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Crystal Growth of Calcium Oxalate Monohydrate in Presence of Amino Acids

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The crystal growth of calcium oxalate monohydrate has been investigated in aqueous solutions at 37 °C by constant composition method. Over a range of super-saturation 1.8-3.0, the calculation of order of reaction from logarithmic plots of growth rate *versus* super-saturations has been appears to be governed by a surface process. The influence of a number of amino acids on the rate of reaction has been investigated, the order of the degree of inhibition is phenyl alanine > arginine > glycine > alanine. Retardation effect was enhanced with increases in the additive content and as the relative super-saturation decreases. Application to a kinetic Langmuir-type model suggests that adsorption of the amino acids at the active growth sites is the cause of the reduction in the growth rates.

Key Words: Crystal growth, Calcium oxalate, Amino acids, Inhibition.

INTRODUCTION

Oxalates are the main inorganic component of sparingly soluble calcium salts in pathological deposits which play a key role in the formation of kidney stones[1-3]. Calcium oxalate (CaOxa) is the main component of uroliths. There are three different hydrated forms of calcium oxalate, oxalate monohydrate (COM, whewellite), oxalate dihydrate (COD, weddillite) and oxalate trihydrate (COT). The monoclinic COM is the thermodynamically most stable phase, followed by the triclinic COT and the tetragonal COD. Monohydrate COM and dihydrate COD together with calcium phosphate (hydroxyl apatite) are the major components of most of the urinary calculi. COT has been rarely found in urines and kidney stones[4].

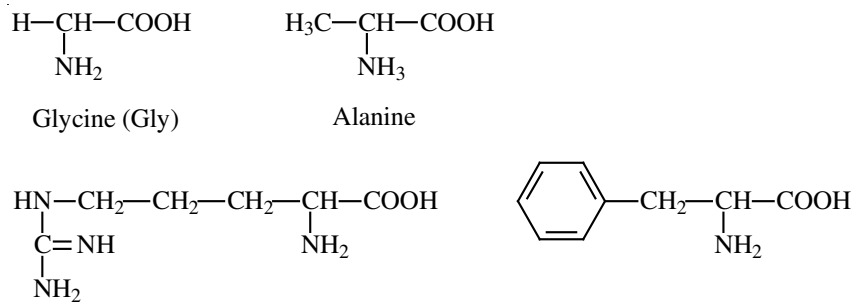
The interaction between crystals and cell membranes in urolithiasis appears to be mediated by specific interactions between stone crystal surface structures and molecular arrays at the surface of cell membranes[5]. The exact nature of this crystal-membrane interaction is still unknown. The main difficulty in elucidating the molecular mechanisms of the crystal-membrane interaction is the complex and dynamic nature of the cell membranes[6,7].

In urolithiasis or renal stone formation, it is now generally accepted that urine is ordinary super-saturated with respect to calcium oxalate hydrate[8]. Renal stones may result from the failure of natural inhibitors which, in normal subjects, prevent

the formation of crystalluria, most humans do not form stones. Precipitation and dissolution studies of calcium oxalate and the influence caused on such processes by foreign substance are of great interest in view of their potential application in the urolithiasis therapy[9-11]. Inhibitors are among the multiple factors that may influence the complex process of stone formation. The factors which govern the mechanism of precipitation of these oxalate salts are therefore of considerable interest, especially the influence of foreign substances which may exert a marked effect on the rates of crystallization either through adsorption at the surface of the crystals or by lattice substitution.

Amino acids are the basic structural building units of proteins. They are compounds of major importance for living organisms, moving freely in blood circulation after digestion of proteins. Also, it has been proven that they enter into the cell environment by simple diffusion[12,13] and thus their concentration is controlled by physiological mechanisms. It is obvious that studies of such molecules of biological relevance onto renal stone crystal growth can be related directly to important processes of desirable or pathological calcification. The influence of amino acids on renal stone formation has been subject of several studies[14-16].

An attempt is made to study the kinetics of crystal growth of calcium oxalate in aqueous solutions by a constant composition technique[17,18]. The rate of growth have been studied as a function of different parameters such as super-saturation, amount of seed, hydrodynamics and presence of trace quantities of three amino acids with neutral polar side groups, glycine (Gly), alanine (Ala), phenylalanine (Phe) and one polar strongly basic amino acid, arginine (Arg).



Scheme-I: Structure and formula of amino acids

EXPERIMENTAL

Solutions of calcium chloride, sodium oxalate and sodium chloride were prepared using both ultra pure (Alfa Chemical) and reagent grade (J.T. Baker) chemicals with triply distilled deionized water. Solutions were filtered through 0.22 μm

Millipore filters which had been pre-washed in order to remove any residual wetting agents or surfactants. Then, they were analyzed by passing aliquots through an ion-exchange resin (Dowex-50) in the hydrogen form and titrating the liberated acid with standard sodium hydroxide solution.

Seed crystals were synthesized by a method similar to that reported by Millan *et al.*[19], by dropping 1 L of 0.1 M $CaCl_2$ and 1 L of 0.1 M NaC_2O_4 stock solutions, with a constant rate of 250 mL/h, through separate inlets, into a vessel containing 0.5 L of water, under mechanical stirring and at room temperature. Then, the slurry was concentrated by decantation and stored for one month before used. The seed crystals were examined by powder X-ray diffraction and they were composed of calcium oxalate monohydrate exclusively. The specific surface determined by single-point nitrogen adsorption was 3.73 m^2/g.

The experiments were performed in a 500 mL capacity double-walled Pyrex glass vessel fitted with a Perspex lid with holes for the electrodes and for sampling. The cell contents were maintained at 37 ± 0.1 °C by circulated thermo-stated water through the outer jacket. The ionic strength was maintained at 0.15 mol L^{-1} during the reaction by the addition of sodium chloride (Ultra-pure) solution. Subsequently, super-saturation solutions of the desired concentration were prepared by slow addition of calcium chloride, sodium oxalate to sodium chloride solutions. Following the introduction of oxalate seed crystals, the activities of calcium and oxalate ions were maintained at constant levels by the addition of those solutions monitored by means of a pH-stat (Methrom Co.). The pH of the working solution was maintained at 6.3 ± 0.01.

Additive solutions were also introduced as titrants in order to compensate for dilution effects. In addition samples were periodically withdrawn and filtered prior to solution and solid-phase analyses. The data confirmed that the lattice ion and inhibitor concentrations were kept constant to within ± 1 %.

RESULTS AND DISCUSSION

The concentrations of ionic species in the solutions were calculated from mass-balance and electro-neutrality expressions. Activity coefficients were calculated from the extended form the Debye-Huckel equation proposed by Davies[20].

For many sparingly soluble salts, M_aA_b, the rate of crystallization, normalized for seed surface area can be expressed by the equation:

$$R = d\,[M_aA_b]/dt = KS\,\sigma^n \quad (1)$$

In which k is the crystallization rate constant, s is proportional to the number of growth sites available on the seed crystals, n is the effective order of reaction and σ is the relative super-saturation, which may be expressed by:

$$\sigma = \left(\left[a_{Ca} - a_{C_2O_4} \right]^{1/2} - K_{sp} \right) / K_{sp}^{1/2} \quad (2)$$

where a_{Ca} and $a_{C_2O_4}$ are the ionic activities of Ca^{2+} and $C_2O_4^{2-}$ in the solutions and K_{sp} is the solubility value at equilibrium. The conditional solubility product, K_{sp}, of calcium oxalate monohydrate[21] was 2.2×10^{-9} mol^2 L^{-2} at the working ionic strength 0.15 mol L^{-2}.

The results of crystallization experiments from super-saturation solutions are compiled in Table-1. Typical plots of the amount of calcium oxalate precipitated as a function of time are shown in Fig. 1. The rate of crystallization (R) in Table-1 was calculated from the slopes of linear plots. Table-1 shows that the rates of crystallization were proportional to the mass of seed crystals used to initiate the reactions (expts. 1-10). The crystal growth rate, normalized for the initial surface area of each inoculation seed, is constant confirming that crystallization takes place on the seed crystals without additional nucleation or spontaneous precipitation. Assuming as first approximation, simple particles, corrections were made for changes in surface area during the crystal growth reactions by introducing a factor $(w_i/w_t)^{2/3}$, where w_i and w_t are the masses of solid phase present initially at time t, respectively. Many studies of crystal growth of calcium oxalate monohydrate give a value of 2 for the order of reaction, although, higher and lower values have been occasionally reported. In the present study, the effective order of reaction, determined from the slopes of typical plots of - log R *versus* -log σ shown in Fig. 2, confirms a poly-nucleation dependence upon relative super-saturation (n = 4.29) in eqn. 2. The suggestion of a predominantly surface-controlled process over a range relative super-saturation may also be supported by the observed independence of the experimental rate of crystal growth changes in fluid dynamics, as shown in Table-1 (compare expts. 11, 12 and 15, 16).

TABLE-1
CRYSTALLIZATION OF CALCIUM OXALATE MONOHYDRATE CRYSTALS AT 37 ºC, T_{Ca}: T_{Ox} = 1:1, IONIC STRENGTH = 0.15 mol L^{-1} (NaCl)

Exp. No.	T_{Ca} /10^{-4} mol L^{-1}	σ	Seed (mg)	Rate/10^{-8} mol min^{-1} m^{-2}
1-5	4.26	1.8	10-100	2.09
6-10	5.17	2.4	10-100	6.70
11	4.26	1.8	20	2.11
12	4.57	2.0	20	4.04
13	4.87	2.2	20	4.85
14	5.48	2.6	20	8.94
15†	4.26	1.8	20	2.10
16†	4.57	2.0	20	4.08
17	4.57	2.0	50	4.06
18†	4.87	2.2	20	4.85
19	5.17	2.4	20	6.71
20†	5.48	2.6	20	8.94
21	5.78	2.8	20	13.95
22	6.09	3.0	20	23.80
23	5.48	2.6	20	8.92

†Stirring speed = 200 rpm for all experiments. While experiments 15,16,18 and 20 = 300 rpm

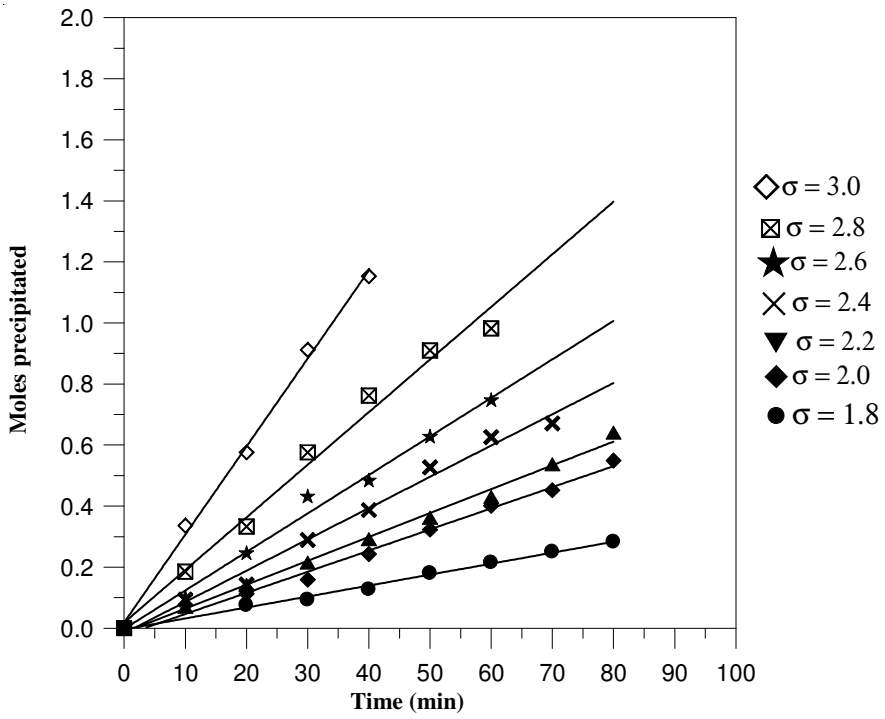


Fig. 1. Moles precipitated against time at different degree of super-saturation

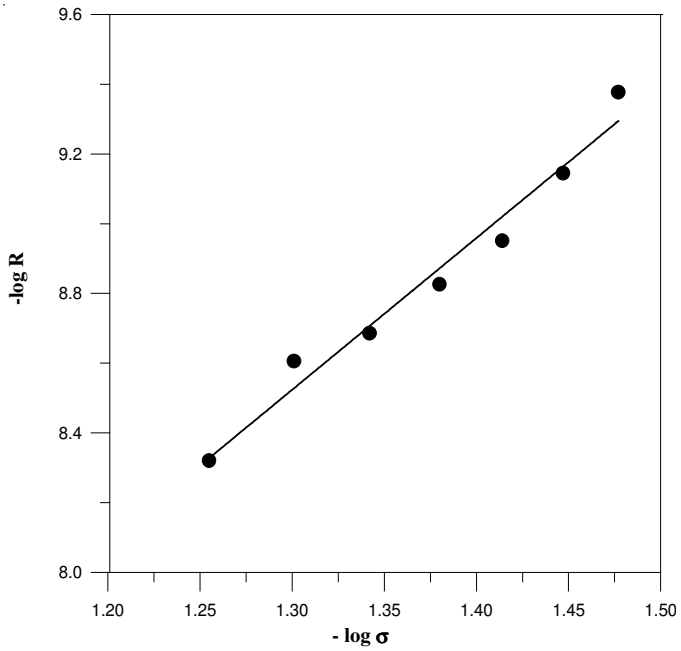


Fig. 2. Plots of -log σ against -log R for crystallization of calcium oxalate

When a solute crystallizes from its super-saturated solution, the presence of a third component can often have a dramatic effect on the crystal growth kinetics and habit form of the crystalline phase. Such a third component is effective at relatively low concentrations (10^{-9} < X mol L^{-1} > 10^{-3}) and exhibit a marked specificity in their action, factors which have led to the generally held conclusion that they are adsorbed onto growing crystal surfaces, Fig. 3 investigate the difference between the morphology of the crystal surface of calcium oxalate monohydrate seed in absence and presence of phenyl alanine as an inhibitor.

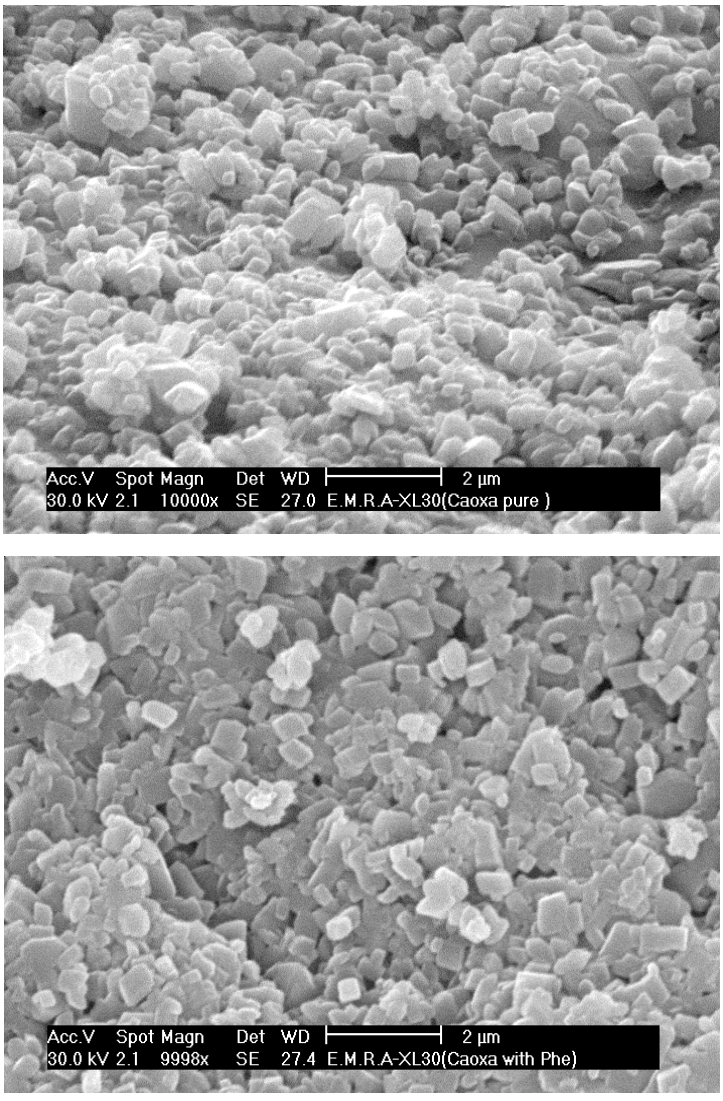


Fig. 3. Scanning electron microscopy of calcium oxalate crystal in absence and presence of phe

Adsorption of impurities onto crystal faces changes the relative surface free energies of the faces and may block sites essential to the incorporation of new solute into the crystal lattice. These effects may result in changes in growth kinetics.

The results of this work indicate that inhibition of calcium oxalate monohydrate crystal growth caused by additives, glycine, phenyl alanine, alanine and arginine. Table-2 summarizes the data at the same super-saturation for each additive. Each experiment was made in duplicate or triplicate for reproducibility. Concentrations as 9×10^{-5} mol L^{-1} for alanine and glycine reduced the rate of crystallization as much as 46.45 and 74, 57 %, respectively, compared to those solutions without additives at the same relative super-saturation (expts. 47 and 53). Arginine and phenyl alanine concentrations as 9×10^{-6} mol L^{-1} (expts. 34 and 41), were very effective in retardation of crystal growth rate of calcium oxalate monohydrate than alanine and glycine at the same concentration of additive at the same degree of

TABLE-2
EFFECT OF SOME AMINO ACIDS ON THE RATE OF CRYSTALLIZATION OF CALCIUM OXALATE CRYSTAL AT 37 ºC AND $\sigma = 2.0$

Exp. No.	Additive / 10^{-5} mol L^{-3}	Rate / 10^{-8} mol min^{-1} m^{-2}	Inhibition (%)
17	–	4.06	–
24	0.1 Arginine	3.59	11.58
25	0.2 Arginine	3.10	23.65
26	0.3 Arginine	2.72	33.00
27	0.6 Arginine	2.45	39.76
28	0.8 Arginine	2.18	46.45
29	0.9 Arginine	1.96	51.81
30	1.0 Arginine	1.96	51.81
31	0.1 Phenylalanine	3.26	19.88
32	0.2 Phenylalanine	2.88	29.04
33	0.4 Phenylalanine	2.45	39.76
34	0.5 Phenylalanine	1.96	51.81
35	0.7 Phenylalanine	1.79	55.83
36	0.9 Phenylalanine	1.47	63.86
37	1.0 Phenylalanine	1.47	63.86
38	1.0 Alanine	3.48	14.34
39	3.0 Alanine	3.10	23.67
40	5.0 Alanine	2.72	33.13
41	7.0 Alanine	2.39	41.09
42	9.0 Alanine	2.18	46.45
43	11.0 Alanine	1.90	53.15
44	1.0 Glycine	3.05	25.06
45	3.0 Glycine	2.28	43.78
46	5.0 Glycine	1.79	55.83
47	7.0 Glycine	1.36	66.53
48	9.0 Glycine	1.03	74.57
49	11.0 Glycine	0.71	82.59

saturation. Crystallization rates of calcium oxalate in presence of arginine and phenylalanine were reduced by as much as 51.81 and 63.86 %, respectively. It can be seen in Table-2 that the rate of crystallization of calcium oxalate in the presence of amino acids decreases with successive additions of additives and the effectiveness of inhibitors is: phenyl alanine > arginine > glycine > alanine. For instance, the real inhibitory action of the additive is carried out by interaction with the crystal surface. This interaction has an electrostatic nature in present case and an environment of ions will be surrounding the crystal surface. It is likely that the large presence of foreign ions will modify the interactions between the additive ions and the crystal surface. As the concentration of additive molecules increases, the active growth sites on the crystal surfaces may be blocked through adsorption and the rate of crystallization decreases.

The factors that might govern the efficiency of amino acids are: (a) the nitrogen content, (b) the number of amino acid groups (c) the pK_a value (d) the molecular size. The first three factors can be ruled out since (a) the nitrogen content of amino acids is not a decisive factor with regard to its reactivity (b) the reactivity is not dependent upon the number of amino groups (the amino acids in their decreasing order of reactivity possess the following number of amino groups: 1, 2, 1 and 1). The pK_a value has no bearing on the reactivity. Since the degree of dissociation has to be taken into account[22] *cf.* Table-3. The values of pK_a for the present amino acids fall in the following order: Arg < Gly ≈ Ala < Phe, which is not the same order of reactivity in inhibiting the crystal growth rate of calcium oxalate monohydrate.

TABLE-3
ACIDITY CONSTANTS OF AMINO ACIDS

Acid	pKa_1	pK_2	pK_3
Ala	2.35	9.87	-
Arg	2.01	9.04	12.48
Gly	2.35	9.60	-
Phe	2.58	9.24	-

A better assessment could only be gained by a comparison of the molecular size of these amino acids. The molecular size for the present amino acids fall in the following order: Phe > Arg > Gly > Ala, which is the same order of reactivity in inhibiting the crystallization of calcium oxalate monohydrate. Therefore the order of increasing reactivity of the amino acid is related to its molecular size.

In general, inhibitor molecules exert their influence through adsorption at active sites on the crystal surfaces. Chelating anions may be adsorbed at cationic sites and inhibit the precipitation when present at very low levels.The adsorption can be interpreted in terms of a Langmuir-type isotherm[22] leading to an equation of the form:

$$R_o/(R_o–R_i) = 1 + 1/K_LC \quad (3)$$

in which R_i and R_o are the rates of crystal growth in presence and absence of inhibitor respectively, K_L is the adsorption affinity and C is the concentration of additive. The values of the adsorption constants K_L are 1.63×10^5, 1.22×10^5, 1.96×10^4 and 0.80×10^4 L mol^{-1} for Phe, Arg, Gly and Ala, respectively. These values reflect the high adsorption affinity at the same relative super-saturation ($\sigma = 2.0$) in order Phe > Arg > Gly > Ala, which is the same order of reactivity of these additives related to the kinetic studies.

Typical adsorption plots according to eqn. 3 (Fig. 4) confirm the applicability of this simple adsorption isotherm at all super-saturations studied. The values of the adsorption affinity constants K_L are 2.05×10^5, 1.63×10^5 and 1.09×10^5 L mol for phenyl alanine at a relative super-saturation, $\sigma = 1.8$, 2.0 and 2.2, respectively. These values reflect the high adsorption affinity at low super-saturation in the presence of phenyl alanine as inhibitor. That is to say that the rate decrease caused by the additives augmented as super-saturation decreased. In fact, this is quite reasonable. Growth inhibition is considered to be caused by the adsorption of additive ions onto the crystal surface in competition with reactant ions. Therefore, according to the Langmuir model the degree of inhibition will be proportional to the ratio of additive/reactant concentrations. But this ratio is increasing as the precipitation proceeds, thus, the effect of the additives will also increase. A similar dependence of the degree of inhibition with change in driving force has been observed for the influence of additives on the rate of precipitation and dissolution of sparingly soluble salts in aqueous solution[24-27].

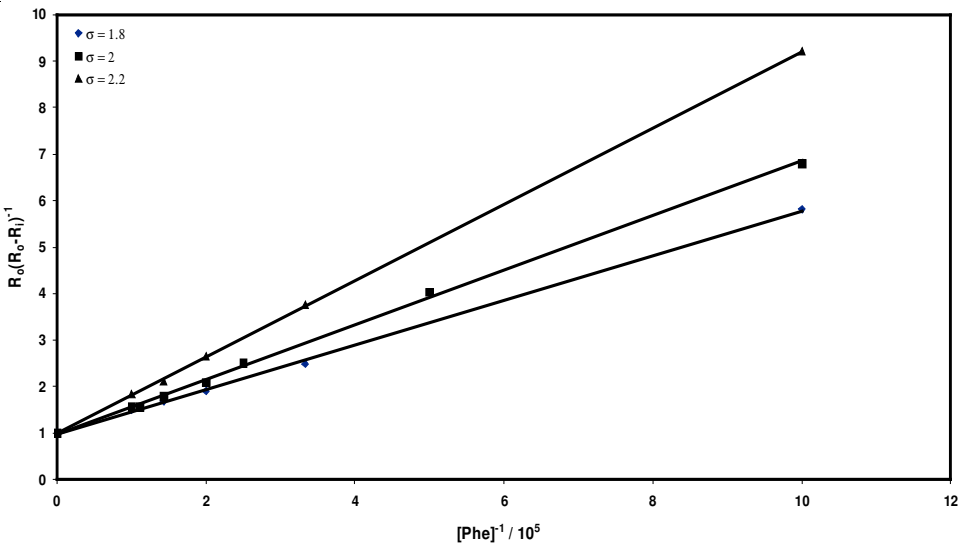


Fig. 4. $R_o(R_o-R_I)^{-1}$ against [phenyl alanine]$^{-1}$ at different relative super-sautration 1.8, 2.0 and 2.2

Conclusion

The analysis of the results show that the rate of precipitation of calcium oxalate monohydrate depends on the initial degree of saturation and on the concentration of the additive amino acids. Over a range of super-saturation, the precipitation reaction appears to be controlled by a surface process. The additions of amino acids, even at relatively low concentration, markedly retard the rate of growth of calcium oxalate monohydrate. The retardation effect was enhanced with increases in the additives content and as the relative super-saturation decreases. Complete inhibition of precipitation of calcium oxalate was not found in any concentration of these additives or any degree of saturation.

REFERENCES

1. R.W. Marshal and G.H. Nancollas, *J. Phys. Chem.*, **73**, 3838 (1970).
2. R.P. Singh, S.S. Gaur, M.E. Sheehan and G.H. Nancolas, *J. Cryst. Growth*, **87**, 318 (1988).
3. F. Grases, A. Maillan and A. Garcia-Raso, *J. Cryst. Growth*, **89**, 496 (1988).
4. F.J. Opalko and S.R. Khan, *J. Cryst. Growth*, **181**, 410 (1997).
5. J.H. Wiessner, A.T. Hasegawa, L.Y. Hung, G.S. Mandel and N.S. Manndel, *Kidney Int.*, **59**, 637 (2001).
6. J.M. Ouyang, X. Yao, Z. Su and F. Cui, *Sci. China, Ser, B*, **46**, 234 (2003).
7. J.M. Ouyang, J. Zhong, Z. Su, L. Kuan and W. Liang, *Chin. J. Urol.*, **24**, 138 (2003).
8. W. Roberson, M. Peacock, R. Marshall, D. Marshal and R. Nordin, *J. Med.*, **294**, 249 (1976).
9. S.M. Hamza, A. Mashaiy and S. Eid, *Indian J. Chem.*, **32A**, 364 (1993).
10. R. Zauner and A. Jones, *Chem. Eng. Sci.*, **55**, 4219 (2000).
11. C. Bouropoulos, N. Vagenas, P. Klepetsanis, N. Stavropoulos and N. Bouropoulos, *Cryst. Res. Technol.*, **39**, 8, 699 (2004).
12. A.L. Lehninger, Biochemistry, Worth Publishing: New York, edn. 2 (1975).
13. A. Meister, Biochemistry of Amino Acids, Academic Press Inc, New York, edn. 2 (1965).
14. H. Tanaka, K. Mijajima, M. Nakagaki and A. Simabayashi, *Chem. Pharm. Bull.*, **37**, 2897 (1989).
15. S. Koutsopoulos and E. Daias, *J. Cryst. Growth*, **216**, 443 (2000).
16. L. Wang, S. Qiu, W. Zachowicz, X. Guan, J. De Yoreo, G.H. Nancollas and J. Hoyer, *Langmuir*, **22**, 7279 (2006).
17. M. Tomson and G.H. Nancollas, *Science*, **200**, 1059 (1978).
18. P.G. Koutsoukos, Z. Amjd, M. Tomson and G.H. Nancollas, *J. Am. Chem. Soc.*, **102**, 1553 (1980).
19. A. Millan, M. Pavelkova, O. Sohnel and F. Grases, *Cryst. Res. Technol.*, **33**, 777 (1998).
20. C.W. Davies, Ion Association, Butterworth, London (1962).
21. B. Tomazic and G.H. Nancollas, *J. Cryst. Growth*, **46**, 355 (1979).
22. P. Koutsoukos, Z. Amjad and G.H. Nancollas, *J. Colloid. Interface Sci.*, **83**, 599 (1981).
23. P. Atkins, The Elements of Physical Chemistry, Oxford, edn. 3 (2001).
24. S.H. Hamza and G.H. Nancollas, *Langmuir*, **1**, 573 (1985).
25. A. Millan, M. Pavelkova, O. Sohnel and F. Grases, *Cryst. Res. Technol.*, **33**, 777 (1998).
26. S.K. Hamdona, M.M. Seif and S.M. Hamza, *Desalination*, **133**, 205 (2001).
27. S.K. Hamdona and U. Al-Haddad, *Desalination*, **228**, 277 (2008).

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