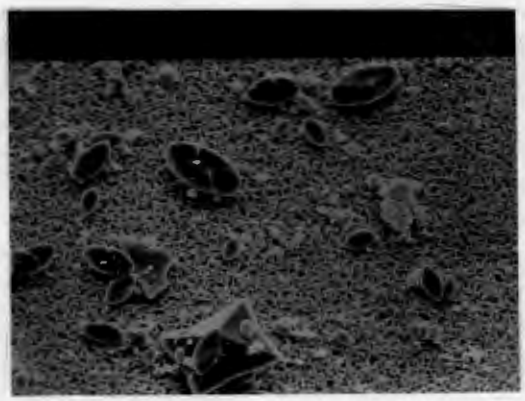
25C: [Mg] = 5.781mmol/dm^3
mag: 544x
max crystal size = 28μm



25D: [Mg] = 7.708mmol/dm^3
mag: 549x
max crystal size = 15μm

Figure 25: Survey photographs at similar magnifications of sample 26 showing the effect of varying magnesium concentrations.





13A: no citrate
mag:1.58Kx
ave COD crystal size = 20μm

13B: [citrate]=1.530mmol/dm^3
mag:1.32Kx
ave COD crystal size = 10μm

Figure 13: SEM micrographs of CaOx deposition in the absence and presence of citrate.

Inhibitor dosing via a separate feed into the crystalliser

Table 4 indicates the growth and nucleation rates which were determined as a function of the concentration of citrate, the latter being admitted to the crystalliser via a separate feed.

		GROWTH μm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	[Citrate] mmol/dm^3	Coulter	Malvern	Coulter	Malvern
23	1.071	0.8586	0.7479	2.328	2.065
	1.785	0.7850	0.7429	1.884	2.031
	3.570	0.7890	0.4439	1.884	1.928
	7.140	0.7512	0.6364	0.1370	0.1037
24 †	0.8925	0.7626	0.7431	2.259	4.942
	1.071	0.6653	0.5901	3.892	4.696
	1.785	0.6659	0.6122	1.355	2.080
	3.570	0.6500	0.5901	1.514	1.416
25	1.071	0.7114	0.6910	4.053	5.304
	1.785	0.6978	0.6855	2.000	1.861
	3.570	0.5472	0.5760	3.029	3.627
	7.140	0.9229	0.5127	0.05146	0.3371

Table 4: CaOx growth and nucleation rates in the presence of various citrate concentrations introduced into the crystalliser via a separate feed. Exogenous NaOx concentration inside the crystalliser was kept constant at 2.5mmol/dm^3. (* denotes significant difference $p < 0.05$).

† citrate concentrations in this sample were different from those in 23 and 25 as the composition of specimen 24 was significantly different

Concentrations shown in Table 4 indicate the final concentration of exogenous citrate in the crystalliser.

Figure 14 shows a graphical representation of CaOx growth and nucleation rates as a function of citrate concentration. Growth rates demonstrate an initial decrease at low citrate concentrations, but then tend to level off (exceptions: sample 23, Malvern data; sample 25, Coulter data).

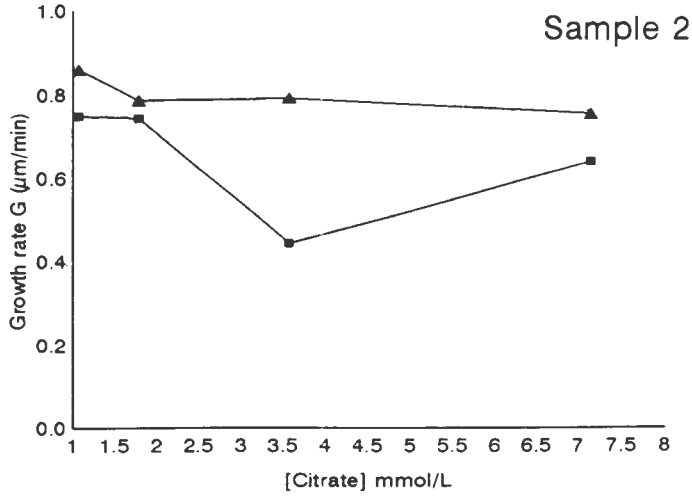
In two cases (samples 23 and 25) the two instruments gave contradictory results at higher citrate concentrations. With respect to nucleation rates, both instruments showed a general decrease with increasing citrate concentrations (Figure 14).

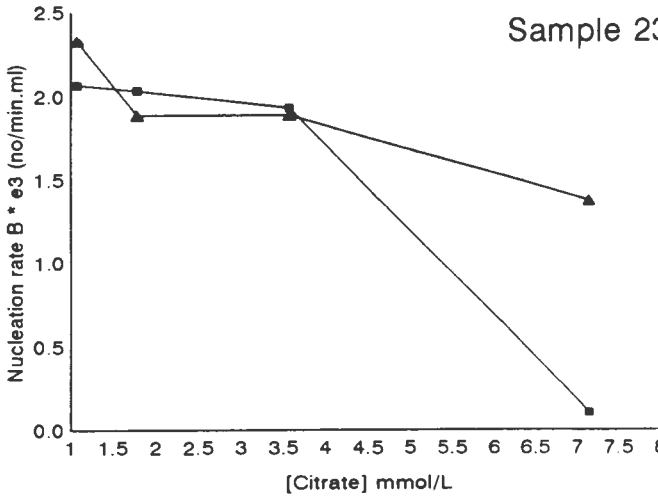
Figure 15 indicates that both the growth and nucleation rates increase with increasing supersaturation ratio for both instruments. The change in growth rates is however much smaller than the change in nucleation rates with increasing supersaturation. Individual values for the Coulter and Malvern are in good agreement.

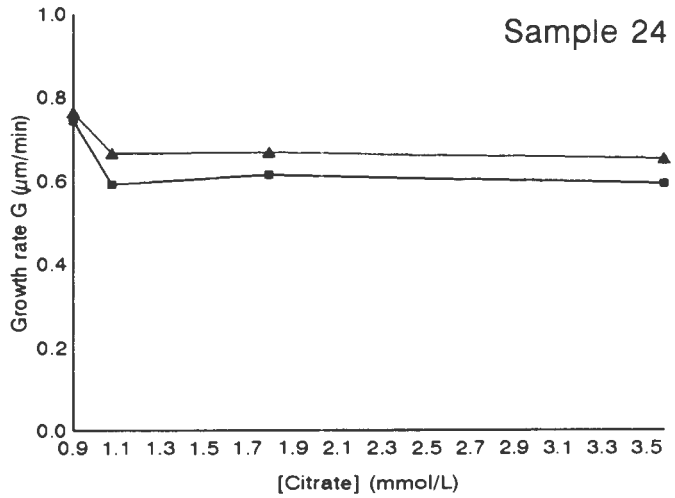
The variation in nucleation rates with growth rates is indicated in Figure 16; no trend is obvious.

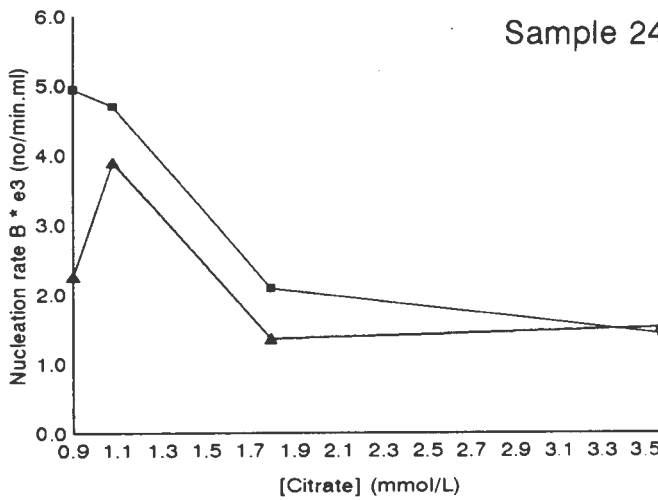
SEM of samples 23 and 24 revealed decreases in the number of both COM and COD crystals as the citrate concentration increased (Figure 17). A decrease in the size of these crystals was noted in the physiological range of citrate concentrations (Figure 17A,B); however, little change in the size of COM was seen above this range (Figure 17C,D) (note the magnification of Figure 17C is 776x). Aggregation was not noted in these samples and intergrowth was not typical at any citrate concentration. The morphology of the COD in the presence of citrate was not in its common octahedral form and is shown in Figure 17E.

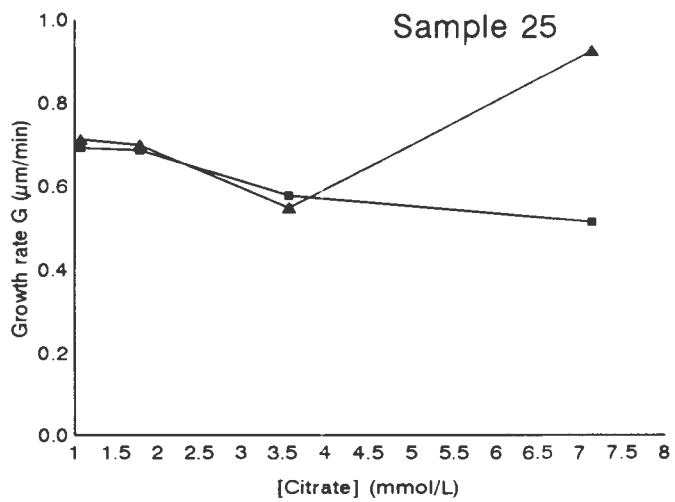
Although COD (25μm) and aggregates thereof and also COM (20μm) were observed in sample 25 at the lowest citrate concentration, they were absent at the highest citrate concentration. At this latter concentration, only tiny crystals of COM and COD 1-2μm were identified; aggregates (up to 10μm in size) of these small crystals were occasionally observed.











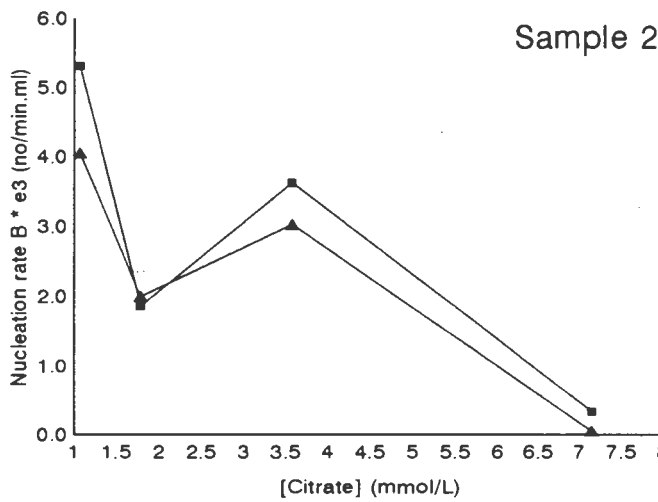
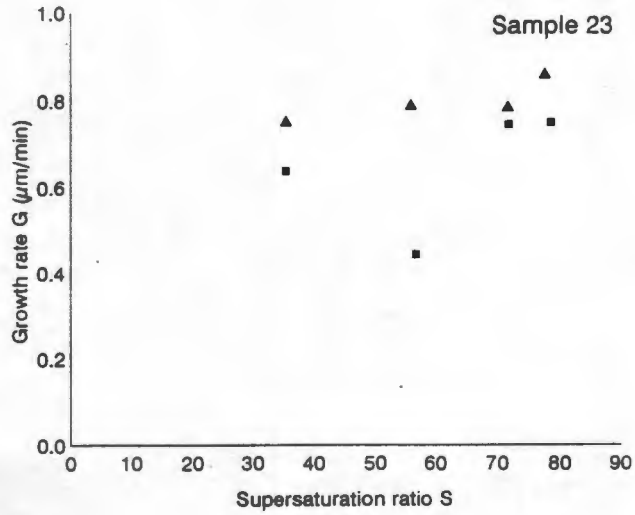
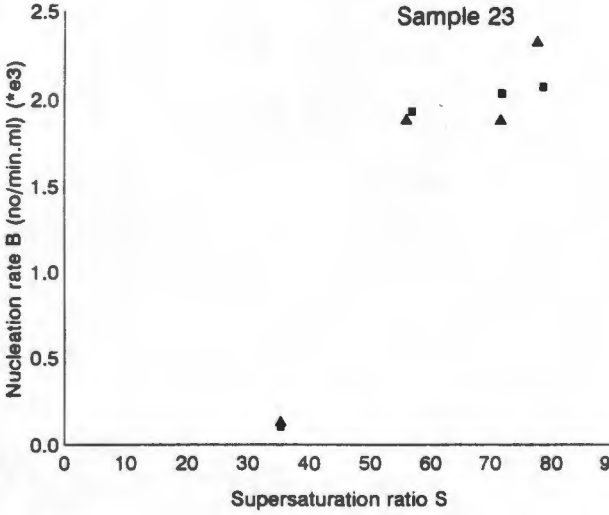
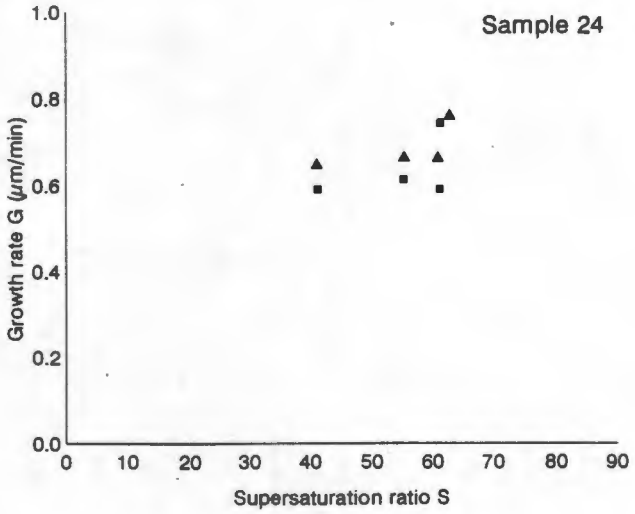
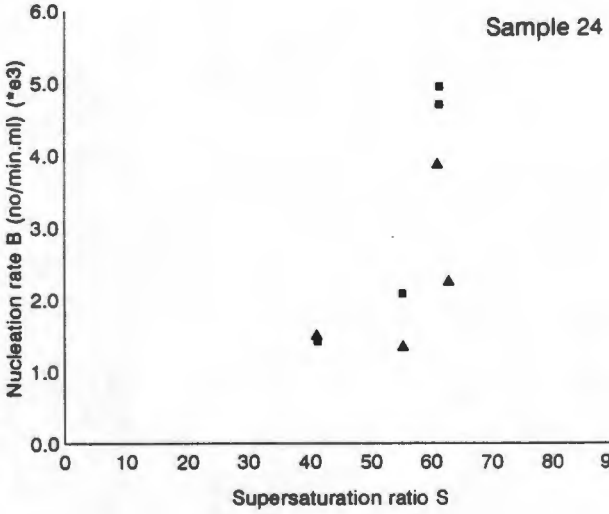


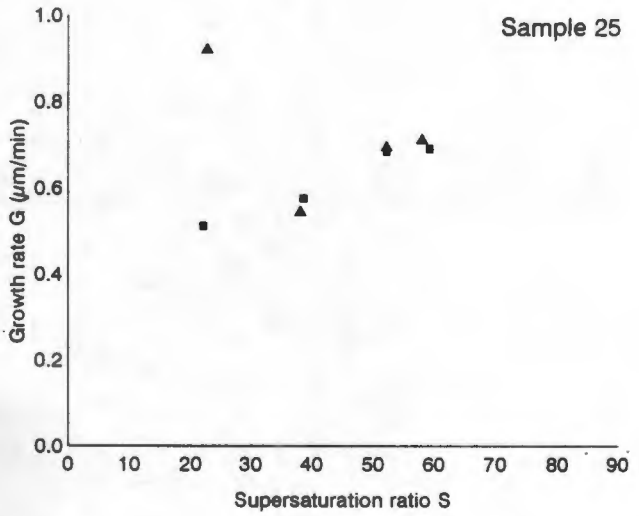
Figure 14: Growth and nucleation rates as a function of citrate when added into the crystalliser separately.











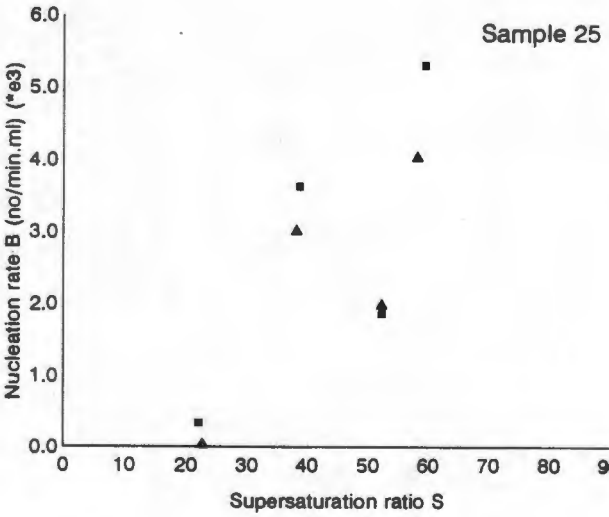


Figure 15: Growth and nucleation rates as a function of supersaturation ratio S for varying concentrations of citrate added to the crystalliser via a separate feed.

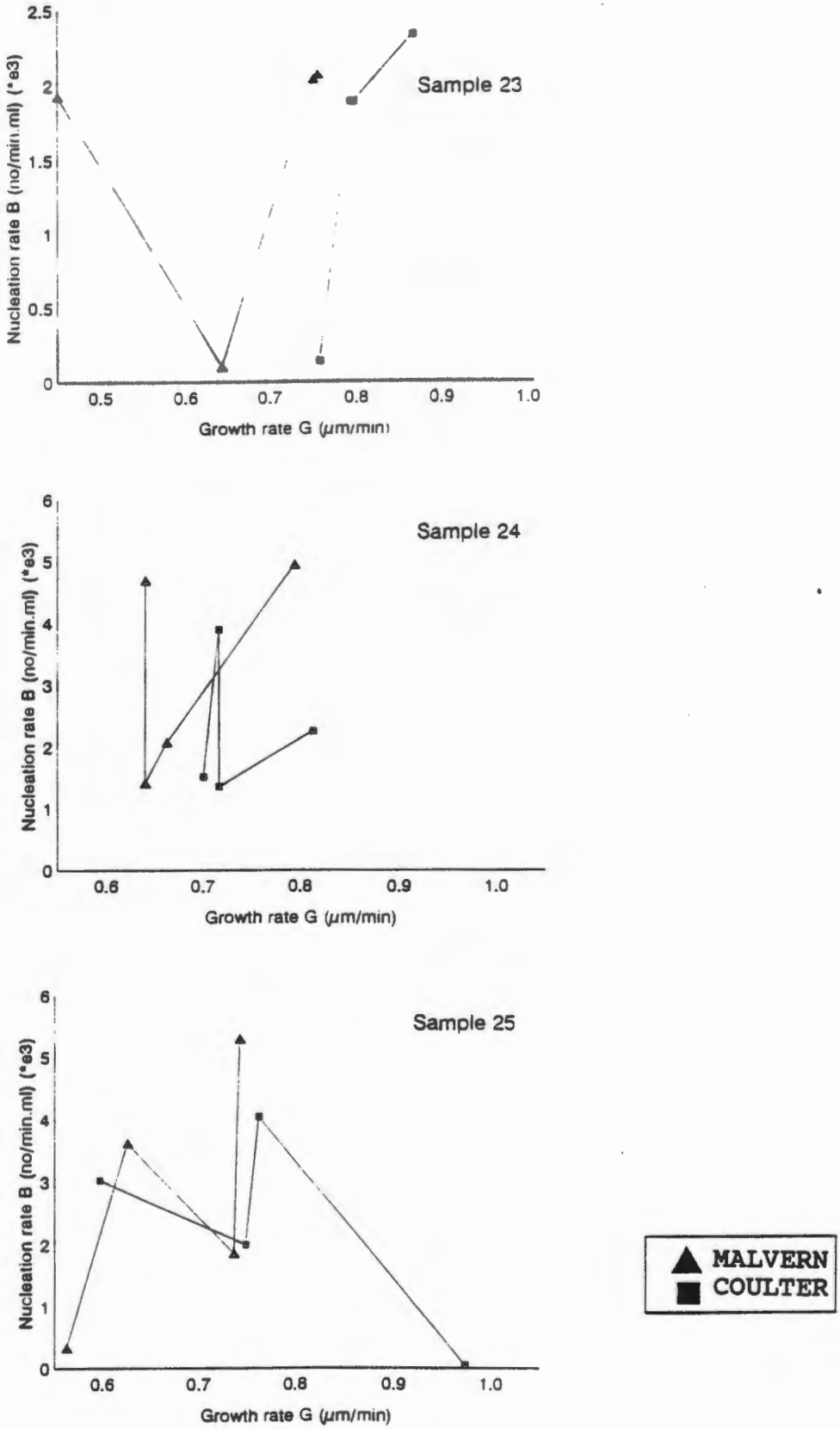
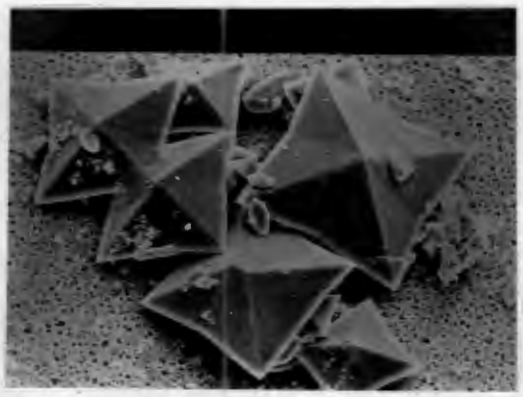
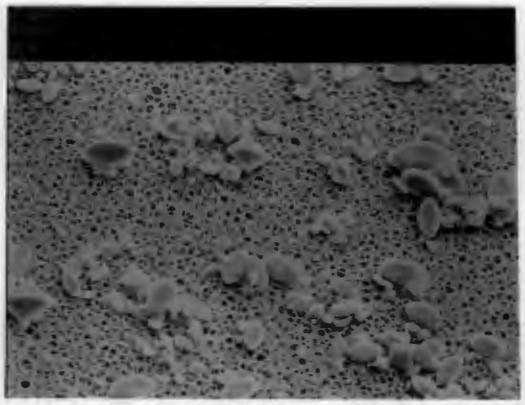


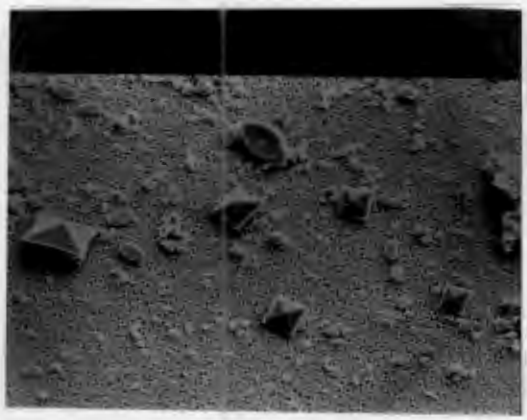
Figure 16: Nucleation rate as a function of growth rate for varying concentrations of citrate admitted to the crystalliser via a separate feed.



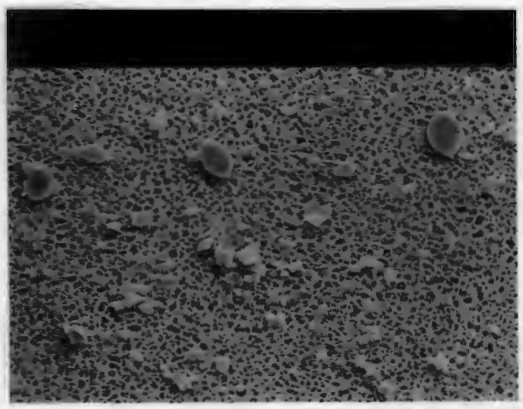
17A: [cit] = 1.071mmol/dm^3
mag: 1.41Kx
max COD size = 28μm



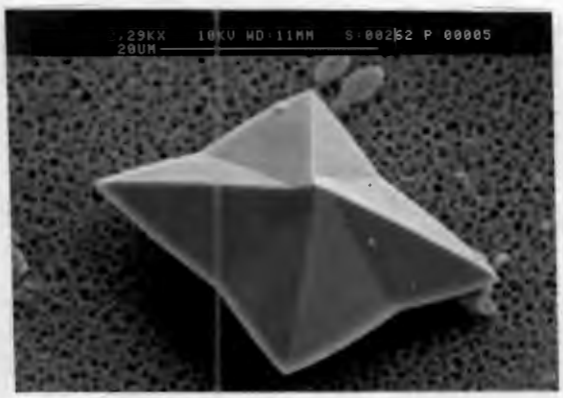
17B: [cit]= 1.785mmol/dm^3
mag: 1.55Kx
max size COD,COM = 10μm



17C: [cit] = 3.570mmol/dm^3
mag: 776x
max size COM,COD = 10μm



17D: [cit] = 7.140mmol/dm^3
mag: 1.36Kx
max size COM = 7μm



17E: Typical COD morphology in the presence of citrate.

Figure 17: SEM micrographs of CaOx deposition in the presence of varying citrate concentrations.

Comparison of the two citrate experiments

A comparison of the general trends in growth and nucleation rates for the experiments in which citrate was introduced into the test urine via different routes can be made. In making this comparison, it is noted that citrate concentrations in experiment 2 (where citrate was introduced into the crystalliser via a separate feed) overlapped those of experiment 1 (where citrate was added to the urine pool) and also included levels above the normal physiological range.

Growth and nucleation rates decreased with increasing citrate concentration in both experiments to a similar extent. Increasingly high concentrations of citrate in experiment 2 continued to show the decreasing trend in nucleation rates observed in experiment 1.

The trend of increasing growth rate with increasing supersaturation is observed in both experiments with experiment 2 having a larger range of supersaturation due to the larger range of citrate concentrations used. The range of growth rates for each experiment is comparable. Other comparisons are not possible because no obvious trends were observed.

Effect of magnesium

Inhibitor dosing of pooled urine prior to introduction into the crystalliser

Physiological concentrations of magnesium (1-10mmol/dm^3) [Takasaki 1972] were used in experiments to investigate its effect on CaOx crystallisation (Hesse et al. (1986) quote 1-5mmol/dm^3 as the physiological range for magnesium). Table 5 presents the values of growth and nucleation rates of CaOx in the presence of various magnesium concentrations. The concentrations shown indicate the final concentration of exogenous magnesium in the crystalliser.

		GROWTH μm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	[Magnesium] mmol/dm^3	Coulter	Malvern	Coulter	Malvern
14	0	0.9114	0.7682	1.526	2.087
	2.478	0.8944	0.7675	1.280	2.097
	3.304	0.8776	0.6120	2.226	1.829
	4.955	0.7757	0.6541	4.326	5.823
15	0	0.6717	0.7789	2.783	0.6666
	2.478	0.6961	0.7201	1.339	1.014
	3.304	0.6413	0.6988	1.209	1.974
	4.130	0.6127	0.6854	1.197	2.548
16	0	0.8345	0.8521	4.946	2.906
	2.478	0.8241	0.8338	2.037	1.989
	3.304	0.8185	0.8053	2.883	5.314
	6.607	0.5203	0.6802	8.085	4.750

Table 5: CaOx growth and nucleation rates in the presence of various exogenous concentrations of magnesium. The concentration of the feed NaOx was kept constant at 2.5mmol/dm^3 inside the crystalliser. (* denotes significant difference $p < 0.05$).

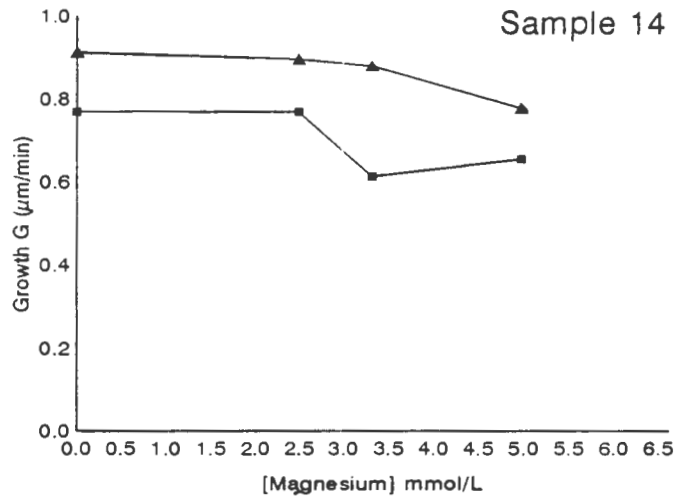
Growth and nucleation rates are plotted against magnesium concentrations in Figure 18. The trend of decreasing growth rate with increasing magnesium concentration is indicated by both the Coulter and Malvern in all three samples. Nucleation rates in samples 14 and 16 decrease initially but then increase at higher magnesium concentrations. Sample 15 shows inconsistent results for the Malvern. Overall, the variation in nucleation rate is larger than the variation in the growth rate as a function of increasing magnesium concentration.

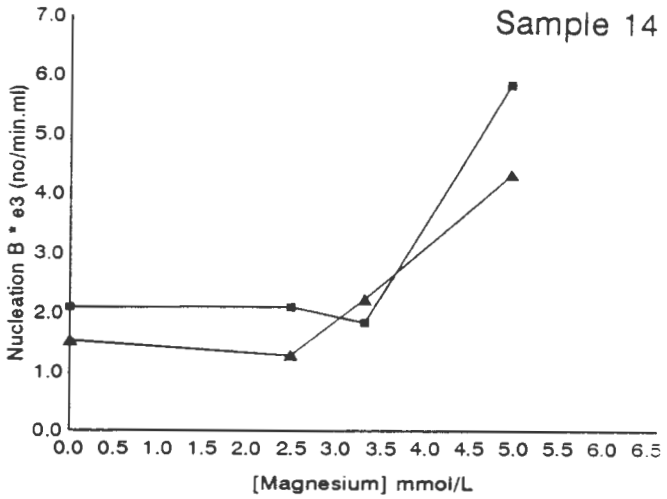
Growth and nucleation rates are plotted against supersaturation ratios for each sample in Figure 19. All samples indicate an increase in growth rates with an increase in the supersaturation ratio, with samples 14 and 16 showing little variation above a supersaturation of 60. With respect to the trends in nucleation with supersaturation, sample 15

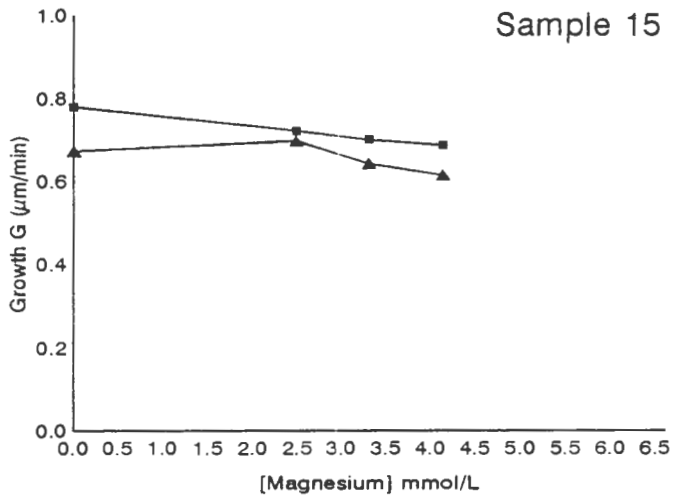
shows conflicting trends for the Coulter and Malvern. On the other hand, samples 14 and 16 indicate a decrease in nucleation above a supersaturation ratio of 47.

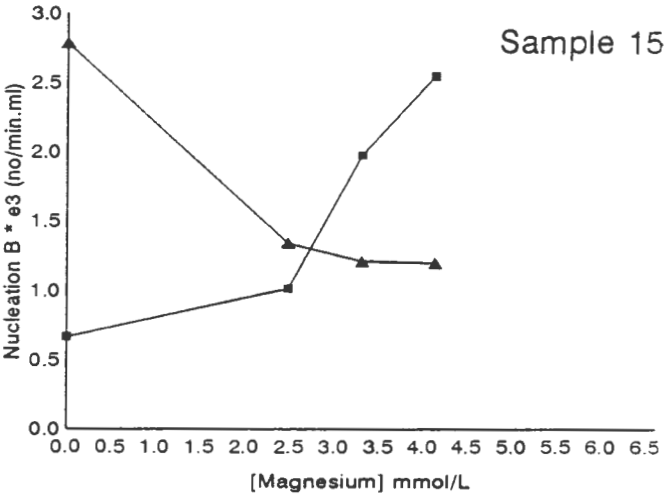
Plots of nucleation rate vs growth rate are given in Figure 20; no trends were apparent.

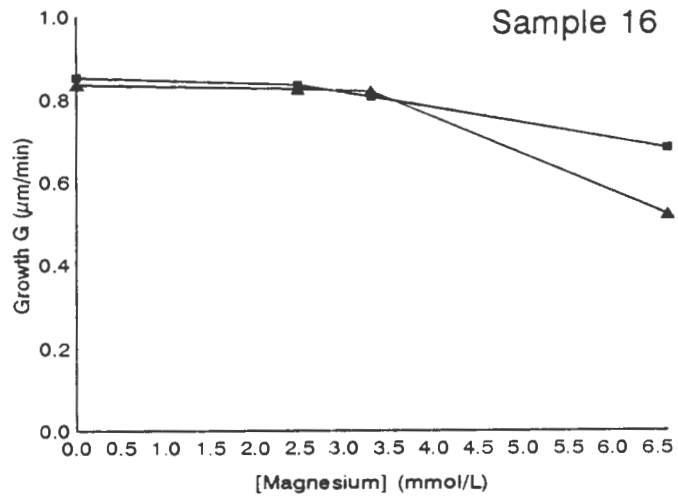
SEM micrographs of sample 16 at various magnesium concentrations (0 - 6.607mmol/dm^3) are shown in Figure 21(A-D). Observations for sample 16 are typical of all the samples. Figure 21A,B at the lower magnesium concentrations show mostly large COD and COM crystals of sizes in excess of 20μm. Figure 21C,D at higher magnesium concentrations reveal an increase in the number of tiny crystals, thereby confirming the increase in the nucleation rate with increasing magnesium concentration (depicted by the graphs in Figure 18). These crystals were commonly COM of size less than 5μm (Figure 21C,D,E). Sample 16 showed a reduction in the number of crystals in the size range of 20-30μm as the concentration of magnesium increased (Figures 21A-D) confirming the kinetic observation of lower growth rates with increasing magnesium concentration. In general, the morphology of the COD was the same as that observed in the presence of citrate (Figure 17E). Qualitatively, COM was as common as COD with the former in the size range 5-10μm. In general, the number of crystals appeared to decrease as the concentration of magnesium increased. Aggregates (20μm) were only noted in all samples in which magnesium was absent (Figure 21A) suggesting that magnesium may be an inhibitor of CaOx aggregation.











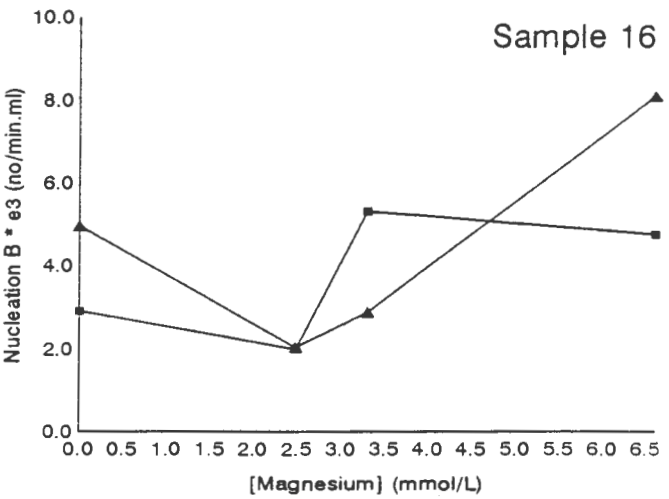
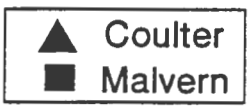
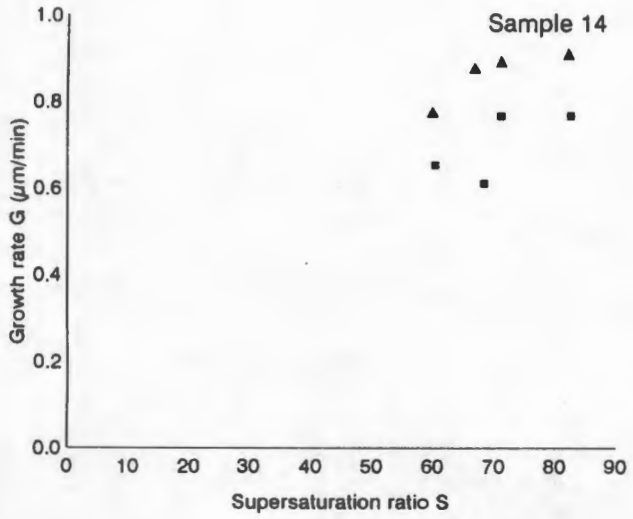
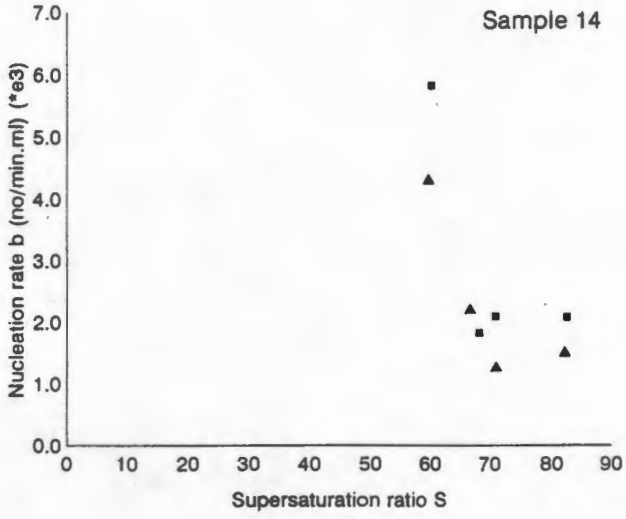
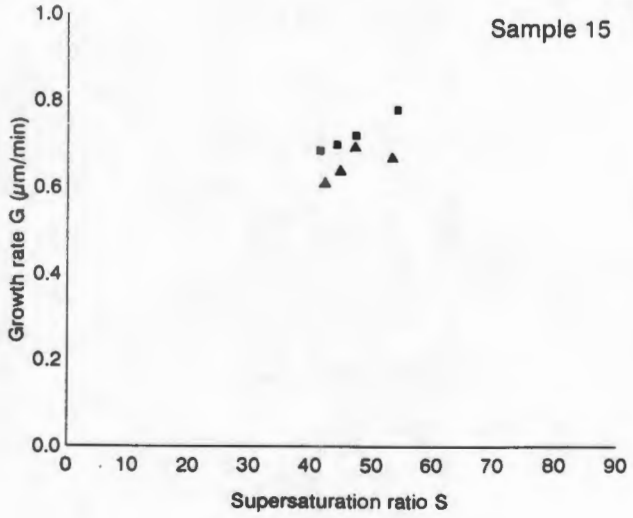


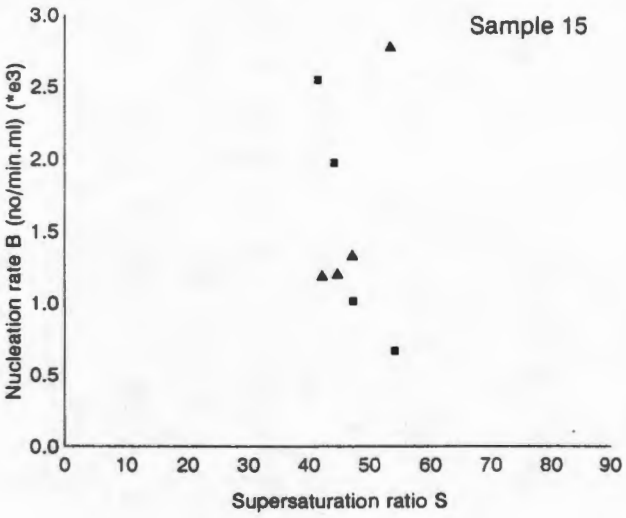
Figure 18: Plots of growth and nucleation for varying magnesium concentrations added to the urine pool.

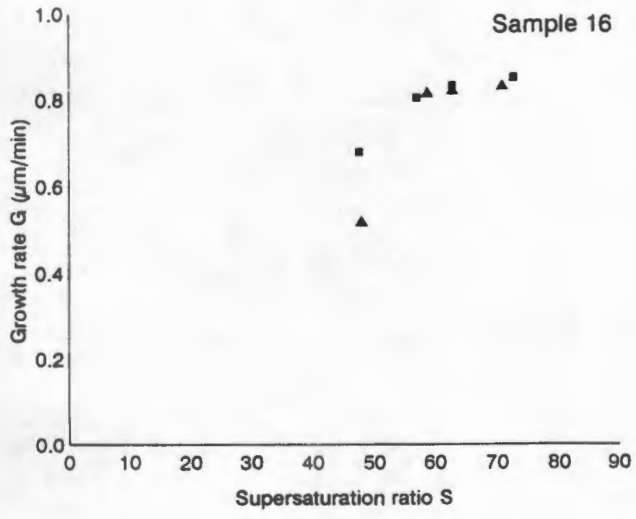












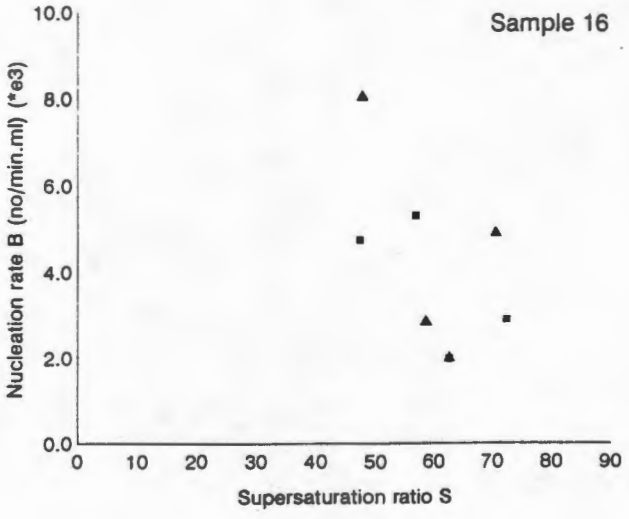
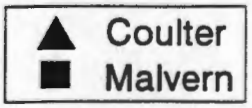


Figure 19: Nucleation and growth rates as a function of supersaturation ratio S for varying concentrations of magnesium added to the urine pool.



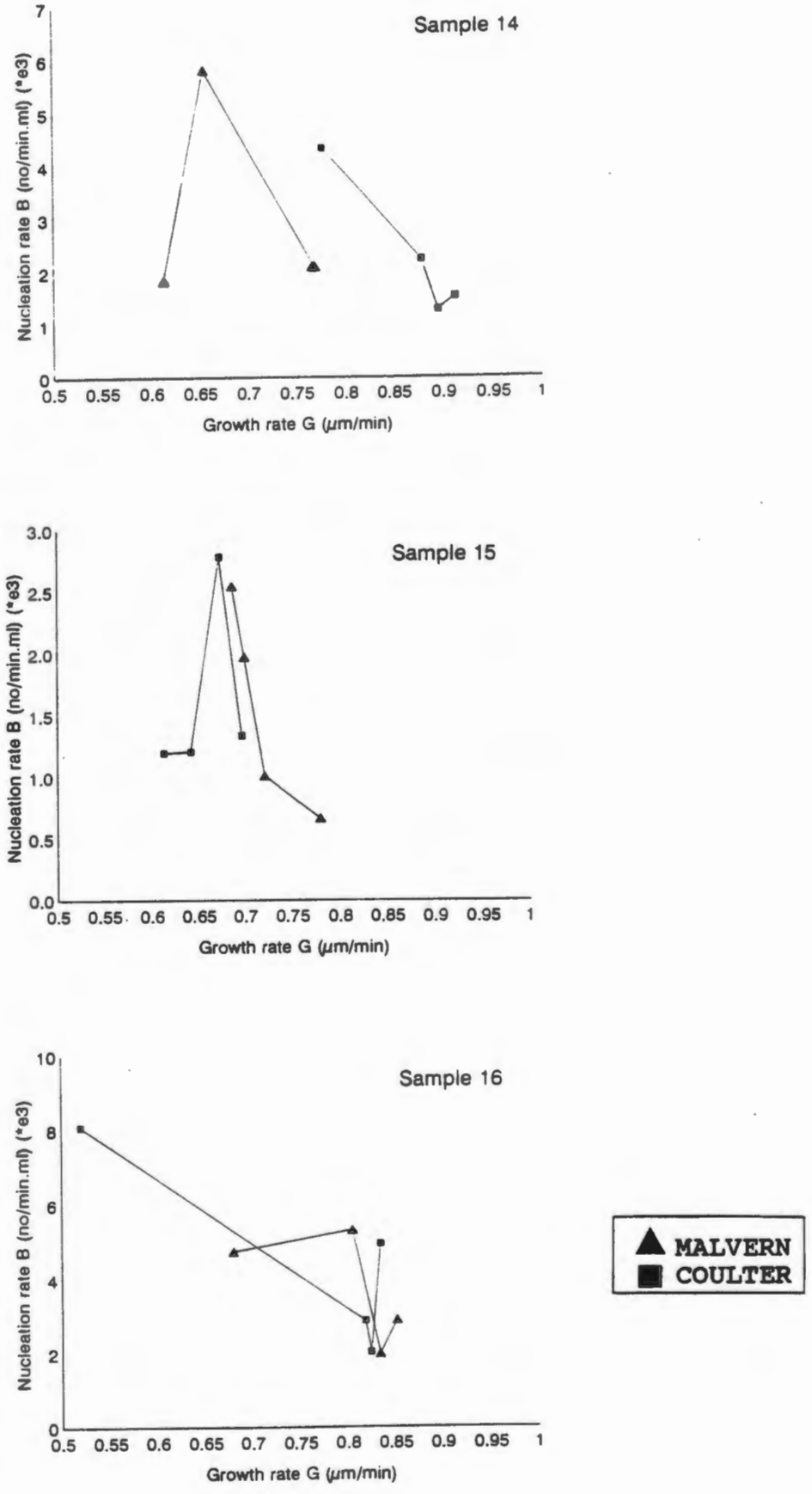
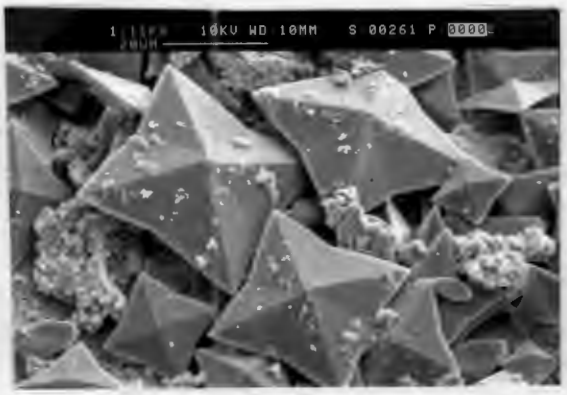
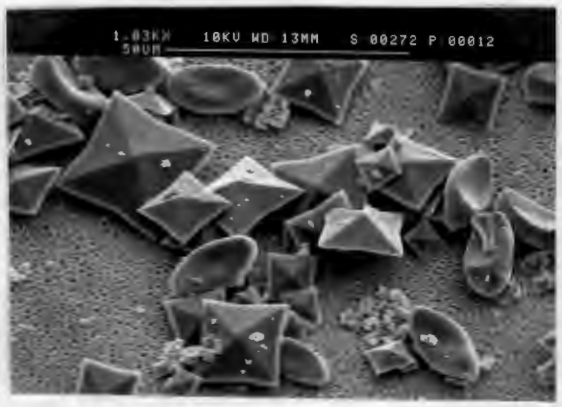


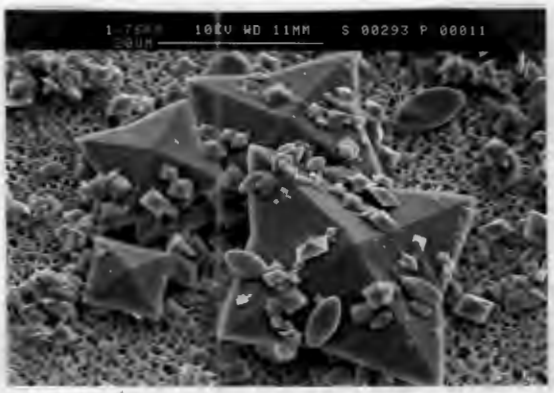
Figure 20: Nucleation rate as a function of growth rate for varying concentrations of magnesium added to the urine pool.



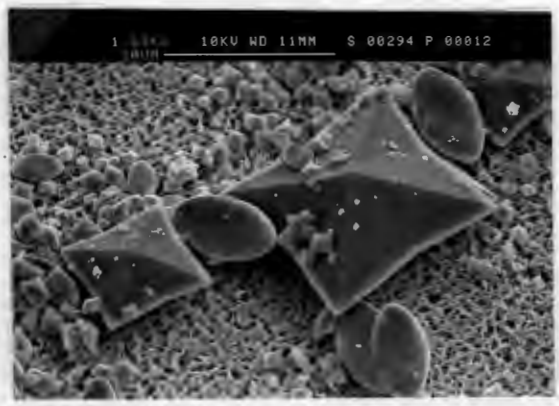
21A: no exogenous magnesium
mag: 1.11Kx
max COD = 30μm



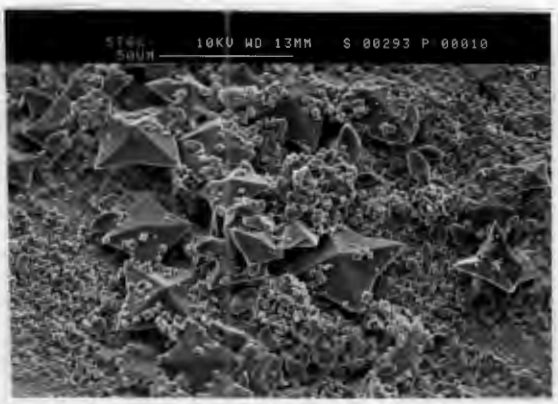
21B: [Mg] = 2.478mmol/dm^3
mag:1.03Kx
max COD = 25μm, COM = 20μm



21C: [Mg] =3.304mmol/dm^3
mag: 1.76Kx
max COD = 25μm
larger number of COM < 5μm



21D: [Mg] = 6.607mmol/dm^3
mag: 1.83Kx
max COD = 22μm, COM = 13μm



21E: Sample 14
[Mg] = 4.955mmol/dm^3
mag 570x
max COD = 27μm
heavy deposits of COM <5μm

Figure 21: SEM micrographs of CaOx deposition in the presence of varying concentrations of magnesium added to the urine pool; micrographs A-D are from sample 16 and micrograph E is from sample 14.

Inhibitor dosing via a separate feed into the crystalliser

Table 6 indicates the growth and nucleation rates for varying concentrations of magnesium introduced into the crystalliser via a separate feed. The magnesium concentrations shown indicate the final concentration of exogenous magnesium in the crystalliser.

		GROWTH μm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	[Magnesium] mmol/dm^3	Coulter	Malvern	Coulter	Malvern
26	1.927	0.8416	0.8775	2.598	2.749
	3.850	0.8564	0.8171	1.532	1.523
	5.781	0.7126 *	0.7730 *	1.226 *	1.656 *
	7.708	0.6997	0.7580	0.9888	0.5449
27	1.927	0.6782	0.7239	4.330	3.927
	3.850	0.6312	0.6729	3.101	3.026
	5.781	0.5852 *	0.5881 *	1.830 *	2.115 *
	7.708	0.5790	0.5282	0.8748	1.009
28	1.927	0.7634	0.6338	3.382	4.173
	3.850	0.7957	0.5707	1.802	3.196
	5.781	0.6848 *	0.5896 *	1.292 *	1.918 *
	7.708	0.6470	0.7028	1.343	0.5378

Table 6: CaOx growth and nucleation rates in the presence of various magnesium concentrations introduced into the crystalliser via a separate feed. Exogenous NaOx concentration inside the crystalliser was kept constant at 2.5 mmol/dm^3. (* denotes significant difference $p < 0.05$).

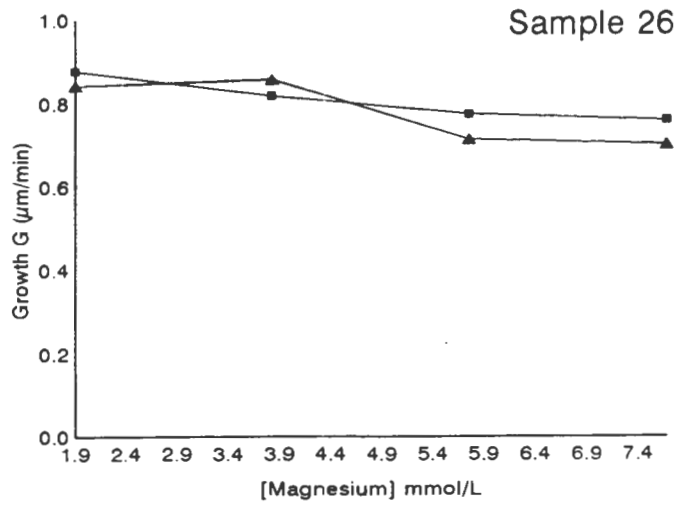
The growth and nucleation rates are plotted against the concentration of magnesium in Figure 22. Growth and nucleation rates decrease with increasing concentrations of magnesium; the decrease in nucleation rate is greater than that of the decrease in growth rate. Nucleation and growth rate trends demonstrated by Coulter and Malvern measurements show good agreement. The exception is sample 28 where the trends disagree at the highest magnesium concentration.

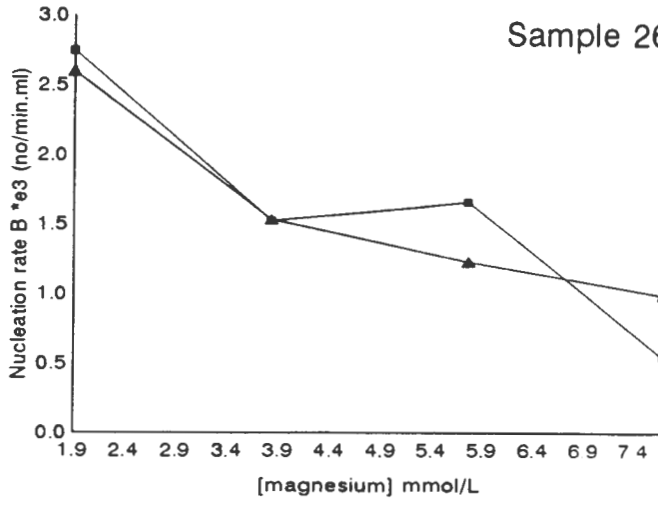
Growth and nucleation rates are plotted as a function of supersaturation in Figure 23. Values for the Coulter and Malvern are in agreement. Both the growth and nucleation rates increase with increasing supersaturation.

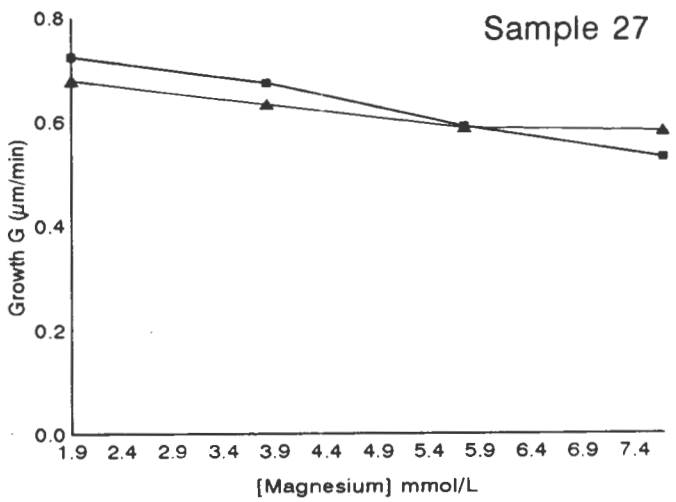
Figure 24 shows plots of nucleation rate versus growth rate with samples 26 and 27 indicating a general increase in nucleation with increasing growth.

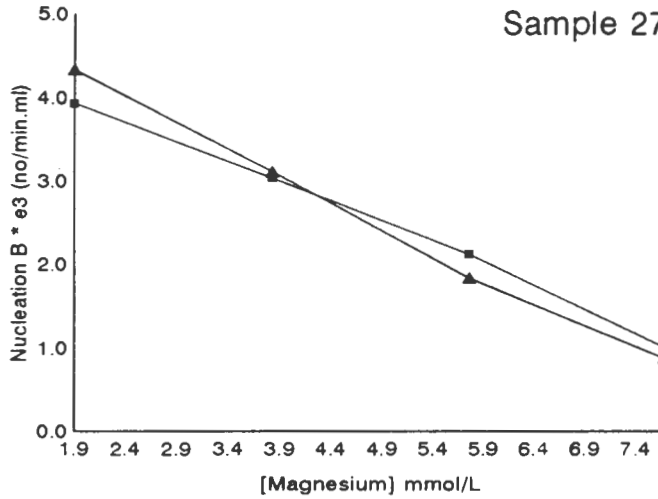
SEM confirmed growth inhibition in all samples as shown in the survey photographs in Figure 25. COD crystals decreased in size from 30μm in Figure 25A at a magnesium concentration of 1.927mmol/dm^3 to 15μm in Figure 25D at a magnesium concentration of 7.708mmol/dm^3. Extremely large COD crystals of size 50μm were rare and are noted in Figure 25B. Also the uncommon morphology of COD crystals (previously observed in both citrate and magnesium experiments) is evident in all the micrographs in Figure 25. Tiny COM crystals of sizes below 10μm were common at the two lower magnesium concentrations; however, they were rarely noted at the two higher magnesium concentrations.

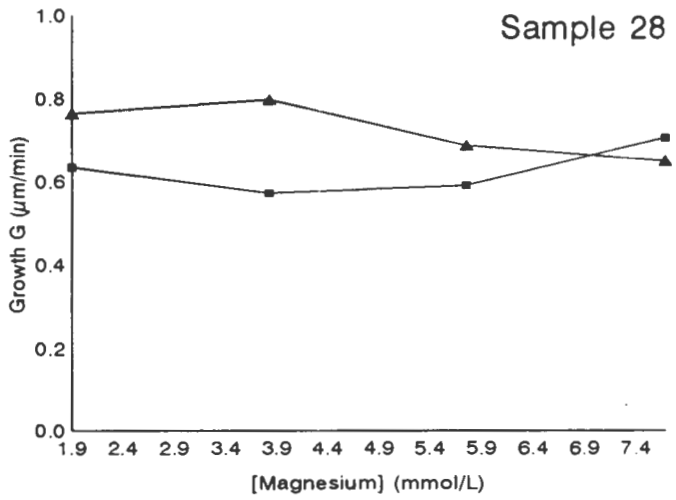
All samples at the lowest magnesium concentration (Figure 25A) showed heavy deposits of single COD and COM crystals; only sample 28 showed marked intergrowth of COD crystals. In samples 26 and 28 the COM phase was dominant at the two lower magnesium concentrations. Aggregates of COD crystals were of the order of 15μm (Figure 25A,B); the average size of these decreased to below 10μm with increase in magnesium concentration (Figure 25C,D) suggesting that magnesium might be an inhibitor of aggregation. In general, the total number of large crystals decreased with increasing magnesium concentration as is evident in the series of micrographs in Figure 25, all of which were recorded at similar magnifications. This tends to support the kinetic observations of nucleation inhibition.











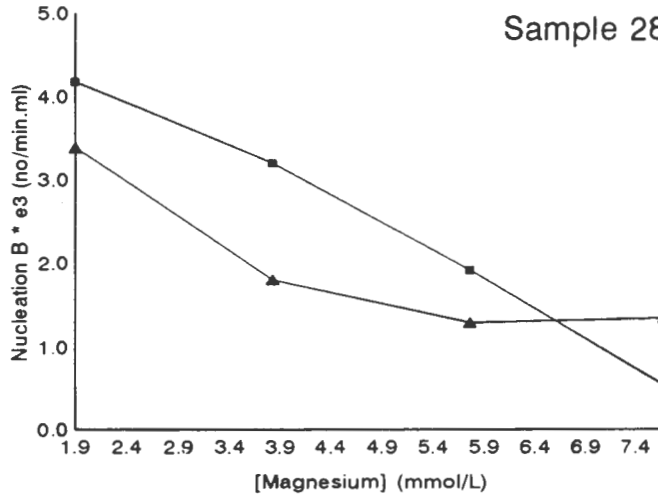


Figure 22: Growth and nucleation rates of CaOx in the presence of magnesium when admitted to the crystalliser via a separate feed.

▲ Coulter
■ Malvern

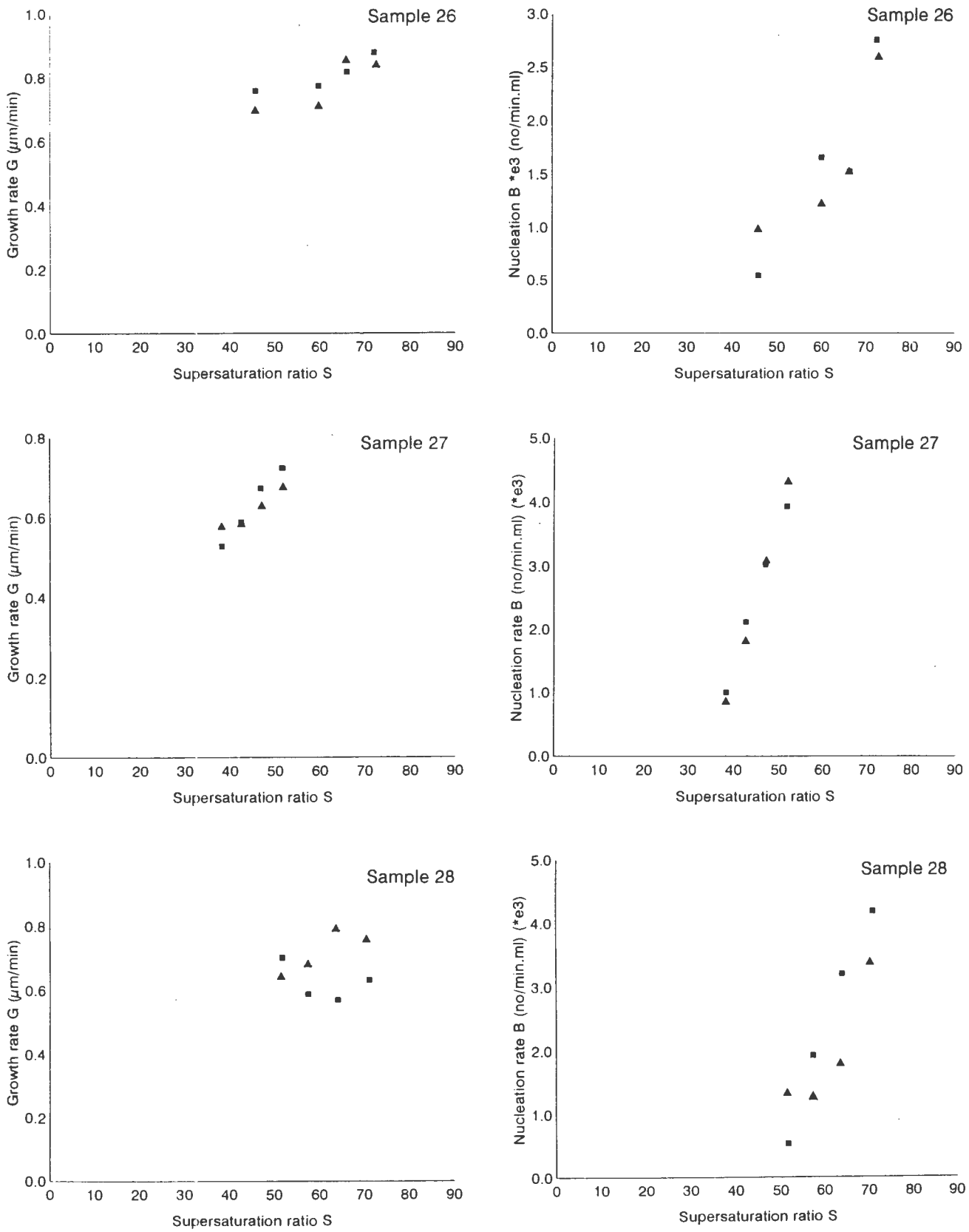
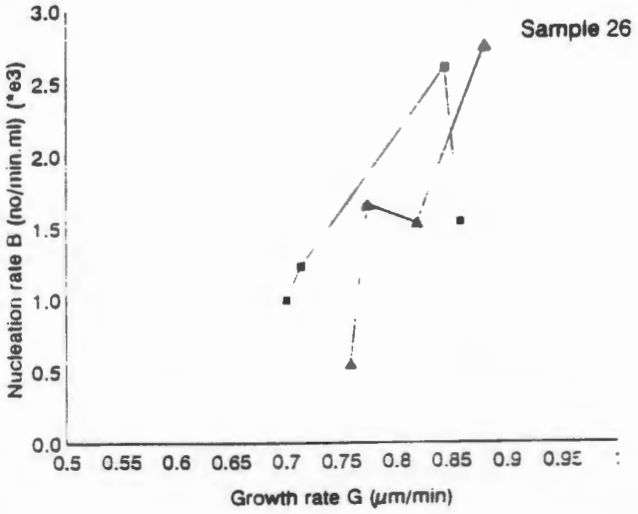
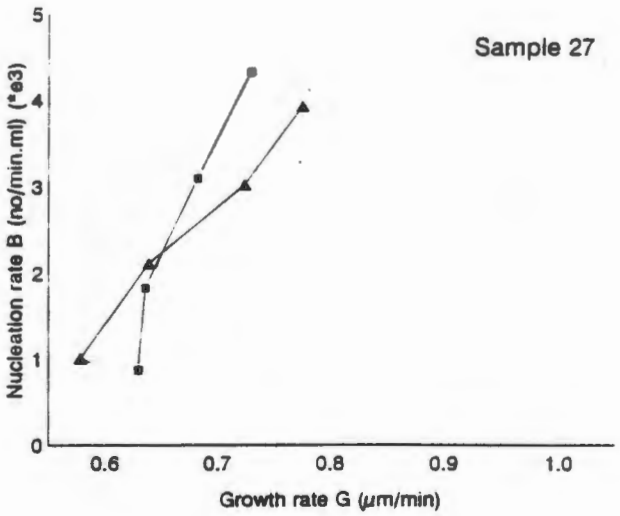


Figure 23: Growth and nucleation rates as a function of supersaturation ratio S at different magnesium concentrations added to the crystalliser via a separate feed.





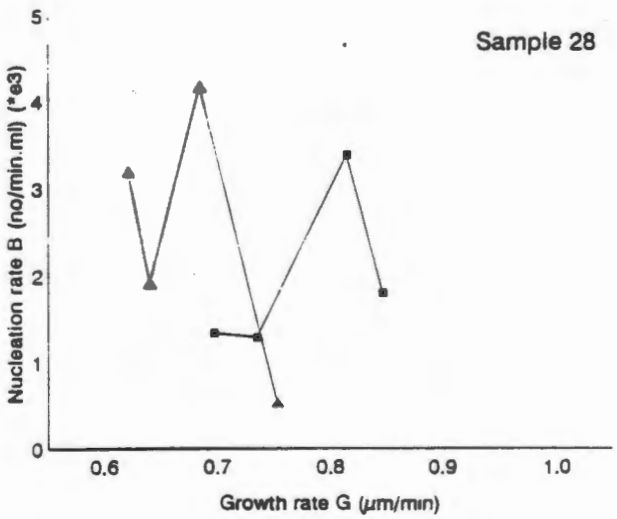


Figure 24: Nucleation rate as a function of growth rate at different magnesium concentrations added to the crystalliser via a separate feed.

Comparison of the two magnesium experiments

A comparison of the general trends in growth and nucleation rates for the experiments in which magnesium was added to the urine pool prior to being admitted into the crystalliser (in experiment 1) and was added as a separate feed into the crystalliser (in experiment 2) can be made. The ranges of the magnesium concentrations in the two experiments are comparable.

Growth rates decreased with increasing magnesium concentration to a similar extent in both experiments. Nucleation rates also decreased as the concentration of magnesium increased; however, in experiment 1, nucleation rates showed an increase at the higher magnesium concentrations.

Trends of increasing growth rate with increasing supersaturation are in agreement for the two experiments. Comparison of nucleation rate with increasing supersaturation showed opposite trends in the two experiments. Other comparisons are not possible because no obvious trends were observed.

Effect of a combination of citrate and magnesium

Inhibitor dosing of pooled urine prior to introduction into the crystalliser

Results are shown in Table 7. Final exogenous inhibitor concentrations in the crystalliser are presented.

Sample	[Inhibitor] mmol/dm^3	GROWTH µm/Min Coulter		GROWTH µm/Min Malvern		NUCLEATION no/min.ml (*10^3) Coulter		NUCLEATION no/min.ml (*10^3) Malvern	
17	0	0.8410		0.7791		3.204		4.430	
	citrate 1.020	0.7600		0.7150		1.110		2.536	
	magnesium 3.304	0.8028	*	0.7055		1.899	*	2.738	*
	citrate & magnesium 1.020 3.304	0.6250		0.6790		2.746		1.361	
18	0	0.7044		0.6782		5.835		6.455	
	citrate 1.020	0.6241		0.6100		1.569		2.237	
	magnesium 3.304	0.6892	*	0.6640		6.478		5.743	
	citrate & magnesium 1.020 3.304	0.5519		0.6052		4.180		3.314	
19	0	0.8394		0.7588		2.058		2.433	
	citrate 1.020	0.7126		0.7288		1.226		0.9213	
	magnesium 3.304	0.7950	*	0.7682		1.802		2.087	
	citrate & magnesium 1.020 3.304	0.6451		0.7000		1.999		1.167	

Table 7: CaOx growth and nucleation rates in the presence of various exogenous concentrations of citrate, magnesium and combinations thereof. The concentration of the feed NaOx was kept constant at 2.5mmol/dm^3 inside the crystalliser. (* denotes significant difference $p < 0.05$).

Figure 26 shows that the introduction of citrate or magnesium or combinations thereof caused a decrease in growth and nucleation rates in all samples. This inhibitory effect was more pronounced in the presence of citrate than in the presence of magnesium. The mixture of citrate and magnesium inhibited CaOx growth rates in all three samples to a greater extent than was achieved by either citrate or magnesium alone. Nucleation rates in the presence of a citrate and magnesium mixture were lower than those for magnesium alone, but higher than those calculated for citrate alone.

Figure 27 indicates the relationship of growth and nucleation rates with respect to supersaturation. An increase in growth

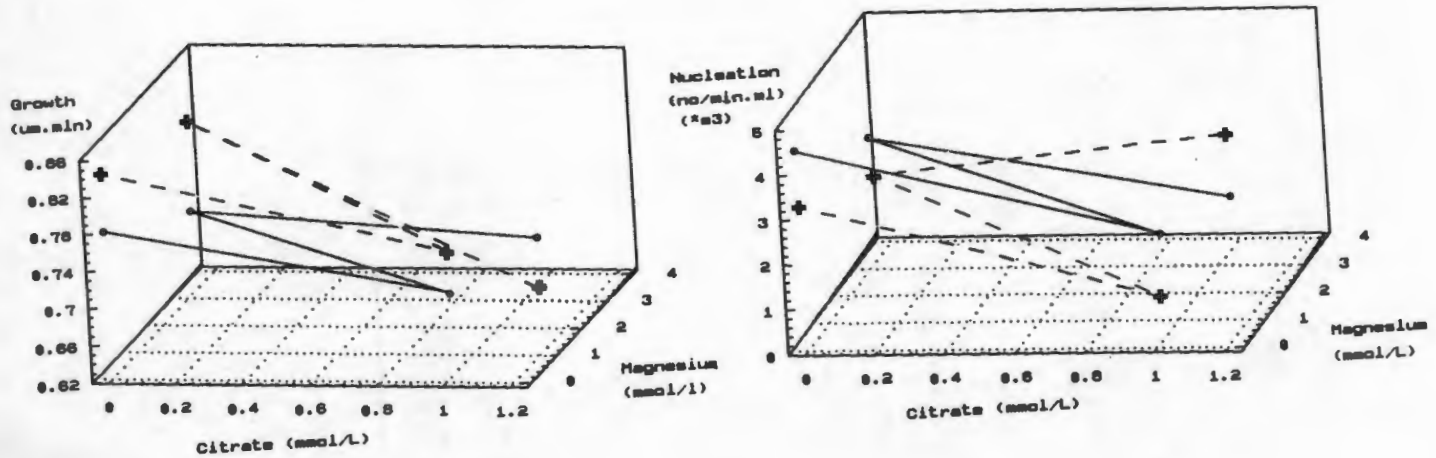
rate with increasing supersaturation ratio is indicated for all samples. The trend and individual values of the growth rate are in good agreement for the Coulter and Malvern. However, large differences in individual values of the nucleation rate for the two instruments are seen in Table 7 and also when they are plotted as a function of supersaturation in Figure 27. No trend in the nucleation rate as a function of supersaturation is apparent.

Figure 28 is a plot of nucleation rate vs growth rate. Although values for the two instruments tend to indicate differences, the trends show an overall increase in nucleation with increasing growth rate.

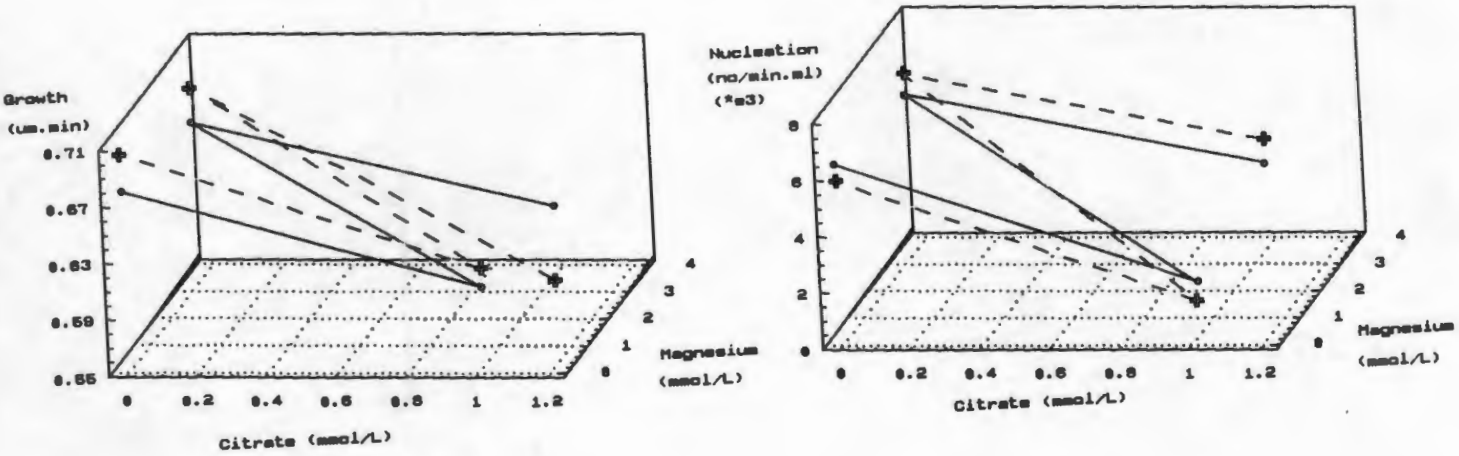
SEM showed that all 3 CaOx crystal phases were present in the control samples and that intergrowth of COD-COD and COM-COD was common (Figure 29). COT crystals were extremely large 30-50μm but were rare in all samples.

The series of micrographs in Figure 30 shows some interesting features. The inclusion of only citrate in the test urine decreased both the size and number of COM and COD crystals observed (Figure 30B). The urine sample which was treated with magnesium only also showed a decrease in the size of COM and COD but there was no change in the number of crystals (Figure 30C). Aggregation of COD crystals only was also present in these samples; COD aggregates having sizes up to 20μm were observed. COT crystals were not detected in the presence of citrate or magnesium alone. The mixture of citrate and magnesium decreased the size of COD crystals (from 20μm to 15μm) (Figure 30D) relative to the control and individual magnesium and citrate samples. The size of the COM crystals in all samples was unchanged at 10μm. No COT crystals were noted in sample 17 while no intergrowth was noted in any sample. Samples 18 and 19 showed two large COD crystals at 50μm. The number of small crystals (<5μm) increased when compared to samples treated with magnesium or citrate alone for pools 17 and 19.

SAMPLE 17



SAMPLE 18



SAMPLE 19

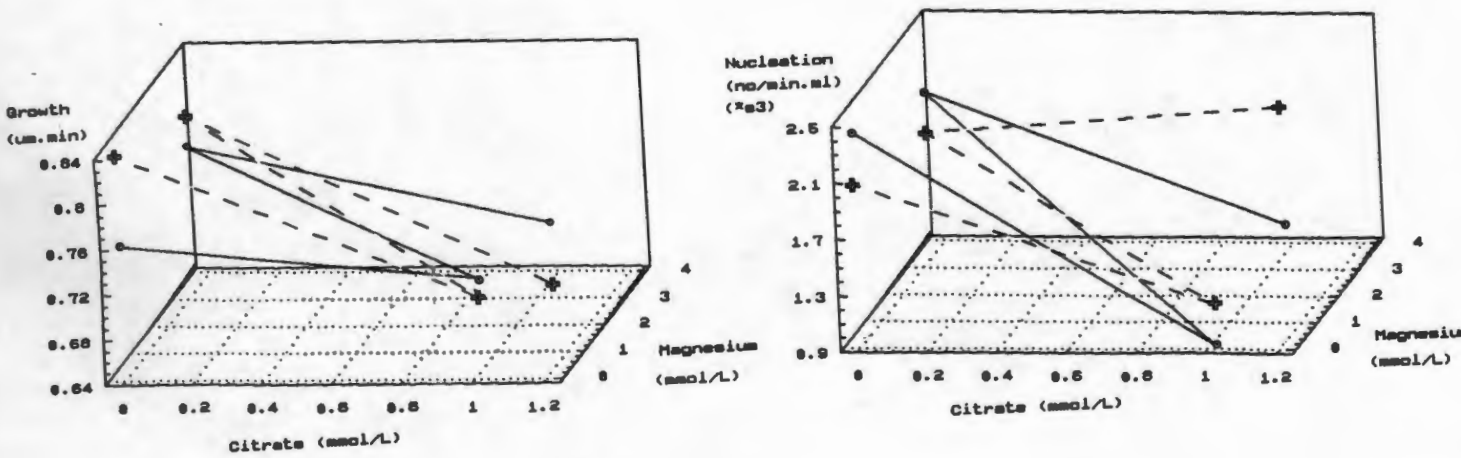


Figure 26: Growth and nucleation of CaOx in the presence of varying concentrations of citrate and magnesium in the pooled urine.

+ – – + MALVERN
● – – ● COULTER

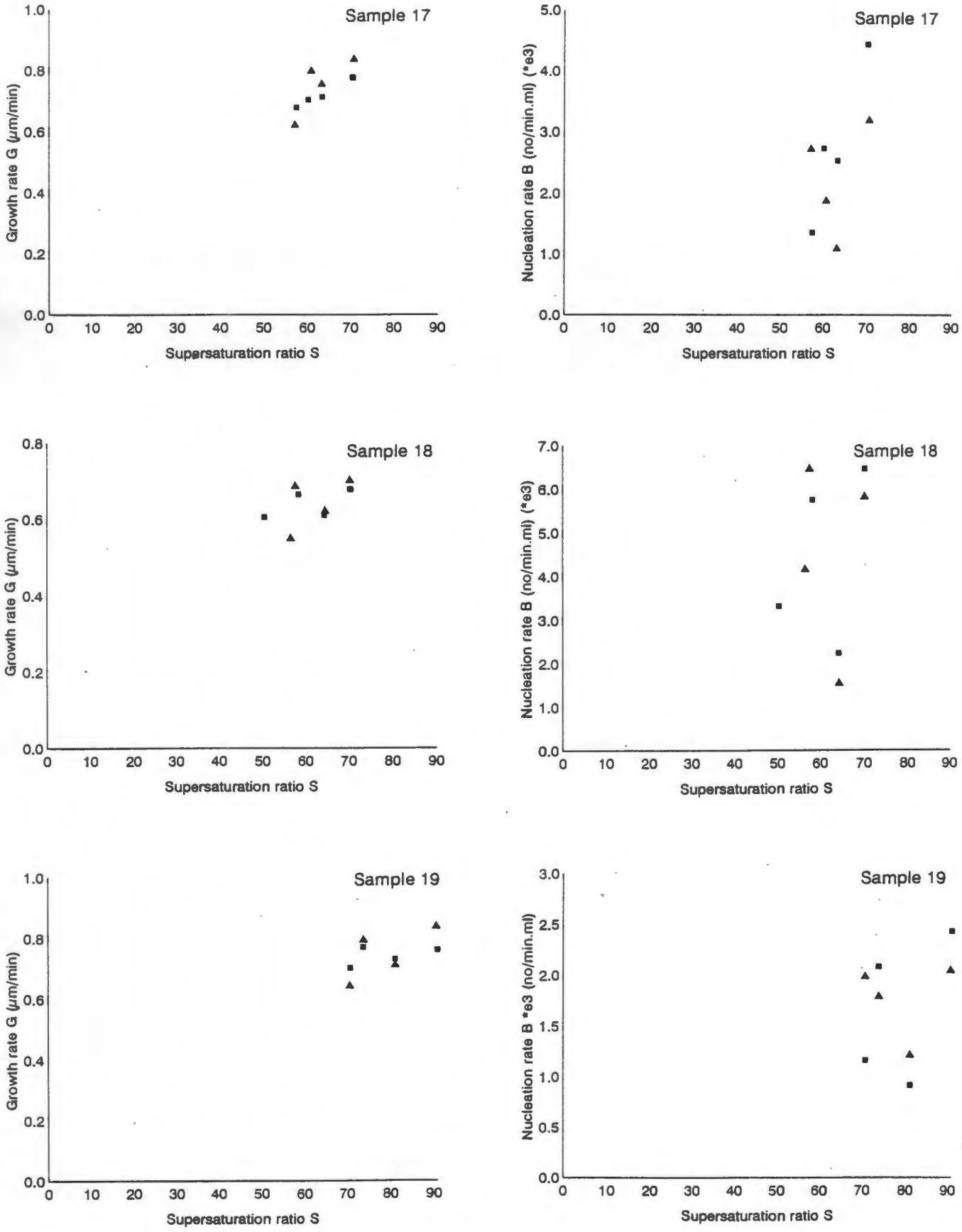
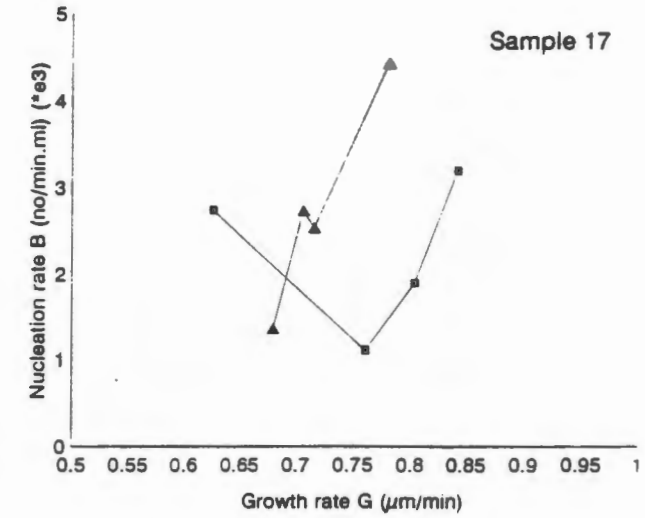
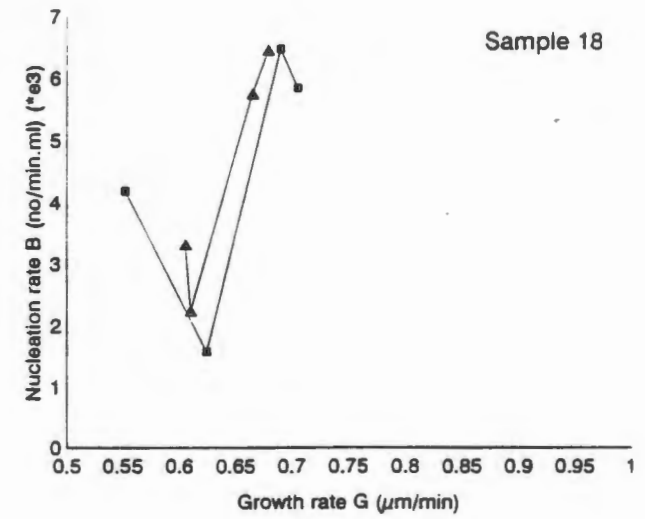


Figure 27: Nucleation and growth rate as a function of supersaturation ratio S for varying concentrations of citrate and magnesium added to the urine pool.





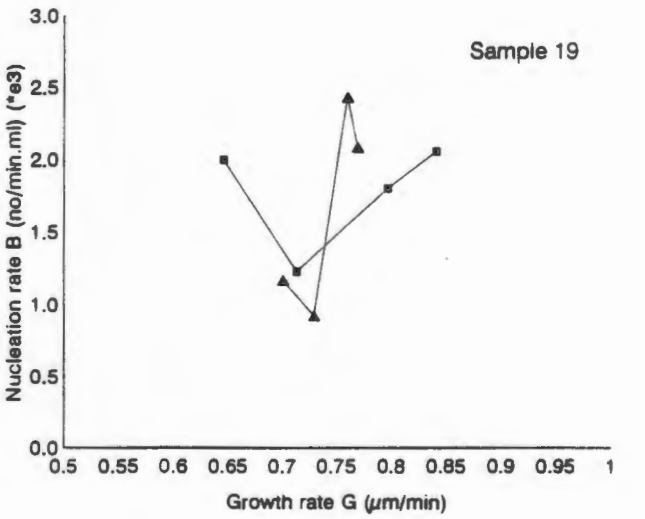
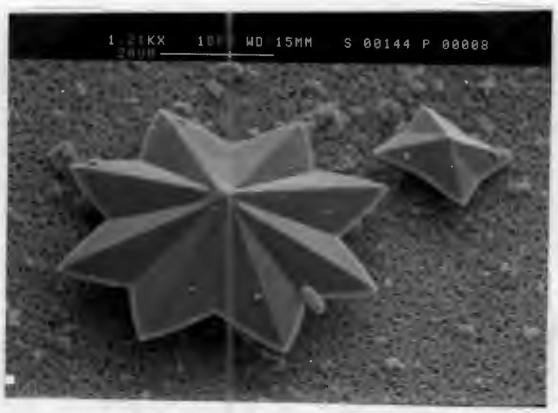
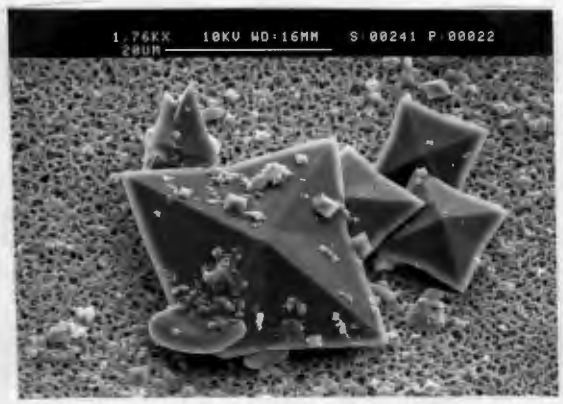


Figure 28: Nucleation rate vs growth rate for varying concentrations of citrate and magnesium added to the urine pool.



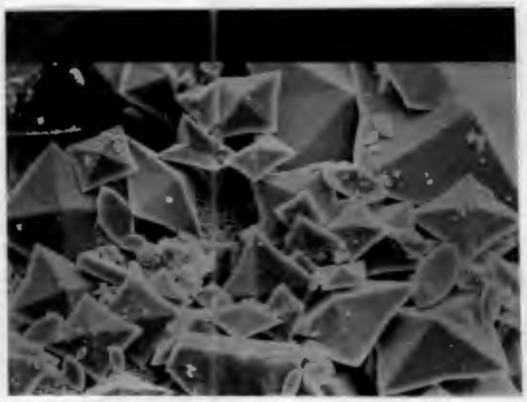
29A: Intergrowth of COD into a star shape
mag: 1.21KX



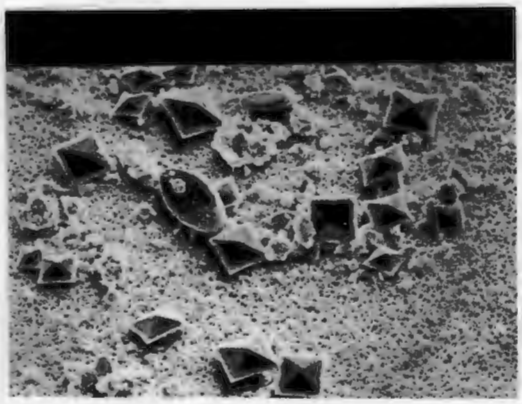
29B: COM-COD intergrowth
mag: 1.76KX

29C:
COD-COD intergrowth
mag:1.43KX

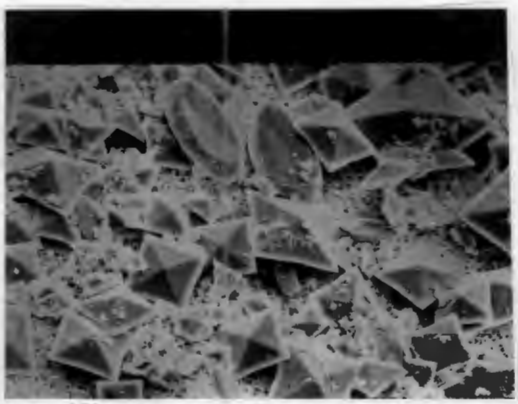
Figure 29: SEM micrographs showing intergrowth that was common in the control samples (ie exogenous magnesium or citrate absent).



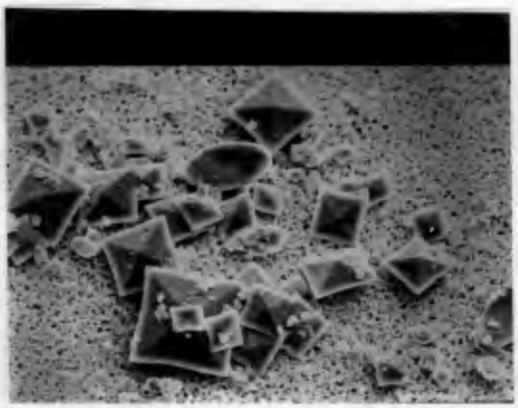
30A:control sample
no citrate or magnesium
mag: 1.32Kx
max COD = 30μm



30B: [cit] = 1.020mmol/dm^3
mag: 120Kx
max COD = 6μm, COM = 13μm



30C: [Mg] = 3.304mmol/dm^3
mag = 132Kx
max COD,COM = 17μm



30D: [cit] = 1.020mmol/dm^3
[Mg] = 3.304mmol/dm^3
mag: 135x
max COD = 15μm

Figure 30: SEM micrographs showing crystallisation in the absence and presence of citrate, magnesium and a mixture thereof.

Inhibitor dosing via a separate feed into the crystalliser

Introducing the citrate-magnesium combination into the crystalliser via a separate feed gave the growth and nucleation rates shown in Table 8. Final exogenous inhibitor concentrations in the crystalliser are shown.

			GROWTH µm/Min		NUCLEATION no/min.ml (*10^3)	
Sample	[Inhibitor] mmol/dm^3		Coulter	Malvern	Coulter	Malvern
29	citrate	2.678	0.8416	0.6394	2.598	2.614
	magnesium	5.781	1.228	0.7060	0.4104	0.2590
	citrate & magnesium	1.339 2.891	0.5266 *	0.5696	1.767	0.9161 *
	citrate & magnesium	2.678 5.781	0.6754	0.7108	0.3828	0.1035
30	citrate	2.678	0.5382	0.5325	1.080	2.513
	magnesium	5.781	0.5161	0.5736	2.278	5.387
	citrate & magnesium	1.339 2.891	0.4767 *	0.5144	8.779 *	3.907
	citrate & magnesium	2.678 5.781	0.3595	0.4689	5.357	4.908
31	citrate	2.678	1.387	0.6724	0.3945	1.484
	magnesium	5.781	1.355	0.7730	0.7424	1.656
	citrate & magnesium	1.339 2.891	1.483	0.7864	0.4817	1.375
	citrate & magnesium	2.678 5.781	1.132	0.7322	0.5932	1.571

Table 8: CaOx growth and nucleation rates in the presence of various citrate and magnesium concentrations and combinations thereof introduced into the crystalliser via a separate feed. Exogneous NaOx concentration inside the crystalliser was kept constant at 2.5mmol/dm^3. (* denotes significant difference $p < 0.05$).

Figure 31 shows that the growth rate in the presence of citrate alone was always lower than when only magnesium was introduced into the crystalliser. This also occurred with

nucleation rates in samples 30 and 31; however sample 29 showed that magnesium had a greater effect on nucleation.

The trends in growth in samples 29 and 30 were individually confirmed by both techniques. In both samples, the mixtures (both high and low concentrations) produced lower growth rates than either citrate and magnesium respectively. In sample 31, Coulter and Malvern data were not in agreement and hence general trends were not obvious.

Trends in the nucleation rates for samples 29 and 31 were individually confirmed by both techniques. In general, nucleation rates in the presence of citrate were always lower than those in the presence of magnesium. The nucleation rate in the presence of the higher concentration mixture was lower than that of magnesium in all cases except the Coulter determination of sample 30. No other clear trends were obvious.

Figure 32 shows the variation of growth and nucleation rates with supersaturation. Trends for the Coulter and Malvern are in agreement. Individual values for sample 31 however show an increase for the Coulter when compared to the Malvern. Trends for the growth rate show a maximum between supersaturation ratios of 55 and 60 for samples 29 and 30 while sample 31 indicates an increase in the growth rate. No trend in nucleation rate with increasing supersaturation can be deduced.

Plots of nucleation rates vs growth rates are shown in Figure 33. No trends are apparent for the three samples.

Survey micrographs (all recorded at approximately the same magnification) in Figure 34 show the effect on the size and number of crystals of the addition of citrate alone (Figure 34A), magnesium alone (Figure 34B) and in combination (Figure 34C,D).

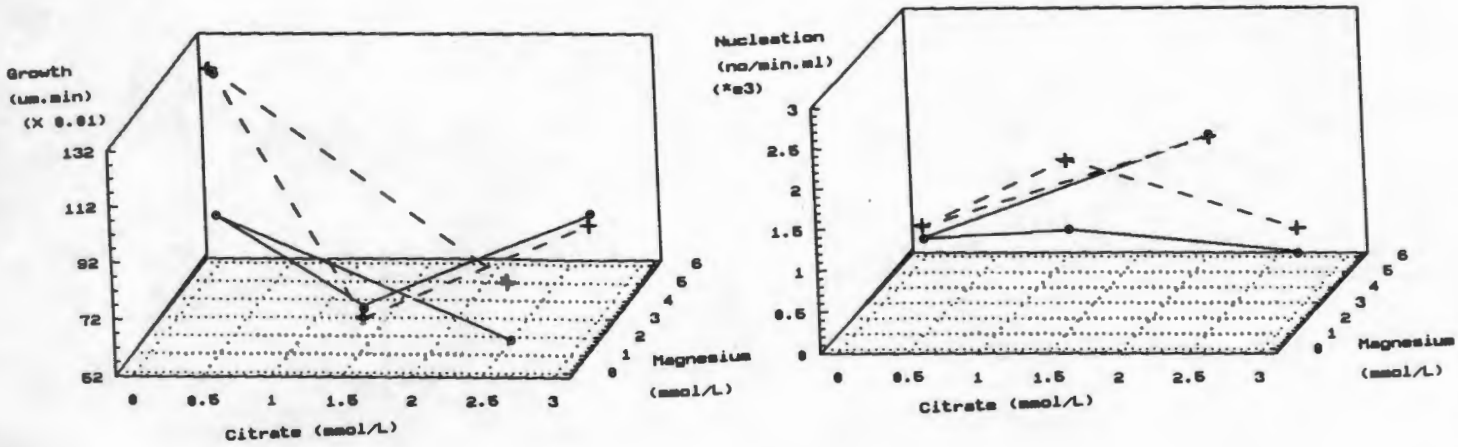
The samples treated with citrate only, showed a large amount of COD crystals and varying amounts of COM's (Figure 34A).

Samples treated with magnesium alone (Figure 34B) showed no change in the size of the COD crystals in samples 30 and 31; however, sample 29 showed larger COD crystals relative to those of the citrate sample. A few aggregates were seen in these samples. The number and size of COM crystals was lower in the samples containing magnesium only when compared to the samples containing citrate only.

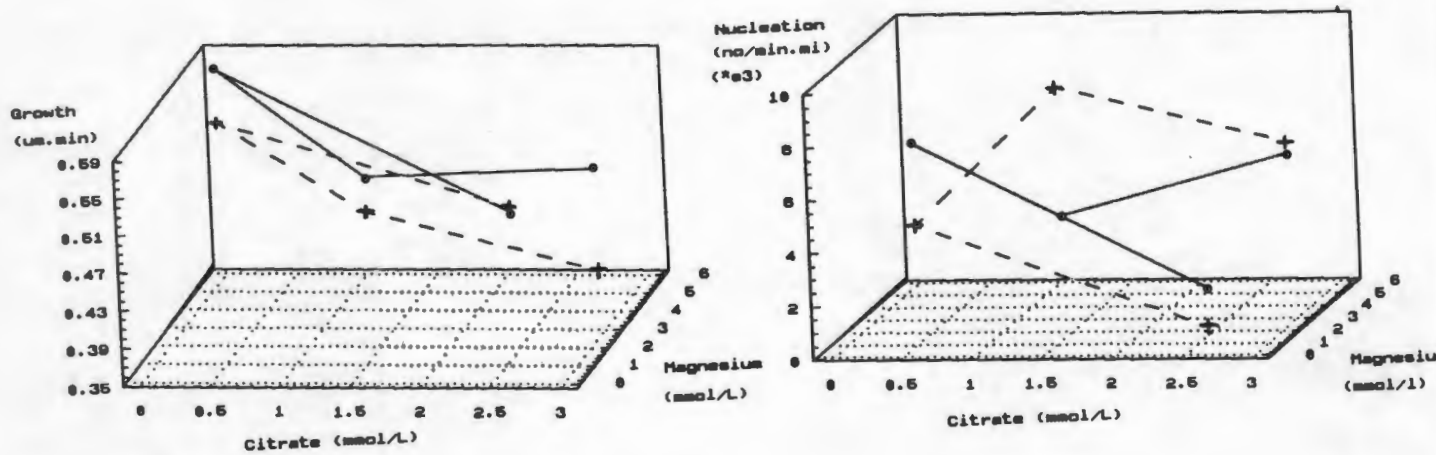
Generally the samples treated with the citrate and magnesium mixture showed an increase in the number of small crystals (COM and COD). A few aggregates (20μm) of tiny crystals were seen only in sample 29 (Figure 34C).

When the citrate and magnesium concentrations were doubled, tiny crystals only (invariably COD - Figure 34D) were observed. The exception was sample 31 which showed COD crystals of similar size to the samples with citrate and magnesium alone (Figure 34D). A small amount of aggregation was noted in sample 30 only. In general, the number of large crystals was reduced in samples 29 and 30 while sample 31 showed small differences when compared to samples treated with citrate or magnesium alone.

SAMPLE 29



SAMPLE 30



SAMPLE 31

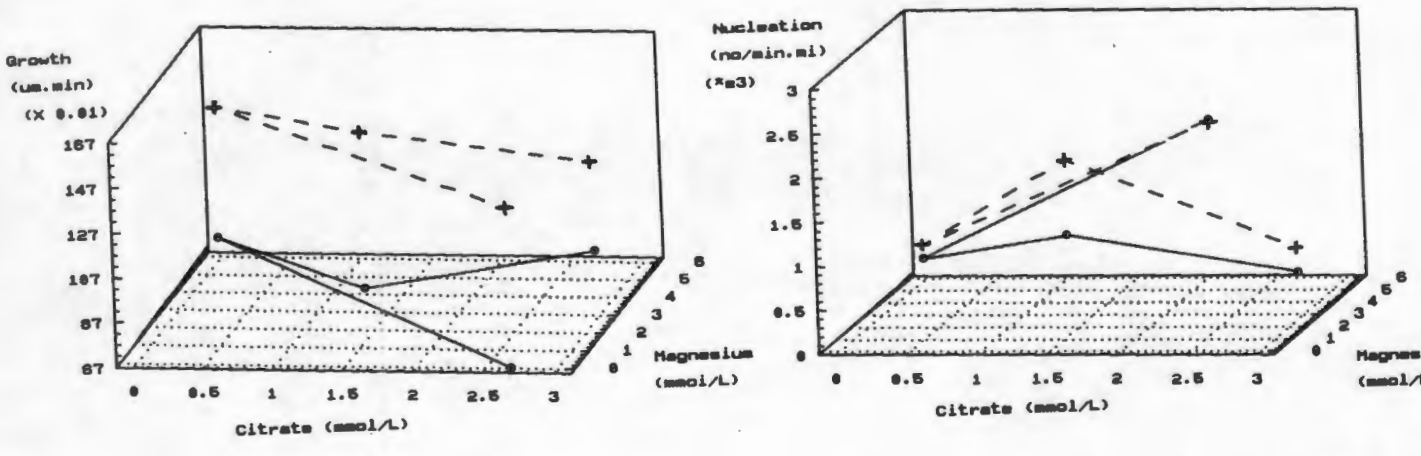


Figure 31: Growth and nucleation rates as a function of citrate and magnesium concentrations when introduced into the crystalliser via a separate feed.

+– –+ MALVERN
●– –● COULTER

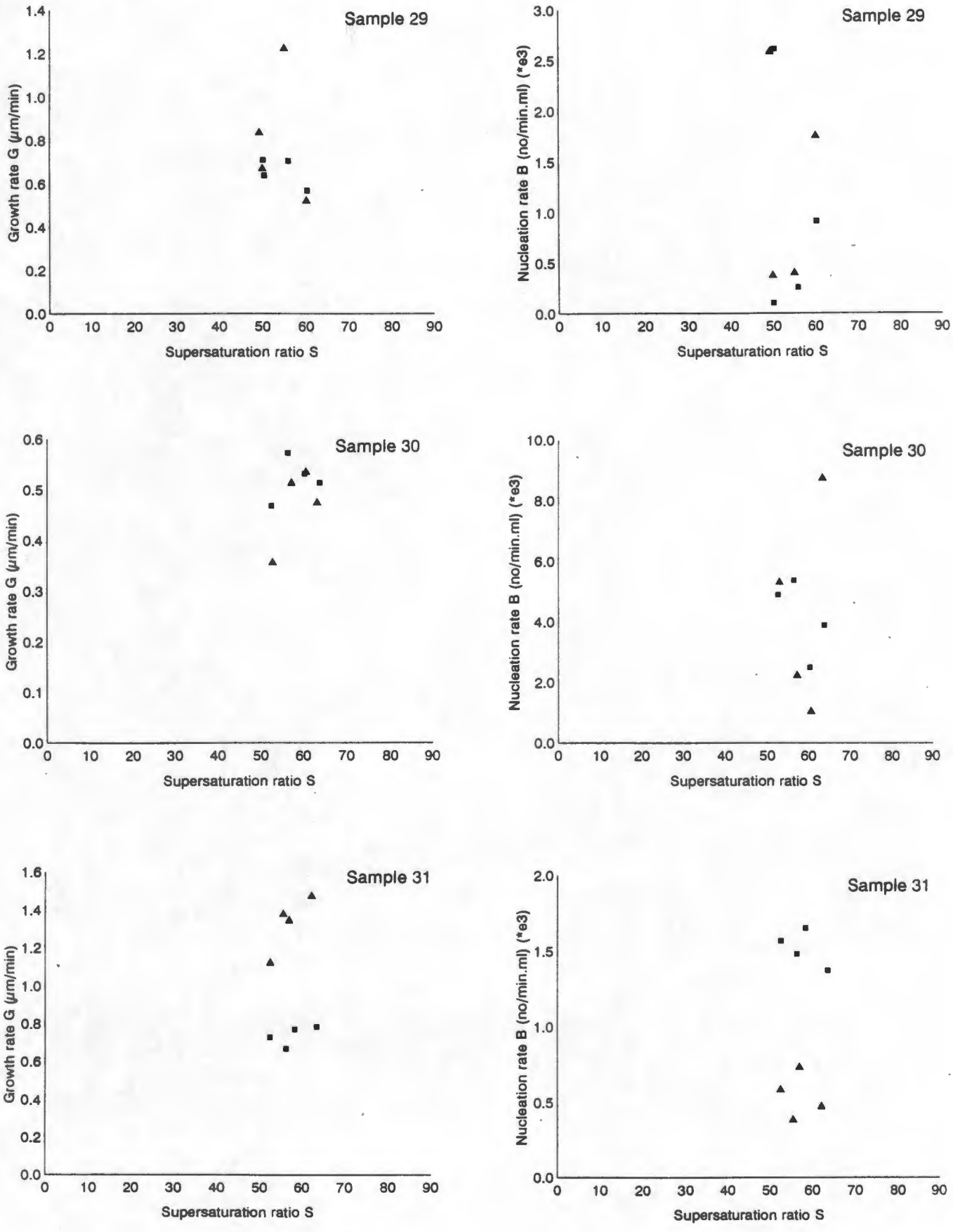


Figure 32: Nucleation and growth rate as a function of supersaturation ratio S at different citrate and magnesium concentrations added to the crystalliser via a separate feed.

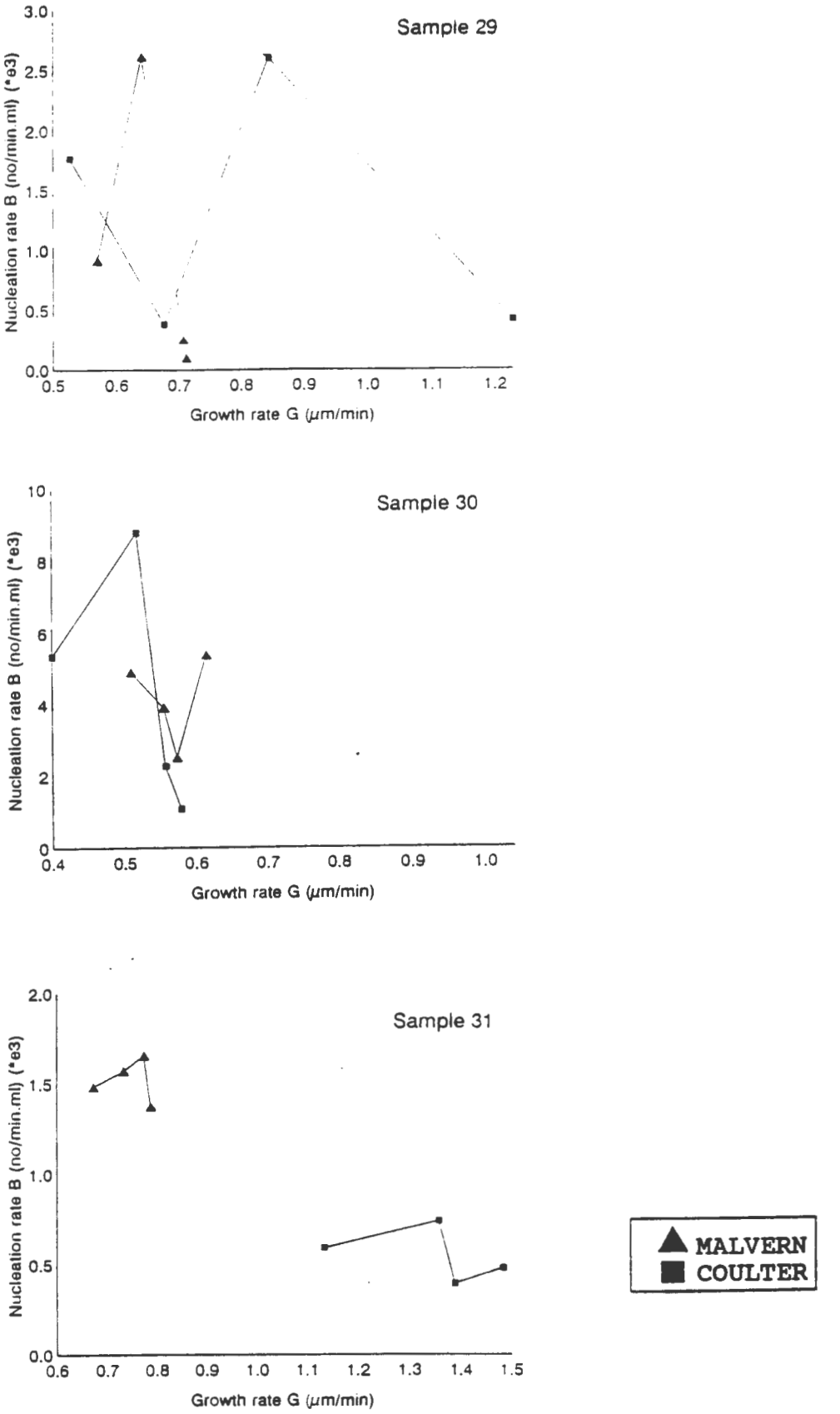
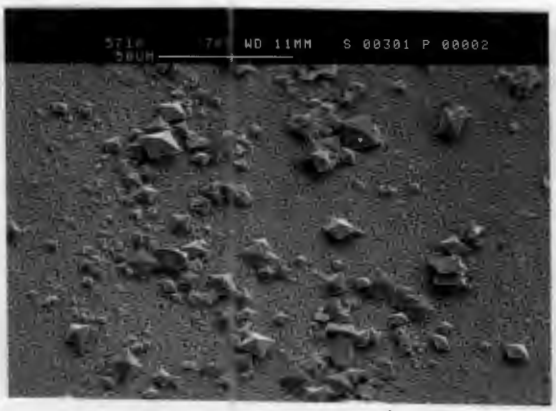
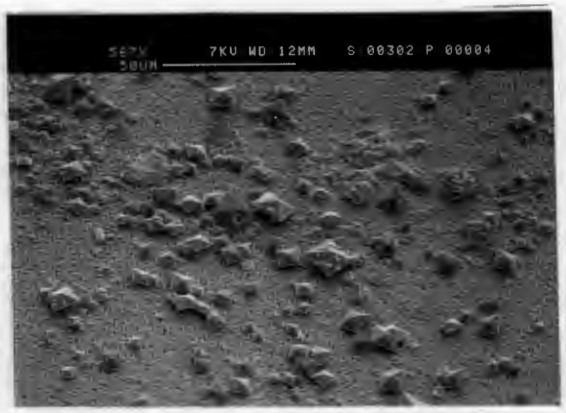


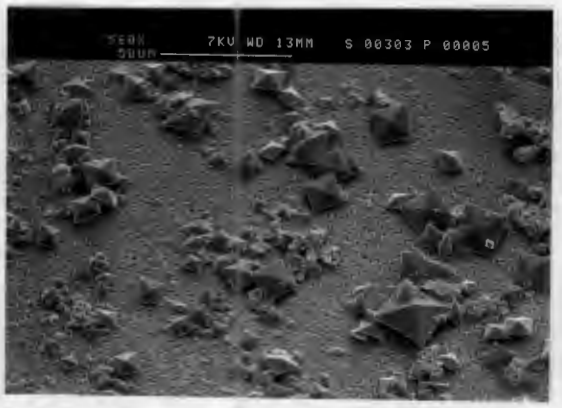
Figure 33: Nucleation rate as a function of growth rate for different concentrations of citrate and magnesium added to the crystalliser via a separate feed.





34A: [cit]=2.678mmol/dm^3
mag: 571x
max COD,COM = 15μm

34B: [Mg] = 5.781mmol/dm^3
mag: 567x aggregate = 10μm
max COD = 15μm



34C: [cit] = 1.339mmol/dm^3
[Mg] = 2.891mmol/dm^3
mag: 560x
max COD = 20μm

34D: [cit] = 2.678mmol/dm^3
[Mg] = 5.781mmol/dm^3
mag: 553x
max COD = 15μm

Figure 34: Survey photographs at similar magnifications showing the effect of varying exogenous citrate and magnesium concentrations in mmol/dm^3 inside the crystalliser.

Comparison of the citrate-magnesium combination experiments

A comparison of the general trends in nucleation and growth rates for the addition of citrate and magnesium in the pooled urine (experiment 1) and the addition into the crystalliser via a separate feed (experiment 2) can be made.

Experiment 2 did not include a control sample (ie a sample in which exogenous citrate or magnesium was absent). As such a comparison of the mixture can only be made with the individual additions of citrate and magnesium and not to a control sample.

The concentrations of citrate and magnesium used in experiment 2 were higher than those used in experiment 1. Trends due to the citrate and magnesium mixture in which their respective concentrations were the same as when their individual effects were examined separately can be compared. A combination of the inhibitors at the concentrations used in the experiments examining their individual effects show that growth rates were generally lower than those for the individual citrate or magnesium in both experiments. Nucleation rates for the combination were generally higher than those for citrate only and lower than those for magnesium only for experiment 1 and some samples in experiment 2.

Growth rates generally increase with increasing supersaturation while trends for nucleation rates are in agreement for both experiments. No trends for nucleation rate with respect to growth rates is apparent for the two experiments.

Effect of chondroitin sulphate A

Inhibitor dosing of pooled urine prior to introduction into the crystalliser

An aqueous solution of chondroitin sulphate sodium salt, type A (Sigma Chemical CO. serial no:C8529) was added to the urine pool which was subsequently thoroughly mixed. Concentrations were in the range 7.6 - 21.6 mg/dm^3, corresponding to that

occurring for GAGs in normal urine [Ryall *et al.* 1981]. Growth and nucleation rates of CaOx in urines containing various concentrations of chondroitin sulphate (CSA) are given in Table 9. Final concentrations of CSA inside the crystalliser are expressed in mg/dm^3 (and not in $mmol/dm^3$ as an accurate molecular mass was not available).

		GROWTH μm/Min		NUCLEATION no/min.ml ($*10^3$)	
Sample	[CSA] (mg/dm^3)	Coulter	Malvern	Coulter	Malvern
20	0	0.6210	0.6980	2.140	2.967
	10.69	0.7550	0.6565	1.992	1.882
	16.04	0.6070 *	0.6265	1.684 *	1.779 *
	21.38	0.6575	0.6563	0.9812	1.205
21	0	0.6787	0.6244	5.324	6.432
	10.69	0.7069	0.6625	5.296	4.393
	16.04	0.6769 *	0.6398 *	4.173	4.029 *
	21.38	0.6786	0.6849	3.412	3.795
22	0	0.6366	0.6563	5.584	6.027
	10.69	0.6431	0.6755	4.730	4.436
	16.04	0.6905	0.6712	4.461	3.575 *
	21.38	0.6868	0.6750	2.261	2.822

Table 9: CaOx growth and nucleation rates in the presence of various exogenous concentrations of chondroitin sulphate A. The concentration of the feed NaOx was kept constant at $2.5mmol/dm^3$ inside the crystalliser. (* denotes significant difference $p < 0.05$).

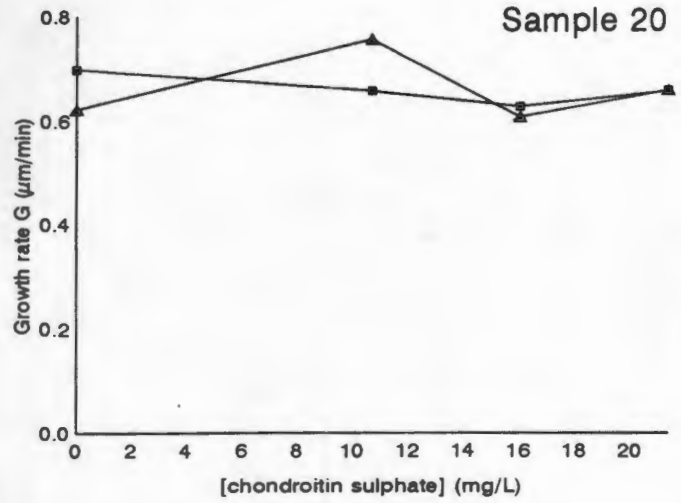
CaOx growth and nucleation rates are plotted against the concentration of CSA in Figure 35. Values calculated from the Malvern and Coulter data show good agreement in the trends for both nucleation and growth. Growth rates did not vary. However, nucleation rates decreased with increasing concentrations of CSA for all three samples.

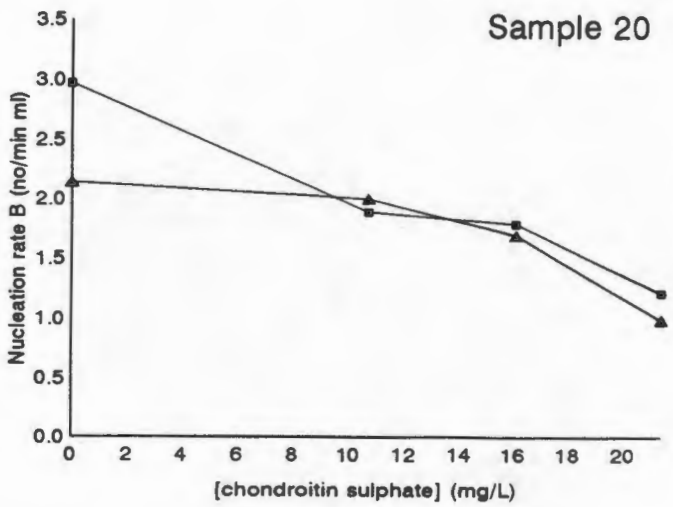
Figure 36 indicates that there is little variation in either the growth rate or the supersaturation ratio within each sample. However, nucleation rates decreased with small

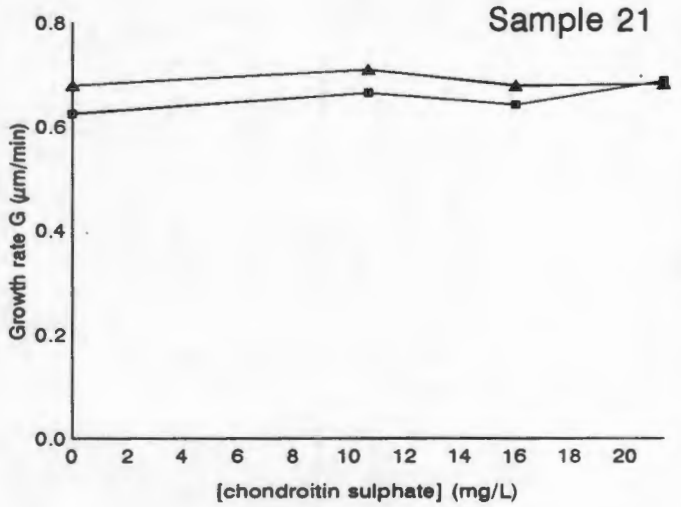
increases in the supersaturation. Individual values for the Coulter and Malvern are in agreement.

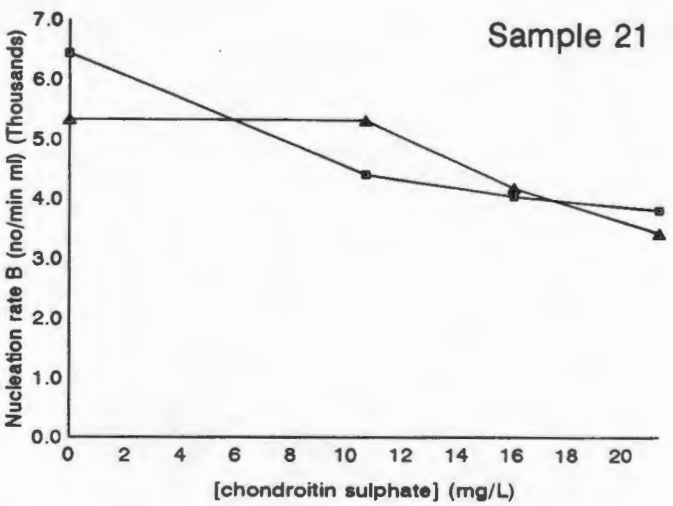
Figure 37 shows the relationship between the nucleation rate and growth rate. Sample 22 shows a decrease in nucleation rate above a growth rate of 0.64 while smaller variations are shown in samples 20 and 21.

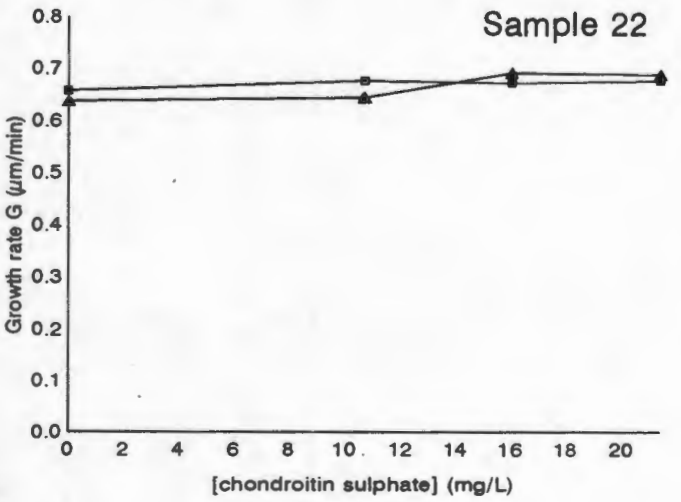
A small increase in the size of COM crystals but not of COD crystals with increasing CSA concentration was seen using the SEM. A few COT crystals were present at extremely large sizes (Figure 38A,B) in the control sample but these were either absent or significantly smaller at the highest concentration of CSA. Intergrowth and aggregation were noted in the control sample (Figure 38A,B,C) but the extent of both was reduced after treatment with CSA. Figures 38D,E,F, are micrographs of a sequence of samples in which 38D does not contain CSA while 38E,F contain increasing concentrations of CSA. A reduction in the total number of crystals is seen with increasing CSA concentration. The absence of tiny crystals at the highest CSA concentration (Figure 38F) confirmed inhibition of nucleation.











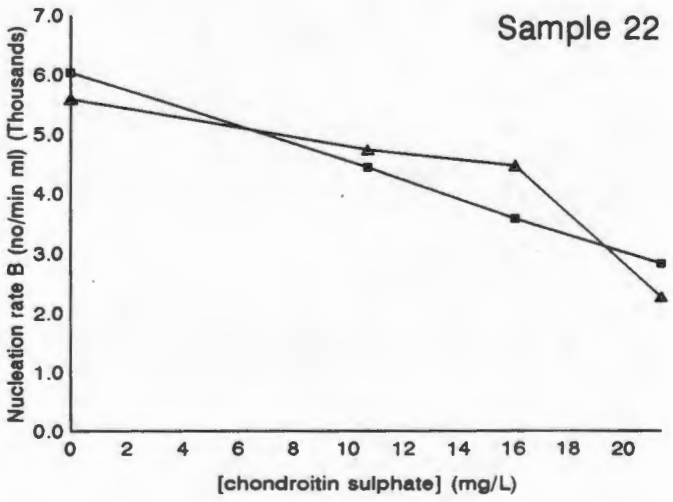
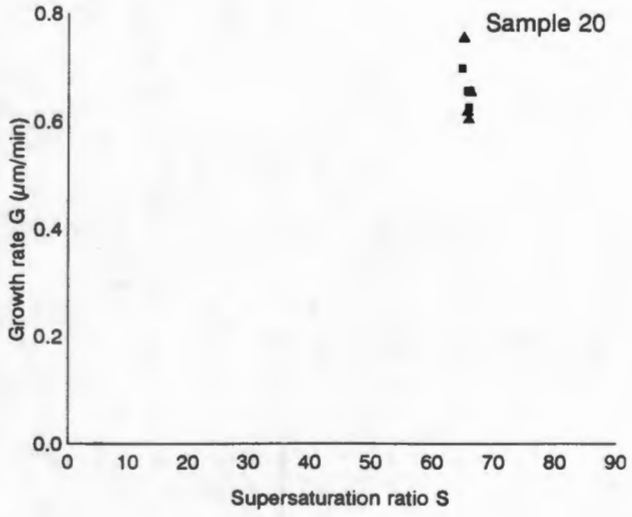
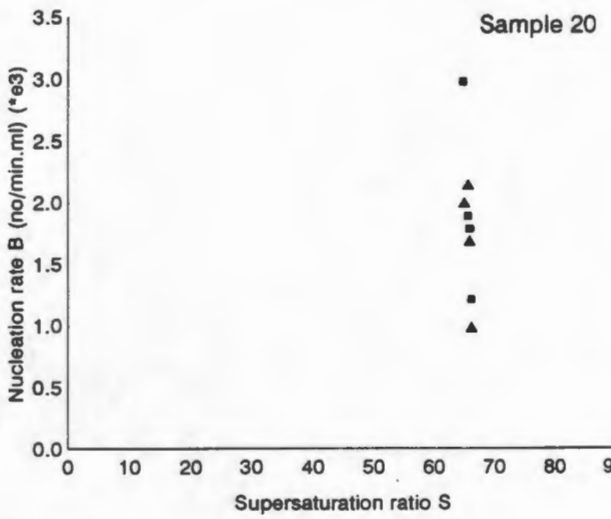
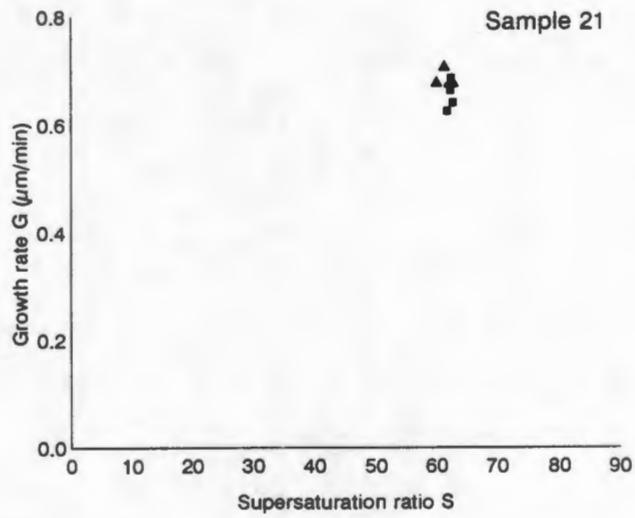
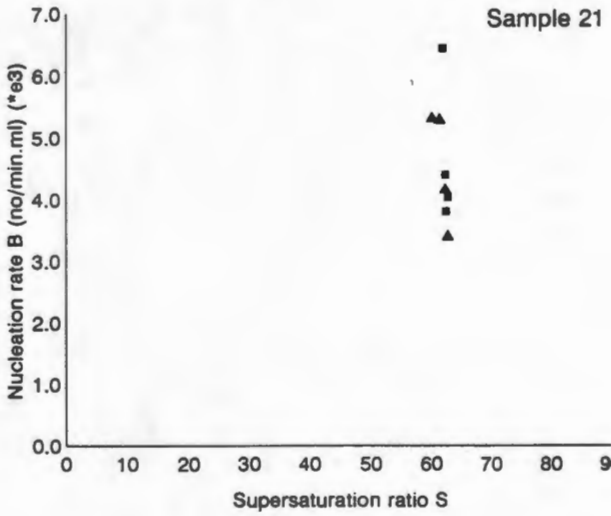


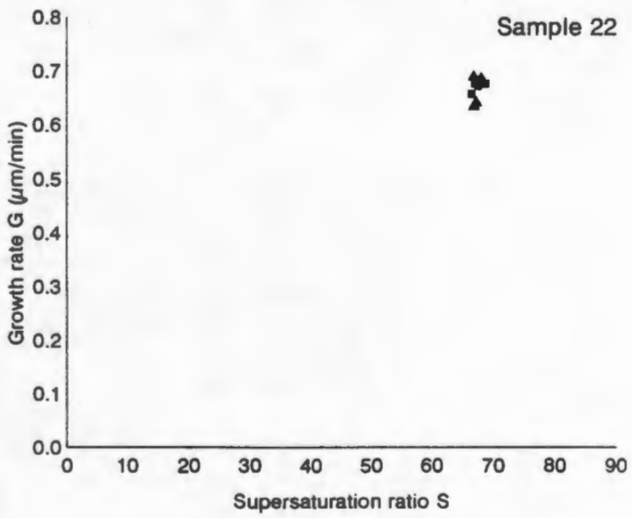
Figure 35: Growth and nucleation rates as a function of CSA concentration in the urine pool.











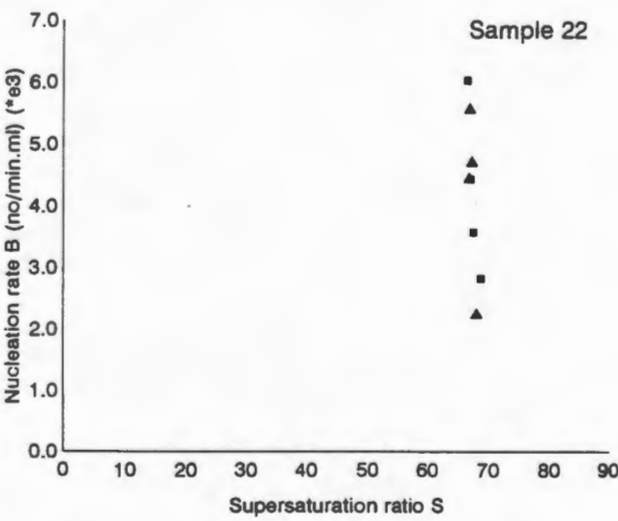


Figure 36: Nucleation and growth rate as a function of supersaturation ratio S at different concentrations of CSA in the urine pool.

▲ Coulter
■ Malvern

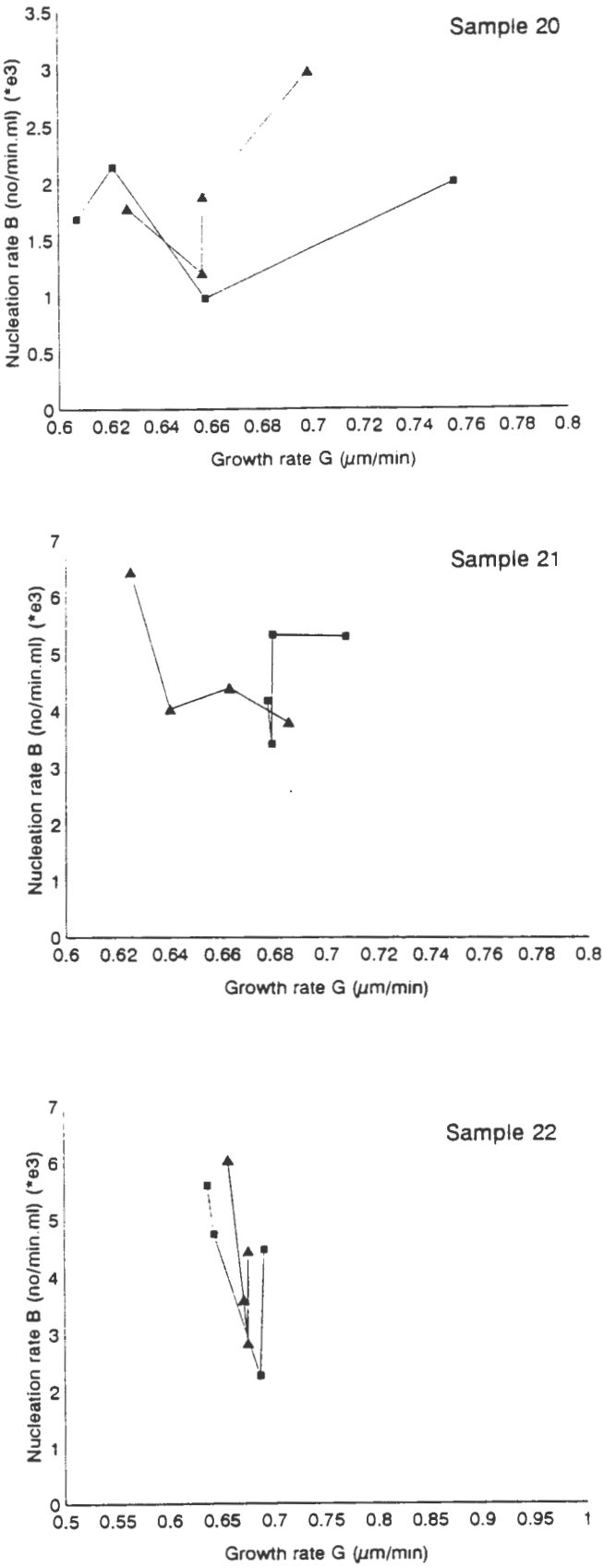
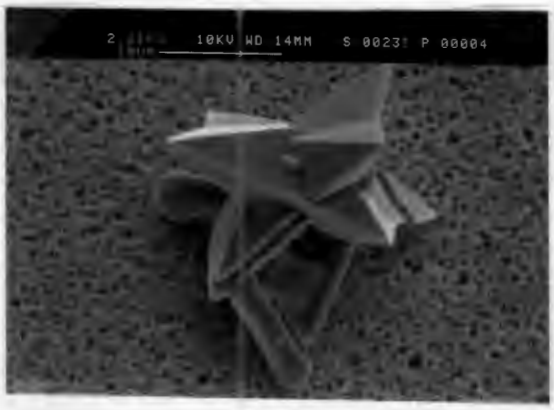


Figure 37: Nucleation rate as a function of growth rate for different concentrations of CSA in the urine pool.

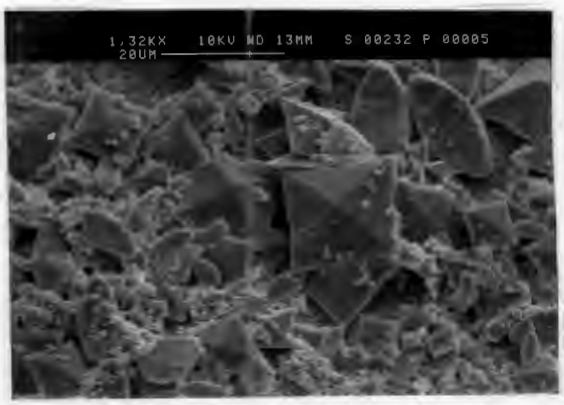
38A: Control
Large COT showing
growth into COD
mag: 1.02KX

38B: Control
Large COT
mag:925x

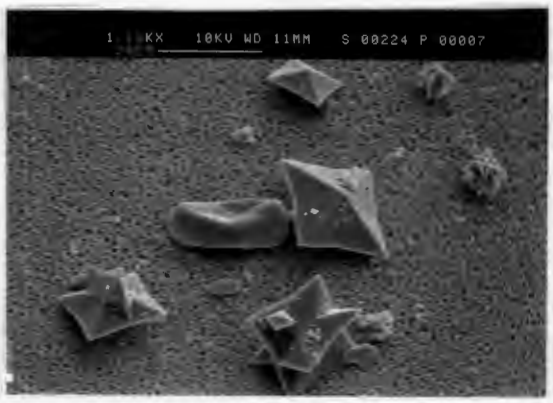


38C: Control
Intergrowth of COD
mag:2.61KX

38D: Control, 0mg/dm^3 CSA
mag: 1.08KX
max COD,COM = 15μm



38E: 16.04mg/dm^3 CSA
mag:1.32KX
max COD = 16μm, COM = 20μm



38F: 21.38mg/dm^3 CSA
mag: 1.11KX
max COD = 15μm, COM = 20μm

Figure 38: SEM micrographs of CaOx crystallisation in the presence of varying concentrations of CSA.

Inhibitor dosing via a separate feed into the crystalliser

These experiments were not performed for the following reasons: firstly, in order that this series of experiments would be performed under precisely the same conditions as the previous set (especially with respect to the ratio of whole urine to inhibitor), a large amount of CSA would have been required; secondly, such quantities of CSA were prohibitively expensive.

REFERENCES: CHAPTER IV

Bayvel,L.P.; Jones,A.R.
"Electromagnetic scattering and its applications"
London. Applied Science (1981), 289

Berg,C.; Tiselius,H.G.
"The effects of citrate on hydroxyapatite induced Calcium Oxalate crystallisation and on the formation of Calcium Phosphate crystals"
Urol.Res. (1989), **17**, 167-172

Brecevic,L.; Garside,J.
"On the measurement of crystal size distributions in the micrometer size range"
Chem.Eng.Sci. (1981), **36**, 867-869

"Coulter Multisizer manual (software level 2.1 and 3.1) Issue C", Coulter Electronics Ltd, England, (May 1987)

de Boer,G.B.J.; de Weerd,C.; Thoenes,D.; Goossens,H.W.J.
"Laser diffraction spectrometry: Fraunhofer versus Mie scattering"
Part.Charact. (1987), **4**, 14-19

Felton,P.G.
"A review of the Fraunhofer diffractions particle sizing technique"
in: "Liquid particle size measurement techniques" 2nd Vol. eds: Hirleman,E.D.; Bachelo,W.D.; Felton,P.G.; American society for testing and materials. Philadelphia (1990), 47-59

Hesse,A.; Classen,A.; Knoll,M.; Timmerman,F.; Vahlensieck,W.
"Dependence of urine composition on the age and sex of healthy subjects"
Clin.Chim.Acta (1986), **160**, 79-86

Hounslow,M.J.; Ryall,R.L.; Marshall,V.R.
"A discretized population balance for nucleation, growth and aggregation"
AIChE.J. (1988), **34** (11), 1821-1832

Hounslow,M.J.
"A descretized population balance for continuous system at steady state"
AIChE.J. (1990a), **36** (1), 106-116

Hounslow,M.J.
"Nucleation, growth and aggregation rates from steady state experimental data"
AIChE.J. (1990b), **36** (11), 1748-1752

Jappie,D.
PhD Thesis "Evaluation of the relative inhibitory or promotional potential of fractionated urinary macromolecules on calcium oxalate crystallisation processes in human urine"
Chemistry Department UCT, 1996

Kohri,K.; Garside,J.; Blacklock,N.J.
"The role of magnesium in calcium oxalate urolithiasis"
Br.J.Urol. (1988), **61**, 107-115

McCave,I.N.; Bryant,R.S.; Cook,H.F.; Coughanour,C.A.
"Evaluation of a laser diffraction-size analyser for use with natural sediments"
J.Sedimentary Petrology (1986), **56**, 561-564

"Operating manual for the Coulter Multisizer (Software level 2.1 and 3.1) Issue C" Coulter Electronics Ltd, England, (May 1987)

Randolph,A.D.; Larson,M.A.
"Theory of particulate processes"
Academic Press, New York, London (1971), 15

Ryall,R.L.; Harnett,R.M.; Marshall,V.R.
"The effect of urine, pyrophosphate, citrate and magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals in vitro"
Clin.Chim.Acta (1981), **112**, 349-356

Seymore,L.
PhD Thesis "A computer model of water-in-oil emulsion coagulation" Chemistry Department UCT, 1996

Takasaki,E.
"The magnesium:calcium ratio in the concentrated urines of patients with calcium oxalate calculi"
Invest.Urol. (1972), **10** (2), 147-150

Tietz,N.W.
"Fundamentals of clinical chemistry"
eds: Tietz,N.W.; Caraway,W.T.; Freier,E.F.; Kachmar,J.F.; Rawnsley,H.M. W.B.Saunder Co. Philadelphia, 1976, pgs: 883, 915, 996, 999

Werness,P.G.; Brown,C.M.; Smith,L.H.; Finlayson,B.
"Equil 2. A basic computer program for the calculation of urinary saturation"
J.Urol. (1985), **134**, 1242-1244

Willis,J.B.
"Determination of calcium and magnesium in urine by atomic absorption spectroscopy"
Anal.Chem. (1961), **33** (4), 556-559

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

V.1 IN VITRO EXPERIMENTS

There are two major aetiologic theories of calcium oxalate (CaOx) urinary stone formation. The first is based upon the supersaturation of urine with respect to calcium and oxalate, while the second focusses upon the presence or absence of inhibitors of CaOx crystallisation. The present work has investigated aspects of both of these.

Calcium and oxalate

Early research by Flocks (1939), showed that stone formers excreted more calcium than controls over a 24 hour period. As a result, he postulated the importance of calcium over oxalate in stone formation. This finding has been supported by various workers [Hodgkinson and Pyrah 1958; Robertson and Morgan 1972; Hodgkinson 1974; Baumann *et al.* 1977; Robertson *et al.* 1978]. However, just as many investigators have not found any difference in calcium excretion between the two groups [King *et al.* 1968; Welshman and McGeown 1975; Jorgensen 1975; Tiselius *et al.* 1978; Ryall and Marshall 1983; Ryall *et al.* 1984].

Ryall and Marshall (1990) have reviewed reports of daily urinary calcium excretion in controls and stone formers. They suggest that normal calcium excretion needs to be properly defined before classifying patients as hypercalciuric. According to these workers there is no definite link between calcium excretion in the urine and the number of stone episodes.

Investigations into the urinary excretion of oxalate by numerous authors have shown higher excretion in stone formers when compared to controls [Hodgkinson 1974; Pinto *et al.* 1974; Robertson *et al.* 1971,1976a,1978]; however, as with calcium

excretion, other studies have been unable to find a difference [Tiselius 1977; Galosy *et al.* 1980]. Contradictions are possibly due to numerous and somewhat unsatisfactory methods for oxalate determination [Robertson *et al.* 1981b]. Ryall and Marshall (1990) point out that the significance of the spontaneous conversion of ascorbate to oxalate in urinary oxalate determinations was only recognised in 1983-1984 by various workers, and that this factor would have to be taken into account when considering earlier studies.

In 1969, Robertson and Nordin found that the calcium concentration required to induce crystallisation of CaOx in urine was abnormally high whereas that of oxalate was within the normal urinary range. This has formed part of the growing evidence that a small increase in the urinary oxalate concentration is more of a risk factor than a larger increase in urinary calcium concentration in CaOx stone formation [Robertson and Nordin 1976; Robertson *et al.* 1981a; Hallson and Rose 1989].

Calcium can react with oxalate to form both an insoluble CaOx salt and a soluble non-ionised CaOx complex [Robertson and Peacock 1980]. The increase in urinary calcium concentration causes only a small increase in the urinary saturation of CaOx whereas an increase in urinary oxalate concentration produces a larger increase in CaOx saturation that could lead to crystallisation [Robertson and Peacock 1980].

Since oxalate is now commonly regarded as being a more important determinant of stone formation than calcium, crystallisation experiments were performed in the present study using the addition of oxalate rather than calcium. The concentrations of oxalate required to produce measureable crystallisation were higher than physiological urinary oxalate levels. Nevertheless, addition of high oxalate concentrations to produce crystallisation has been used previously [Kavanagh *et al.* 1993].

Factors which lead to a high urinary supersaturation of CaOx (such as an increase in calcium or oxalate concentrations) are

generally considered to be risk factors in CaOx urolithiasis. This is a consequence of supersaturation being a pre-requisite for crystallisation. However, although high supersaturation of CaOx increases the risk of crystal formation, it does not necessarily increase the risk of stone formation since small crystals can be excreted harmlessly from the renal tract.

This implies that the important mechanisms in stone formation are growth and aggregation of crystals rather than nucleation. The promotion or impaired inhibition of these two processes is therefore a risk factor in urolithiasis.

In the present study, experiments have shown that increasing concentrations of oxalate increase both the nucleation and growth of CaOx crystals. This confirms findings of Kohri *et al.* (1988) and is also in agreement with those of Randolph and Drach (1981) who reported decreasing growth and nucleation of CaOx with decreasing oxalate concentrations at constant calcium:oxalate ratio in an MSMPR crystalliser using synthetic urine.

CaOx forms 3 hydrates: the thermodynamically stable monohydrate (COM), the metastable dihydrate (COD) and the unstable trihydrate (COT) [Gardner 1975; Garside *et al.* 1982; Brecevic *et al.* 1986]. For a given set of conditions, one of these phases will represent a minimum in the free energy of the system and will therefore be thermodynamically stable [Gardner 1975; Brecevic *et al.* 1986]. Other phases appearing under the same given conditions will be metastable with respect to the stable phase.

The increase in growth and nucleation noted in the present study was confirmed by SEM which showed larger sizes and increased crystal numbers of COD as a function of increasing oxalate concentrations. The presence of COD as the predominant phase in this study (performed at 37°C) supports the findings of Dent and Sutor (1971) who showed that COD crystallisation is the critical factor in the initiation of oxalate urinary stone disease. Using an MSMPR crystalliser with artificial urine as the crystallising medium, Miller *et*

al. (1977) also found COD crystals to be the only product formed.

The present system does not allow the measurement of aggregation. SEM however, confirmed the occurrence of aggregates in some samples.

The results of the present study are therefore in agreement with the general view that an increase in the urinary oxalate concentration increases the supersaturation leading to a greater risk of stone formation through a promotion of nucleation, growth and possibly aggregation [Robertson and Nordin 1976; Robertson and Peacock 1983].

Temperature

The effect of temperature on the crystallisation of CaOx was investigated using a constant initiating concentration of NaOx of 3mmol/dm^3. The results indicate that growth rates increase and nucleation rates decrease with increasing temperature. The trends of increasing growth rates and decreasing nucleation rates with increasing temperature are in agreement with the findings of Garside *et al.* (1982). They explain these trends as being due to the presence of both stable and unstable phases of CaOx. In the present study, the favoured CaOx phases were: COT at 18°C, COD at 38°C and COM at 58°C. (COT and COD are thermodynamically unstable phases with respect to COM, and as such, have higher solubilities, therefore making them the kinetically favoured phases [Skrtic *et al.* 1987]). Using an MSMPR crystalliser with $CaCl_2$ and NaOx as the crystallising medium, Garside *et al.* (1982) found that the nucleation of COT crystals at 9°C was higher than the nucleation of COM crystals at 37°C and 45°C. In further support of the present study, Brecevic *et al.* (1989), using a batch crystalliser, found that COD crystals had a lower nucleation rate than COT crystals when precipitated from a solution of $CaCl_2$ and NaOx.

There is little variation of supersaturation with a change in temperature. This is not surprising since (a) temperature is

not taken into account in the calculation of supersaturation and (b) crystallisation is initiated in each experiment via the same NaOx concentration. Therefore, within a sample, only two variables in the supersaturation calculation change at the different temperatures: the concentration of calcium and the concentration of oxalate. These changes are dependent only upon the change in the extent to which crystallisation occurs at the different temperatures. Since the latter is small, a narrow range of supersaturation values occurs in these experiments.

The presence of different CaOx hydrates has been reported at varying levels of supersaturation by Gardner (1978) and Gardner and Doremus (1978). They found that the main growth phase at low and medium supersaturation was COT while COD was the important growth phase at high supersaturation. Although there was little change in the supersaturation in the present study, different hydrates were nevertheless observed by SEM as mentioned earlier. It is therefore concluded that the distribution of hydrates in the present system is governed by temperature.

In support of these findings are those of Gardner (1975) who noted that COT crystals precipitated first at temperatures of 10-37°C using a precipitating solution of NaOx and $CaCl_2$. Brecevic and Garside (1981) also noted the precipitation of only COT crystals from a solution of NaOx and $CaCl_2$ in an MSMPR crystalliser operating at 25°C. Using the same crystallising medium, Garside *et al.* (1982) reported that COT was the predominant phase below 25°C with COM dominating above 30°C. In addition, they showed that the nucleation of COT was greater than that of COM and concluded that the hydrate formed is determined by kinetic rather than thermodynamic factors. The suggestion by Gardner (1975) that COT undergoes a solid state transformation to COM is in contrast to the studies of Brecevic *et al.* (1986) whose batch experiments, using equal volumes of NaOx and $CaCl_2$, suggest a solution mediated process. More recently, Kavanagh *et al.* (1993) found a mixture of COT and COD crystals at 37°C in minimally diluted (92%) urine using an MSMPR crystalliser.

The mixture of hydrates obtained in the present study precipitated from weakly acidic solutions (pH 5) with total calcium and oxalate concentrations each of 10^{-3}M. The presence of hydrate mixtures is in agreement with the results of Gardner (1975) who reported that such mixtures were formed in solutions of pH 4-8 with total calcium and oxalate concentrations in the range 10^{-4}M - 10^{-2}M. (The pH and concentrations used in the present study were within these ranges).

The transformation of COT to COM is temperature sensitive; high activation energies of 188 and 109 KJ/mol for this transformation have been recorded by Gardner (1976) and Tomazic and Nancollas (1979) respectively. Skrtic *et al.* (1987) have also qualitatively demonstrated the strong temperature dependence of the COT to COM transformation process in batch experiments when they identified significant transformation within one hour. However, the time required for this transformation is much longer than the time spent by the crystals in an MSMPR crystalliser. This implies that the different crystal phases are preferentially nucleating at different temperatures which is in agreement with the results of Garside *et al.* (1982).

The conditions of steady state precipitation in the MSMPR crystalliser have been shown to produce all 3 hydrates of CaOx in the present study. It has also been shown that the hydrate precipitated is strongly temperature dependent and that formation of COT (rarely found in kidney stones) is favoured at lower temperatures and COM at higher temperatures. Furthermore, an increase in temperature causes a greater increase in nucleation and growth rates than that of an increase in the concentration of NaOx at the temperatures and concentrations used.

Citrate

The presence of citrate in urine was first reported by Amberg and McClure in 1917. An association of low citrate excretion and urinary stone formation was later shown by Boothby and

Adams (1934). Various investigators have therefore attempted to find a difference in urinary citrate excretion between stone formers and controls. Early research indicated that there was no apparent difference [Thomas and Howard 1959; Canary *et al.* 1964; Robertson *et al.* 1968,1978; Pak *et al.* 1978]. More recently however, reduced levels of citrate excretion have indeed been reported in stone formers [Welshman and McGeown 1976; Schwille *et al.* 1979,1982; Menon and Mahle 1983; Nikkila *et al.* 1989; Sakhaee *et al.* 1989; Laminski *et al.* 1990; Alaverz Arroyo *et al.* 1992; Trinchieri *et al.* 1992] thus implying the importance of hypocitraturia as a risk factor in calcium stone formation.

Citrate-to-calcium ratios have been measured by Parks and Coe (1986) and also Conte *et al.* (1989). Both studies showed a reduction in this ratio in stone formers. Hobarth and Hofbauer (1991) have also shown lower citrate excretions in recurrent stone formers when compared to single stone formers and controls. However no significant difference between the latter two groups was found. Cupisti *et al.* (1992) have measured 24 hour urinary levels of calcium, oxalate and citrate. They concluded that the most important abnormality distinguishing recurrent from single stone formers was the low excretion of citrate in the former group.

The factors responsible for decreased citrate excretion in stone formers have been investigated by Burr and Nuseibeh (1990) and also by Nicar *et al.* (1983). Burr and Nuseibeh (1990) found that the single most important factor related to hypocitraturia was low urinary potassium. They also found that variations in urinary volume significantly contributed to variations in urinary citrate and that this was linked to stone disease. Nicar *et al.* (1983) found a positive correlation between urinary citrate and urinary oxalate, calcium and magnesium. They suggest that the divalent cations, calcium and magnesium, enhance citrate excretion by complexing citrate and therefore inhibiting its reabsorption.

Citrate is known to form complexes with calcium [Hastings *et al.* 1934; Nicar *et al.* 1983; Parks and Coe 1986]. This

complexation is dependent upon urinary pH and causes a decrease in the concentration of ionic calcium [Tiselius *et al.* 1993a]. Hypocitraturia would therefore enhance the saturation of calcium salts as it would produce a greater free calcium ion concentration; the risk of CaOx crystallisation would therefore increase [Meyer and Smith 1975].

Citrate is also known to be an inhibitor of CaOx crystallisation [Meyer and Smith 1975; Hallson *et al.* 1983; Ryall *et al.* 1985; Kok *et al.* 1986; Tiselius *et al.* 1993a] *in vitro*. Inhibition is thought to occur as a result of the binding of citrate onto the growth sites on the surface of the CaOx crystals [Kok *et al.* 1986; Berg and Tiselius 1989; Grases and Costa-Bauza 1990]. The heterogeneous growth of CaOx on calcium phosphate is also counteracted by citrate [Berg and Tiselius 1989]. Ryall *et al.* (1981) showed that inhibition of CaOx growth by citrate occurred at physiological citrate concentrations. However, much higher citrate concentrations were necessary to achieve aggregation inhibition to the same extent. They therefore concluded that appreciable inhibition of CaOx aggregation by citrate at physiological concentrations was unlikely.

On the other hand, Kok *et al.* (1986,1987) have shown reduced inhibition of aggregation, but not of growth with a decreased citrate concentration. In later work, Kok *et al.* (1990) showed that inhibition of CaOx aggregation in urine from controls and stone formers seeded with COM crystals, was related to the citrate content in the urine. In support of this, Tiselius *et al.* (1993a) have shown inhibition of CaOx crystal aggregation by citrate. They have suggested that citrate might efficiently inhibit the aggregation of COM crystals [Tiselius *et al.* 1993b]. Using a suspension of COM crystals, they measured the rate of sedimentation in the presence of varying concentrations of citrate and found that at physiological concentrations, it reduced the rate of COM crystal sedimentation. They concluded that the reduced rate of sedimentation reflected an inhibition of crystal aggregation since large crystal aggregates sediment more rapidly than small ones [Hess *et al.* 1989].

On the other hand, Robertson and Scurr (1986) found weak inhibition of growth and aggregation by citrate at physiological concentrations using an MSMPR crystalliser with simulated whole urine conditions. Appreciable inhibitory activity was only noted at extremely high citrate concentrations.

The results of the present experiments indicate that, above physiological urine oxalate concentrations, citrate (at physiological urine concentrations) inhibits both the growth and nucleation of CaOx, irrespective of how the citrate is introduced into the crystalliser. The inhibition of growth was more pronounced however in experiment 1 (when the citrate was added to the urine pool prior to it being pumped into the crystalliser). The results are in agreement with those of Ryall *et al.* (1981) who found growth inhibition at physiological citrate concentrations. The results are not in agreement with those of Rodgers and Garside (1981) who found higher growth rates with increasing citrate concentration in an MSMPR crystalliser; however they used synthetic urine whereas real urine was used in the present study. Nevertheless, a decrease in growth rate is expected in the presence of citrate; Rodgers and Garside accounted for their discrepancy as being due to the increasing solution supersaturation. In contrast to Robertson and Scurr (1986), no significant increase in the inhibition of growth above physiological citrate concentrations was noted in the present study.

Nucleation inhibition occurred at all citrate concentrations in the present study, with the exception of one sample. The decrease in the nucleation rates was more pronounced at the high concentrations of citrate used in experiment 2 (when citrate was introduced into the crystalliser via a separate feed). The depressed nucleation rates confirm the results of Rodgers and Garside (1981).

Citrate also had a significantly favorable effect on CaOx supersaturation in the present study. Experiment 1 (citrate concentration range: 0 to 1.530 mmol/dm^3) indicated a 25%

reduction in the CaOx supersaturation ratio for the three samples which were tested. Experiment 2 (citrate concentration range: 1.071 to 7.140 mmol/dm^3) led to a 35% - 60% reduction in the supersaturation for the various samples. Since the present study has also shown that nucleation and growth rates both increase with increasing CaOx supersaturation, it is concluded that citrate has a favorable effect on the risk of CaOx crystallisation in urine.

The reduction in supersaturation observed in the present study is in agreement with the results of Robertson and Scurr (1986) and Tiselius *et al.* (1993a) who showed that increasing concentrations of citrate decreased the ion-activity product of CaOx. Tiselius *et al.* (1993a) found that this effect occurred in the acidic range but was more pronounced at alkaline pH. (The range of pH in the present study was 5.8 - 6.2). According to these workers, this occurs as a result of greater complex formation of citrate with calcium due to an increased dissociation of citrate at higher pH.

However, this decrease in CaOx crystallisation at alkaline pH occurs at the risk of increased calcium phosphate (CaP) crystallisation [Tiselius 1993a]. This is due to the preferential precipitation of CaP at alkaline pH. This in turn increases the risk of heterogeneous crystallisation of CaOx on CaP. The effect of citrate on this process has been investigated by Berg and Tiselius (1989). They concluded that alkaline citrate significantly inhibited CaOx growth on hydroxyapatite and also reduced the growth of CaP.

SEM in the present study showed both COM and COD as the dominant crystal phases in contrast to Rodgers and Garside (1981) who, using XRD, noted predominantly COT crystals with only small traces of COD crystals. However, these workers conducted their experiments in a synthetic urine. In support of the decreasing growth and nucleation rates with increasing concentrations of citrate, SEM showed that both the total number of crystals and their size (COD) decreased. An increase in the number and size of COM crystals (<10μm) when the citrate was added to the pooled urine was also noted. The

increase in the number of smaller crystals together with the decrease in the size of COD crystals with increasing citrate concentration in the present study is in support of the findings of Tiselius et al. (1993a). They found that increasing concentrations of citrate shifted their size distributions towards smaller crystals.

Aggregation of COD and COM crystals was only observed in the control samples to which citrate had not been added, implying that citrate inhibited aggregation. This is in support of results reported by Tiselius *et al.* (1993a,b), Kok *et al.* (1986,1987,1990), Grases and Costa-Bauza (1990) and also Robertson and Scurr (1986).

The altered morphology of the COD crystals obtained in the presence of citrate (and sometimes magnesium) in this study (Chapter IV, Figure 17E) has also been reported by Werness *et al.* (1981b) in the urines of patients with urolithiasis. They suggest that this change in crystal morphology from the typical tetragonal bipyramidal (ie octahedral) habit (to a twinned crystal) may be due to the presence or absence of inhibitors. (The altered morphology arises from the twinning of four crystals to form a single crystal with a four-fold rotational symmetry).

The results of the present study have therefore demonstrated the inhibitory potential of citrate on both the nucleation and growth of CaOx, and also its ability to significantly reduce the supersaturation with respect to CaOx.

Magnesium

Interest in the inhibitory role of magnesium in CaOx stone formation can be traced back to Hammarsten (1929) [cited by Takasaki *et al.* 1972]. Shortly thereafter, Cramer (1932) [cited by Resnick *et al.* 1982a] demonstrated experimentally that dietary magnesium restriction induced urolithiasis in rats. Further work using animals has shown that stones induced by magnesium and vitamin B_6 deficiency can be prevented by dietary magnesium supplementation [Heaton and

Anderson 1965; Lyon *et al.* 1966; MacManus and Heaton 1969; Rushton and Spector 1982].

Unlike citrate however, most studies have been unable to show lower magnesium excretion in stone formers. Various investigators have reported no difference in 24 hour magnesium excretion between stone formers and controls [Welshman and McGeown 1975; Robertson *et al.* 1978; Johansson *et al.* 1980a; Bach *et al.* 1981]. However more recently, Trinchieri *et al.* (1992) have shown magnesium excretion was significantly reduced in their stone formers.

Investigators have attempted to explain the inhibitory role of magnesium as a function of the ratio of magnesium to calcium concentration [Takasaki 1972; Johansson *et al.* 1980b]. Indeed, Johansson (1982) lists numerous papers reporting low magnesium:calcium ratios in stone formers when compared to controls. They found that this ratio declined with increasing calcium values for both groups, but was lower in stone formers than in controls for corresponding calcium values.

The role of magnesium in CaOx crystallisation processes is unknown. Depending upon its concentration, it can either promote or inhibit growth [Angell and Resnick 1987]. At physiologic concentrations, it promotes growth but inhibits aggregation [Ryall and Marshall 1981]. As far as CaOx stone *prevention* is concerned, it is thought that magnesium plays a dual role [Resnick *et al.* 1982a]. Firstly, *in vitro* studies have shown that magnesium can increase the solubility of CaOx by forming soluble complexes with oxalate [Hammersten (1929) cited by Kohri *et al.* 1988; Miller *et al.* 1958; Elliot *et al.* 1965;]. Secondly, the inhibitory effect of magnesium on CaOx crystallisation appears to be related to adsorption onto the crystal surface thereby preventing growth through competition for a lattice position [Smith *et al.* 1973]. The first effect is important, as a decrease in urinary magnesium would suggest an increase in urinary oxalate levels and, as already discussed, a small increase in this parameter is more effective in increasing CaOx supersaturation than a similar increase in urinary calcium concentration.

The effect of physiological concentrations of magnesium on CaOx crystallisation has been investigated *in vitro* using artificial and real urine. In most cases, magnesium has been shown to decrease the formation of CaOx [Randolph and Drach 1981; Hallson *et al.* 1982; Li *et al.* 1985; Robertson and Scurr 1986; Kohri *et al.* 1988; Rodgers and Wandt 1991]. However, neither Robertson *et al.* (1973) nor Tiselius and Fornander (1981) could find any inhibition of CaOx growth in the presence of magnesium. In addition, Robertson *et al.* (1973) did not observe inhibition of CaOx aggregation in the presence of magnesium.

Desmars and Tawashi (1973), investigated the effects of physiological concentrations of magnesium on CaOx crystallisation in a pH adjusted saline solution of $CaCl_2$ and oxalic acid. They found that within the pH range 4.5 - 8.2, magnesium was a powerful inhibitor of COM crystal growth. They also found that alkalinization favours the growth of CaOx.

Ryall et al. (1981) used several inhibitors (including magnesium) to determine their effect on the growth and aggregation of CaOx seed crystals using a Coulter Counter. They found that magnesium at concentrations between 10^{-7}-10^{-4} mol/dm^3 promoted growth while concentrations of 10^{-3} mol/dm^3 inhibited growth. They also noted an increase in aggregation inhibition in the presence of magnesium at concentrations between 10^{-6}-10^{-3} mol/dm^3.

Various investigators have studied the role of magnesium on CaOx crystallisation in synthetic urine using an MSMPR crystalliser [Li *et al.* 1985; Robertson and Scurr 1986; Kohri *et al.* 1988]. Li *et al.* (1985) found a reduction in the nucleation rate at magnesium concentrations above 2 mmol/dm^3 and a reduction in the growth rate above 3 mmol/dm^3. Meanwhile Robertson and Scurr (1986), found that magnesium inhibited both the nucleation and growth of CaOx at magnesium concentrations above the physiological level but that no inhibition of aggregation occurred at any concentration of magnesium. Kohri *et al.* (1988) confirmed the findings of Li

et al. (1985) by showing that magnesium inhibited both the nucleation and growth of CaOx at physiological concentrations of oxalate and magnesium. However, they found that CaOx growth increased with increasing magnesium concentrations at high oxalate concentrations.

The results of the present study suggest that increasing the magnesium concentration retards the growth of CaOx at oxalate concentrations above the physiological range. The effect occurred irrespective of the manner in which the magnesium was introduced into the crystalliser. The decrease in the growth rate of CaOx was however greater in experiment 1 (when the magnesium was introduced into the pooled urine) rather than experiment 2 (when the magnesium was introduced into the crystalliser via a separate feed). This was also observed for citrate.

No comparison can be made of the present results with those of Robertson and Scurr (1986) due to the large range of magnesium concentrations used (10^{-4}-10^{-2} mol/dm^3) in their study relative to the smaller range used in the present study (0 - 6.607 mmol/dm^3). Comparisons can however be made with Kohri *et al.* (1988) and Li *et al.* (1985) both of whom used magnesium concentrations in the range 0.5 - 15 mmol/dm^3.

The inhibition of growth found in the present study is in agreement with the results of Li *et al.* (1985) but is contrary to the findings of Kohri *et al.* (1988). The latter showed growth promotion at the same oxalate concentration of 2.5 mmol/dm^3 as that used in this study. This concentration is in the middle of the range (0.6 - 3.6 mmol/dm^3) investigated by Kohri *et al.* (1988) and occurs where the effect of increasing magnesium concentration changes from an inhibitory to a promotory role in their study. It is possible therefore that the trends reported by these workers might also have occurred in the present system if oxalate concentrations greater than 2.5 mmol/dm^3 had been employed.

In agreement with Kohri *et al.* (1988) and Robertson and Scurr (1986), the nucleation rate in the present study decreased at

all concentrations of magnesium in experiment 2. However, experiment 1 indicated a promotion of nucleation above a magnesium concentration of 2.5mmol/dm^3. The increase in supersaturation that occurs during the rapid phase of crystal growth is relieved by an increase in the nucleation of crystals and possibly explains the observed promotion of nucleation [Robertson and Scurr 1986].

Like citrate, magnesium also had a favorable effect on the CaOx supersaturation. Experiment 1 (magnesium concentration range: 0 to 6.607 mmol/dm^3) indicated a reduction of 27% in the supersaturation in the three samples. Experiment 2 (magnesium concentration range: 1.927 to 7.708 mmol/dm^3) led to a reduction of 26% to 37% in the supersaturation in the three samples. The decrease in supersaturation with increasing magnesium concentrations is in agreement with the findings of Robertson and Scurr (1986) and of Kohri *et al.* (1988) and is due to complexation of oxalate by magnesium ions.

Both the trends in growth and nucleation rates with supersaturation appear to be in agreement with Kohri *et al.* (1988). The growth rate increased with increasing supersaturation irrespective of how the magnesium was introduced. The nucleation rate however, decreased with increasing supersaturation in experiment 1 and increased with increasing supersaturation in experiment 2. The decrease in nucleation with increasing supersaturation in experiment 1 is unexpected. However, it is noted that Kohri *et al.* (1988) also found a decrease in nucleation at supersaturations above 60.

No obvious trends between nucleation and growth rates occurred. However, it is possible that the nucleation rate peaks at growth rates between 0.65 and 0.75. On the other hand, the nucleation rate appears to correlate with the inverse of the growth rate at growth rates above 0.75. This is in agreement with the results of Kohri *et al.* (1988).

SEM in the present study indicated mostly COD crystals, which is in agreement with the observations of Kohri *et al.* (1988) who suggested that at high magnesium concentrations, a high supersaturation may encourage the formation of the CaOx dihydrate rather than the less stable trihydrate. The absence of COT crystals in the present study is supported by the findings of Li *et al.* (1985) who reported that magnesium decreases the nucleation and growth rates of COT. It is also known that urinary trace elements, in particular magnesium, stabilize COD [Rodgers and Wandt 1991].

The results of the present study have shown that magnesium inhibits both the nucleation and growth of CaOx. The ability of magnesium to reduce the risk of stone formation by lowering the supersaturation with respect to CaOx has also been demonstrated.

Citrate and magnesium

A comparison of the individual effects of citrate and magnesium additions on the same sample indicates that citrate has a greater inhibitory effect on both the growth and nucleation of CaOx. This effect was noted in both cases where the inhibitor was either added to the pooled urine prior to being admitted to the crystalliser (experiment 1) or where it was incorporated via a separate feed (experiment 2). However, one sample indicated that magnesium lowered the nucleation rate more than citrate in experiment 2.

Both citrate and magnesium individually reduced the level of supersaturation from that of the control sample in experiment 1. Since the concentrations of citrate and magnesium were not the same in the two experiments, no conclusion can be made as to which inhibitor had a greater effect on the supersaturation. However, Tiselius (1991) has reported that citrate was more efficient than magnesium in lowering the ion activity product of CaOx.

SEM showed that in the presence of citrate or magnesium alone (in both experiments) COD was the predominant phase; varying

amounts of COM crystals were also observed. COT and the intergrowth of crystals were only present in the control samples which did not contain any added citrate or magnesium. The absence of COT crystals in the presence of magnesium is in agreement with the observations of Li *et al.* (1985) and Kohri *et al.* (1988) and suggests that COD crystals are preferentially formed in the presence of citrate and also magnesium.

Lower concentration mixture (experiment 2)

When a mixture of citrate and magnesium was used in concentrations at 50% of their original values, growth and nucleation rates in two of the three samples were found to be lower than those recorded for citrate and magnesium alone.

SEM supported the results of the kinetic experiments; on those occasions when SEM indicated a slight decrease in the number of small crystals compared to that occurring in the presence of citrate or magnesium alone, the corresponding nucleation rates from the kinetic experiments were lower, and visa versa. SEM showed that the addition of both citrate and magnesium produced COD as the predominant phase with varying amounts of COM crystals.

Higher concentration mixture (experiments 1 and 2)

When the concentration of citrate and magnesium in the mixture was doubled (so that they were the same as those when they were individually investigated), further decreases were observed in the growth rate in all six samples for the two experiments. For experiment 2 however, this decrease was not as pronounced as the decrease using the lower concentration mixture in the same experiment.

The nucleation rates for all six samples in the two experiments were generally lower than those when magnesium alone was present. The nucleation rates were lowered with respect to the nucleation rates in the presence of citrate only one of the three samples in experiment 2 (but not in

experiment 1). This indicates that a combination of citrate and magnesium is more beneficial than using either one alone.

In experiment 2, both instruments showed that the nucleation rate decreased in one sample, showed contradictory results in another sample, and showed an increase in the third sample compared to the lower concentration mixture.

SEM revealed that the mixture in experiment 1 caused a reduction in the size of COD crystals relative to those of the control and individual citrate and magnesium in experiment 1. This evidence supports the kinetically observed decrease in the growth rates for the mixture compared to the growth rates of the control and also those of the samples involving citrate and magnesium alone. As was the case with the lower concentration mixture, SEM supported the results of the kinetic experiments; on those occasions when SEM indicated a decrease in the number of small crystals compared to citrate or magnesium alone, the corresponding nucleation rates from the kinetic experiments were lower than those of the latter, and visa versa.

SEM revealed that the mixture in experiment 2 caused a reduction in the number of large crystals with a corresponding small reduction in the number of small crystals. This supports the experimentally observed decreasing growth and nucleation rates for the mixture when compared to those obtained in experiments involving citrate and magnesium individually.

Comment

The lower inhibitory power of the higher concentration mixture implies that the inhibitory effect on both nucleation and growth of a combination of citrate and magnesium is greater at lower concentrations of the two inhibitors.

The decreases in both the growth and nucleation rates with a combination of citrate and magnesium is in agreement with the results of Werness *et al.* (1981a). Using hydroxyapatite

seeded crystal growth experiments they showed that the inhibitory activity of citrate and magnesium was additive, albeit in calcium phosphate and not CaOx crystallisation.

Chondroitin sulphate A

High molecular weight molecules (macromolecules) are present in urine and urinary stones [Rodgers and Jappie 1996] and include glycoproteins (eg Tamm-Horsfall mucoprotein, nephrocalcin [Hess 1991]) and glycosaminoglycans (GAGs).

GAGs are polysaccharide chains composed of repeating disaccharides of identical compositions [Cao *et al.* 1993]. In total there are 7 types of GAG, which are all sulphated except for hyaluronic acid [Gianotti *et al.* 1989]. Several of these are present in urine occurring in both a free form or in association with proteins such as glycoproteins [Cao *et al.* 1993].

Most studies have ascribed a major portion of the inhibitory activity of urine in *in vitro* experiments to these urinary GAGs [Robertson *et al.* 1976b; Bowyer *et al.* 1979; Ryall *et al.* 1981; Resnick *et al.* 1982b; Scurr and Robertson 1986; Edyvane *et al.* 1987; Shum *et al.* 1988; Ryall *et al.* 1991; Gohel *et al.* 1992] while other studies have shown urinary GAGs to be promoters of CaOx crystal nucleation [Hallson and Rose 1979; Drach *et al.* 1982; Rose and Sulaiman 1982,1984; Rodgers *et al.* 1993].

It has been suggested that most of the inhibitory activity of GAGs is due to chondroitin sulphate (CS) [Bowyer *et al.* 1979; Ryall *et al.* 1981]. (CS occurs as a number of isomers with "A" and "C" differing in the positioning of the sulfate on carbon 4 and carbon 6 respectively). CS (in both A and C forms) is the most predominant GAG found in urine and constitutes about 55% of the total GAG content [Nishio *et al.* 1985]. This has led to investigations of whether urinary GAG excretion is lower in stone formers relative to normals. Results have been conflicting with some authors confirming lower excretions in stone formers [Robertson *et al.* 1978,1984]

while others have failed to find a difference [Sallis *et al.* 1981; Samuell 1981; Ryall and Marshall 1983; Ryall *et al.* 1987; Hwang *et al.* 1988]. Trinchieri *et al.* (1992) even report *higher* GAG excretion in their stone formers compared to their controls. They suggest this could be due to the higher urine volumes and protein intakes of their stone formers. Meanwhile, Gianotti *et al.* (1989) investigated whether it is more important to consider 24 hour GAG excretion or 24 hour GAG concentration and showed significant differences in both of these between their stone formers and their controls.

The inhibitory potential of GAGs has been ascribed to either their degree of sulphation [Foye *et al.* 1976] or to their polyvalent anion structure [Sallis and Lumley 1979]. The basis for the former lies in the belief that acidic sulphates have a strong affinity for calcium ions. Of particular interest is that both hyaluronic acid and heparan sulphate are the main GAGs in the matrix component of urinary calculi, while CS is absent [Nishio *et al.* 1985; Roberts and Resnick 1986]. This implies that the former are promoters and the latter is an inhibitor of CaOx stone formation.

The second approach is supported by Fellström *et al.* (1986) who have shown that the differences in the charge densities of the polyanions can explain the differences in their inhibitory activities. They found that the polyanions with the highest charge density (heparin and pentosan polysulphate) produced the strongest inhibition of CaOx crystal growth while weaker inhibition was found with CS and Tamm-Horsfall glycoprotein.

On the other hand, Angell and Resnick (1989) obtained different results to those of Fellström *et al.* (1986). These workers showed that the binding affinity (of the GAGs tested) for CaOx decreased in the order: heparin > hyaluronic acid > CS > pentosan polysulphate. More recently, Suzuki *et al.* (1994) showed that CS is included into CaOx crystals only in the absence of heparan sulphate. They concluded that the selective inclusion of GAGs into crystals and stones is a function of their relative binding affinity rather than of their concentration. They further concluded that heparan

sulphate and CS compete for specific binding sites on the crystal surface.

Both seeded [Robertson *et al.* 1973; Fellström *et al.* 1986; Kok *et al.* 1988; Shirane *et al.* 1990] and MSMPR crystalliser systems [Robertson and Scurr 1986; Kohri *et al.* 1989] have been used to show inhibition of CaOx growth and aggregation by GAGs. In 1986, Springmann *et al.* investigated the effect of urine on the aggregation of CaOx crystals in a flow system using an MSMPR crystalliser with an on-line Couette agglomerator. The agglomerator was continuously seeded with crystals generated in the MSMPR crystalliser and a particle size distribution (PSD) of the product from the agglomerator was measured using a zone-sensing particle counting analyser. Using a synthetic urine control sample and samples containing 5% volume in volume of urine from controls and stone formers, they found that stone formers had a greater number of large particles compared to controls. The effect of GAGs was not tested in their system. Nevertheless, they proposed that the lowered aggregation inhibition found in the stone formers was due to a deficiency of high molecular weight macromolecules.

Shum and Gohel (1993) point out that GAGs tested in crystallisation studies are commercially prepared from the tissues of origin [eg Kohri *et al.* 1989] and contain CS isomers and heparin. The GAGs found in urine consist mainly of CS and heparan sulphate. Therefore they argue against using commercially available GAGs but recognise that until preparatory scale separation of these GAG classes is achieved, commercially available GAGs will have to be used to represent urinary GAG activities.

Consensus on the role of CS in CaOx growth and nucleation has not been achieved. In some studies it has been shown to inhibit growth [Crawford *et al.* 1968; Robertson *et al.* 1973; Ryall *et al.* 1981; Robertson and Scurr 1986], while others [Gjaldbaek 1982; Ryall *et al.* 1991] have failed to confirm its inhibitory role. Pak *et al.* (1979) and Kohri *et al.* (1989) have shown it inhibits nucleation while Robertson and Scurr (1986) found promotion of nucleation.

Rodgers *et al.* (1994) investigated the role of chondroitin sulphate A (CSA) in ultrafiltered urine. Using turbidimetry and Malvern particle size analysis, they compared crystallisation in macrofiltered and ultrafiltered samples and observed a promotory effect for urinary macromolecules (UMM). However, this effect was not ascribed specifically to CSA since the latter was found to decrease the crystallisation rate, crystal numbers and crystal sizes of CaOx. They therefore suggested that CSA was an inhibitor of both CaOx nucleation and growth. Unlike the present study, their results were obtained in ultrafiltered samples in which UMM > 10kD had been removed.

Despite the lack of consensus concerning the role of CS in CaOx nucleation and growth, many investigators have agreed that CS is an inhibitor of CaOx aggregation [Bowyer *et al.* 1979; Ryall *et al.* 1981; Gjaldbaek 1982; Robertson and Scurr 1986; Scurr and Robertson 1986; Rodgers *et al.* 1994]. Using concentrations of CS in 1% urine, Ryall *et al.* (1981) concluded that a major portion of the inhibitory effect of urine on crystal aggregation could be ascribed to CS by comparing the inhibition in the presence of CS to that in the presence of normal 1% urine. It's effect on growth, however, was suggested as being due to the synergistic effects of other urinary constituents. Later work by Ryall *et al.* (1986,1991) highlighted the need to conduct inhibition experiments in whole urine. Contrary to their previous reports, they concluded in 1991 that CS was unlikely to contribute a significant proportion of the inhibitory activity of CaOx crystallisation *in vitro*.

Using an MSMPR crystalliser with artificial urine as the crystallising medium, Robertson and Scurr (1986) observed weak inhibition of growth and aggregation and a small decrease in crystal mass due to CSA. Above physiological concentrations however, there was an increase in inhibition of growth and aggregation with a promotion of nucleation. This latter effect, which had also been noted by Resnick *et al.* (1982b) in their study of UMM's on CaOx crystallisation, occurs as a

result of the need to relieve the increase in supersaturation during the rapid phase of crystal growth.

Contrary to these findings, Kohri *et al.* (1989), also using an MSMPR crystalliser and artificial urine, found that CSA decreased the nucleation rate and increased both the growth rate and suspension density.

The experiments in the present study showed little change in the growth rate with increasing concentrations of CSA; however there was a sharp decrease in the nucleation rate. The latter finding is therefore in agreement with the results of Kohri *et al.* (1989). A possible explanation for the small change in growth rates may lie in the small concentration range of CSA used in this study. If a relative molecular mass of 50,000 is assumed, then the concentration range used in this study was $2x10^{-7}$ - $4x10^{-7}$ mol/dm^3 which falls into a narrow band compared to the ranges of 10^{-6}-10^{-8} mol/dm^3 used by Kohri *et al.* (1989) and 10^{-9}-10^{-5} mol/dm^3 used by Robertson and Scurr (1986). The range used corresponded to that occurring for GAGs in normal urine (as stated earlier on page 201)

An increase in the CaOx supersaturation can increase the rate of nucleation and growth of CaOx crystals. In the present study, the small variation in the growth rate is reflected in the small change in supersaturation with increasing concentrations of CSA. In fact, the results indicate an increase in supersaturation with increasing CSA concentration. This latter effect possibly explains the small increase in the growth rates noted.

SEM revealed no change in the size of COD but a small decrease in the size of COT with increasing concentrations of CSA. This suggests that growth might be inhibited by increasing the concentration of CSA. Meanwhile, the observation that tiny crystals (<5μm) were absent (ie only large crystals were noted) in the sample containing the highest concentration of CSA confirms the increasing growth rates. The decrease in the total number of crystals with increasing concentrations of CSA confirms the decreasing nucleation rates observed in the crystallisation experiments.

Aggregation was not observed at any of the concentrations of CSA thereby supporting the widely held view that CSA is an inhibitor of CaOx aggregation. There was no change in the morphology of COM and little change in that of COD with increasing CSA concentrations. It is interesting to note that Shirane *et al.* (1994) also observed that, in the presence of chondroitin sulphate C (CSC), there was no change in the morphology of COM.

The present study has attempted to investigate the individual role of CSA which is the most abundantly occurring GAG in urine. At the concentrations tested, the study was unable to conclusively show the role of CSA with respect to its effect on the growth of CaOx. Similarly CSA had little effect on the supersaturation of CaOx. However, the inhibitory effect of CSA on the nucleation of CaOx was conclusive. Although the present system is unable to measure aggregation, the SEM observations suggest that CSA is indeed a possible inhibitor of this process.

Concluding remarks

It is generally thought that citrate and magnesium exert their effects primarily through their ability to complex with calcium and oxalate respectively and thereby reduce the level of supersaturation of CaOx. In the present study these inhibitors were shown to be active within their normal physiological concentrations. Citrate was found to be a stronger inhibitor of both CaOx growth and nucleation than magnesium. Combining the two inhibitors had a favorable inhibitory effect on both the growth and nucleation within and above physiological concentrations of the two inhibitors. This indicates that a combination of magnesium and citrate is a stronger inhibitor than either citrate or magnesium alone.

Both citrate and magnesium are well defined molecules and their urinary levels are easily manipulated by prophylactic treatment [Fetner *et al.* 1978; Johansson *et al.* 1980a; Hesse *et al.* 1992; Pak *et al.* 1992; Abdulhadi *et al.* 1993; Hofbauer *et al.* 1994]. The inhibitory roles of both citrate and

magnesium on CaOx crystallisation and their effect in increasing CaOx solubility as shown in the present study are therefore important.

CSA on the other hand inhibited nucleation without a significant reduction in supersaturation. Since it is likely that GAGs act by adsorbing onto the surfaces of the forming crystals creating an energy barrier to both crystal growth and aggregation [Leal and Finlayson 1977; Brockis *et al.* 1980; Fellström *et al.* 1985] rather than by forming soluble complexes, the small reduction in supersaturation is expected. No trend in the growth rate with increasing CSA concentration was indicated in the present study. However, results from the SEM suggest that with increasing concentrations of CSA, inhibition of growth might occur.

As discussed by Rodgers *et al.* (1994), the charge density and degree of sulphation of CSA can influence the role played in the crystallisation of CaOx [Fellström et al. 1986]. In that light, it is more meaningful to use CS fractions isolated from fresh human urine than to use commercially prepared samples from different sources. Nevertheless, information can be gained from the use of commercial preparations of CS (as in the present study); indeed, they have been used by many workers in previous studies [Ryall *et al.* 1981,1986,1991; Fellström *et al.* 1986; Grases *et al.* 1988,1989; Kohri *et al.* 1989; Shum and Gohel 1993; Rodgers *et al.* 1994].

Synergism is likely to be an important factor in determining the role of urinary macromolecules, and as such, the role of CS might differ in the presence and absence of other urinary macromolecules. This might explain the reasons for the differing roles of CS proposed by various workers. Although the present study of the role of CSA was conducted in a macrofiltered urine sample in which most of the urinary macromolecules were present, cognizance must be taken of the absence of Tamm-Horsfall mucoprotein which might have influenced the ultimate conclusions.

V.2 COMPARISON OF THE MALVERN AND COULTER TECHNIQUES

The present study has revealed that the Malvern Multisizer has several advantages over the Coulter Counter for particle aize distribution analysis. These are the following.

1. The Coulter Counter has apertures that can become blocked; the Malvern Multisizer has no apertures.

2. The Coulter Counter requires sample matching with a suitable electrolyte; the Malvern has no such requirement.

3. The Coulter Counter requires expensive calibration standards which change their size in distilled water and electrolyte [Allen 1992] leading to the potential introduction of small errors in the calibration. The Malvern instrument does not require calibration as it is based on the fundamental physical properties of light scattering. It uses conventional Fourier optics to transform the scattered light incident on the receiver lens into a diffraction pattern which ultimately provides a particle size distribution.

4. The Malvern method is non-destructive and non-intrusive because the light intensity and wavelength are too low to damage the particles. Samples can thus be recovered. In the Malvern, samples can be measured in-situ; therefore they do not have to be isolated from their natural environments. On the other hand, the Coulter Counter involves immersion of the aperture in the sample to be measured; this has been shown to disrupt fragile aggregates [Gibbs 1982; Gibbs and Konwar 1982].

5. Fast and precise measurements (requiring one second) can be achieved with the Malvern, while the Coulter Counter requires a minimum of 7 seconds for a measurement (if no blockage or other complicating factors occur).

6. In the Malvern, the entire sample is measured (ie all the particles pass through the laser beam); thus the diffraction

pattern is representative of all the particles. Continuous measurement allows temporal changes in the particle characteristics to be determined. However, the Coulter Counter measures only a small volume of the sample and an average of a number of measurements yields the closest to a continuous measurement. (In practice, a continuous measurement is not a viable option because too much sample would be required).

7. The Malvern is more versatile as it can be used with different types of samples that do not need to be conducting (or do not need to be compatible with a conducting medium).

8. The Malvern is easier to operate than the Coulter Counter due to pre-programmed commands available in the Easy Mode operation.

9. In the Malvern, a volume distribution is generated automatically; furthermore, if the density is constant, the volume distribution is equivalent to the weight distribution. The Coulter Counter on the other hand calculates a number distribution which can be transformed to a volume distribution.

10. The lower limit of detection of the Coulter Counter is higher than that of the Malvern.

11. Dense or large particles are difficult to force through the aperture of the Coulter Counter. Such a problem does not arise in the Malvern.

Both the Coulter Counter and the Malvern Multisizer have a wide size range. However extending the available range on both machines is time consuming. For the Coulter Counter, this would involve changing the orifice tube while for the Malvern it involves changing the lens. In both cases either re-calibration or re-focussing respectively is necessary; this is not always possible in kinetic measurements when time is a limiting factor.

The major advantage of the Coulter Counter is that it counts actual praticle numbers, thereby allowing the population density to be determined. The actual number density cannot be determined by the Malvern.

Another advantage that the Coulter Counter has over the Malvern Multisizer is that data in the former are manipulated more easily. In the Malvern instrument, complex mathematical programming is needed. Both techniques are highly reproducible.

Experimental work using both Malvern and Coulter principles has recently been performed. Davies and Collins (1988) compared the size distribution of boron powders using both instruments and concluded that the Malvern yielded larger sizes by as much as 30%. The present study supported this finding with the Malvern indicating larger sizes than the Coulter Counter by 15%. In their studies of clays, Agrawal *et al*. (1991) reported difficulties in comparison of monomodal results from the two techniques. On the other hand, Garside and Brecevic (1981), working on CaOx crystallisation in inorganic solutions, demonstrated good agreement in nucleation and growth rates as determined by the two instruments.

In the *in vitro* experiments of the present study, trends in the nucleation and growth rates for the two techniques showed good aggreement. In general, individual results showed agreement but values calculated from the Coulter Counter data were sometimes larger than those calculated from the corresponding Malvern measurements. Again, these elevated nucleation and growth rates can be attributed to possible crystallisation during the time delay associated with measurements using the Coulter Counter.

Chigier (1984) has reviewed the difficulties of comparing data obtained from more than one instrument. He points out that measurements should be made simultaneously in the different instruments. If this is not possible then separate independent monitoring is needed to ensure that the conditions within the measurement volume are on average identical when taking consecutive readings.

V.3 CONCLUDING COMMENTS

In concluding this thesis, there are two questions to be addressed:

(i) to what extent have the objectives been achieved?
(ii) has this project made a contribution to existing knowledge in urolithiasis research?

The first two objectives dealt with the design, construction and development of an MSMPR crystalliser and a thermostatted flow cell. Tests using the Coulter Counter showed that the MSMPR crystalliser conformed to the requirements necessary to satisfy fundamental MSMPR crystalliser constraints. Several flow system designs were developed to allow for the withdrawal of representative samples from the crystalliser. The final flow system design enabled non-invasive continuous measurement of the sample dispersion from the crystalliser to be effected.

Coulter Counter and Malvern particle sizing were used successfully in the present study for the measurement of crystal size distributions in CaOx crystallisation experiments in undiluted urine. The on-line application of the flow cell is unique in this field of study. The use of undiluted urine in *in vitro*, MSMPR crystalliser experiments is also unique. Furthermore, the initiation of crystallisation by the addition into the crystalliser of only NaOx rather than both NaOx and $CaCl_2$, represents yet another new approach.

Individual and synergistic effects of the various inhibitors were confirmed by two different methods, thereby lending confidence to the results and highlighting the potential therapeutic value of both citrate and magnesium in preventing CaOx kidney stone disease. That the Malvern and Coulter Counter results from the *in vitro* experiments showed general agreement and further that they supported the findings of other workers, indicate that the on-line Malvern flow system represents a satisfactory model for studying crystallisation in urine.

The objectives defined for the present study have therefore been achieved.

It is suggested that the method outlined in this study for the use of an MSMPR crystalliser with an on-line Malvern Particle Sizer as a non-invasive, non-destructive sizing technique will be of benefit to other scientists involved in the study of CaOx crystallisation. In particular, CaOx crystallisation with respect to nucleation and growth kinetics; the system lends itself very well for the investigation of the effects of a wide variety of crystallisation inhibitors and promotors. This design and its successful application using minimally diluted urine, suggests that the work described in this thesis will indeed make a meaningful contribution to existing knowledge in the field of urolithiasis research.

REFERENCES: CHAPTER V

Abdulhadi,M.H.; Hall,P.M.; Streem,S.B.
"Can citrate therapy prevent nephrolithiasis?"
Urology (1993), **41** (3), 221-224

Agrawal,Y.C.; McCave,I.N.; Riley,J.B.
"Laser diffraction size analysis"
in: "Principles, methods and applications of particle size analysis" ed: Syvitski,J.P.M. Cambridge Univ. Press (1991), 119-128

Allen,T.
"Particle size measurement", Chapman & Hall, 4th edition. (1992), 414-431

Alvarez Arroyo,M.V.; Traba,M.L.; Rapado,A.
"Hypocitraturia as a pathogenic risk factor in the mixed (calcium oxalate/uric acid) renal stones"
Urol.Int. (1992), **48**, 342-346

Amberg,S.; McClure,W.B.
"Occurrence of citric acid in urine"
Am.J.Physiol. (1917), **44**, 453-462

Angell,A.H.; Resnick,M.I.
"Surface interaction between glycosaminoglycans and calcium oxalate"
J.Urol. (1989), **141**, 1255-1258

Angell,A.H.; Resnick,M.I.
"Urinary inhibitors and promoters of stone formation"
in:"Stone Disease: Diagnosis and Management" ed:Rous,S.N. Grune and Stratton Inc. (1987), 19-40

Bach,D.; Hesse,A.; Strenge,A.; Vahlensieck,W.
"Magnesium excretion in urine on condition of individual as well as standard diet in healthy controls and calcium oxalate stone formers"
in:"Urolithiasis: clinical and basic research"
eds:Smith,L.H.; Robertson,W.G.; Finlayson,B.
Plenum Press, New York, (1981), 45-49

Baumann,J.M.; Bisaz,S.; Felix,R.; Fleisch,H.; Ganz,U.; Russell,R.G.G.
"The role of inhibitors and other factors in the pathogenesis of recurrent calcium-containing renal stones"
Clin.Sci.Molec.Med. (1977), **53**, 141-148

Berg,C.; Tiselius,H.G.
"The effects of citrate on hydroxyapatite induced calcium oxalate crystallisation and on the formation of calcium phosphate crystals"
Urol.Res. (1989), **17**, 167-172

Boothby,W.H.; Adams,M.
"Occurrence of citric acid in urine and body fluids"
Am.J.Physiol. (1934), **107**, 471-479

Bowyer,R.C.; Brockis,J.G.; McCulloch,R.K.
"Glycosaminoglycans as inhibitors of calcium oxalate crystal growth and aggregation"
Clin.Chim.Acta (1979), **95**, 23-28

Brecevic,L.; Garside,J.
"On the measurement of crystal size distributions in the micrometer size range"
Chem.Eng.Sci. (1981), **36**, 867-869

Brecevic,Lj.; Krajl,D.; Garside,J.
"Factors influencing the distribution of hydrates in calcium oxalate precipitation"
J.Cryst.G. (1989), **970**, 460-468

Brecevic,Lj.; Skrtic,D.; Garside,J.
"Transformation of calcium oxalate hydrates"
J.Cryst.Growth (1986), **74**, 399-408

Brockis,J.G.; Bowyer,R.C.; McCulloch,R.K.
"Macromolecular inhibitors of calcium oxalate crystal growth and aggregation"
Scand.J.Urol.Nephrol. (Suppl) (1980), **53**, 67

Burr,R.G.; Nuseibeh,I.
"Citrate excretion in spinal cord patients"
Paraplegia (1990), **28** (8), 496-504

Canary,J.J.; Meloni,C.R.; Cline,D.; Grossman,E.
"The renal clearance of citrate in man"
Metabolism (1964), **13**, 21-30

Cao,L.C.; Boeve,E.R.; de Bruijn,W.C.; Robertson,W.G.; Schroder,F.H.
"A review of new concepts in renal stone research"
Scann.Micros. (1993), **7** (3), 1049-1065

Chigier,N.
"Comparative measurements using different particle size instruments"
in: "Liquid Particle Size Techniques" Vol 1
eds: Tishkoff,J.M.; Ingebo,R.D.; Kennedy,J.B.
Am.Soc.Testing and Materials (1984), 169-186

Conte,A.; Roca,P.; Gianotti,M.; Grases,F.
"On the relation between citrate and calcium in normal and stone-former subjects"
Int.Urol.Nephrol. (1989), **21** (4), 369-373

Cowley,D.M.; McWhinney,B.C.; Brown,J.M.; Chalmers,A.H.
"Effect of citrate on the urinary excretion of calcium and oxalate: relevance to calcium oxalate urolithiasis"
Clin.Chem. (1989), **35** (1), 23-28

Crawford,J.E.; Crematy,E.P.; Alexander,A.E.
"The effect of natural and synthetic polyelectrolytes on the crystallisation of calcium oxalate"
Aust.J.Chem. (1968), **21**, 1067

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** (1), 73-76

Davies,J.A.; Collins,D.L.
"Comparison of the size distribution of Boron powders as measured by Malvern Diffractometer and Coulter counter"
Part.Part.Syst.Charact. (1988), **5**, 116-121

Dent,C.E.; Sutor,D.J.
"Presence or absence of inhibitor of calcium oxalate crystal growth in urines of normals and of stone formers"
Lancet (1971), **2**, 775

Desmars,J.F.; Tawashi,R.
"Dissolution and growth of calcium oxalate monohydrate I. Effect of magnesium and pH"
Biochim.Biophys.Acta (1973), **313**, 256-267

Drach,G.W.; Kraljevich,Z.; Randolph,A.D.
"Effects of high molecular weight urinary macromolecules on crystallisation of calcium oxalate dihydrate"
J.Urol. (1982), **127**, 805-810

Edyvane,K.A.; Hibberd,C.M.; Harnett,R.M.; Marshall,V.R.; Ryall,R.L.
"Macromolecules inhibit calcium oxalate crystal growth and aggregation in whole human urine"
Clin.Chim.Acta (1987), 329-338

Elliot,J.S.; Eusebio,E.
"Calcium oxalate solubility: the effect of inorganic salts, urea, creatine and organic acids"
Invest.Urol. (1965), **3**, 72

Fellström,B.; Danielson,B.G.; Karlsson,F.A.; Ljunghall,S.
"Crystal inhibition: binding of heparin and chondroitin sulphate to calcium oxalate, sodium urate and uric acid crystals"
in:"Urolithiasis and related clinical research"
eds:Schwille,P.; Smith,L.H.; Robertson,W.G.; Vahlensieck,W.
New York, Plenum Publishing Co. (1985), 831-834

Fellström,B.; Danielson,B.G.; Ljunghall,S.; Wikström,B.
"Crystal inhibition: the effects of polyanions on calcium oxalate crystal growth"
Clin.Chim.Acta (1986), **158**, 229-235

Fetner,C.D.; Barilla,D.E.; Townsend,J.; Pak,C.Y.C.
"Effects of magnesium oxide on the crystallisation of calcium salts in urine in patients with recurrent nephrolithiasis"
J.Urol. (1978), **120**, 399-401

Flocks,R.H.
"Calcium and phosphorous excretion in the urine of patients with renal or ureteral calculi"
J.Amer.Med.Assoc. (1939), **113**, 1466-1471

Foye,W.O.; Hong,H.S.; Kim,C.M.; Prien,E.L.
"Degree of sulfation in mucopolysaccharide sulfates in normal and stone forming urines"
Invest.Urol. (1976), **14**, 33

Galosy,R.; Clarke,L.; Ward,D.I.; Pak,C.Y.C.
"Renal oxalate excretion in calcium urolithiasis"
J.Urol. (1980), **123**, 320-323

Gardner,G.L.
"Nucleation and crystal growth of calcium oxalate trihydrate"
J.Cryst.Growth (1975), **30**, 158-168

Gardner,G.L.
"Kinetics of dehydration of calcium oxalate trihydrate crystals in aqueous solution"
J.Colloid Int.Sci. (1976), **54** (2), 298-310

Gardner,G.L.
"Effect of pyrophosphate and phosphonate anions on the crystal growth kinetics of calcium oxalate hydrates"
J.Phys.Chem. (1978), **82**, 864-870

Gardner,G.L.; Doremus,R.H.
"Crystal growth inhibitors in human urine"
Invest.Urol. (1978), **15**, 478-485

Garside,J.; Brecevic,L.
"On the measurement of crystal size distributions in the micrometer size range"
Chem.Eng.Sci. (1981), **36**, 867-869

Garside,J.; Brecevic,Lj.; Mullin,J.W.
"The effect of temperature on the precipitation of calcium oxalate"
J.Cryst.Growth (1982), **57**, 233-240

Gianotti,M.; Genestar,C.; Palou,A.; Pons,A.; Conte,A.; Grases,F.
"Investigation of GAGs on 24-hour and 2-hour urines from calcium oxalate stone formers and healthy subjects"
Int.Urol.Nephrol. (1989), **21** (3), 281-288

Gibbs,R.J.
"Floc stability during Coulter counter analysis"
J.Sedimentary Petrology (1982), **52**, 657-670

Gibbs,R.J.; Konwar,L.
"Effect of pipetting on mineral flocs"
Enviro.Sci.Technol. (1982), **16**, 119-121

Gjaldbeak,J.Chr.
"Inhibition of chondroitin sulphate and heparin on the growth and agglomeration of calcium oxalate monohydrate crystals in vitro"
Clin.Chim.Acta (1982), **120**, 363-365

Gohel,M.D.; Shum,M.K.; Li,M.K.
"The dual effect of urinary macromolecules on the crystallisation of calcium oxalate endogeneous in urine"
Urol.Res. (1992), **20**, 13-17

Grases,F.; Costa-Bauza,A.
"Study of factors affecting calcium oxalate crystalline aggregation"
Br.J.Urol. (1990), **66**, 240-244

Grases,F.; Genestar,C.; Conte,A.; March,P.; Costa-Bauza,A.
"Inhibitory effect of pyrophosphate, citrate, magnesium and chondroitin sulphate in calcium oxalate urolithiasis"
Br.J.Urol. (1989), **64**, 235-237

Grases,F.; Genestar,C.; March,P.; Conte,A.
"Variations in the activity of urinary inhibitors in calcium oxalate urolithiasis"
Br.J.Urol. (1988), **62**, 515-520

Hallson,P.C.; Rose,G.A.
"Uromucoids and urinary stone formation"
Lancet (1979), **1**, 1000-1002

Hallson,P.C.; Rose,G.A.
"Risk factors for urinary calcium oxalate crystals as revealed by their specific enzymic assay"
Br.J.Urol. (1989), **64**, 451-457

Hallson,P.C.; Rose,G.A.; Sulaiman,S.
"Magnesium reduces calcium oxalate crystal formation in human whole urine"
Clin.Sci. (1982), **62**, 17-19

Hallson,P.C.; Rose,G.A.; Sulaiman,S.
"Raising urinary citrate lowers calcium oxalate and calcium phosphate crystal formation in whole urine"
Urol.Int. (1983), **38**, 179-181

Hastings,A.B.; McLean,F.C.; Eichelberger,L.; Hall,J.L.; Da Costa,E.
"The ionization of calcium, magnesium, and strontium citrates"
J.Biolog.Chem. (1934), **107**, 351-370

Heaton,F.W.; Anderson,C.K.
"The mechanism of renal calcification induced by magnesium deficiency"
Clin.Sci. (1965), **28**, 99

Hess,B.
"The role of Tamm-Horsfall glycoprotein and nephrocalcin in calcium oxalate monohydrate crystallisation processes"
Scann.Micros. (1991), **5** (3), 689-696

Hess,B.; Nakagawa,Y.; Coe,F.L.
"Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins"
Am.J.Physiol. (1989), **F99**, 257

Hesse,A.; Klocke,K.; Nolde,A.; Vahlensieck,W.
"Advances in medical treatment of renal stones"
Urol.Res. (1992), **20**, 90-93

Hobarth,K.; Hofbauer,J.
"Value of routine citrate analysis and calcium/citrate ratio in calcium urolithiasis"
Eur.Urol. (1991), **19**, 165-168

Hodgkinson,A.
"Relation between oxalic acid, calcium, magnesium, and creatinine excretion in normal men and male patients with calcium oxalate kidney stones"
Clin.Sci.Molec.Med. (1974), **46**, 357-367

Hodgkinson,A.; Pyrah,L.N.
"The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin"
Br.J.Surg. (1958), **46**, 10-18

Hofbauer,J.; Hobarth,K.; Szabo,N.; Marberger,M.
"Alkali citrate prophylazis in idiopathic recurrent calcium oxalate nephrolithiasis - a prospective randomized study":
Br.J.Urol. (1994), **73**, 362-365

Hwang,T.I.S.; Preminger,G.M.; Poindexter,J.; Pak,C.Y.C.
"Urinary glycosaminoglycans in normal subjects and patients with stones"
J.Urol. (1988), **139**, 995-997

Johansson,G.
"Magnesium and renal stone disease"
Acta Medica Scand. (suppl), (1982), **16**, 13-18

Johansson,G.; Backman,U.; Danielson,B.G.; Fellström,B.; Ljunghall,S; Wikström,B.
"Biochemical and clinical effects of the prophylactic treatment of renal calcium stones with magnesium hydroxide"
J.Urol. (1980a), **124**, 770-774

Johansson,G.; Backman,U.; Danielson,B.G.; Ljunghall,S.; Wikström,B.
"Magnesium metabolism in renal stone disease"
Invest.Urol. (1980b), **18** (2), 93-96

Jorgensen,F.S.
"The urinary excretion and serum concentration of calcium magnesium, sodium and phosphate in male patients with recurring renal stone formation"
Scand.J.Urol.Nephrol. (1975), **9**, 243-248

Kavanagh,J.P.; Nishio,S.; Garside,J.; Blacklock,N.J.
"Crystallisation kinetics of calcium oxalate in fresh minimally diluted urine: comparison of recurrent stone formers and healthy controls in a continuous mixed suspension mixed product removal crystalliser"
J.Urol. (1993), **149**, 614-617

King,J.S.; O'Connor,F.L.; Smith,M.J.U.; Crouse,L.
"The urinary calcium/magnesium ratio in calcigerous stone formers"
Invest.Urol. (1968), **6**, 60-65

Kohri,K.; Garside,J.; Blacklock,N.J.
"The role of magnesium in calcium oxalate urolithiasis"
Br.J.Urol. (1988), **61**, 107-115

Kohri,K.; Garside,J.; Blacklock,N.J.
"The effect of glycosaminoglycans on the crystallisation of calcium oxalate"
Br.J.Urol. (1989), **63**, 584-590

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.
"Excessive crystal agglomeration with low citrate excretion in recurrent stone formers"
Lancet (1986), **1**, 1056-1058

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.
"Low inhibition of crystal agglomeration and citrate excretion in recurrent calcium oxalate stone formers"
Contr.Nephrol. (1987), **58**, 73-77

Kok,D.J.; Papapoulos,S.E.; Bijvoet,O.L.
"Crystal agglomeration is a major element in calcium oxalate urinary stone formation"
Kid.Int. (1990), **37**, 51

Kok,D.J.; Papapoulos,S.E.; Blomen,L.J.M.J.; Bijvoet,O.L.
"Modulation of calcium oxalate monohydrate crystallisation kinetics in vitro"
Kid.Int. (1988), **34**, 346-350

Laminski,N.A.; Meyers,A.M.; Sonnekus,M.I.; Smyth,A.E.
"Prevalence of hypocitraturia and hypopyrophosphaturia in recurrent calcium stone formers: as isolated defects or associated with other metabolic abnormalities"
Nephron (1990), **56**, 379-386

Leal,J.J.; Finlayson,B.
"Adsorption of naturally occurring polymers onto calcium oxalate crystal surfaces"
Invest.Urol. (1977), **14**, 278

Li,M.K.; Blacklock,N.J.; Garside,J.
"Effects of magnesium on calcium oxalate crystallisation"
J.Urol. (1985), **133**, 123-125

Lyon,E.S.; Borden,T.A.; Ellis,J.E.; Vermeulen,C.W.
"Calcium oxalate lithiasis produced by pyridoxine deficiency and inhibition with high magnesium diets"
Invest.Urol. (1966), **4**, 133

MacManus,J.; Heaton,F.W.
"The effect of magnesium deficiency on calcium homeostasis in the rat"
Clin.Sci. (1969), **36**, 297

Menon,M.; Mahle,C.J.
"Urinary citrate excretion in patients with renal calculi"
J.Urol. (1983), **129**, 1158-1160

Meyer,J.L.; Smith,L.H.
"Growth of calcium oxalate crystals II. Inhibition by natural growth inhibitors"
Invest.Urol. (1975), **13**, 36-39

Miller,J.D.; Randolph,A.D.; Drach,G.W.
"Observations upon calcium oxalate crystallisation kinetics in simulated urine"
J.Urol. (1977), **117**, 342-345

Miller,G.H.; Vermuelen,C.W.; Moore,J.D.
"Calcium oxalate solubility in urine: experimental urolithiasis XIV"
J.Urol. (1958), **79**, 607

Nicar,M.J.; Skurla,C.; Sakhaee,K.; Pak,C.Y.C.
"Low urinary citrate excretion in nephrolithiasis"
Urology (1983), **XXI** (1), 8-14

Nikkila,M.; Koivula,T.; Jokela,H.
"Urinary citrate excretion in patients with urolithiasis and normal controls"
Eur.Urol. (1989), **16**, 382-385

Nishio,S.; Abe,Y.; Wakatsuki,A.; Iwata,H.; Ochi,K.; Takeuchi,M.; Matsumoto,A.
"Matrix glycosaminoglycan in urinary stones"
J.Urol. (1985), **134**, 503-505

Nishio,S.; Kavanagh,J.P.; Garside,J.
"A small-scale continuous mixed suspension mixed product removal crystalliser"
Chem.Eng.Sci. (1991), **46** (2), 709-711

Pak,C.Y.C.; Holt,K.; Zerwekh,J.E.
"Attenuation by monosodium urate of glycosaminoglycan on calcium oxalate nucleation"
Invest.Urol. (1979), **17**, 138-140

Pak,C.Y.C.; Holt,K.; Zerwekh,J.E.; Barilla,D.E.
"Effects of orthophosphate therapy on the crystallisation of calcium salts in urine"
Min.Elect.Metab. (1978), **2**, 147-154

Pak,C.Y.C.; Koenig,K.; Khan,R.; Haynes,S.; Padalino,P.
"Physiochemical action of potassium - magnesium citrate in nephrolithiasis"
J.Bone Min.Res. (1992), **7** (3), 281-285

Parks,J.H.; Coe,F.L.
"A urinary calcium-citrate index for the evaluation of nephrolithiasis"
Kid.Int. (1986), 30, 85-90

Pinto,B.; Crespi,G.; Sole-Balcells,F.; Barcelo,P.
"Patterns of oxalate metabolism in recurrent oxalate stone formers"
Kid.Int. (1974), **5**, 285-291

Randolph,A.D.; Drach,G.W.
"Some measurements of calcium oxalate nucleation and growth rates in urine-like liquors"
J.Cryst.Growth (1981), **53**, 195-201

Randolph,A.D.; Larson,M.A.
"Theory of particulate processes"
Academic Press, New York, London (1971)

Resnick,M.I.; Munday,D.; Boyce,W.H.
"Magnesium excretion and calcium oxalate urolithiasis"
Urology (1982a), **XX** (4), 385-389

Resnick,M.I.; Sorrell,M.E.; Bailey,J.A.; Boyce,W.H.
"Inhibitory effects of urinary calcium binding substances on calcium oxalate crystallisation"
J.Urol. (1982b), **127** (March), 568-571

Roberts,S.D.; Resnick,M.
"Glycosaminoglycans content of stone matrix"
J.Urol. (1986), **135**, 1078-1083

Robertson,W.G.; Knowles,F.; Peacock,M.
"Urinary acid mucopolysaccharide inhibitors of calcium oxalate crystallisation"
in:"Urolithiasis Research"
Plenum Press, New York, (1976b), 331-334

Robertson,W.G.; Latif,A.B.; Scurr,D.S.; Caswell,A.M.; Drach,G.W.; Randolph,A.D.
"Inhibitors of calcium oxalate crystallisation in urine from stone formers and normals"
in:"Urinary Stone" eds:Ryall,R.; Brockis,J.G.; Marshall,V.; Finlayson,B. Churchill Livingstone, Melbourne Edinburgh, London, New York, (1984), 167-172

Robertson,W.G.; Morgan,D.B.
"The distribution of urinary calcium in normal persons and stone formers"
Clin.Chim.Acta (1972), **37**, 503-508

Robertson,W.G.; Nordin,B.E.C.
Renal Stone Research Symp.
eds:Hodgkinson,A.; Nordin,B.E.C.
London, Churchill Livingstone (1969), 221

Robertson,W.G.; Nordin,B.E.C.
"Physiochemical factors governing stone formation"
in:"Scientific Foundations of Urology" eds:Williams,D.I.; Chisholm,G.D. London, Heinemann (1976), **1**, 254-267

Robertson,W.G.; Peacock,M.
"The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria?"
Nephron (1980), **26**, 105-110

Robertson,W.G.; Peacock,M.
"Review of risk factors in calcium oxalate urolithiasis"
World J.Urol. (1983), **1**, 114-118

Robertson,W.G.; Peacock,M.; Heyburn,P.J.; Marshall,D.H.; Clark,P.B.
"Risk factors in calcium stone disease of the urinary tract"
Br.J.Urol. (1978), **50**, 449-454

Robertson,W.G.; Peacock,M.; Marshall,R.W.; Marshall,D.H.; Nordin,B.E.C.
"Saturation inhibition index as a measure of the risk of calcium oxalate stone-formation in the urinary tract"
N.Engl.J.Med. (1976a), **294**, 249-252

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Activity products in stone-forming and non-stone forming urine"
Clin.Sci. (1968), **34**, 579-594

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Calcium oxalate crystalluria and urine saturation in recurrent renal stone formers"
Clin.Sci. (1971), **40**, 365-374

Robertson,W.G.; Peacock,M.; Nordin,B.E.C.
"Inhibitors of the growth and aggregation of calcium oxalate crystals in vitro"
Clin.Chim.Acta (1973), **43**, 31

Robertson,W.G.; Peacock,M.; Ouimet,D.; Heyburn,P.J.; Rutherford,A.
"The main risk factor for calcium oxalate stone disease in man: hypercalciuria or mild hyperoxaluria?"
in:"Urolithiasis: Clinical and Basic Research"
eds:Smith,L.H.; Robertson,W.G.; Finlayson,B.
Plenum Press, New York, London (1981b), 3-12

Robertson,W.G.; Scurr,D.S.
"Modifiers of calcium oxalate crystallisation found in urine I. Studies with a continuous crystalliser using an artificial urine"
J.Urol. (1986), **135** (June), 1322-1326

Robertson,W.G.; Scurr,D.S.; Bridge,C.M.
"Factors influencing the crystallisation of calcium oxalate in urine - critique"
J.Cryst.Growth (1981a), **53**, 182-194

Rodgers,A.L.; Ball,D.; Harper,W.
"Urinary macromolecules are promoters of calcium oxalate urolithiasis in human urine: turbidimetric studies"
Clin.Chim.Acta (1993), **220**, 125-134

Rodgers,A.L.; Ball,D.; Harper,W.
"Effect of urinary macromolecules and chondroitin sulphate on calcium oxalate crystallisation in urine"
Scann.Micros. (1994), **8** (1), 71-77

Rodgers,A.L.; Garside,J.
"The nucleation and growth kinetics of calcium oxalate in the presence of some synthetic urine constituents"
Invest.Urol. (1981), **18** (6), 484-488

Rodgers,A.L.; Jappie,D.
"Studies on the role of urinary macromolecules in urolithiasis: review of methodologies and a proposal for a standard reference crystallisation system"
Scann.Micros. (1996), **10** (1) in press

Rodgers,A.L.; Wandt,M.A.E.
"Influence of ageing, pH and various additives on crystal formation in artificial urine"
Scann.Micros. (1991), **5** (3), 697-706

Rose,G.A.; Sulaiman,S.
"Tamm-Horsfall mucoproteins promote calcium oxalate crystal formation in urine: quantitative studies"
J.Urol. (1982), **127**, 177-179

Rose,G.A.; Sulaiman,S.
"Effect of different fractions of macromolecules upon triggering of calcium oxalate and calcium phosphate crystal formation in whole urine"
Urol.Int. (1984), **39**, 147-149

Rushton,H.G.; Spector,M.
"Effects of magnesium deficiency on intratubular calcium oxalate formation and crystalluria in hyperoxaluric rats"
J.Urol. (1982), **127**, 598-604

Ryall,R.L.; Darroch,J.N.; Marshall,V.R.
"The evaluation of risk factors in male stone-formers attending a general hospital out-patient clinic"
Br.J.Urol. (1984), **56**, 116-121

Ryall,R.L.; Harnett,R.M.; Hibberd,C.M.; Edyvane,K.A.; Marshall,V.R.
"Effects of chondroitin sulphate, human serum albumin and Tamm-Horsfall mucoprotein on calcium oxalate crystallisation in undiluted human urine"
Urol.Res. (1991), **19**, 181-188

Ryall,R.L.; Harnett,R.M.; Hibberd,C.M.; Mazzachi,B.C.; Mazzachi,R.D.; Marshall,V.R.
"Urinary risk factors in calcium oxalate stone disease: comparison of men and women"
Br.J.Urol. (1987), **60**, 480-488

Ryall,R.L.; Harnett,R.M.; Marshall,V.R.
"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**, 349-356

Ryall,R.L.; Harnett,R.M.; Marshall,V.R.
"The effect of monosodium urate on the capacity of urine, chondroitin sulphate and heparin to inhibit calcium oxalate crystal growth and aggregation"
J.Urol. (1986), **135**, 174-177

Ryall,R.L.; Hibberd,C.M.; Marshall,V.R.
"A method for studying inhibitory activity in whole urine"
Urol.Res. (1985), **13**, 285-289

Ryall,R.L.; Marshall,V.R.
"The effect of urine and other inhibitors on the growth and aggregation of calcium oxalate crystals in vitro"
in"Urolithiasis, Clinical and Basic Research"
eds:Smith,L.H.; Robertson,W.G.; Finlayson,B.
New York, Plenum Press, (1981), 631-635

Ryall,R.L.; Marshall,V.R.
"The value of the 24-hour urine analysis in male stone formers attending a general hospital outpatients clinic"
Br.J.Urol. (1983), **55**, 1-5

Ryall,R.L.; Marshall,V.R.
"The investigation and management of idiopathic urolithiasis"
in:"Metabolic basis and clinical practice"
eds: Wickham,J.E.A.; Colin Buck,A. Churchill Livingstone
(1990), 307-331

Sakhaee,K.; Poindexter,J.R.; Pak,C.Y.C.
"The spectrum of metabolic abnormalities in patients with cystine nephrolithiasis"
J.Urol. (1989), **141**, 819

Sallis,J.D.; Bichler,K.H.; Korn,S.; Haussman,A.
"Urinary glycosaminoglycan excretion in patients with urolithiasis"
in:"Urolithiasis: clinical and basic research"
eds:Smith,L.H.; Robertson,W.G.; Finlayson,B.
New York, Plenum Press, (1981), 619-622

Sallis,J.D.; Lumley,M.F.
"On the possible role of glycosaminoglycans as natural inhibitors of calcium oxalate stones"
Invest.Urol. (1979), **16**, 296-299

Samuell,C.T.
"A study of glycosaminoglycan excretion in normal and stone-forming subjects using a modified cetylpyridinium chloride technique"
Clin.Chim.Acta (1981), **117**, 63-73

Schwille,P.O.; Scholtz,D.; Paulus,M.; Engelhardt,W.; Segel,A.
"Citrate in daily and fasting urine. Results of controls, patients with recurrent idiopathic calcium urolithiasis and primary hyperparathyroidism"
Invest.Urol. (1979), **16**, 457-462

Schwille,P.O.; Scholtz,D.; Schwille,K.; Leutschaft,R.; Goldberg,I.; Segel,A.
"Citrate in urine and serum and associated variables in subgroups of urolithiasis"
Nephron (1982), **31**, 194-202

Scurr,D.S.; Robertson,W.G.
"Modifiers of calcium oxalate crystallisation found in urine III. Studies on their mode of action in artificial urine"
J.Urol. (1986), **136** (July), 128-131

Shirane,Y.; Yamamoto,A.; Kagawa,S.
"The effect of glycosaminoglycans on calcium oxalate crystal formation"
Jap.J.Urol. (1990), **81**, 1065-1070

Shirane,Y.; Yamamoto,A.; Kagawa,S.
"The effect of glycosaminoglycans on calcium oxalate monohydrate crystal morphology"
in:"Urolithiasis 2" Proc.7th Int.Symp.Urolithiasis eds:Ryall,R.; Bais,R.; Marshall,V.R.; Rofe,A.M.; Smith,L.H.; Walker,V.R. New York, Plenum Press (1994), 189-191

Shum,D.K.Y.; Gohel,M.D.I.
"Separate effects of urinary chondroitin sulphate and heparan sulphate on the crystallisation of urinary calcium oxalate: differences between stone formers and normal control subjects"
Clin.Sci. (1993), **85**, 33-39

Shum,D.K.Y.; Gohel,M.D.I.; Li,M.K.
"The influence of urinary acidic macromolecules on the crystallisation of calcium oxalate in whole urine"
in: Inhibitors of crystallisation in renal lithiasis and their clinical application" eds:Martelli,A.; Buli,B.; Marchesini,B. Rome, Acta Medica (1988), 115-120

Skrtic,D.; Furedi-Milhofer,H.; Markovic,M.
"Precipitation of calcium oxalate from high ionic strength solutions V. The influence of precipitation conditions and some additives on the nucleating phase"
J.Cryst.Growth (1987), **80**, 113-120

Smith,L.H.; Meyer,J.L.; McCall,J.T.
"Chemical nature of crystal inhibitors isolated from human urine"
in:"Urinary Calculi, Recent Advances in Aetiology, Stone Structure, and Treatment" eds:Cifuentes Delatte,L.; Rapado,A.; Hodgkinson,A.
Basel,S Karger, (1973), 318-327

Springmann,K.E.; Drach,G.W.; Gottung,B.; Randolph,A.D.
"Effects of human urine on aggregation of calcium oxalate crystals"
J.Urol. (1986), **135**, 69-71

Suzuki,K.; Mayne,K.; Doyle,I.R.; Ryall,R.L.
"Urinary glycosaminoglycans are selectively included into calcium oxalate crystals precipitated from whole human urine"
Scann.Micros. (1994), **8** (3), 523-530

Takasaki,E.
"The magnesium:calcium ratio in the concentrated urines of patients with calcium oxalate calculi"
Invest.Urol. (1972), **10** (2), 147-150

Thomas,W.C.; Howard,J.E.
"Studies on the mineralizing propensity of urine from patients with and without renal calculi"
Trans.Assoc.Amer.Physicians (1959), **72**, 181-187

Tiselius,H.G.
"Excretion of 4-pyridoxic acid and oxalic acid in patients with urinary calculi"
Invest.Urol. (1977), **15**, 5-8

Tiselius,H.G.
"Aspects on estimation of risk of CaOx crystallisation in urine"
Urol.Int. (1991), **47**, 255-259

Tiselius,H.G.; Almgard,L.E.; Larsson,L.; Sorbo,B.
"A biochemical basis for grouping of patients with urolithiasis"
Eur.Urol. (1978), **4**, 241-249

Tiselius,H.G.; Berg,C.; Fornander,A.M.; Nilsson,M.A.
"Effects of citrate on the different phases of calcium oxalate crystallisation"
Scann.Micros. (1993a), **7** (1), 381-390

Tiselius,H.G.; Fornander,A.M.
"Evaluation of a routine method for determination of calcium oxalate crystal growth inhibition in diluted urine samples"
Clin.Chem. (1981), **27** (4), 565-568

Tiselius,H.G.; Fornander,A.M.; Nilsson,M.A.
"The effects of citrate and urine on calcium oxalate crystal aggregation"
Urol.Res. (1993b), **21**, 363-366

Tomazic,B.B.; Nancollas,G.H.
"A study of the phase transformation of calcium oxalate trihydrate-monohydrate"
Invest.Urol. (1979), **16** (5), 329-335

Trinchieri,A.; Mandressi,A.; Luongo,P.; Rovera,F.; Longo,G.
"Urinary excretion of citrate, glycosaminoglycans, magnesium and zinc in relation to age and sex in normal subjects and in patients who form calcium stones"
Scand.J.Urol.Nephrol. (1992), **26** (4), 379-386

Welshman,S.G.; McGeown,M.G.
"The relationship of urinary cations calcium, magnesium, sodium and potassium in patients with renal calculi"
Br.J.Urol. (1975), **47**, 237-242

Welshman,S.G.; McGeown,M.G.
"Urinary citrate excretion in stone-formers and normal controls"
Br.J.Urol. (1976), **48**, 7-11

Werness,P.G.; Bergert,J.H.; Lee,K.E.
"Urinary crystal growth: effect of inhibitor mixtures"
Clin.Sci. (1981a), **61**, 487-491

Werness,P.G.; Bergert,J.H.; Smith,L.H.
"Crystalluria"
J.Cryst.G. (1981b), **53**, 166-181

APPENDIX 1

Table of probability values (p) for the results from multiple regression analyses showing significant differences ($p < 0.05$) in the intercept and slope (from which nucleation and growth rates of CaOx were calculated) between the various experiments within each pool. p-Values are indicated only where a significant difference was found.

	Coulter		Malvern	
Sample	Intercept	Slope	Intercept	Slope
5	0.00001	0.00001		
6		0.0002	0.0317	
7		0.0005	0.0457	
8	0.00001	0.00001		0.0086
9	0.00001	0.00001	0.00001	0.00001
10	0.0405		0.0007	0.0005
11	0.0007			
12		0.00001		0.0338
13	0.0084	0.00001		
14			0.0433	
15			0.00001	0.0011
16	0.0033		0.0223	0.0064
17		0.0170	0.0003	
18	0.0352	0.0001		
19		0.00001		
20	0.0070	0.0082	0.0213	
21			0.0015	0.0111
22			0.00001	
23			0.0041	
24	0.00001			
25	0.0078		0.00001	
26	0.0005	0.0006	0.00001	0.0480
27			0.0016	0.00001
28		0.0261	0.0080	
29		0.0005	0.0219	
30	0.0056	0.0124		
31				

APPENDIX 2

Suspension density (M_T), calcium ($[Ca]_{sol}$ $*10^{-3}$M), oxalate ($[Ox]_{sol}$ $*10^{-3}$M) and supersaturation ratio (S) for the Coulter and Malvern in each experiment. Experiment numbers shown correspond directly to the order of experiments shown in the tables in Chapter IV.

		Coulter				Malvern			
Sampl	Expt	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S
5	1	0.04	2.21	2.797	63.46	0.048	2.202	2.789	63.10
	2	0.046	2.204	3.291	72.06	0.033	2.217	3.304	72.68
	3	0.061	2.189	3.776	79.56	0.047	2.203	3.790	78.41
6	1	0.039	1.761	2.393	56.06	0.072	1.782	2.360	54.43
	2	0.075	1.725	2.857	62.76	0.068	1.732	2.864	63.13
	3	0.098	1.702	3.334	69.12	0.092	1.708	3.340	69.46
7	1	0.046	3.194	2.404	67.88	0.032	3.208	2.418	68.48
	2	0.035	3.205	2.915	80.20	0.041	3.199	2.909	92.00
	3	0.084	3.156	3.366	93.33	0.058	3.182	3.392	90.25
8	1	0.032	2.038	3.562	78.24	0.050	2.020	3.544	77.28
	2	0.058	2.012	3.536	76.85	0.018	2.052	3.576	79.00
	3	0.024	0.046	3.570	78.67	0.035	2.035	3.559	78.08
9	1	0.101	2.869	3.367	82.84	0.039	2.931	3.429	85.68
	2	0.065	2.905	3.403	84.49	0.039	2.931	3.429	85.68
	3	0.048	2.922	3.420	85.27	0.041	2.929	3.427	85.59
10	1	0.081	1.989	3.594	76.45	0.061	2.009	3.614	74.49
	2	0.030	2.040	3.645	79.12	0.065	2.005	3.610	77.28
	3	0.061	0.009	3.614	77.49	0.002	2.068	3.673	80.59
11	1	0.125	1.405	2.717	44.48	0.084	1.446	2.758	46.32
	2	0.030	1.500	2.812	38.05	0.024	1.506	2.818	38.52
	3	0.050	1.480	2.792	35.29	0.035	1.495	2.807	35.84
	4	0.048	1.482	2.794	33.28	0.075	1.455	2.767	32.35
12	1	0.047	1.303	2.777	40.94	0.062	1.288	2.762	40.31
	2	0.025	1.325	2.799	33.73	0.028	1.322	2.796	33.62
	3	0.015	1.335	2.809	32.26	0.016	1.334	2.808	32.23
	4	0.017	1.333	2.807	30.46	0.020	1.330	2.804	30.46

Appendix 2 (cont)

		Coulter				Malvern			
Sampl	Expt	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S
13	1	0.110	1.510	2.759	49.78	0.011	1.510	2.759	49.78
	2	0.060	1.560	2.809	42.24	0.055	1.565	2.814	42.44
	3	0.031	1.589	2.838	41.11	0.034	1.586	2.835	41.00
	4	0.058	1.562	2.811	37.91	0.056	1.564	2.813	37.98
14	1	0.039	2.751	2.929	82.18	0.032	2.758	2.936	82.52
	2	0.031	2.759	2.937	71.04	0.032	2.758	2.936	71.00
	3	0.051	2.739	2.917	66.72	0.014	2.776	2.954	68.24
	4	0.068	2.722	2.900	59.80	0.055	2.735	2.913	60.29
15	1	0.028	1.322	2.886	53.25	0.011	1.339	2.903	54.14
	2	0.015	1.335	2.899	47.14	0.013	1.337	2.901	47.23
	3	0.011	1.339	2.903	44.65	0.023	1.327	2.891	44.13
	4	0.009	1.341	2.905	42.15	0.028	1.322	2.886	41.37
16	1	0.097	2.063	2.880	70.66	0.061	2.099	2.916	72.46
	2	0.038	2.122	2.939	62.65	0.039	2.121	2.938	62.61
	3	0.053	2.107	2.924	58.62	0.094	2.066	2.883	56.90
	4	0.038	2.122	2.939	47.82	0.050	2.110	2.927	47.40
17	1	0.064	2.186	3.012	70.56	0.071	2.179	3.005	70.22
	2	0.033	2.217	3.043	63.17	0.032	2.218	3.044	63.22
	3	0.016	2.234	3.060	60.68	0.031	2.219	3.045	60.06
	4	0.023	2.227	3.053	57.03	0.014	2.236	3.062	57.38
18	1	0.069	2.181	2.944	70.02	0.068	2.182	2.945	70.07
	2	0.013	2.237	3.000	64.28	0.017	2.233	2.996	64.10
	3	0.072	2.178	2.941	57.39	0.057	2.193	2.956	58.10
	4	0.024	2.226	2.989	56.29	0.025	2.225	2.988	50.25
19	1	0.041	2.479	3.026	90.48	0.036	2.484	3.031	90.76
	2	0.015	2.520	3.067	80.96	0.012	2.520	3.067	80.96
	3	0.031	2.489	3.036	73.74	0.032	2.488	3.035	73.69
	4	0.018	2.502	3.049	70.50	0.014	2.506	3.053	70.67

Appendix 2 (cont)

		Coulter				Malvern			
Sampl	Expt	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S
20	1	0.017	1.693	2.897	65.52	0.034	1.676	2.880	64.65
	2	0.029	1.681	2.885	64.90	0.018	1.692	2.896	65.47
	3	0.013	1.697	2.901	65.73	0.015	1.695	2.899	65.74
	4	0.009	1.701	2.905	66.05	0.011	1.699	2.903	65.94
21	1	0.056	1.654	2.813	59.98	0.053	1.657	2.816	61.77
	2	0.063	1.647	2.806	61.26	0.043	1.667	2.826	62.28
	3	0.044	1.666	2.825	62.22	0.036	1.674	2.833	62.64
	4	0.036	1.674	2.833	62.64	0.041	1.669	2.828	62.38
22	1	0.049	1.841	2.820	66.74	0.057	1.833	2.812	66.33
	2	0.042	1.848	2.827	67.09	0.046	1.844	2.823	66.89
	3	0.050	1.840	2.819	66.68	0.036	1.854	2.833	67.39
	4	0.025	1.865	2.844	67.95	0.029	1.861	2.840	68.63
23	1	0.050	2.83	2.837	77.45	0.029	2.851	2.858	78.44
	2	0.031	2.849	2.856	71.55	0.028	2.852	2.859	71.68
	3	0.031	2.849	2.856	55.75	0.006	2.874	2.881	56.70
	4	0.002	2.878	2.885	35.35	0.001	2.879	2.886	35.37
24	1	0.034	2.036	3.033	62.78	0.068	2.002	2.999	61.22
	2	0.039	2.031	3.028	60.83	0.032	2.038	3.035	61.15
	3	0.014	2.056	3.053	55.23	0.016	2.054	3.051	55.15
	4	0.014	2.056	3.053	41.06	0.010	2.060	3.057	41.19
25	1	0.049	2.201	2.892	57.92	0.020	2.230	2.921	59.16
	2	0.023	2.227	2.918	52.08	0.023	2.227	2.918	52.08
	3	0.017	2.233	2.924	38.01	0.0002	2.250	2.941	38.52
	4	0.001	2.249	2.940	22.62	0.032	2.218	2.909	22.05

Appendix 2(cont)

		Coulter				Malvern			
Sampl	Expt	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S	M_T	$[Ca]_{sol}$	$[Ox]_{sol}$	S
26	1	0.052	2.738	2.880	72.86	0.063	2.727	2.869	72.37
	2	0.032	2.758	2.900	66.14	0.028	2.762	2.904	66.30
	3	0.015	2.775	2.917	59.98	0.026	2.764	2.906	59.97
	4	0.011	2.779	2.921	45.71	0.008	2.782	2.924	45.81
27	1	0.046	1.754	2.940	51.92	0.051	1.749	2.935	51.72
	2	0.026	1.774	2.960	47.11	0.031	1.769	2.955	46.92
	3	0.012	1.788	2.974	42.45	0.014	1.786	2.972	42.39
	4	0.006	1.794	2.980	38.19	0.005	1.795	2.981	38.22
28	1	0.051	2.559	2.917	70.32	0.036	2.574	2.932	70.98
	2	0.031	2.579	2.937	63.51	0.020	2.590	2.948	63.96
	3	0.014	2.596	2.954	57.30	0.013	2.597	2.955	57.34
	4	0.012	2.598	2.956	51.47	0.006	2.604	2.962	51.67
29	1	0.052	2.198	3.024	49.16	0.023	2.227	3.053	50.25
	2	0.026	2.224	3.050	54.83	0.003	2.247	3.073	55.71
	3	0.009	2.241	3.067	59.98	0.006	2.244	3.070	60.11
	4	0.004	2.246	3.072	49.84	0.001	2.249	3.075	49.94
30	1	0.006	2.424	3.106	60.50	0.013	2.417	3.099	60.21
	2	0.011	2.419	3.101	57.13	0.034	2.396	3.078	56.27
	3	0.032	2.398	3.080	63.11	0.018	2.412	3.094	63.68
	4	0.008	2.422	3.104	52.74	0.017	2.413	3.095	52.43
31	1	0.036	2.394	3.130	55.29	0.015	2.415	3.151	56.11
	2	0.061	2.369	3.105	56.76	0.026	2.404	3.140	58.09
	3	0.053	2.377	3.113	62.01	0.022	2.408	3.144	63.28
	4	0.029	2.401	3.137	52.31	0.021	2.409	3.145	52.59

APPENDIX 3

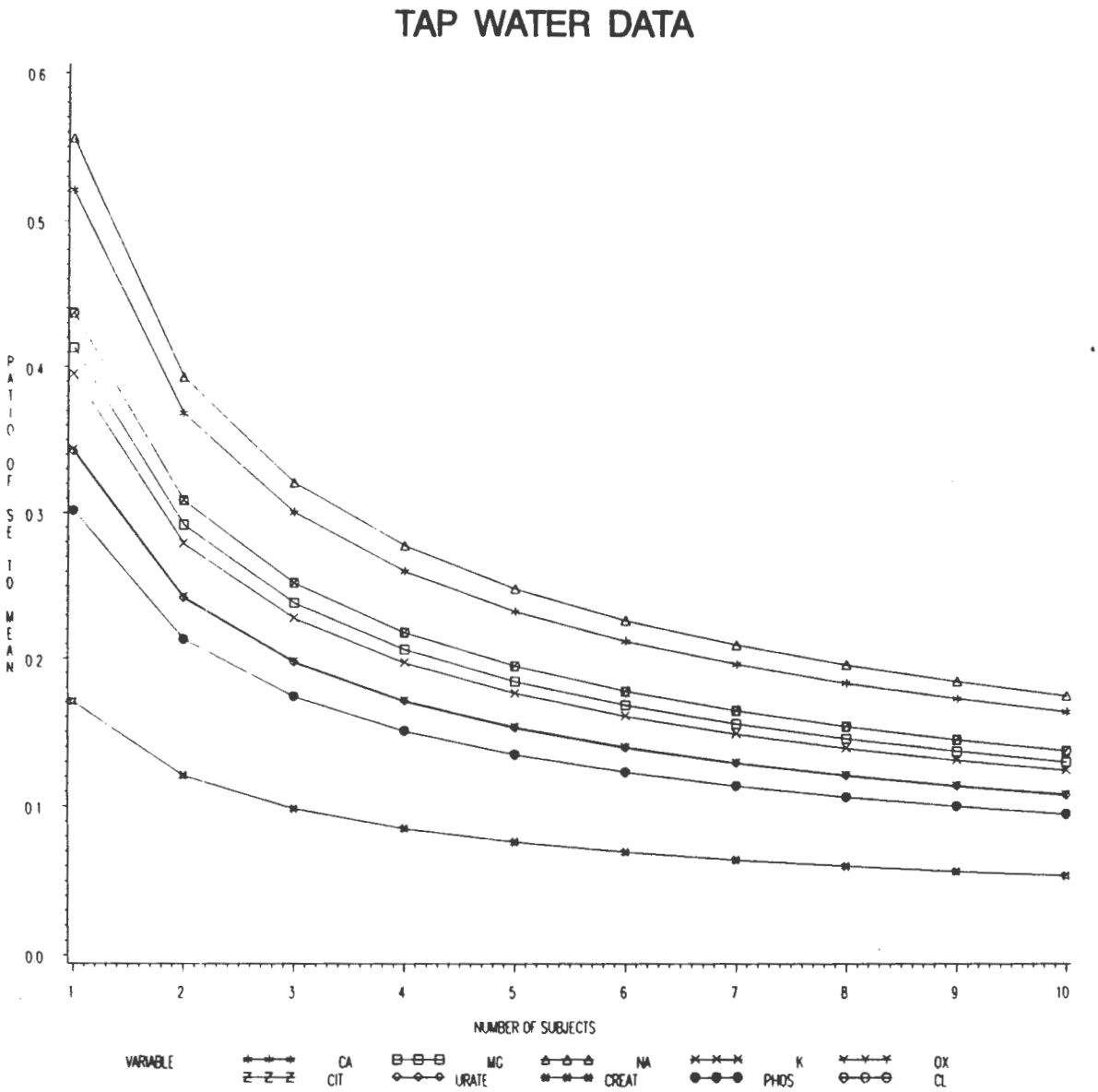
Statistical requirements for pooled urines [Jappie PhD Thesis UCT, 1996].

It is assumed that having pooled the urines from N subjects, a measurement of a variable from a sample of the pooled urine would produce a value equal to the mean of the variable from measurements made on the N separate urines. The variances would also be expected to be the same.

The statistic used to determine the number of subjects for a particular variable is the standard deviation of the mean, also called the standard error (SE). The SE is defined as the root of (the variance of variable)/N. Clearly, the more subjects participating in the study, the smaller the SE and the more reliable the mean. As a guide to determining the required number of subjects, the SE is plotted against N. It is useful to have the plots for all variables on the same set of axes, since the required number for one variable may be quite different from that for another. However, since the SE's of the variables may differ markedly, they may be expressed relative to the corresponding means, which produces a ratio, SE/mean, which is independent of units. The plot shows the relationship between this statistic and N for all variables using experimental data.

From the plot it is apparent that Na (sodium) shows the greatest variation, relative to the mean, and CREAT the least variation. Note that the results for CIT and Cl are virtually identical, so that their symbols overlap. From the plot it is seen that once more than 5 urines are pooled, there is a smaller error in the measured variables when compared to pooling less than 5 samples. Also, there is little change in this error (ie graphs level off) after 5 urines and therefore this must be the minimum number of urines that must be pooled to ensure that the variation of the measured variables will be at a minimum and will also be the same between pools.

Appendix 3 (cont)



APPENDIX 4

Composition of each of the pooled samples. Results shown are in mmol/dm^3.

Sample	pH	citrate	oxalate	calcium	magnesium
5	6.25	1.51	0.93	2.5	2.3
6	6.35	1.92	0.48	2.0	2.3
7	6.15	2.71	0.50	3.6	3.4
8	6.35	2.01	0.66	2.3	2.4
9	5.95	2.00	0.52	3.3	3.8
10	5.85	2.00	0.75	2.3	2.7
11	6.25	1.60	0.38	1.7	1.7
12	6.20	1.34	0.36	1.5	2.0
13	6.20	1.53	0.41	1.8	2.2
14	6.20	1.12	0.52	3.1	3.5
15	6.35	1.02	0.46	1.5	1.9
16	6.25	1.19	0.53	2.4	3.5
17	6.15	1.45	0.64	2.5	3.2
18	6.15	1.37	0.57	2.5	3.4
19	6.00	1.43	0.63	2.8	2.6
20	6.35	0.57	0.46	1.9	2.3
21	5.90	1.04	0.41	1.9	2.5
22	6.00	0.78	0.41	2.1	2.7
23	5.95	1.26	0.43	3.2	2.6
24	6.00	1.22	0.63	2.3	2.5
25	6.25	1.52	0.49	2.5	2.4
26	6.25	1.28	0.48	3.1	2.7
27	6.10	1.16	0.54	2.0	2.4
28	6.10	1.50	0.52	2.9	3.0
29	5.90	1.80	0.64	2.5	2.5
30	5.90	1.21	0.68	2.7	3.4
31	5.85	1.75	0.74	2.7	2.7

Sample	sodium	potassium	urate	creatinine	phosphate	Cl^-
5	78.2	24.1	2.1	8.6	21.2	85
6	21.6	13.1	1.5	5.9	11.6	64
7	66.7	16.6	2.2	9.2	22.9	115
8	28.2	9.6	1.9	6.7	18.7	70
9	84.9	40.7	2.0	8.2	23.0	85
10	60.1	23.1	1.8	7.2	17.5	53
11	54.4	37.1	1.6	6.2	14.6	55
12	68.5	39.6	1.0	6.2	16.1	87
13	56.4	29.8	1.7	6.6	14.8	57
14	66.8	37.7	1.4	6.8	16.8	82
15	41.5	28.0	1.0	5.4	11.9	53
16	45.3	27.5	1.7	5.5	14.8	54
17	52.6	38.7	1.6	7.1	17.5	73
18	62.0	33.0	1.8	7.7	15.7	64
19	53.7	24.6	1.7	7.7	13.7	47
20	59.8	21.6	1.5	7.3	13.8	75
21	48.8	25.8	1.8	6.6	15.0	74
22	53.8	27.5	1.7	7.0	16.4	73
23	59.8	29.5	2.0	8.0	20.9	82
24	60.4	26.1	1.9	7.8	18.8	78
25	59.8	25.6	1.9	8.0	19.5	75
26	56.3	39.7	1.7	8.3	21.2	88
27	77.3	26.9	1.9	8.3	20.8	82
28	52.4	41.8	1.6	9.2	16.4	95
29	49.9	32.4	1.6	9.5	18.3	64
30	58.2	27.0	1.8	9.1	20.4	63
31	55.8	38.0	1.8	9.1	18.8	72