Inhibition of Mineralisation of Urinary Stone Forming Minerals by EDTA Salts and Effect of Macro and Micro Nutrient Metal Ions on the Inhibition

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Disodium, dipotassium and magnesium salts of ethylenediamine tetraacetic acid (EDTA) 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. Effect of some macro- and micro-nutrient metal ions on the inhibition efficiency of disodium EDTA has also been investigated. Utility of the results in urolithiasis inhibition has been discussed.

INTRODUCTION

Urinary calculogenesis is a result of mineralisation of insoluble calcium and magnesium salts in the urinary tract. Urinary stones contain both crystalloid and colloid components. The crystalloids are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium ammonium phosphate, uric acid and cysteine.[1] Stone formation is apparently related to level of urinary crystalloid and also to the level of inhibitors of calculogenesis in urine.[2–4] Calcium chelating agents might form suitable inhibitors in urolithiasis. As a part of our systemetic study on inhibitors of urinary calculogenesis we are presently reporting on the inhibition efficiency of some EDTA salts on the mineralisation of calcium phosphate, oxalate and carbonate in different experimental models. Effect of some macro- and micro-nutrient metal ions on the inhibition efficiency of disodium EDTA has also been studied.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solution of calcium chloride, trisodium phosphate, disodium oxalate and sodium carbonate were prepared in distilled water. Four experimental models, namely, 'simultaneous flow static model' (SSM), 'simultaneous flow dynamic model' (SDM) 'reservoir static model' (RSM) and 'reservoir dynamic model' (RDM) were designed. In the SSM model the two salt forming solutions, *e.g.*, sodium phosphate and calcium chloride (for calcium phosphate) and the inhibitor (solution of EDTA salt) were taken in three separate burettes (50 mL) and were allowed to fall simultaneously into a 250 mL

beaker in 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 pre-weighed 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 over 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 continuously 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).

The effect of macro- and micro-nutrient metal ions on the inhibition efficiency of sodium salt of EDTA was studied in the reservoir dynamic model (RDM). In the inhibitor reservoir (50 mL 0.001 M Na_2H_2EDTA), calculated quantity of solid metal salts (chlorides in case of Na, K and Mg and sulphates in case of Zn, Fe, Ni and Cu) were added so that the concentration of metal salt was 0.0003 M in the reservoir. Rest of the process was same as in other R.D. models.

RESULTS AND DISCUSSION

pHs 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 EDTA salts towards the mineralisation of calcium phosphate, calcium oxalate and calcium carbonate are recorded in Tables 1–3. Effects of macro and micro nutrient metal ions on the inhibition efficiency of disodium EDTA are recorded in Table-4 and Table-5 respectively. A study of the Tables 1–3 suggests that the EDTA salts are good inhibitors of calcium phosphate and carbonate mineralisation but are moderate inhibitors of calcium oxalate mineralisation. Out of different salts, the potassium and magnesium salts of EDTA seem to be more effective inhibitors even at low concentration. Magnesium EDTA has very low solubility (0.0003 M) and even at this very low concentration it shows 15% inhibition (in R.D. model) of calcium oxalate, the highly insoluble and stubborn constituent of urinary stones.

Sequestering of the insoluble calcium salts by the EDTA salts might be due to effective single or mixed ligand chelation. It is observed that the inhibitory

capacity decreases with a decrease in the strength of inhibitor solution. This may be due to 'mass effect'. As the concentration of inhibitor decreases the equilibrium might be favouring the precipitation of insoluble salt. A comparative study of different models indicates that the reservoir dynamic model is the most effective one in the inhibition of mineralisation. This may also be due to the 'mass effect'. An *ab-initio* presence of large concentration of inhibitor (in the reservoir) coupled with continuous stirring might be effectively chelating the Ca^{2+} and screening from precipitating anions like phosphate, oxalate or carbonate.

TABLE-1
INHIBITION OF CALCIUM PHOSPHATE MINERALISATION BY EDTA SALTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M Na_3PO_4

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		SSM	SDM	RSM	RDM
Disodium EDTA	0.0100	100	100	100	100
Disodium EDTA	0.0010	46	47	56	58
Dipotassium EDTA	0.0100	100	100	100	100
Dipotassium EDTA	0.0010	58	60	60	62
Magnesium EDTA	0.0003 (saturated solution)	16	18	20	20

TABLE-2
INHIBITION OF CALCIUM OXALATE MINERALISATION BY EDTA SALTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M $Na_2C_2O_4$

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		SSM	SDM	RSM	RDM
Disodium EDTA	0.0100	48	48	50	52
Disodium EDTA	0.0010	17	19	20	20
Dipotassium EDTA	0.0100	51	53	56	58
Dipotassium EDTA	0.0010	18	20	22	23
Magnesium EDTA	0.0003 (saturated solution)	11	11	14	15

Macro- and micro-nutrient metal ions form part of urinary system. Coordinating abilities of these, particularly of micro-nutrient metal ions, towards the

calcium precipitating/dissolving ligands present in the urinary system might affect the mineralisation-inhibition efficiency of complexons. Study of Tables 4 and 5 suggests that in general K^+ and Mg^{2+} are better additional inhibitors towards calcium salt mineralisation. Marked increase of inhibition is generally shown for oxalates. Inhibition of phospate by disodium EDTA is not much affected by the additional inhibitors. Micro-nutrient metal ions too have been found to enhance the inhibition of calcium oxalate mineralisation more than phosphate or carbonate. This may be due to additional complexation equilibria of oxalate ions by the metal ions. It may also be due to some mixed metal-mixed ligand complex equilibria in solution involving the EDTA and oxalate ligands.

TABLE-3
INHIBITION OF CALCIUM CARBONATE MINERALISATION BY EDTA SALTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M Na_2CO_3

Inhibitor	Concentration (M)	Inhibition efficiency (%)			
		SSM	SDM	RSM	RDM
Disodium EDTA	0.0100	100	100	100	100
Disodium EDTA	0.0010	52	54	60	60
Dipotassium EDTA	0.0100	100	100	100	100
Dipotassium EDTA	0.0010	59	60	66	68
Magnesium EDTA	0.0003 (saturated solution)	20	20	21	22

TABLE-4
EFFECT OF MACRO-NUTRIENT METAL IONS ON THE INHIBITION EFFICIENCY OF DISODIUM EDTA

Main inhibitor (50 mL 0.001 M soln.)	Additional inhibitor (0.0003 M with respect to main inhibitor soln)	Mineralisation inhibition (%) of			Increase (+) or decrease (–) or inhibition (%) over disodium EDTA		
		$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$	$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$
Disodium EDTA	—	58	20	60	—	—	—
Disodium EDTA	NaCl	65	55	91	+7	+35	+31
Disodium EDTA	KCl	69	71	93	+11	+51	+33
Disodium EDTA	$MgCl_2$	71	73	95	+13	+53	+35

EDTA is a well known chelating agent. Its salts find diverse analytical applications. Higher concentrations of these would be physiologically toxic. As indicated by our present studies, that even at low concentrations the EDTA salts moderately inhibit the mineralisation of calcium salts including oxalate, the EDTA salts might find application in chemodissolution of urinary stones and prophylaxis of

urolithiasis, a disease that exists in 'endemic' proportion in some parts of our country.[5–8]

TABLE-5
EFFECT OF MICRO-NUTRIENT METAL IONS ON THE INHIBITION EFFICIENCY OF DISODIUM EDTA

Main inhibitor (50 mL 0.001 M soln.)	Additional inhibitor (0.0003 M with respect to main inhibitor soln)	Mineralisation inhibition (%) of			Increase (+) or decrease (–) of inhibition (%) over disodium EDTA		
		$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$	$Ca_3(PO_4)_2$	CaC_2O_4	$CaCO_3$
Disodium EDTA	—	58	20	60	—	—	—
Disodium EDTA	$ZnSO_4$	66	30	46	+8	+10	–14
Disodium EDTA	$FeSO_4$	50	47	73	–8	+27	+13
Disodium EDTA	$NiSO_4$	62	40	66	+4	+20	+6
Disodium EDTA	$CuSO_4$	57	42	64	–1	+22	+4

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