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Reactions of Calcium Phosphate with Dihydrogen Calcium Ethylenediamine Tetraacetate

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An attempt has been made to synthesise an important calcium complex, dihydrogen calcium ethylenediamine tetraacetate with a view to study its reactivity with urinary stones.

INTRODUCTION

Ethylenediamine tetraacetic acid (EDTA) is a potential chelating agent as it forms a stable complex with calcium. The disodium salt of calcium EDTA is used as an antidote in lead poisoning. Salts of EDTA act as a strong sequestering agent (inhibitor) towards the insoluble precipitate of calcium phosphate and calcium oxalate.

It has been reported that EDTA is more toxic than any other solution of the compounds of EDTA[1]. The calcium chelating ability[2] of EDTA early raised expectations that EDTA would dissolve stones lodged in the urinary tract.[3, 4]

In the present paper, we have synthesised a protonated complex of EDTA and studied its applicability in the dissolution of calcium phosphate as well as its inhibitory effect on the mineralisation of calcium phosphate by a new model system. Our view is that this protonated calcium complex of EDTA, [H_2CaEDTA], being less toxic than [Na_2H_2EDTA], can act as a complexon for further calcium ions of calcium phosphate.

EXPERIMENTAL

Calcium hydroxide and EDTA were mixed in 1 : 1 mole ratio in a conical flask and stirred well over a magnetic stirrer. Whole of calcium hydroxide went into solution. The solution was then concentrated on a water bath and the concentrate was left for crystallisation at room temperature. Crystals formed were washed with cold distilled water and dried. Analytical results were found to be in close agreement to the values required for dihydrogen calcium ethylenediaminetetraacetate [H_2CaEDTA].

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Solubility of calcium phosphate in [$H_2CaEDTA$]

A saturated solution of [$H_2CaEDTA$] was prepared (250 mg of [$H_2CaEDTA$] was dissolved in 100 mL of distilled water) and was treated with known weight of calcium phosphate. The reaction mixture was stirred for a few minutes over a magnetic stirrer at room temperature. The suspension was then filtered through a G_3 sintered glass crucible to remove the insoluble calcium phosphate. The insoluble part of calcium phosphate remaining in crucible after drying was weighed out. The amount of calcium phosphate dissolved was calculated. Solubility was calculated out for 1000 mL of [$H_2CaEDTA$] solution.

Inhibitory activity of [$H_2CaEDTA$] on the mineralisation of calcium phospate

The model system used to study the inhibitory effect of [$H_2CaEDTA$] solution on the mineralisation of calcium phosphate bears some resemblance to the one (model) used by Kabra.[5] The model consisted of two beakers of 100 mL capacity. In one of them 70 mL of 0.1 M solution of calcium chloride was placed; to it 20 mL of 0.0066 M (M/150) solution of [$H_2CaEDTA$] (inhibitor) was added. In the second beaker 70 mL of 0.1 M solution of trisodium phosphate (Na_3PO_4) was added and 20 mL of 0.0066 M solution of [$H_2CaEDTA$] was added to it. Two filter papers (Whatman 41) were folded in a square shape such that both the wicks and equal surface area. Both of them were separately weighed, these filter paper wicks were then suspended separately into the solution of the above two beakers. This set of two beakers was termed 'Experimental set'. A similar assembly of two beakers with suspended filter paper wicks was arranged at the site of the experimental set. One of the beakers of this set contained 90 mL of 0.1 M solution of $CaCl_2$ and the other beaker contained 90 mL of 0.1 M Na_3PO_4 solution. No inhibitor was added in this case. This set was termed 'Blank set'.

Next, two filter paper wicks suspended in the two beakers of experimental set were interchanged at the end of every five minutes interval. This interchange of wicks, at the end of every five minutes, was continued for 4 h. Similarly, it was also repeated for 'Blank set'. At the end of the experimentation the filter paper wicks of experimental as well as blank sets, after careful washing, were dried at 90°C in an air oven, cooled to room temperature and weighed separately. From the difference in weights of filter papers, the amount of crystalloid $Ca_3(PO_4)_2$ deposited on each paper was determined. Average deposition of phosphate in the experimental set was calculated by adding the deposition on both the papers of experimental set and dividing it by two. Similarly average deposition on the blank set papers was also calculated out. The difference between the average deposition of phosphate in experimental and blank sets was calculated; this was termed 'net inhibition'. The net inhibition value was divided by the average amount deposited in the blank set and multiplied by 100 to get 'percentage inhibition' value.

Similar experimental and blank sets under all similar experimental conditions were run for different concentrations, *viz.*, 0.05 M and 0.04 M of salt forming solutions, *viz.*, calcium chloride and trisodium phosphate solutions. Inhibitor concentration was, however, kept the same in all the runs.

The colour, solubility and decomposition temperatures of all the complexes isolated are recorded in Table-1.

TABLE-1

Compound	Colour	Aqueous solubility	Decomposition temperature (°C)
Dihydrogen calcium ethylenediamine tetraacetate	White	Soluble in water	245

RESULTS AND DISCUSSION

Analytical Results of [H_2CaEDTA] Complex

A. [H_2CaEDTA] complex (1 : 1)

Found %	:	Ca, 10.82	N, 7.40	C, 30.80	H, 2.23
(Required %)	:	(10.90)	(7.10)	(32.70)	(4.30)

B. Solubility of [H_2CaEDTA] in water = 2.5 g/L

C. Solubility of calcium phosphate in saturated solution of [H_2CaEDTA] = 0.87 g/L

D. Inhibitory values of 0.0066 M (M/150) aqueous solution of [H_2CaEDTA] on the mineralisation of calcium phosphate.

E. [H_2 CaEDTA] on the mineralisation of calcium phosphate:

Contents of Beakers

Experimental set:	Beaker I	70 mL $CaCl_2$ solution + 20 mL inhibitor solution H_2CaEDTA
	Beaker II	70 mL of Na_3PO_4 solution + 20 mL inhibitor solution H_2CaEDTA
Blank set:	Beaker I	90 mL $CaCl_2$ solution
	Beaker II	90 mL $Na_2C_2O_4$ solution

TABLE–2

No. of obs.	Strength of salt forming solutions	Average accretion of calcium phosphate (mg)		Net inhibition (difference) (mg)	Percentage inhibition
		Blank set	Expt. set		
1.	0.10 M	597.0	474.0	123.0	20.60
2.	0.05 M	187.5	84.1	103.4	55.20
3.	0.04 M	195.0	58.5	136.5	70.00

IR spectra of (H_2CaEDTA)

It has been shown that study of infrared spectra generally makes it possible to identify complexed carbonyl groups and carboxylic acid groups in the presence of each other, while the distinction between complexed carbonyl groups and free carboxylate ions may be definite only when the metal ion has a great tendency to form covalent bonds.

The bonds of primary concern are those associated with the carbonyl group. Five absorptions are considered in this connection, and three of these appear only in the case of carboxylic acid group. These are the bands of 2800–2600 cm^{-1} (—OH stretch hydrogen bonded), 1700 cm^{-1} (C═O asymmetric), 1400 cm^{-1} (C═O symmetric), and 1225 cm^{-1} (associated with carboxylic acid group).

By using the frequency difference between the asymmetric and symmetric COO^- group as the criterion for the degree of covalent character for the metal carboxylate bonds, an arbitrary designation has been made that the bonds are primarily covalent when the difference is 225 cm^{-1} or more; when the difference is less than 225 cm^{-1}, the bonding is primarily ionic.

For the compound [$H_2CaEDTA$], it is expected that two protons from carboxylic acid groups have been replaced by divalent calcium ion. The other two carboxylic acid groups are free, may be ionic. The IR spectra of [$H_2CaEDTA$] show no peak around 1760 cm^{-1}; instead two peaks appear, one at 1645 cm^{-1} and the other at 1605 cm^{-1}. These peaks are perhaps of C═O group—asymmetric C═O of coordinated Ca(II) at 1645 cm^{-1} and 1605 cm^{-1} being the ionic C═O.

Again, the symmetic C═O shows two peaks, one at 1410 cm^{-1} and the other at 1308 cm^{-1}.

In the spectra of [$H_2CaEDTA$] two sets of C═O frequency exist. One set is 1645 cm^{-1} and 1398 cm^{-1} and the other is 1605 cm^{-1} and 1410 cm^{-1}, which can be assigned to asymmetric and symmetric C═O frequencies respectively. For the first set, the difference is 247 cm^{-1} and falls clearly in the covalent category, whereas for the second set, a difference of 195 cm^{-1} suggests that it is ionic.

Thus it is quite apparent that in [$H_2CaEDTA$] compound, two types of carboxylate groups are present, one covalently bounded to Ca(II), and the other ionic. Absence of any band around 1700 cm^{-1} clearly shows that undissociated COOH groups do not exist in the compound.

For [$H_2CaEDTA$] compound two bands are observed, one at 1100 cm^{-1} and the other at 1080 cm^{-1}. These two values are comparable to those for covalently and ionically bunded cationic salts.

Conclusion

[$H_2CaEDTA$] has a moderate solubility in water and its solution has some solubility towards certain calcium phosphate. This compound [$H_2CaEDTA$], thus, would be effective in the demineralisation of calcium phosphate of urinary stones. In the studies of the inhibitory activity of [$H_2CaEDTA$] on the mineralisation of calcium phosphate, it has been found the the average deposition of calcium phosphate on the filter papers in the experimental sets are always less than the corresponding blank sets. Presence of the inhibitor [$H_2CaEDTA$] in the system in experimental sets is probably the cause of this decreased deposition of phosphate. [$H_2CaEDTA$] thus probably shows this inhibition of phosphate mineralisation either by removing calcium ions as Ca_2EDTA or by forming some mixed aqueous soluble complex with a portion of calcium phosphate deposited.

One experimental result suggests that $H_2CaEDTA$ affects 20 to 70% inhibition of phosphate mineralisation, depending on the concentration of the salt forming solutions. With more dilute solutions of calcium ions, the effectivity is more. These results suggest that in animal system, even a highly dilute solution of [$H_2CaEDTA$] would serve as a potent inhibitor in the formation of calcium phosphate urinary stones. This fosters the possibilities of applying this compound [$H_2CaEDTA$] as a chemotherapeutic agent in the urolithiosis patients.

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