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DISSOLUTION BEHAVIOUR OF CARBONATED APATITES IN THE PRESENCE OF SOME IONS

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ABSTRACT

As a part of the long-term goal of understanding the site of dissolution initiation of hydroxyapatite (HAP), main constituent of the teeth, and for more understanding the reasons for some enamel being more susceptible to dissolution than the other, we had undertaken a systematic investigation of the dissolution of four types of synthetic apatites prepared in our laboratory in absence and presence of some ions. Investigation has been done by determining the dissolution rate of the four powders at different pH's, various percentages of partial saturation and in the presence of calcium, phosphate, strontium and fluoride ions. Data obtained revealed that the combined effect of fluoride ions with 50% or 75% partial saturation with calcium and phosphate ions was more significant in minimizing the dissolution rate of all the prepared apatites. Moreover, the inclusion of zinc, strontium or fluoride ions during the process of preparation of the carbonated apatite powder

reduced the dissolution rate at all the conditions studied. This has been attributed to the possible alteration of the crystal imperfections caused by the presence of carbonate in the prepared apatites.

INTRODUCTION

Apatite is basic calcium phosphate typified by hydroxyapatite (HAP), $Ca_{10}(PO_4)_6(OH)_2$. Its physicochemical properties, in particular solubility and detailed crystallographic structure, are directly related to the mineralization process in biological systems. The various reports in the literature on solubility of HAP are contradictory. Clark[1] concluded that HAP has a definite solubility product. Thermodynamic functions for HAP from which a solubility product can be calculated have been reported[2,3]. Other reports[4-6], however, claimed that systems saturated with respect to HAP don't conform to the principle of a solubility product constant based on the stoichiometry of the salt. Among the models that have been advanced to explain the apparent anomalous behaviour of HAP in aqueous solution,those advocating formation of complexes on the surface of the solid have received considerable attention[5]. Furthermore, various physical models for dissolution of the apatites were explored to determine the factors governing the rate of dissolution[7]. In addition, recent studies under partial saturation conditions have shown that the dissolution process could be largely zonal involving a region of 50-100 μm thickness of the surface[8]. Recently, a model involving two distinct types of dissolution sites[9] has been proposed to explain the dissolution behaviour of HAP.

The inorganic phase of teeth and bone belongs to the apatite group. Biological apatites are modified by the presence of other ions such as Na, K, Mg, CO_3, F, Zn, Cl and P_2O_7[10,11]. Carbonate is the third most abundant ion (after calcium and phosphate) in bone and dental enamel, although the mode of its substitution in apatite both structurally and compositionally is subject to controversy[11]. Carbonate in dental enamel has been implicated in the caries process and is thought to act by increasing the acid reactivity. Moreover, the substitution of carbonate in precipitated apatite is accompanied by changes in the size and shape of the apatite crystals[12].

The aim of the present study was to prepare and characterize carbonated HAP with or without foreign metals usually present in the enamel, namely, zinc, strontium and fluoride. A thorough investigation of the effect of common ions, pH, partial saturation and fluoride on the dissolution was undertaken.

MATERIALS AND METHODS

Synthetic carbonate-containing HAP was prepared according to the method of LeGeros[11]. The carbonate content being about 5%. The product was checked by I.R. and elemental analysis. Three other samples were prepared using the same method with the addition of zinc sulphate, strontium chloride or sodium fluoride to the reactants.

Buffer Solutions: All the chemicals used for the preparation of buffer solutions were of analytical grade. Acetate buffer solution, 0.1 M, was prepared by mixing the calculated amounts of acetic acid and sodium acetate. Partially saturated buffer solutions were made by adding predetermined amounts of calcium chloride and sodium dihydrogen phosphate. Sodium fluoride was also added to provide 1,2 and 5 p.p.m. Calculated amounts of sodium chloride were added to give an ionic strength of 0.4. The pH was adjusted to the required values (4.4, 5 or 5.6). The solution ionic activity product of hydroxyapatite:

$$K_{HAP} = a^{10}_{Ca^{2+}} \cdot a^{6}_{PO_4^{3-}} \cdot a^{2}_{OH^-}$$ and of fluoroapatite

$$K_{FAP} = a^{10}_{Ca^{2+}} \cdot a^{6}_{PO_4^{3-}} \cdot a^{2}_{F^-}$$ were calculated with

the help of the ionic activity data of the individual species as shown in Tables 1 and 2[13].

Powder Dissolution Method: Forty milligrams of carbonated HAP was suspended in 20 ml of double distilled water. The slurry was stirred with a magnetic stirrer (400 r.p.m.). Stirring was resumed and 20 ml of the dissolution medium of double concentration were added. Samples of 0.5 or 1 ml were withdrawn at proper time intervals and were immediately passed through a millipore filter (GSU, 0.22 μ). Sampling and filtration were done within 4-6 seconds. Filtrates were analyzed for phosphate content. Temperature was maintained at 30°C.

Analytical Method: Phosphate concentration was determined using the ammonium molybdate-stannous chloride colorimetric method[14]. The phosphate-ammonium molybdate complex was reduced by stannous chloride. Absorbence of the color produced was determined after 10 minutes at 700 nm.

Solubility Product Calculation: When the amount of HAP in an acidic buffer solution reaches an apparent equilibrium, the apparent solubility product K_{ap} can be determined by knowing the concentration of total calcium and phosphate in the bulk. Such data can be obtained for each dissolution rate experiment. The equation applicable to the bulk equilibrium conditions is:

$$K_{ap} = (Ca^{2+})^{10} (PO_4^{3-})^{6} (OH^-)^{2}$$

TABLE 1

Equilibrium Constants and Activity Coefficients for Theoretical Calculations[13]

K^*_{2P}	6.40×10^{-8}	K^*_{3P}	4.73×10^{-13}
H^+	0.800	F^-	0.632
$H_2PO_4^-$	0.550	$HPO_4^=$	0.230
PO_4^{3-}	0.095	Sr^{2+}	0.360
Ca^{2+}	0.360	OH^-	0.700

K_{2P} and K_{3P} are the second and third dissociation constants, respectively, of phosphoric acid.

TABLE 2

Composition and Ionic Activity Products for the Dissolution Media Used.

Ratio of calcium to phosphate ions	Total concentrations of			Ionic activity products	
	calcium $10^3M.L^{-1}$	phosphate $10^3M.L^{-1}$	fluoride $10^5M.L^{-1}$	pK_{HAP}	pK_{FAP}
1.67	2.47	1.5	5.26	132	122
1.67	4.94	3.0	5.26	128	118
1.67	7.41	4.5	5.26	125	115
1.67	2.47	1.5	10.52	132	121
1.67	4.94	3.0	10.52	128	117
1.67	7.41	4.5	10.52	125	114
1.67	2.47	1.5	26.30	132	120
1.67	4.94	3.0	26.30	128	116

where the quantities in parentheses are concentrations in the bulk. Calcium and total phosphate can be easily analyzed and phosphate can be calculated[13] according to the equation:

$$(PO_4^{3-}) = \frac{TP}{\left(1 + \frac{(H^+)}{K_{3P}} + \frac{(H^+)^2}{K_{2P}\ K_{3P}}\right)}$$

where TP is the total phosphate analyzed in the bulk. If pH of the bulk solution is known, (OH^-) can be calculated by the equation:

$$K_w = (H^+)\ (OH^-)$$

then,

$$K_{2P} = \frac{(H^+)\ (HPO_4^=)}{(H_2PO_4^-)}$$

$$K_{3P} = \frac{(H^+)\ (PO_4^{3-})}{(HPO_4^=)}$$

Thus, from the determination of TP, calcium and phosphate concentrations, the solubility product, K_{ap}, can be calculated.

Crystallographic Investigation: Electron micrographs were obtained for the four types of carbonated apatite powders, the plain powder and those containing strontium, zinc and fluoride ions, using a Scanning Microscope (JSM-2:SII) equipped with a fine coat ion sputter (Joel, JFC-1100).

RESULTS AND DISCUSSION

Dissolution Rates and Solubilities of Carbonated Hydroxyapatite:

Dissolution experiments were performed under varying degrees of partial saturation and various calcium, phosphate and fluoride concentrations. Degrees of partial saturation are denoted by the ionic activities, as described in Table 2. Figure 1 shows typical raw dissolution data

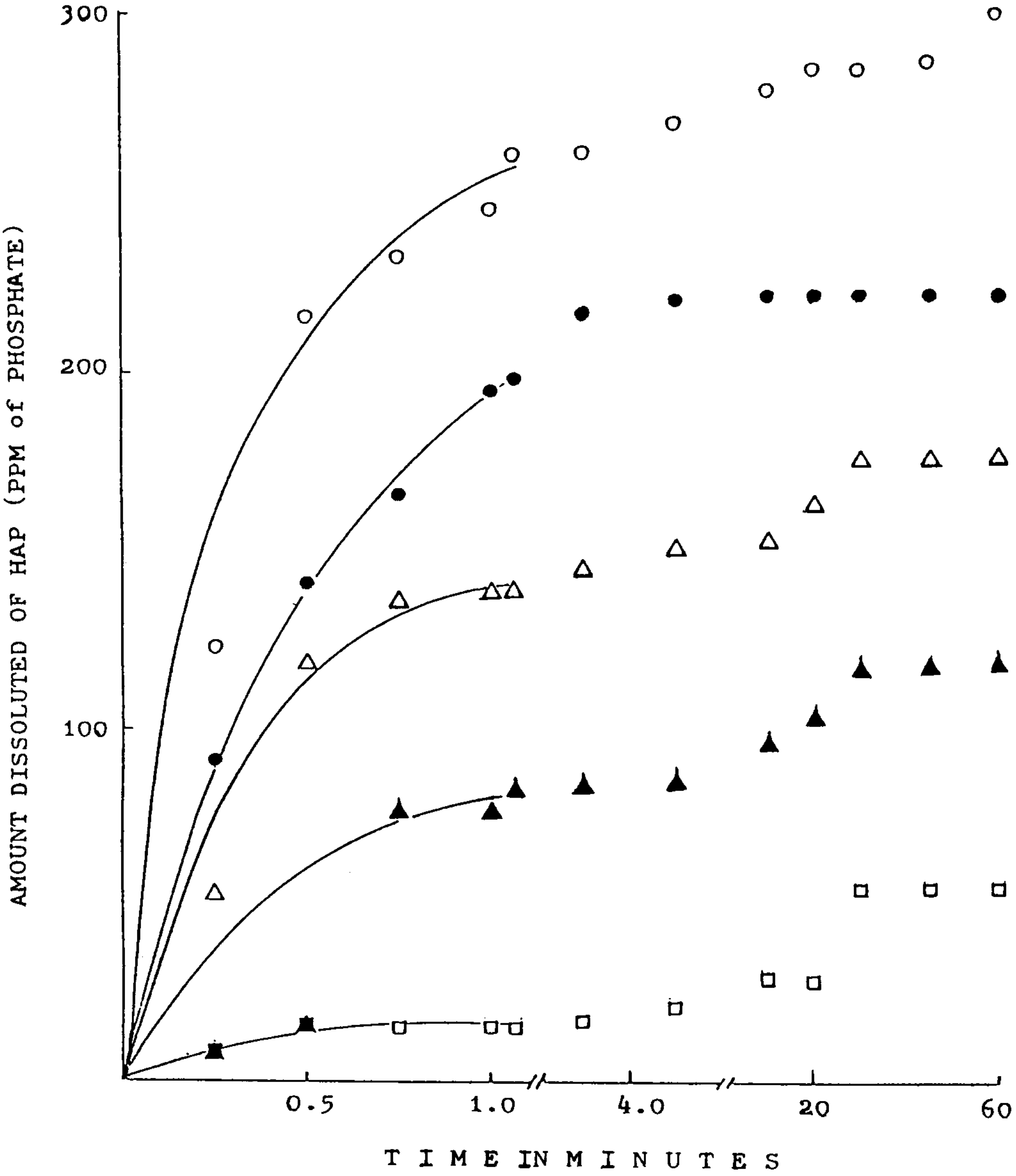
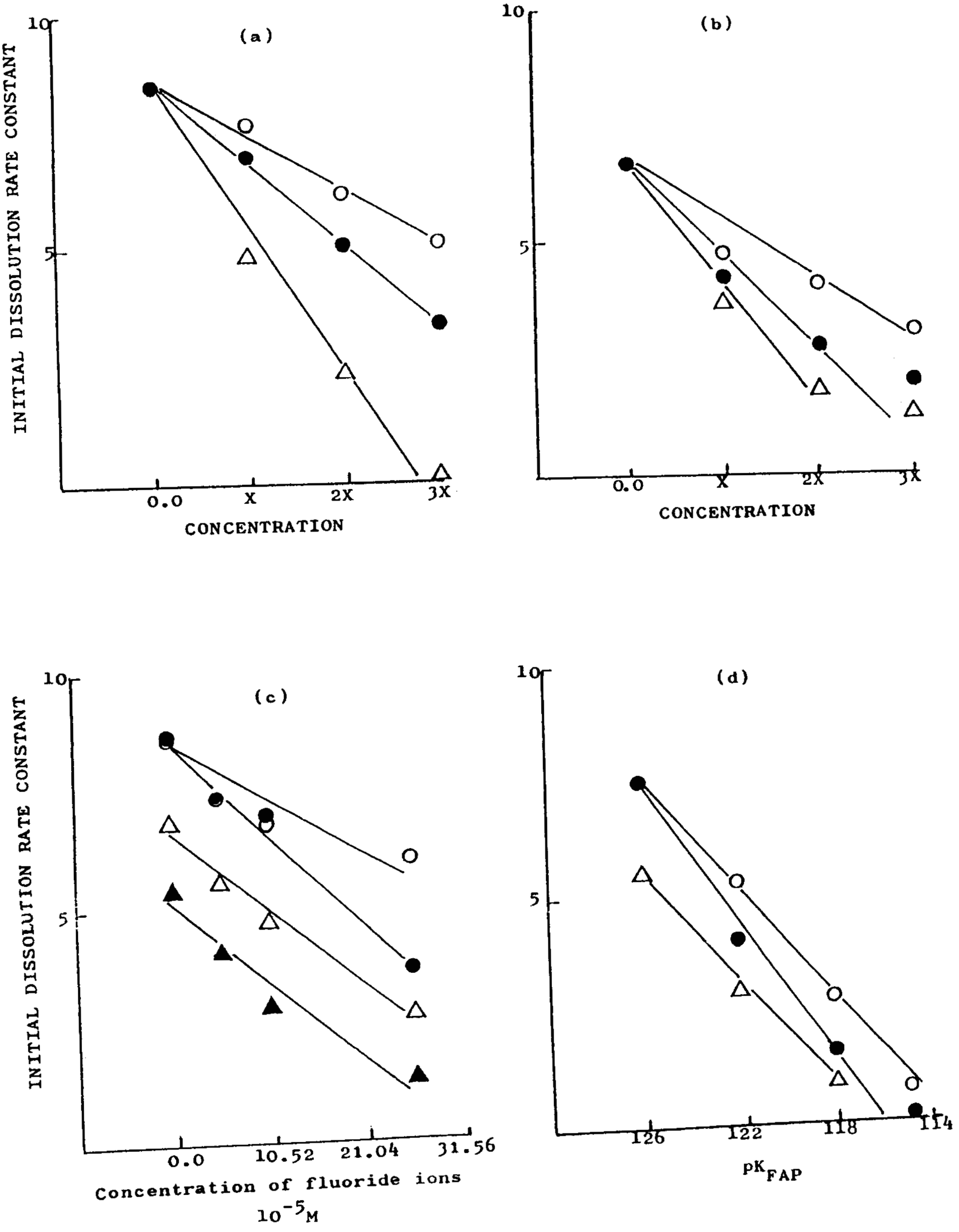


FIGURE 1

Effect of calcium, phosphate and/or fluoride ions on the dissolution of carbonated HAP at pH 4.4.

Key: HAP alone (○); HAP in the presence of $4.94\times10^{-3}M$ Ca^{2+} (●); $4.5\times10^{-3}M$ PO_4^{3-} (△); $7.41\times10^{-3}M$ $Ca^{2+}+4.5\times10^{-3}M$ PO_4^{3-} (▲) and $4.94\times10^{-3}M$ $Ca^{2+}+3.0\times10^{-3}M$ $PO_4^{3-}+26.3\times10^{-5}M$ F^- (□).


(a)
10
5
INITIAL DISSOLUTION RATE CONSTANT
0.0
X
2X
3X
CONCENTRATION
(b)
10
5
0.0
X
2X
3X
CONCENTRATION
(c)
10
5
INITIAL DISSOLUTION RATE CONSTANT
0.0
10.52
21.04
31.56
Concentration of fluoride ions
10-5M
(d)
10
5
126
122
118
114
pKFAP

in buffer solutions, of the amounts of HAP dissoluted in terms of phosphate (p.p.m.) versus time. It is noticed that calcium or phosphate significantly inhibited the carbonated apatite dissolution. Generally, dissolution rates decreased with increasing the concentration of either calcium or phosphate (Figure 2a and b). It is also noticed that dissolution rates decrease with increasing K_{FAP} of the initial solution (Figure 2c). The initial rates are shown to decrease to a low value when pK_{FAP}= 114 (Figure 2d). Quantitative estimation of the initial rates was somewhat difficult because of the high degree of curvature of the plotted data near zero times. Tangent lines were drawn in order to calculate the initial rates. The plateau regions of the dissolution curves represent apparent saturation of the medium with the

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FIGURE 2

Plots of the initial rates of dissolution versus:

(a) calcium and/or phosphate concentrations using carbonated HAP.
Key: calcium (○) where X= 2.47×10^{-3}M; phosphate (●) where X=1.5×10^{-3}M and both calcium and phosphate (△) where X=2.47×10^{-3}M Ca^{2+}+1.5×10^{-3}M PO_4^{3-}.

(b) calcium and/or phosphate concentrations using carbonated HAP containing zinc ions.
Keys: the same as for (a)

(c) Fluoride concentrations using the four types of carbonated HAP.
Key: HAP alone (○); HAP containing strontium ions(●); HAP containing fluoride ions (▲) and HAP containing zinc ions (△).

(d) pK_{FAP} for three types of carbonated HAP.
Key: the same as for (c).

TABLE 3

Rate of Dissolution, K, and Saturation Solubilities, Cs, of Carbonated HAP at pH 4.4.

Concentration, molar				
Calcium(10^3)	phosphate(10^3)	fluoride(10^5)	K	$10^4 Cs_M$
–	–	–	8.40	5.0
2.47	–	–	7.70	4.2
4.94	–	–	6.13	3.7
7.41	–	–	5.10	3.1
–	1.5	–	7.70	4.6
–	3.0	–	5.10	3.3
–	4.5	–	3.33	2.9
2.47	1.5	–	4.90	4.2
4.94	3.0	–	2.40	2.5
7.41	4.5	–	0.70	2.0
–	–	5.26	7.30	4.2
–	–	10.52	6.80	3.5
–	–	26.30	6.10	3.2
4.94	3.0	5.26	2.80	2.0
7.40	4.5	5.26	0.87	1.5

apatite. Data in these regions are a direct measure of the solubility of apatites in the solvent (Table 3).

The hydrodynamic conditions used and the relatively small particle siz of the apatite powder allow the assumption that the surface microenvironmental conditions are well approximated by the bulk solution conditions. Therefore, solution diffusion both in the aqueous boundary layer and the aqueous pores in the case of crystal aggregates may be neglected and the dissolution rates may be simply related directly to the bulk solution conditions. Plots of the initial rates versus phosphate concentrations produced linear relationships (Figure 2a) which may be expressed as first-order relationships. Thus rate = K C, where C is the unsaturated concentration expressed in molar units of the appropriate apatite and K is the first order rate constant. The intercept values on the abscissas can then be used to calculate the corresponding K_{FAP} of the carbonated HAP used (Fig. 2d).

Dissolution Rates of Carbonated Hydroxyapatites Containing Other Ions:

Hydroxyapatites prepared in the presence of zinc, strontium or fluoride ions exhibited lower dissolution rates than plain HAP. This retardation in dissolution rates was in the order of F > Zn > Sr (Figs. 3 and 4). The extent of retardation in the dissolution of HAP powder precipitated from a solution containing 10 p.p.m. of strontium ions is comparable in magnitude with the effect exerted by a bulk solution containing the same concentration of strontium ions (Fig. 4). This observation may support the physical model proposed by Higuchi and Co-workers[13,15]. These authors assumed that when HAP crystals are exposed to acidic buffer solution containing a sufficiently high concentration of strontium ions, a thin layer of calcium-strontium apatite complex, $Ca_6Sr_4(PO_4)_6(OH)_2$ is formed rapidly around the crystal surfaces. It was also assumed that this complex governs the ambient solution conditions at the crystal-solvent interface. Interestingly, a similar effect was observed with carbonated HAP containing zinc or fluoride ions which may be explained in the same manner as for strontium ion (Fig. 5). Furthermore, the rate of dissolution of the carbonated apatites containing zinc, strontium or fluoride ions decreased markedly with increasing the pH

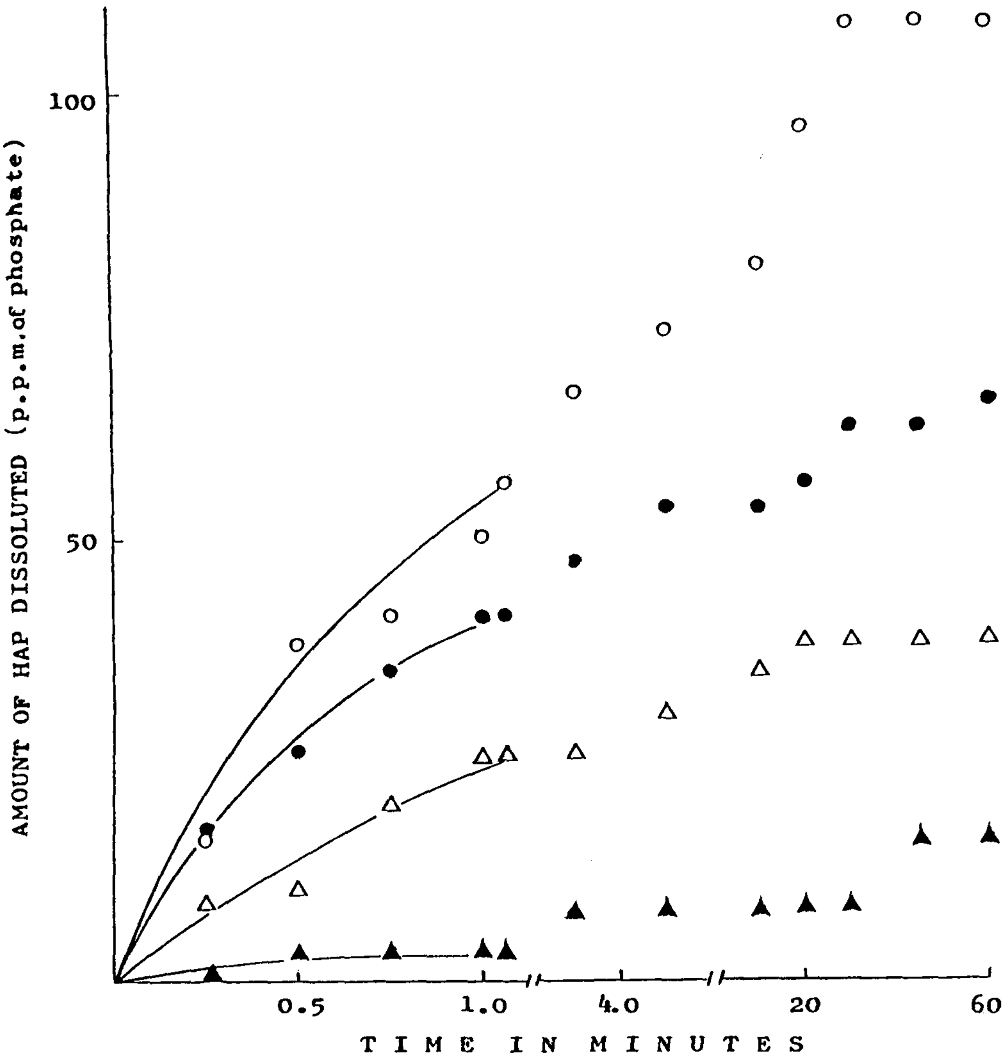

FIGURE 3

Dissolution of the four types of carbonated apatite powders in the presence of $4.94\times10^{-3}M\ Ca^{2+}$, $3.0\times10^{-3}M\ PO_4^{3-}$ and $10.52\times10^{-3}M\ F^-$.

Key: HAP alone (○); HAP containing strontium ions (●); HAP containing zinc ions (△) and HAP containing fluoride ions (▲).

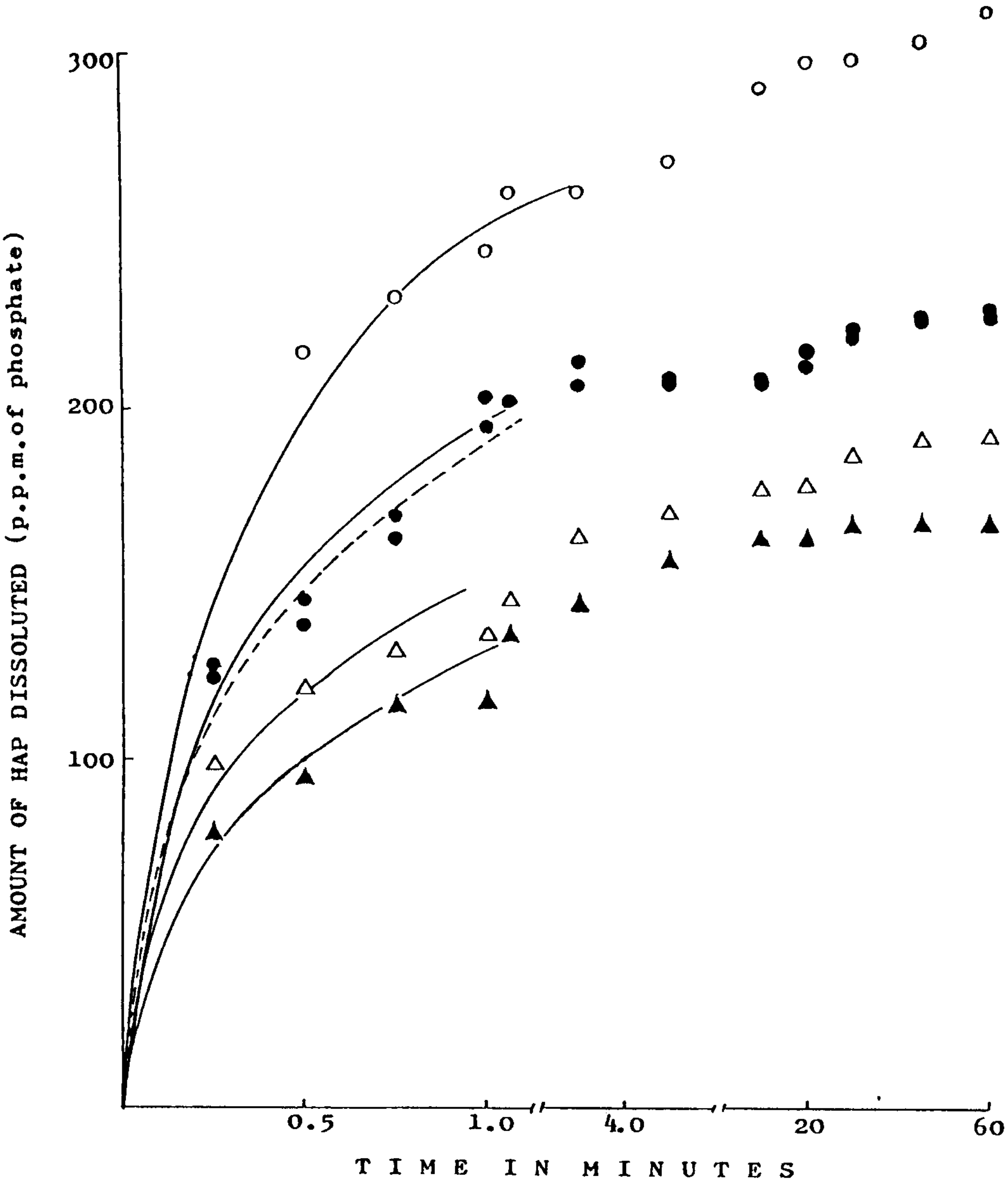

FIGURE 4

Dissolution of the four types of carbonated apatite powders at pH 4.4.

Key: HAP alone (○); HAP containing strontium ions(●—●); HAP containing strontium ions, dissoluted in the presence of 10 p.p.m. Sr^{2+} (●----●); HAP containing zinc ions (△) and HAP containing fluoride ions (▲).

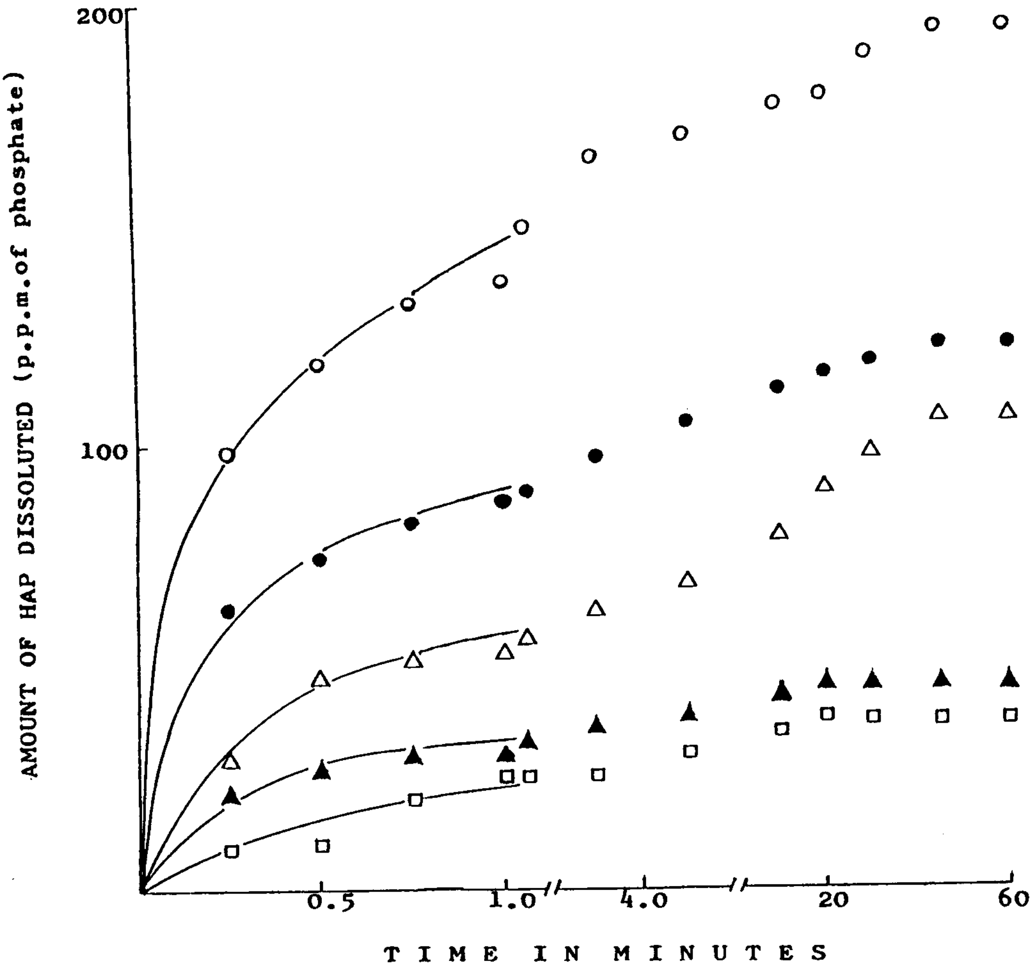

FIGURE 5

Effect of calcium,phosphate and fluoride ions on the dissolution of carbonated HAP containing zinc ions at pH 4.4. Key: HAP containing zinc alone (○); HAP containing zinc ions in the presence of 4.94×10^{-3} Ca^{2+} (●), $4.5\times10^{-3}M$ PO_4^{3-} (△); $7.41\times10^{-3}M$ Ca^{2+}+$4.5\times10^{-3}M$ PO_4^{3-} (▲) and $4.94\times10^{-3}M$ Ca^{2+} + $3\times10^{-3}M$ PO_4^{3-} + $10.52^{-5}M$ F^- (□).

of the solution, calcium, phosphate concentrations and partial saturations of these solutions (Figures 6 and 7). Fluoride has a very dramatic effect on the dissolution of all apatites. Synergistic effects of phosphate ions is evident in most of the results obtained supporting the idea that the rate-determining step in dissolution involves a complex formation of the metal ions added with phosphate. Calculations of the initial rate constants, K, and saturation solubilities, Cs, of the carbonated apatites are shown in Table 3. It is observed that K and Cs of HAP containing strontium, zinc or fluoride ions are less than those of the plain carbonated apatite and these values are reproducible under all the conditions studied (Tables 4, 5 and 6). Calculation of pK_{HAP} for carbonated HAP gave a value of 124 ± 2 and that for HAP containing strontium or zinc was 131 ± 2. The dissolution rate of carbonated apatites containing zinc or strontium becomes very slow when the ionic activity product, $pK_{FAP} = 118$. However, in the case of plain carbonated apatite, some dissolution was observed when pK_{FAP} lied in the range of 115-119. In brief, results of the present work indicate that the presence of strontium, zinc or fluoride ions may reduce the adverse effects caused by the inclusion of carbonate ions on the dissolution of hydroxyapatite.

Electron Microscope Observations: Electron micrographs of plain carbonated apatites showed irregular shaped crystals with imperfections (Fig. 8a). The presence of carbonate ions was reported[12] to cause the bonding in the apatite crystals to become weaker and more isotropic which results in the formation of small spheroidal crystals. On the other hand, the presence of strontium or zinc ions generally improved the crystallinity of the apatites as it is evident from Figures 8b and 8C. More-

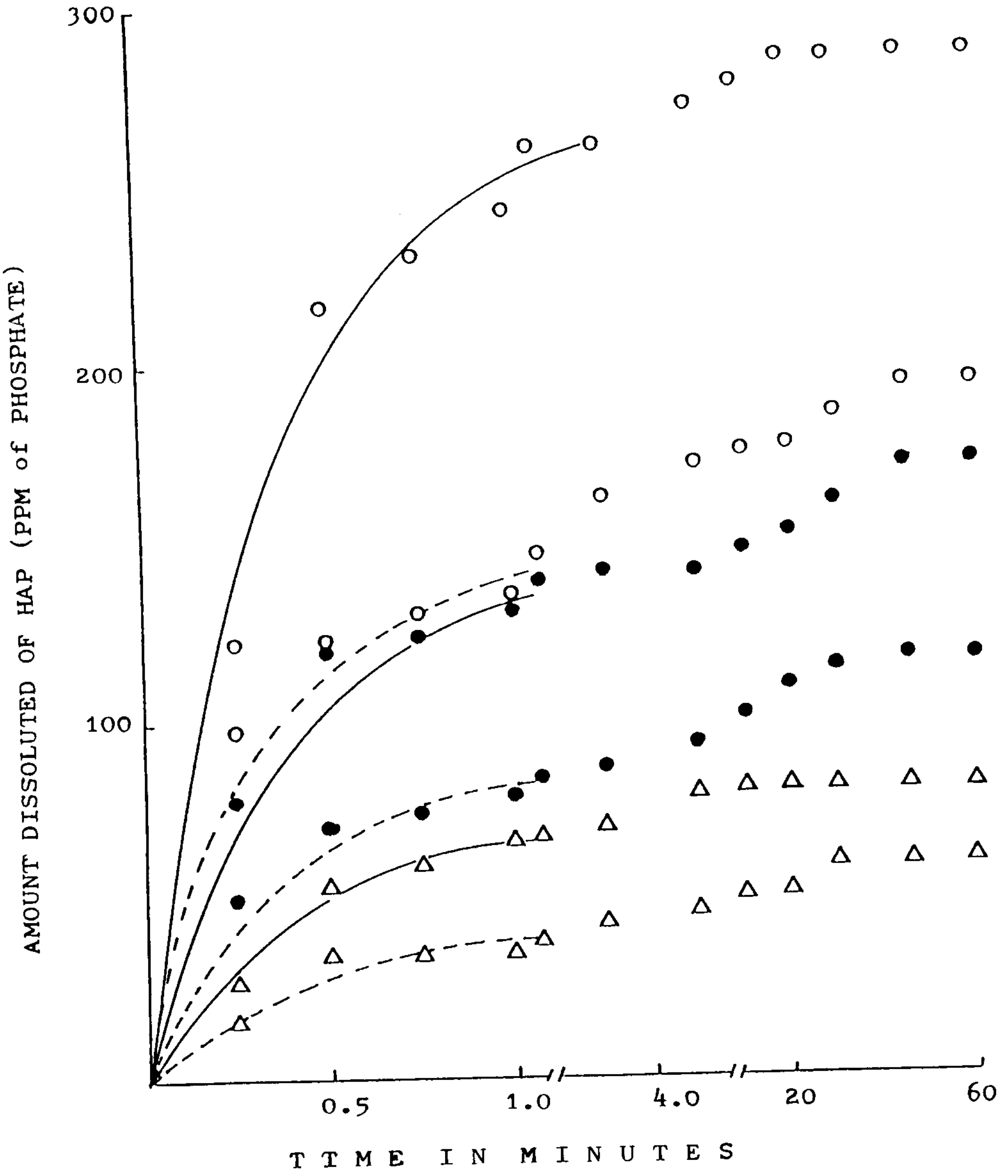

FIGURE 6

Effect of pH on the dissolution of carbonated HAP alone (——) and that containing zinc ions (---).
Key: pH 4.4 (○); pH 5.0 (●) and pH 5.6 (△).

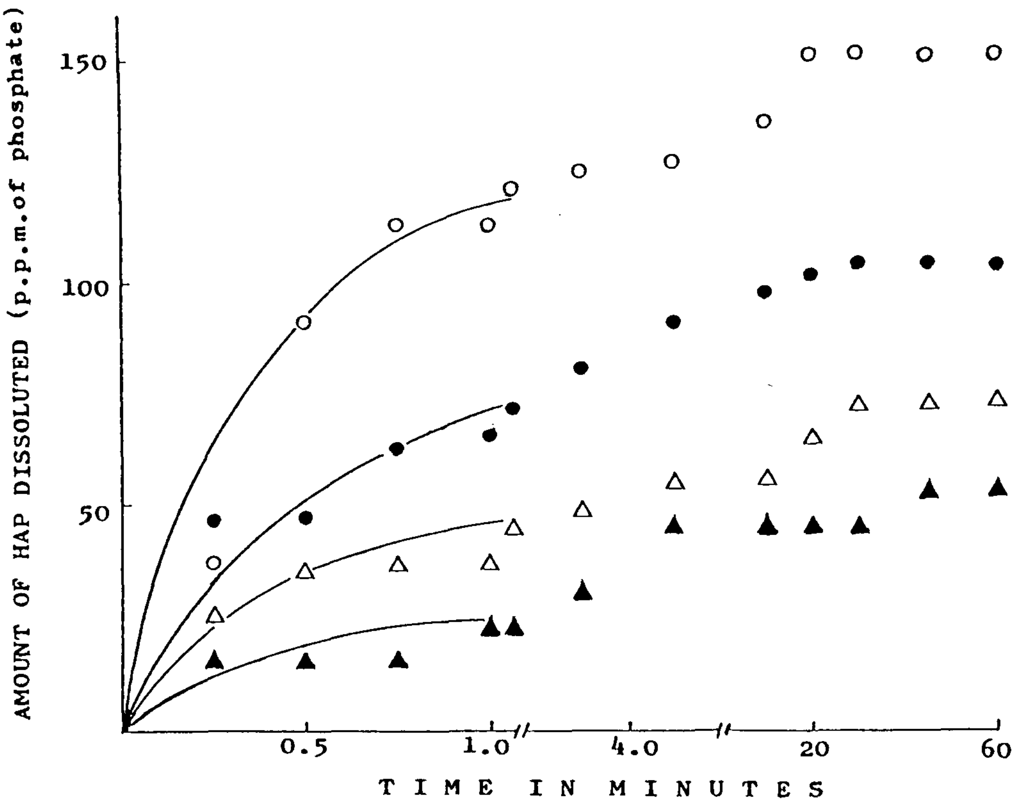

FIGURE 7

Dissolution of the four types of carbonated apatite powders at pH 4.4 in the presence of 4.94×10^{-3}M Ca^{2+} and 3.0×10^{-3}M PO_4^{3-}.

Key: the same as for Figure 3.

over, the presence of fluoride ions produced smaller irregular shaped crystals of high density (Fig. 8d). These observations may support dissolution data obtained in this work.

TABLE 4

Rate of Dissolution, K, and Saturation Solubilities, Cs, of Carbonated HAP containing Strontium ions at pH 4.4.

Concentrations				
calcium.10^3M	phosphate.10^3M	fluoride.10^5M	K	Cs.10^4M
--	--	--	8.50	3.80
2.47	--	--	6.90	3.20
4.94	--	--	5.07	2.80
7.41	--	--	4.00	2.10
--	1.50	--	4.90	2.90
--	3.00	--	4.30	2.50
--	4.50	--	3.50	2.10
2.47	1.50	--	4.70	2.20
4.94	3.00	--	3.30	1.70
7.41	4.50	--	2.10	1.30
--	--	5.26	7.30	2.70
--	--	10.52	5.90	2.20
--	--	26.30	3.60	1.80
4.94	3.00	5.26	1.50	1.30
7.40	4.50	5.26	0.67	0.98

TABLE 5

Rate of Dissolution, K, and Saturation Solubilities, Cs, of Carbonated HAP containing Zinc ions at pH 4.4.

Concentrations				
Calcium.10^3M	Phosphate.10^3M	Fluoride.10^5M	K	Cs.10^5M
--	--	--	6.80	3.20
2.47	--	--	4.80	2.50
4.94	--	--	4.10	2.00
7.41	--	--	3.20	1.50
--	1.50	--	4.10	2.60
--	3.00	--	2.70	2.10
--	4.50	--	2.10	1.75
2.47	1.50	--	3.60	1.75
4.94	3.00	--	1.73	1.20
7.41	4.50	--	1.47	0.70
--	--	5.26	5.50	2.30
--	--	10.52	4.70	1.70
--	--	26.30	2.70	0.14
4.94	3.00	5.26	0.87	0.78
7.40	4.50	5.26	0.80	0.47

TABLE 6

Rate of Dissolution, K, and Saturation Solubilities, Cs, of Carbonated HAP containing Fluoride Ions at pH 4.4.

Concentrations			K	Cs.10^4M
Calcium.10^3M	Phosphate.10^3M	Fluoride.10^5M		
--	--	--	5.30	2.80
2.47	--	--	4.13	2.00
4.94	--	--	2.93	1.50
7.41	--	--	2.30	0.80
--	1.50	--	2.90	2.30
--	3.00	--	1.90	1.60
--	4.50	--	0.93	1.40
2.47	1.50	--	1.90	1.40
4.94	3.00	--	0.90	0.88
7.41	4.50	--	0.00	0.00
--	--	5.26	4.00	1.60
--	--	10.52	2.90	1.10
--	--	26.30	1.50	0.80
4.94	3.00	5.26	0.47	0.50
7.40	4.50	5.26	0.00	0.00

It could, thus, be concluded that the inclusion of fluoride and metal ions increase the acid resistance and crystalline order of carbonated apatite. The availability of such ions, at optimum levels, to the enamel during development would improve the resistance of the fully formed enamel to acid attack. Further, the presence of these ions in the oral environment, as ingredients of appropriate pharmaceutical products, is also expected, by virtue of remineralization, to reduce the susceptibility of dental enamel to caries. Since the acid reactivity of enamel is influenced by ion inclusions, studies relevant to dental caries should be carried out on apatite with included fluoride and trace elements.

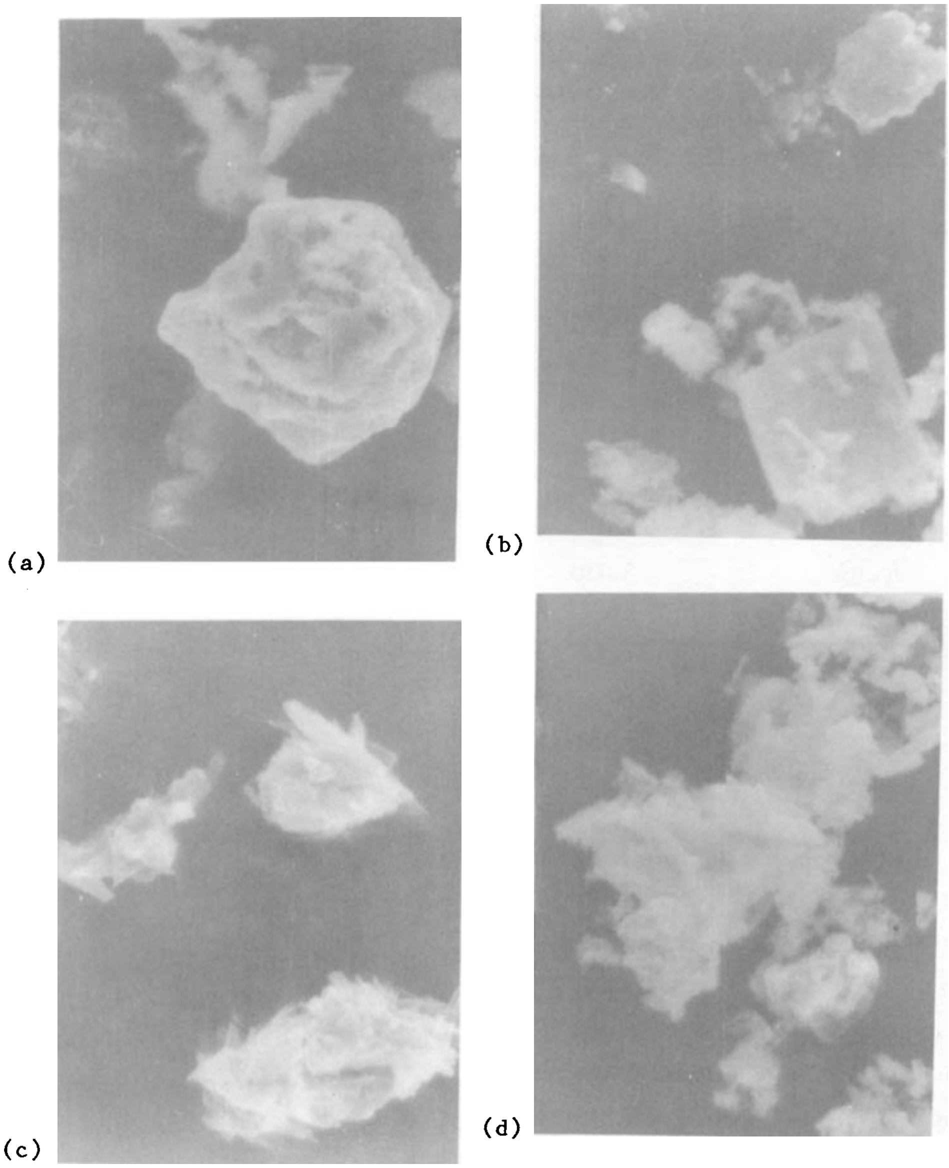

FIGURE 8

Electron micrographs of plain carbonated HAP (a) and those containing zinc (b), strontium (c) and fluoride(d) ions.

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