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In-vitro Chemodissolution of Urinary Stones by Some Chelating Natural Acids

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Chemodissolution of ten surgically removed urinary stones was studied in dilute aqueous solutions of some chelating natural acids, *viz.*, lactic, malic, tartaric, citric or succinic acid. All the stones studied were of pure oxalate or phosphate or mixed oxalate-phosphate types. Kinetics of dissolution was observed over a period of ten days. Results revealed significant *in-vitro* solubility of urinary stones in the natural acids. Surface activity and chelation specificity seem to be the dominant factors in the process of dissolution.

Key Words: Chemodissolution, Urinay stones, chelating natural acids.

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, magnesium ammonium phosphate, uric acid and cystine[2]. Stone formation is apparently related to the 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 compounds for chemodissolution of urinary stones would be helpful in the designing of drugs for this disease. Specific chelation of the cations of the stones by the natural chelating compounds might lead to their (stones) dissolution or crumbling. With these views in mind, we have presently studied the chemodissolution of some surgically removed urinary stones by some chelating natural acids, *viz.*, lactic, malic, tartaric, citric or succinic acid.

EXPERIMENTAL

Surgically removed urinary stones were procured from local hospitals/nursing homes. Small pieces of stones were cut off and subjected to qualitative analysis for crystalloid composition by adopting standard methods[2]. Ten such stone samples were selected which were mainly composed of calcium, magnesium, oxalate and phosphate. Some of them were mixed calculi. The stones were labeled separately as sample S_1–S_{10}. Each stone sample was carefully cut into six pieces. Five of the pieces from each sample were placed, individually, in 100 mL of

0.001 M aqueous solution of natural acids, *viz.*, lactic, malic, tartaric, citric or succinic acid, separately, in 250 mL conical flasks. One pieces of each sample was placed in 100 mL distilled water for blank purpose. Each of the stone piece was weighed out on a sensitive monopan balance before dropping into the solution. Each flask was labelled indicating the number of the sample; initial weight of the stone and the nature of solution. A pinch of sodium chloride was added to each of the flasks, a bit of camphor was also added to each of the flasks as a preservative. After every 24 h the stone pieces were carefully removed from the solution with the help of a stainless steel spatula and washed carefully with distilled water and dried in an air oven at 100°C for 0.5 h. Next the pieces were cooled to room temperature and weighed out carefully. The stone pieces were then dropped back into their respective earlier solutions. The process of weighing after every 24 h was continued till 10 days or till crumbling into small pieces, whichever was earlier. From the weights of stone dissolved every 24 h, the percentage dissolution was calculated out using the formula:

$$\% \text{ dissolution} = \frac{\text{Initial wt. of stone} - \text{wt. of stone dissolved}}{\text{Initial wt. of stone}} \times 100$$

RESULTS AND DISCUSSION

Qualitative crystalloid composition and anatomical location of selected stone samples including patient details are recorded in Table-1. Data of chemodissolution of urinary stone samples by the chelating natural acids is recorded in Table-2.

TABLE-1
QUALITATIVE CRYSTALLOID COMPOSITION AND ANATOMICAL LOCATION OF SELECTED URINARY STONES

Stone sample	Sex	Age (yr.)	Anatomical location	Crystalloid composition
S_1	Male	32	Bladder	Ca^{2+}, PO_4^{3-}
S_2	Female	45	Pelvis	$Ca^{2+}, Ox^-, Mg^{2+}, PO_4^{3-}$
S_3	Male	45	Upper urinary tract	$Ca^{2+}, Mg^{2+}, PO_4^{3-}$
S_4	Female	27	Upper calyceal system	$Ca^{2+}, Mg^{2+}, PO_4^{3-}$
S_5	Female	35	Pelvis	$Ca^{2+}, PO_4^{3-}, Mg^{2+}$ (Traces)
S_6	Male	45	Bladder	Ca^{2+}, PO_4^{3-}
S_7	Male	20	Bladder	Ca^{2+}, Ox^-
S_8	Male	21	Pelvis	Ca^{2+}, PO_4^{3-}, Ox^-
S_9	Male	6	Bladder	Ca^{2+}, PO_4^{3-}, Ox^-
S_{10}	Male	2.5	Bladder	Ca^{2+}, PO_4^{3-}, Ox^-

A study of Table-2 suggests that the rate of dissolution of urinary stone in different natural acids is seemingly a function of many factors, *viz.*, the composition of the calculi, the surface area of the calculi, the cementation of crystals within the stones, the time factor, as well as the composition of the particular layer of the stone (ultrastructure) to which the chelating agent is exposed. However, the net total dissolving power of a chelating agent seems to be a factor of specificity of the agent towards the Ca^{2+} ions. In other words, the

TABLE-2
CHEMODISSOLUTION OF URINARY STONES BY CHELATING NATURAL ACIDS

Stone sample	Dissolving agent (100 mL 0.001 M soln.)	Initial wt. of stone (mg)	% Dissolution										Increase of % dissolution compared to water									
			Time period (days)										Time period (days)									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
S_1	Water	230	1.30	2.20	3.50	6.52	8.70	9.60	10.97	10.97	11.30	12.20	–	–	–	–	–	–	–	–	–	–
	Lactic acid	187	1.60	3.21	5.40	6.42	9.63	13.90	17.75	24.60	27.30	29.94	0.30	1.00	1.90	0.10	0.90	4.30	6.80	13.90	16.00	17.70
	Malic acid	214	3.73	7.94	14.95	19.62	26.25	34.11	36.91	41.12	42.52	48.00	2.43	5.70	11.40	13.10	17.50	23.50	26.00	30.20	31.20	36.40
	Tartaric acid	173	8.09	2.72	25.45	28.32	35.83	45.10	47.40	50.30	51.40	53.20	6.79	10.50	21.90	21.80	27.10	35.50	36.50	39.40	40.10	41.00
	Citric acid	240	5.00	7.92	15.83	22.10	28.30	30.00	35.80	47.80	50.80	55.00	3.70	5.70	12.30	15.60	19.60	20.40	24.90	36.90	39.50	43.20
	Succinic acid	153	1.31	2.61	5.90	14.40	16.99	20.91	22.90	24.80	25.50	27.50	0.01	0.40	2.40	10.40	10.40	11.30	12.00	13.90	14.20	15.30
S_2	Water	163	2.50	3.70	3.70	4.90	7.40	8.60	8.60	9.80	9.80	10.40	–	–	–	–	–	–	–	–	–	–
	Lactic acid	188	3.20	4.30	4.30	7.97	12.80	18.60	24.50	27.70	29.80	31.40	0.70	0.40	0.40	3.00	5.40	10.00	15.90	17.90	10.00	21.00
	Malic acid	182	2.70	3.80	6.04	11.50	18.13	21.40	24.20	26.90	26.90	28.02	0.20	0.10	2.30	6.60	10.70	12.80	15.60	17.10	17.10	17.60
	Tartaric acid	196	1.50	2.04	4.60	9.20	13.30	16.30	19.40	23.97	24.50	24.50	–0.50	–1.70	0.90	4.30	4.30	7.70	10.80	14.70	14.70	14.10
	Citric acid	164	4.90	6.70	10.97	14.60	18.90	28.04	32.30	36.60	37.80	37.80	2.40	3.00	7.20	9.90	9.70	19.40	23.30	28.00	28.00	27.40
	Succinic acid	186	1.10	3.80	8.10	10.80	15.10	17.70	20.96	22.00	22.00	22.60	–1.40	0.10	4.40	5.90	5.90	9.10	12.30	12.20	12.20	12.20
S_3	Water	127	2.50	3.14	4.70	7.10	10.20	11.80	11.70	12.60	14.20	14.20	–	–	–	–	–	–	–			
	Lactic acid	118	4.30	6.80	8.50	11.90	17.80	23.70	30.50	35.60	38.10	38.90	1.80	3.70	3.80	4.80	7.60	11.90	18.80	23.00	23.90	24.70
	Malic acid	102	5.90	9.80	12.70	17.60	23.50	32.40	41.20	50.00	50.98	51.96	3.40	6.70	8.00	10.50	13.30	20.60	29.50	37.40	36.70	37.70
	Tartaric acid	116	6.90	10.30	12.93	18.10	26.70	31.00	39.70	46.50	50.90	53.40	4.40	7.20	8.20	11.00	16.50	19.20	28.00	33.90	36.70	39.20
	Citric acid	132	7.60	10.60	12.10	21.20	24.20	28.80	37.10	43.93	51.50	55.30	5.10	7.50	7.40	14.10	14.00	17.00	25.40	31.30	37.30	41.10
	Succinic acid	96	3.10	6.20	8.30	12.50	18.70	22.90	Crumbled			0.60	3.10	3.60	5.40	8.50	11.10	–	–	–	–	–

Stone sample	Dissolving agent (100 mL 0.001 M soln.)	Initial wt. of stone (mg)	% Dissolution										Increase of % dissolution compared to water									
			Time period (days)										Time period (days)									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
S_4	Water	92	2.20	4.30	5.40	7.60	7.60	9.80	11.95	13.04	13.04	13.04	–	–	–	–	–	–	–			
	Lactic acid	106	3.80	5.70	7.50	8.50	13.20	17.92	24.50	35.80	37.70	37.70	1.60	1.40	2.10	0.90	5.60	8.10	12.60	22.70	22.70	22.70
	Malic acid	121	4.95	6.60	9.91	12.40	21.50	31.40	37.20	46.30	50.40	Crum.	2.70	2.30	4.50	4.80	13.90	21.60	25.30	33.30	37.40	–
	Tartaric acid	110	7.30	9.10	10.00	12.70	10.10	35.50	43.60	51.80	Crumbled		5.10	4.80	5.60	5.10	11.50	25.70	31.70	38.80	–	–
	Citric acid	109	9.20	12.80	16.50	19.30	25.60	30.20	39.40	56.80	70.60	75.20	7.00	8.50	11.10	11.70	18.00	20.40	27.50	43.80	57.60	62.20
	Succinic acid	115	6.90	12.10	13.90	17.30	12.60	26.90	33.04	36.20	37.40	38.20	4.70	7.80	8.50	9.70	15.00	17.10	21.10	23.20	24.40	25.20
	Water	98	2.00	3.10	5.10	5.10	7.10	11.20	11.20	12.20	13.20	13.20	–	–	–	–	–	–	–			
S_5	Lactic acid	96	5.20	8.30	11.40	15.60	18.70	27.00	33.30	37.50	39.60	39.60	–3.20	5.20	6.30	10.50	11.60	6.80	12.10	25.30	26.40	26.40
	Malic acid	100	2.70	4.60	7.40	11.10	17.60	24.10	34.20	40.70	43.50	44.40	0.70	1.50	2.30	6.00	10.50	12.90	23.00	28.50	30.30	31.20
	Tartaric acid	120	5.00	5.80	11.60	17.50	24.20	28.30	38.30	48.30	58.30	30.00	3.00	2.70	6.50	12.40	17.10	7.10	27.10	36.10	45.10	47.80
	Citric acid	126	4.70	7.90	10.30	13.40	20.60	30.20	38.10	52.40	62.60	65.80	2.70	4.80	5.20	8.30	13.50	19.00	26.90	40.20	49.40	52.60
	Succinic acid	112	1.80	5.40	7.10	10.70	16.90	18.70	23.20	25.80	26.70	26.70	–0.20	2.30	2.00	5.60	9.80	7.50	12.00	13.60	13.50	13.50
S_6	Water	180	2.20	2.20	3.80	4.40	6.10	7.70	8.80	9.40	9.40	10.50	–	–	–	–	–	–	–			
	Lactic acid	196	2.60	3.60	5.10	7.10	10.20	14.20	16.80	22.90	25.50	26.00	–0.40	1.40	1.30	2.70	4.10	5.50	8.00	13.50	16.10	15.50
	Malic acid	210	1.90	2.80	7.10	8.60	13.30	18.10	23.30	30.00	34.80	39.00	–0.30	0.60	3.30	4.20	7.20	10.40	14.50	20.60	25.40	28.50
	Tartaric acid	226	4.80	8.40	11.50	19.90	27.90	34.50	Crumbled				2.60	6.20	7.70	15.50	21.80	26.80	–	–	–	–
	Citric acid	243	3.20	6.20	11.50	16.80	23.90	29.60	34.20	Crumbled			1.00	4.00	7.70	12.40	17.80	21.90	25.40	–	–	–
	Succinic acid	188	3.20	6.40	9.60	11.70	16.50	20.20	24.50	24.50	25.50	25.50	1.00	4.20	5.80	7.30	10.40	12.50	15.70	15.10	16.10	15.00

Stone sample	Dissolving agent (100 mL 0.001 M soln.)	Initial wt. of stone (mg)	% Dissolution										Increase of % dissolution compared to water									
			Time period (days)										Time period (days)									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
S_7	Water	192	1.04	1.60	3.10	3.10	4.20	5.20	5.70	7.30	8.30	8.30	–	–	–	–	–	–	–	–	–	–
	Lactic acid	240	1.20	2.50	3.70	5.80	8.70	15.80	16.30	18.30	20.00	20.80	–0.20	0.90	0.60	2.70	4.50	10.60	10.60	11.00	11.70	12.50
	Malic acid	212	0.90	2.80	4.70	7.10	11.30	13.20	16.00	19.30	21.70	23.10	–0.10	1.20	1.60	4.00	7.10	8.00	10.30	12.00	13.40	14.80
	Tartaric acid	198	1.50	2.50	4.00	4.70	8.10	10.60	16.20	20.20	21.20	21.20	0.50	0.90	0.90	1.60	3.90	5.40	10.50	12.90	13.90	12.90
	Citric acid	252	0.80	2.80	3.60	5.50	7.90	11.10	15.10	18.30	20.60	23.40	–0.20	1.20	0.50	2.40	3.70	5.90	9.40	11.00	12.30	15.10
	Succinic acid	173	0.60	2.30	6.90	8.70	15.60	16.20	19.60	20.20	21.40	21.90	–0.40	0.70	3.80	5.60	11.40	11.00	13.90	12.90	13.10	13.60
S_8	Water	152	2.60	3.90	5.30	5.30	6.60	7.20	7.80	7.80	9.20	9.20	–	–	–	–	–	–	–	–	–	–
	Lactic acid	178	1.60	4.50	6.70	11.80	13.50	15.70	17.90	20.20	23.60	26.40	–1.00	0.60	1.40	6.50	6.90	8.50	10.10	12.40	14.40	17.20
	Malic acid	186	2.70	3.70	5.90	10.20	13.90	16.10	19.40	24.20	27.40	28.40	0.10	–0.20	0.60	4.90	7.30	8.90	11.60	16.40	18.20	19.20
	Tartaric acid	192	2.60	4.20	7.30	9.40	14.60	16.10	21.40	24.50	26.00	27.10	0.00	0.30	2.00	4.10	8.00	8.90	13.60	16.70	15.80	17.90
	Citric acid	176	2.30	4.00	5.70	10.20	14.80	18.80	25.00	30.10	35.20	38.60	–0.30	0.10	0.40	4.90	8.20	11.60	17.20	22.30	26.00	29.40
	Succinic acid	188	2.70	3.70	4.80	7.40	9.60	11.70	15.90	21.30	25.50	26.10	0.10	–0.20	–0.50	2.10	3.00	4.50	8.10	13.50	16.30	16.90
S_9	Water	204	0.90	1.90	1.90	2.90	4.40	5.80	6.40	7.80	8.30	8.30	–	–	–	–	–	–	–	–	–	–
	Lactic acid	188	2.10	4.30	5.30	6.90	9.60	13.80	17.00	21.80	25.50	26.10	–1.10	2.40	3.40	4.00	5.20	8.00	10.60	14.00	17.20	17.80
	Malic acid	208	1.40	2.90	8.80	6.70	8.60	11.50	18.30	22.10	24.50	25.00	0.50	1.00	1.90	3.80	4.20	5.70	11.90	14.30	16.20	16.70
	Tartaric acid	212	1.40	2.40	4.20	7.50	9.40	13.20	16.90	21.20	22.60	23.60	0.50	0.50	2.30	4.60	5.00	7.40	10.50	13.40	14.30	15.30
	Citric acid	221	1.80	4.50	8.10	13.10	19.00	20.40	23.10	Crumbled			0.90	2.60	6.20	10.20	14.60	14.60	16.70	–	–	–
	Succinic acid	210	1.40	2.40	2.90	6.20	8.60	11.90	14.70	8.10	21.90	22.90	0.50	0.50	1.00	3.30	4.20	6.10	8.30	10.30	13.60	14.60

Stone sample	Dissolving agent (100 mL 0.001 M soln.)	Initial wt. of stone (mg)	% Dissolution										Increase of % dissolution compared to water									
			Time period (days)										Time period (days)									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
S_{10}	Water	242	0.80	2.50	3.30	3.30	4.50	4.90	6.20	7.00	7.80	7.80	–	–	–	–	–	–	–	–	–	–
	Lactic acid	210	1.90	3.30	6.20	7.10	10.00	13.30	16.20	22.80	24.80	25.20	1.10	0.80	2.90	3.80	5.50	8.40	10.00	15.80	17.00	17.40
	Malic acid	208	1.90	4.80	7.20	9.60	14.90	19.70	23.10	Crumbled			1.10	2.30	3.90	6.30	10.40	14.80	16.90	–	–	–
	Tartaric acid	185	0.40	4.30	7.60	9.70	15.10	16.70	26.50	24.30	27.60	28.60	–0.40	1.80	4.30	6.40	10.60	11.80	20.30	17.30	19.80	20.80
	Citric acid	230	2.60	4.30	7.40	9.60	12.60	16.90	20.40	24.30	26.50	27.80	1.80	1.80	4.10	6.30	8.10	12.00	14.20	17.30	18.70	20.00
	Succinic acid	226	2.20	3.10	5.30	7.50	11.50	14.60	17.70	20.80	22.10	22.50	1.40	0.60	2.00	4.20	7.20	9.70	11.50	13.80	14.30	14.70

chelating ability of the agent with the Ca^{2+} ions and its stabilisation in solution is the most important factor in the process of dissolution.

The non-oxalate stones (S_1, S_3, S_4, S_5 and S_6) have been found to have a better solubility as compared to the oxalate ones (S_2, S_7, S_8, S_9 and S_{10}). The average dissolution of non-oxalate calculi is highest (55.53%) with tartaric acid, closely followed by citric acid (49.07%) and malic acid (45.97%). Lactic acid and succinic acid show rather low dissolution. The higher average dissolution by tartaric acid can be explained on the basis of its high specificity[6] for Ca^{2+}. Citric acid and malic acid too have good chelating ability for calcium ions[7]. The dissolution of calcium phosphate of the stone by the hydroxy acids might also be due to mixed ligand calcium complexation by the phosphate and hydroxy acid anions. In fact, such complexes have been reported[8] in aqueous medium. The aqueous solubility (12.62%) of the stone might be due to slight decementation and crumbling as well as slight salt (of stone) solubility. Amongest the non-oxalate stones, the ones containing magnesium also show a better dissolution percentage, which may be explained on the basis of higher solubility of magnesium salts compared to those of calcium.

The oxalate containing calculi, in general, showed a low dissolution compared to the non-oxalate stones. This might be due to a slightly lower specificity of hydroxy acid anions compared to the oxalate ions for Ca^{2+}. It seems the extraction of Ca^{2+} by the chelating agents from calcium oxalate lattice requires a higher activation energy than from calcium phosphate lattice. The lattice energy values of calcium oxalate and phosphate seem to be important ruling factors in the chemodissolution of urinary stones. For oxalate containing stones, the trend of dissolution power of chelating agents has not been found to be significantly different. The overall range varies only slightly from 23 to 31%. Only citric acid has somewhat better ability (31.9%). The average aqueous dissolution of oxalate stones is comparatively a meagre 8.8%.

The process of dissolution has been observed to be slow in the beginning. The rate picked up after 2 to 3 days, perhaps, after good initial surface activity. In fact, surface activity and chelation specificity seem to be the dominant factors in the process of dissolution. In non-oxalate as well as oxalate stones, there has been crumbling of the calculi in some cases, particularly in citric acid, malic acid or tartaric acid. It seems, continuous removal of calcium ions from the surface layers by the chelating agents effects the cementation of the entire stone. This might be for exposition of new inner Ca^{2+} by the chelating anions. In fact a crumbling agent (if not dissolving) would also serve the purpose in urolithiasis, because the crumbled small pieces can easily be passed out through urine. A suitable crumbling agent would form an alternative to lithotripsy (the present day popular remedy).

All the acids, that we have presently studied, are common plant acids. They are contained by many edible fruits such as orange, sweet orange, lemon, grapes, cherry, etc. A good dissolving power of these natural acids for urinary stones suggests applicability of natural products and fruits (containing these acids) in the management of urolithiasis. *In-vivo* studies would further confirm this aspect.

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