Asian Journal of Chemistry Vol. 12, No. 2 (2000), 403–406

Inhibition of Mineralisation of Urinary Stone Forming Minerals by Naturally Occurring Acids

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Naturally occurring acids, *viz.*, lactic, malic, tartaric, citric or succinic acids have been studied as inhibitors in the mineralisation of urinary stone forming minerals, *viz.*, calcium phosphate, oxalate or carbonate. Inhibition efficiency has been studied in different experimental models. Utility of these acids in urolithiasis inhibition has been discussed.

INTRODUCTION

Urolithiasis disease exists in 'endemic' proportions in some parts of our country[1]. Urinary stones contain both crystalloid and colloid components. The crystalloid components are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium-ammonium phosphate, uric acid and cysteine[2]. Stone formation is apparently related to level of urinary crystalloid and also to the level of inhibitors of calculogenesis in urine[3–5]. Quest for physiologically non-toxic and naturally occurring inhibitors of urolithiasis would be helpful in the prevention of this disease. As a part of our systematic study on inhibitors of urinary calculogenesis, we are presently reporting on the inhibition efficiency of some naturally occurring hydroxy and polybasic acids on the mineralisation of calcium phosphate, oxalate and carbonate in different experimental models.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solution of calcium acetate, trisodium phosphate, disodium oxalate and sodium carbonate, were prepated in distilled water. Four experimental models namely 'simultaneous flow static model' (S.S.M), 'simultaneous flow dynamic model' (S.D.M.), 'reservoir static model' (R.S.M.) and 'reservoir dynamic model' (R.D.M.) were designed. In the S.S.M. model the two salt forming solutions, *e.g.*, sodium phosphate and calcium acetate (for calcium phosphate) and the inhibitor (hydroxy or dibasic acid), were taken in three separate burettes (50 mL) and were allowed to fall simultaneously into a 250 mL beaker with a slow (dropwise) and equal speed. The whole operation took about 40 min. At the end the mixture was digested in a hot water bath for 10 min., cooled to room temperature and the precipitate was collected into a

preweighed centrifuge tube by centrifuging small volumes at a time and rejecting the supernatant liquid. Next, the tube with the precipitate was dried in an air oven at 120°C, cooled to room temperature and weighed till constant weight. Weight of the precipitate was determined.

In the S.D. model, the process was same except that the reaction mixture in the beaker was continuously stirred on a magnetic stirrer during the flow of salt forming solutions and the inhibitor. In the R.S. model, the whole amount of inhibitor solution (50 mL) was placed in the beaker in the beginning itself and the two salt forming solutions were allowed to run into it dropwise through burettes. Thus, a reservoir of inhibitor was created into which the salt forming solutions ran down. Rest of the operation was same as in other models.

In the R.D. model the process was same as R.S. model except that the reaction mixture was stirred continously on a magnetic stirrer during experiment. Simultaneous blank experiments with water in place of inhibitor were also carried out for evaluating the inhibition efficiency of inhibitors compared to water. All experiments were conducted at room temperature (20–25°C).

RESULTS AND DISCUSSION

pH of all the final solutions after experimentation were found to be around 7. Percentage efficiency of inhibition of inhibitor was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{wt. of ppt. in blank set} - \text{wt. of ppt. in exptl. set}}{\text{wt. of ppt. in blank set}} \times 100$$

Inhibition efficiencies of different naturally occurring acid solutions towards the precipitation of calcium phosphate, calcium oxalate and calcium carbonate are recorded in Tables 1–3. Study of the Tables suggests that the hydroxy acids are moderate to good inhibitors of calcium phosphate and carbonate mineralisation. Sequestering of these insoluble calcium salts by the hydroxy acids might be due to effective single or mixed ligand chelation[6] stabilised by effective hydrogen-bonding through the —OH groups. It is observed that the inhibitory capacity decreases with a decrease in the strength of inhibitor solution. Mass effect might be playing a role here. As the concentration of inhibitor decreases the equilibium might be favouring the precipitation of insoluble salts. As shown for citrate,

$$Ca^{2+} + (Cit)^{3-} \rightleftharpoons Ca\,(Cit)^{-}$$

lesser the Cit^{3-} prsent lesser Ca^{2+} ions can be trapped as $Ca(Cit)^{-}$ and more Ca^{2+} ions will be free for precipitation as insoluble salts.

A comparative study of Tables 1–3 suggests that the inhibitors are relatively less effective in the inhibition of calcium oxalate. However, tartaric acid, in higher concentration, is effective in inhibiting even this stubborn crystalloid. Once formed, calcium oxalate has practically no solubility in hydroxy acid solutions but if they are present before the formation of calcium oxalate, they may prevent the precipitation by exerting specificity towards calcium ions. Most of the inhibitors have been found to effectively inhibit calcium carbonate precipitation. A higher pK value of carbonic acid compared to inhibitors might be a factor.

TABLE-1
INHIBITION OF CALCIUM PHOSPHATE MINERALISATION BY NATURALLY OCCURRING ACIDS

Salt forming solutions 0.01 M $(CH_3COO)_2Ca$ and 0.01 M Na_3PO_4

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		S.S.M.	S.D.M.	R.S.M.	R.D.M.
Lactic acid	0.010	100	100	100	100
Lactic acid	0.001	48	50	50	60
Malic acid	0.010	100	100	100	100
Malic acid	0.001	49	49	50	55
Tartaric acid	0.010	85	85.5	86.6	88
Tartaric acid	0.001	30	31	33.3	35
Citric acid	0.010	100	100	100	100
Citric acid	0.001	48	48	50	60
Succinic acid	0.010	95	95	100	100
Succinic acid	0.001	34	36	50	55
Ammonium tartarate	0.010	69.2	71	83	85
Ammonium tartarate	0.001	18.7	20	66	68

TABLE-2
INHIBITION OF CALCIUM OXALATE MINERALISATION BY NATURALLY OCCURRING ACIDS

Salt forming solutions 0.01 M $(CH_3COO)_2Ca$ and 0.01 M $Na_2C_2O_4$

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		S.S.M.	S.D.M.	R.S.M.	R.D.M.
Lactic acid	0.010	33	34	43	44
Lactic acid	0.001	08	08	10	12
Malic acid	0.010	80	81	87.5	88
Malic acid	0.001	38	39	42.5	44
Tartaric acid	0.010	83	83	87.5	87.5
Tartaric acid	0.001	33	34.5	37.5	40
Citric acid	0.010	36	36	43	43
Citric acid	0.001	18	19	23	24
Succinic acid	0.010	50	51	57	57
Succinic acid	0.001	12	12	14	15
Ammonium tartarate	0.010	25	26.5	28.5	29
Ammonium tartarate	0.001	20	20	26.5	27

TABLE-3
INHIBITION OF CALCIUM CARBONATE MINERALISATION BY NATURALLY OCCURRING ACIDS

Salt forming solutions: 0.01 M $(CH_3COO)_2Ca$ and 0.01 M Na_2CO_3

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		S.S.M.	S.D.M.	R.S.M.	R.D.M.
Lactic acid	0.01	80	82	92.5	94
Lactic acid	0.001	60	64	90	90
Malic acid	0.01	97	97	100	100
Malic acid	0.001	78	80	90	92
Tartaric acid	0.01	65	65.5	90	90
Tartaric acid	0.001	50	52	80	82
Citric acid	0.01	100	100	100	100
Citric acid	0.001	60	62	92.5	93
Succinic acid	0.01	100	100	100	100
Succinic acid	0.001	65	66	87.5	88
Ammonium tartarate	0.01	65	68	95	95
Ammonium tartarate	0.001	53	55	90	92

A comparative study of different models indicates that the reservoir dynamic model is the most effective one in the inhibition of mineralisation. This may again be due to the mass effect. An *ab-initio* presence of large concentration of inhibitor (in the reservior) coupled with continuous stirring might be effectively chelating the Ca^{2+} and screening from precipitating anions like phosphate, oxalate and carbonate.

All the acids that we have studied presently are naturally occurring acids. Citric acid is present in various citrus fruits, malic acid occurs in cherries, tartaric acid in grapes, succinic acid in tomatoes while lactic acid is present in milk. Inhibition of calcium phosphate, oxalate and carbonate mineralisation by these acids suggest that increased intake of hydroxy acid containing fruits would be helpful in urinary stone prophylaxis.

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(*Received*: 7 September 1999; *Accepted*: 12 November 1999) AJC-1919