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The influence of ion activity product on remineralization behavior of carbonate-apatite pellets, hydroxyapatite pellets and hydroxyapatite powder

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Summary

The effect of the ion activity product, $K_{FAP}(a^{10}_{Ca^{2+}} \cdot a^{6}_{PO_4^{3-}} \cdot a^{2}_{F^-})$ of the remineralizing solution on the remineralization behavior of carbonate-apatite pellets, hydroxyapatite pellets and hydroxyapatite powder have been studied. The pellets were demineralized in partially saturated 0.1 M acetate buffer, pH = 4.5, then remineralized in 0.1 M acetate buffer containing 10 ppm F and various equimolar levels of ^{45}Ca and phosphate. The hydrodynamics was controlled by using the rotating disk apparatus. The acid-abrasion and acid-etch methods were used to determine F, ^{45}Ca and P levels at different depths. For powder remineralization studies, hydroxyapatite seed crystals were added to similar acetate buffer solutions containing Ca, P and F. The seed crystals were sonicated and maintained in uniform suspension in a thermostated beaker using a magnetic stirring bar.

For carbonate-apatite pellets, there was mainly remineralization when the ion activity product of the remineralizing solution was 1×10^{-108}. When the $K_{FAP} = 10^{-112}$ and 10^{-116}, there was simultaneous demineralization/remineralization. The F concentration profiles in the lesion were steepest at 1×10^{-108} and broadened with decreasing K_{FAP}. The molar F/^{45}Ca ratios obtained with carbonate-apatite pellets were essentially in quantitative agreement with those previously obtained with bovine dental enamel but differed somewhat from those obtained with hy-

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droxyapatite pellets. In the hydroxyapatite powder studies the rates of crystal growth were relatively rapid at an ion activity product of 10^{-108}, but decreased significantly at $K_{FAP} = 10^{-112}$ and were very slow at $K_{FAP} = 10^{-116}$. These powder remineralization results are consistent with remineralization behavior in the surface region of hydroxyapatite and the carbonate-apatite pellets.

Introduction

The influence of ion activity product, K_{FAP}, of the remineralizing solution on the remineralization of predemineralized bovine dental enamel has been studied previously (Fox et al., 1983). The results of these studies led to the hypothesis that K_{FAP} value of 1×10^{-112} marks the demarcation between remineralization only and simultaneous demineralization/remineralization. Recently (Bergstrom et al., 1984) the above hypothesis has been confirmed by a study in which a quantitative microradiographic method has been used. It was found that when the K_{FAP} of the remineralizing solution was 1×10^{-108}, remineralization of the demineralized region was essentially complete. However, when the K_{FAP} value of the remineralizing solution was 1×10^{-112} or less, remineralization of the demineralized region was incomplete. Besides that, there was significant demineralization in the deeper recesses of the originally demineralized region. Because bovine dental enamel used in these experiments contained about 4% carbonate, Ludwig et al. (1982) have conducted chemical kinetic studies to investigate the influence of the presence of carbonate in apatite on the crystal dissolution behavior. Their results have shown that dissolution rate of carbonate-apatite in the presence of solution fluoride was controlled by an apparent solubility corresponding to an ion activity product (K_{FAP}) of around 1×10^{-115}; for hydroxyapatite, they found that the corresponding K_{FAP} value was around $1 \times 10^{-119 \pm 1}$.

The purpose of the present study was to investigate the influence of the ion activity product of the remineralizing solution, K_{FAP}, on the remineralization behavior of predemineralized carbonate-apatite pellets, hydroxyapatite pellets and hydroxyapatite powder.

Materials and Methods

Synthesis of carbonate-apatite. Hydroxyapatite containing 4–5% carbonate was prepared according to a procedure described by Legeros et al. (1971).

Synthesis of hydroxyapatite. Hydroxyapatite was prepared using the Avnimelech et al. (1973) procedure.

Preparation of pellets. About 60 mg of apatite was directly compressed in 0.28 cm diameter die with a force of 10,000 lb. using a laboratory press [1]. The pellet was

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[1] Carver Press.

TABLE 1

THE COMPOSITIONS OF THE REMINERALIZING SOLUTIONS (pH 4.5, μ = 0.5, 0.1 M ACETATE BUFFER)

Total calcium (mM)	Total phosphate (mM)	F (ppm)	pK_{FAP}
12.00	12.00	10	108
6.87	6.87	10	112
3.83	3.83	10	116

then ejected from the die and mounted on a pellet-holder with melted paraffin so that only one flat surface of the pellet was exposed.

Preparation of solutions. A solution 16% saturated (on molar basis) with respect to the thermodynamic solubility of hydroxyapatite was used for demineralization. The solution was 0.1 M acetate buffer containing 3.5 mM each of total calcium and phosphate. The pH was adjusted to 4.5 by NaOH and ionic strength to 0.5 by addition of NaCl.

For remineralization 0.1 M acetate buffer solutions, containing 10 ppm F and various equimolar levels of ^{45}Ca and phosphate, were used (Table 1). The pH was adjusted to 4.5 by NaOH and ionic strength to 0.5 by the addition of NaCl. Although the remineralizing solutions were supersaturated with respect to CaF_2, there was no spontaneous CaF_2 precipitation into the solutions.

Demineralization of the pellet. The pellet was demineralized in 25 ml of the demineralizing solution at 30 °C, using the rotating disk apparatus [2] (Fig. 1) at stirring speed of 5 rpm. At the end of the demineralization process the pellet was washed completely with double-distilled water and dried at room temperature in a desiccator containing saturated potassium nitrate solution.

Remineralization of the pellet. The demineralized pellet was remineralized in 50 ml of the remineralizing solution at 30 ° using the rotating disk apparatus (Fig. 1) at a stirring speed of 5 rpm. At the end of the reminaralization process the pellet was washed completely with double-distilled water.

Powder remineralization method. The remineralization experiments involved adding seed hydroxyapatite preparations to 95 ml of the remineralizing solution containing Ca (^{45}Ca), P and F. The hydroxyapatite was sonicated for 60 s in 5 ml of 0.5 M NaCl solution before adding to the remineralization solution. The seed crystals were maintained in a uniform suspension in a thermostated beaker (at 30 °) using a magnetic stirring bar. The depletion of F ion was monitored intermittently by withdrawing samples. The withdrawn samples were (1 ml) passed through injection syringes fitted with a Swinny filtration assembly containing filter paper [3]. The F content of the filtrate was determined. Most of the remineralization experi-

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[2] Cole Parmer Standard Servodyne System.

[3] Millipore filter, 0.22 μm Pore size.

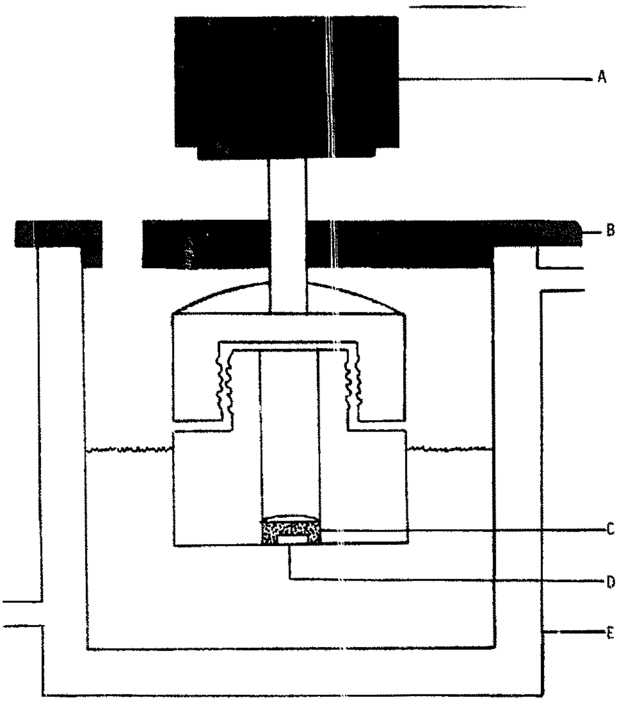

Fig. 1. Constant speed rotating device. Key: A, constant speed motor; B, cover; C, paraffin wax; D, apatite pellet; E, jacketed beaker.

ments were completed in 24 h. Ca and P ions, being in large excess, did not deplete significantly in most cases; however, when useful, ^{45}Ca and P were also determined along with F.

The precipitate was collected over 0.45 μm Millipore filter paper washed thoroughly with double-distilled water and dried at 60–70 °C for 24 h. The dried remineralized powders were analyzed for ^{45}Ca, P and F.

Acid-abrasion method. The procedure which has been developed by Iyer et al. (1983) for the abrasion of dental enamel has been modified so that it would be suitable for pellets. The remineralized pellet was acid-abraded for successive periods of 30, 60, 60, 60, 60, 60 and 60 s, using 5 μl of 0.1 M $HClO_4$ and an abrasion load of 100 g and 1200 rpm. The abraded samples were dissolved in 1.5 ml of 0.5 M $HClO_4$ and assayed to determine F, P and ^{45}Ca concentrations.

Acid-etch-method. For hydroxyapatite pellet (containing no carbonate), the acid-etch procedure described previously (Yonese et al., 1981) was employed. The pellets were very soft, and therefore acid abrasion was not a convenient method.

Analytical procedure for phosphate, fluoride and ^{45}calcium. Phosphate concentration was determined colorimetrically by the method of Gee et al. (1954) in which phosphoammonium molybdate complex formed was reduced by stannous chloride, then the absorbance was measured at 720 nM [4].

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[4] Beckman model 25 Spectrophotometer, Fullerton, CA.

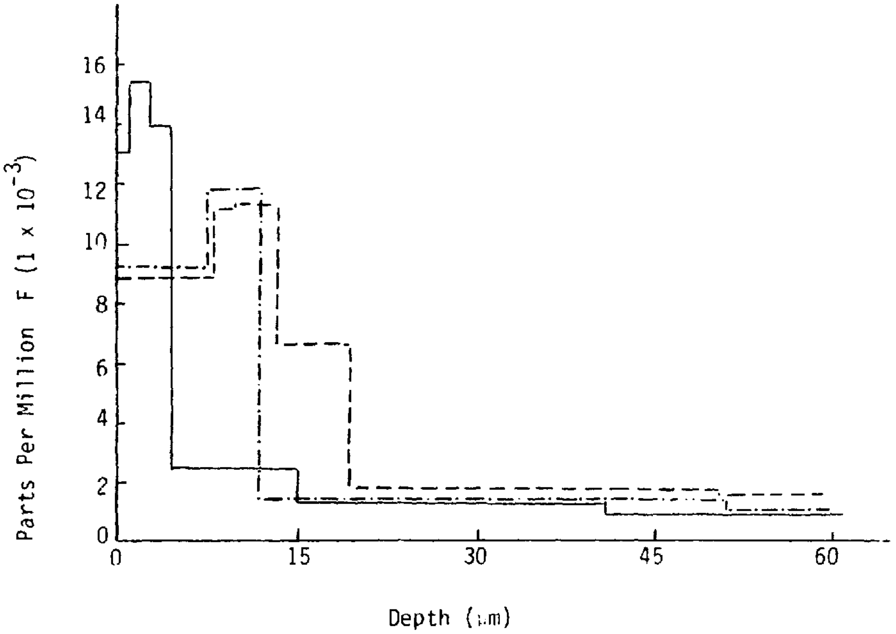

Fig. 2. Influence of the pK_{FAP} of the remineralizing solution on the 48 h F uptake profiles in predemineralized carbonate-apatite pellets as measured by the acid-abrasion method. Key: ———, 108; — — —, 112; and -·-·-, 116.

^{45}Calcium was determined by using scintillation counting[5] and fluoride was determined by a fluoride electrode[6] using low level total ionic strength adjusting buffer. The adjusting buffer was prepared by adding 57 ml of glacial acetic acid and 58 g of sodium chloride to 800 ml of double-distilled water contained in a 1-liter beaker. The pH was then adjusted to 5.25 by addition of sodium hydroxide solution and the final volume was completed to 1-liter with double-distilled water.

Depth calculation. The depths of the abraded or etched layers of the pellets were determined from the amounts of phosphate recovered, assuming the density of the samples to be that of pure hydroxyapatite (2.95 g · cm^{-3}; Rootare, 1973). Based on this information fluoride and ^{45}calcium per unit volume of the mineral were calculated. This density value and therefore the depth calculations are approximations, since the porosity of demineralized pellet layers was not known. This method, however, does provide a convenient means of estimating the F levels as a function of depth.

Results and Discussion

The fluoride concentrations for remineralized carbonate-apatite pellets shown in Fig. 2 decreased with increasing depth and the F gradient in the lesion was steepest

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[5] Beckman model 9000 liquid scintillation system, Beckman Instr., CA.

[6] Orion, Model 94-09, Cambridge, MA.

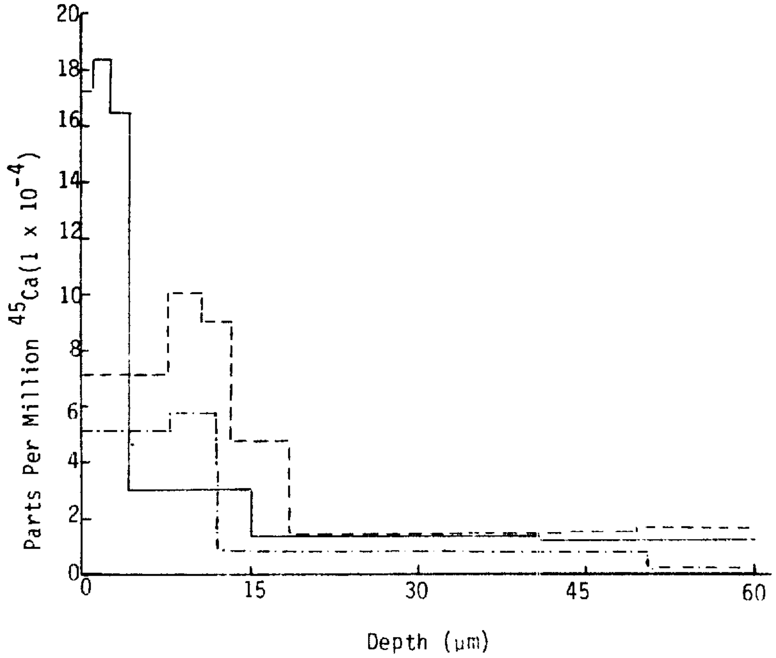

Fig. 3. Influence of the pK_{FAP} of the remineralizing solution on the 48 h ^{45}Ca uptake profiles in predemineralized carbonate-apatite pellets. Key: ———, 108; — — —, 112; and — · — ·, 116.

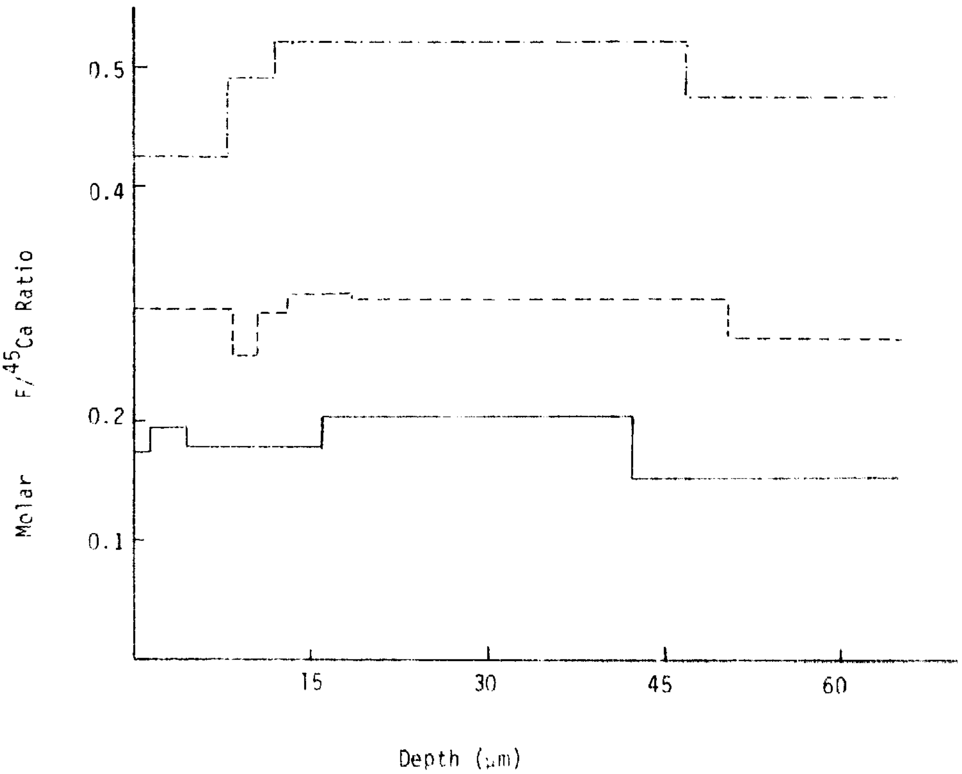

Fig. 4. Influence of the pK_{FAP} of the remineralizing solution on the molar F/^{45}Ca ratio in predemineralized carbonate-apatite pellets. Key: ———, 108; — — —, 112; and — · — ·, 116.

for pK_{FAP} 108 and broadened with increasing pK_{FAP}. For pK_{FAP} of 108, values of 13,000–15,000 ppm F can be seen in the first few μm which then decreased to about 2500 ppm at a depth of 5–15 μm. For pK_{FAP} values of 112 and 116, the patterns were somewhat different from the 108 case in that there was less F in the surface region and more F at the intermediate depths (10–15 μm). The ^{45}Ca uptake as a function of depth in the carbonate-apatite pellets are shown in Fig. 3. The ^{45}Ca uptake data were qualitatively similar to the F uptake results, viz. at $pK_{FAP} = 108$, the levels were high near surface and declined rapidly with depth while at 112 and 116 the patterns were broader. Fig. 4 shows the molar F/^{45}Ca ratio as a function of depth in the remineralized carbonate-apatite pellet. The ratio was increased from about 0.2 to 0.3 as the pK_{FAP} value of the remineralizing solution increased from 108 to 112, and to around 0.5 when the pK_{FAP} was 116.

The result in Fig. 4 also indicates that remineralization occurred through fluorapatite deposition in the lesion rather than CaF_2. If CaF_2 was depositing the F/^{45}Ca molar ratio would be 2 and higher.

Experimental data obtained with carbonate-apatite pellets may be interpreted on the basis of remineralization or simultaneous demineralization/remineralization, and in general the findings are consistent with data obtained with bovine enamel slabs. The results shown in Figs. 2, 3 and 4 suggest that when the pK_{FAP} of the remineralizing solution was 108 remineralization mainly occurred through fluorapatite deposition in the lesion. When the pK_{FAP} of the remineralizing solution was 112 and 116, the results are consistent with a mechanism based upon simultaneous demineralization/remineralization. This shift in mechanism is consistent with the expectation that as the concentration of calcium and phosphate decreases in the solution, the dissolution process increases. The solution with a pK_{FAP} of 108, for example, has a pK_{HAP} ($K_{HAP} = a^{10}_{Ca^{2+}} \cdot a^{6}_{PO_4^{3-}} \cdot a^{2}_{OH^-}$) of 120 which marks the effective driving force for dissolution of hydroxyapatite (Fox et al., 1978). Thus for hydroxyapatite the situation is a clean one in which a solution with a pK_{FAP} of 108 is expected to have no dissolution component whatsoever; with carbonate-apatite, which has a pK_{HAP} of 115, we should expect some dissolution in the $pK_{FAP} = 108$ solution, but much less than in the solutions with pK_{FAP} of 112 or 116. Thus the shift in mechanism from simultaneous demineralization/remineralization to remineralization only might not be expected to be as sharp for carbonate-apatite as for hydroxyapatite. The experimental results, however, indicate that the shift does occur. The molar F/^{45}Ca ratio increases above 0.2 as the pK_{FAP} value of the remineralizing solution was increased to 112 and to 116. As was discussed by Fox et al. (1983), the departure of the ratio from 0.2 could be a measure of decreased microenvironmental ^{45}Ca specific activity arising from dissolution of non-radioactive calcium from the deeper regions in the enamel and is therefore an indication of simultaneous demineralization/remineralization. The results obtained in the present work are also consistent with the quantitative microradiographic data reported by Bergstrom et al. (1984) and the chemical kinetic data obtained by Fox et al. (1983), using bovine enamel slabs. The carbonate levels in bovine dental enamel used in these experiments are approximately 4%. In this work the carbonate-apatite used contained approximately 5% carbonate. The study of Bergstrom et al. (1984) showed that mineral density recovery in the outer 10–20 μm was relatively complete when the pK_{FAP} of remineralizing solution was 108 and was incomplete when the pK_{FAP} was 112 and 116. Also, at a pK_{FAP} of 112 and 116, there was a less dense region in the bovine enamel at a depth slightly greater than that produced by the demineralization treatment alone. This mineral loss from regions deeper than the original lesion indicates that simultaneous dissolution and remineralization was occurring. The results of Fox et al. (1983) also showed that the molar $F/^{45}Ca$ ratio was increased from a value of around 0.2 at pK_{FAP} of 108 to a value of around 0.3 at pK_{FAP} 112 and to a value of around 0.5 at pK_{FAP} 116. This increase in the molar $F/^{45}Ca$ ratio to a value greater than 0.2 at pK_{FAP} 112 indicates that simultaneous demineralization/remineralization had occurred. Also Fox et al. (1983) found that the fluoride uptake at the enamel surface was relatively higher when the pK_{FAP} of the remineralizing solution was 108 compared to that at 112 and 116.

The corresponding experimental results for the hydroxyapatite pellets at pK_{FAP} = 108 and 112 are shown in Figs. 5, 6 and 7. Fig. 5 shows that both the cumulative F uptake and the F profiles shapes are quantitatively similar to those obtained with the carbonate-apatite pellets. Fig. 7 shows, however, that in the case of the hydroxyapatite pellets the $F/^{45}Ca$ ratio does not appear to change significantly in going from $pK_{FAP} = 108$ to $pK_{FAP} = 112$, while there was a significant change in the experiments with carbonate-apatite pellets.

Although the results obtained with hydroxyapatite and carbonate-apatite pellets are consistent, there are also some significant differences. The pellets of both materials behave similarly at pK_{FAP} 108 and both materials showed relatively complete remineralization at the surface when the pK_{FAP} of the remineralizing solution was 108 and less than complete remineralization at pK_{FAP} = 112 and 116.

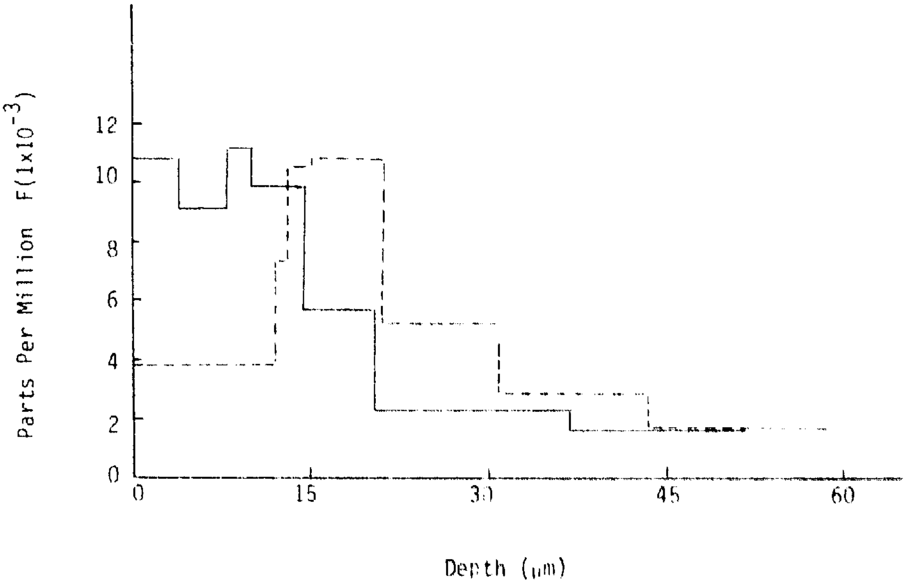

Fig. 5. Influence of the pK_{FAP} of the remineralizing solution on the 48 h F uptake profiles in predemineralized hydroxyapatite pellets. Key: ———, 108; and - - - -, 112.

At 112, however, the increase in the molar F/^{45}Ca ratio which was observed with carbonate-apatite pellets (Fig. 4) did not occur or did not occur to the same extent as with hydroxyapatite pellets. Fox et al (1983) concluded that the onset of simulta-

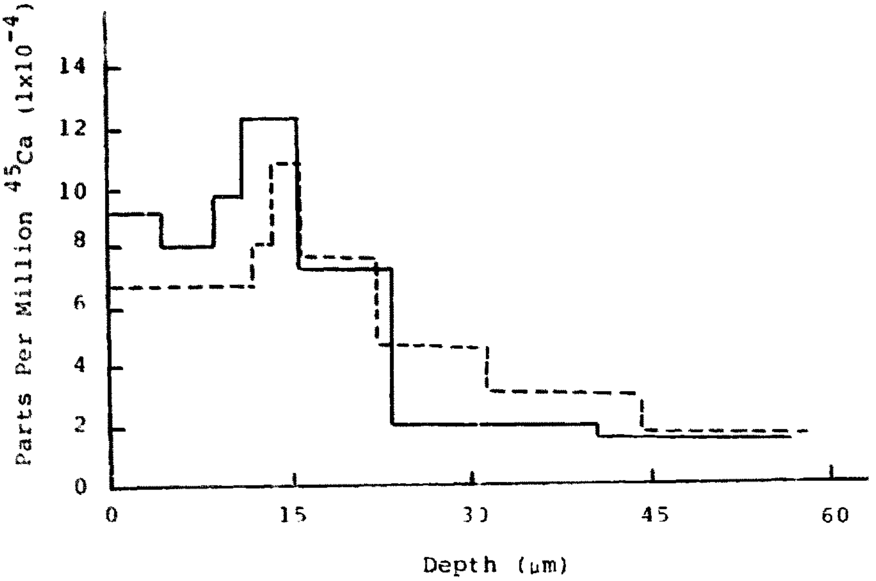

Fig. 6. Influence of the pK$_{FAP}$ of the remineralizing solution on the 48 h ^{45}Ca uptake profiles in predemineralized hydroxyapatite pellets. Key: ———, 108; and — — —, 112.

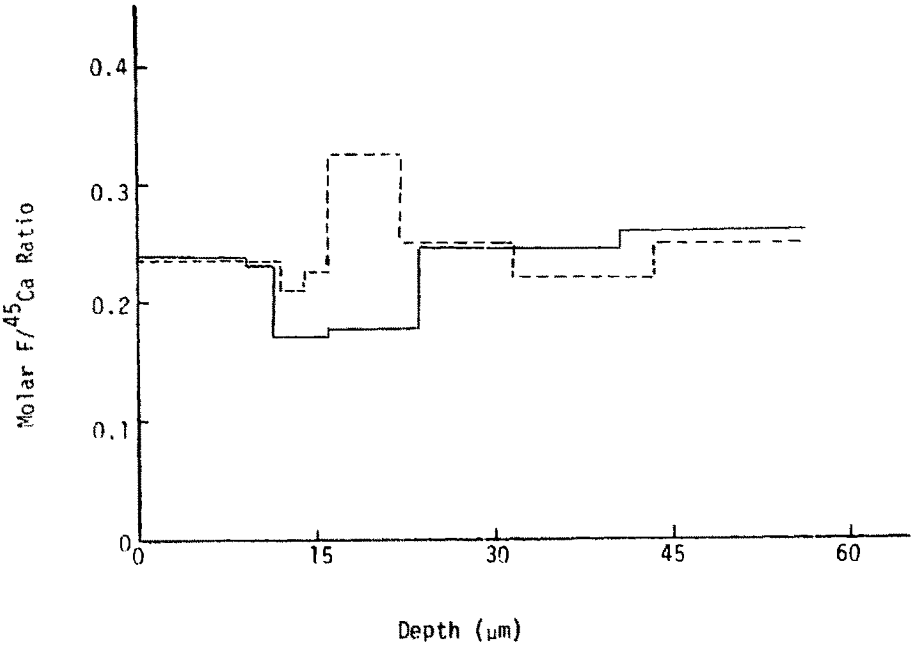

Fig. 7. Influence of the pK$_{FAP}$ of the remineralizing solution on the molar F/^{45}Ca ratio in predemineralized hydroxyapatite pellets. Key: ———, 108; and — — —, 112.

neous demineralization/remineralization for hydroxyapatite pellets occurred at around a pK_{FAP} of 114 rather than 112 and the present findings suggest this interpretation.

The results in Figs. 8 and 9 show the remineralization behavior of hydroxyapatite crystal at two different slurry densities as a function of the pK_{FAP} of the remineralizing solution. At the high slurry density (Fig. 8) the remineralization rates for pK_{FAP} of 108 and 112 were similar but at $pK_{FAP} = 116$, the rate was much slower. At the low slurry density (Fig. 9) the remineralization behavior was different from that shown in Fig. 8 in that both the rate and apparent extent of remineralization of $pK_{FAP} = 112$ was much smaller than those for the case of $pK_{FAP} = 108$.

The crystal suspension studies have revealed an interesting slurry density effect. Although no attempt will be made to explain these results, it should be noted that at the high slurry density the average crystal grows up to a fraction of its original weight at the point of depletion of the solution F. Both 108 and 112 solutions efficiently allowed this fractional growth to occur. At the low slurry density the point of F depletion corresponds to the average crystal having grown to many times its original weight. This difference in the extent of growth may account for the fact that at the low slurry density the behavior at 108 and 112 differ significantly. This can be extended to the remineralization behavior of predemineralized pellets. The mineral density in the surface region of the pellet demineralized with 16% partially saturated solution is about 1/5 of the original mineral density. Since the remineralization of this layer is believed to be controlled by the ion activity product of the bulk solution, remineralization using the 108 solution would be expected to result in almost

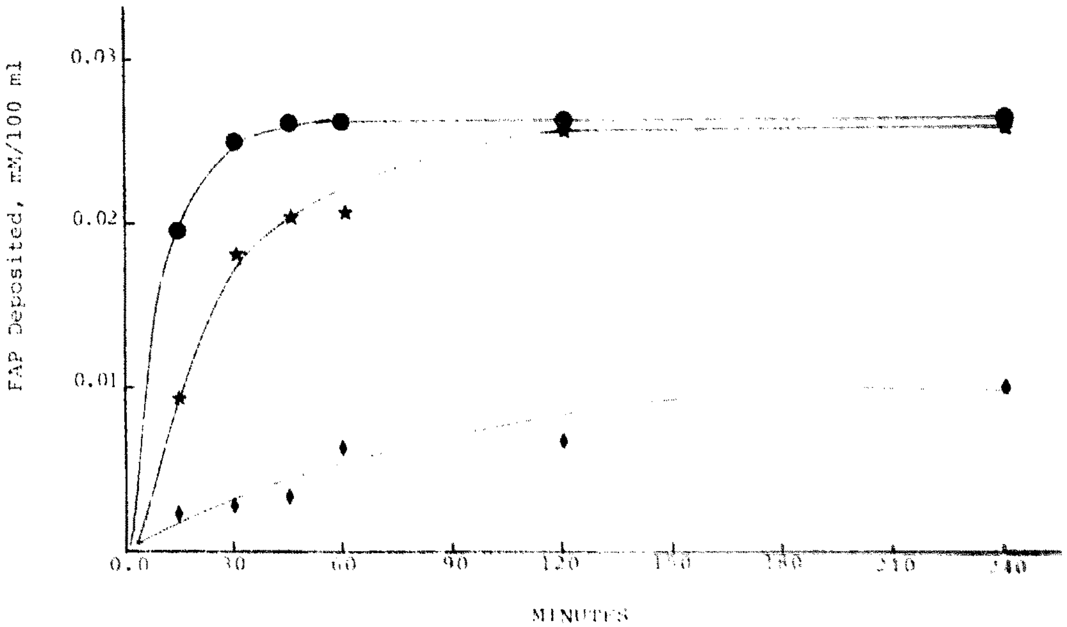

Fig. 8. The influence of the pK_{FAP} of the remineralizing solution on crystal growth of powder suspensions (50 mg/100 ml) as a function of time. Key: ●, 108; ★, 112; and ◆, 116.

complete recovery, while because the average crystal would be required to grow many times its weight complete remineralization in the surface region would not occur when the $pK_{FAP} = 112$ or 116.

The present work also provides more evidence which enables us to explain the dense zone which is usually seen at a region beyond the surface when a solution of pK_{FAP} of 112 is used for remineralization (Bergstrom et al., 1984). This region originally is less demineralized than the surface and both processes of simultaneous demineralization/remineralization from deeper layers and remineralization from bulk solutions could contribute to the mineral deposition at this layer.

Conclusions

The F/^{45}Ca molar ratios seen in experiments with carbonate-apatite pellets were in good quantitative agreement with those obtained with bovine dental enamel (Fox et al., 1983) but differed somewhat from those obtained with hydroxyapatite pellets. For carbonate-apatite pellets there was mainly remineralization at pK_{FAP} of 108 but when pK_{FAP} was 112 and 116 there was simultaneous demineralization/remineralization. For hydroxyapatite pellets the process of simultaneous demineralization/remineralization was less pronounced at pK_{FAP} of 112 than that which occurred with carbonate-apatite pellets. This difference in behavior between hydroxyapatite and carbonate-apatite may be attributed to the presence of carbonate in the latter. The results show that for both carbonate-apatite and hydroxyapatite pellets, fluoride

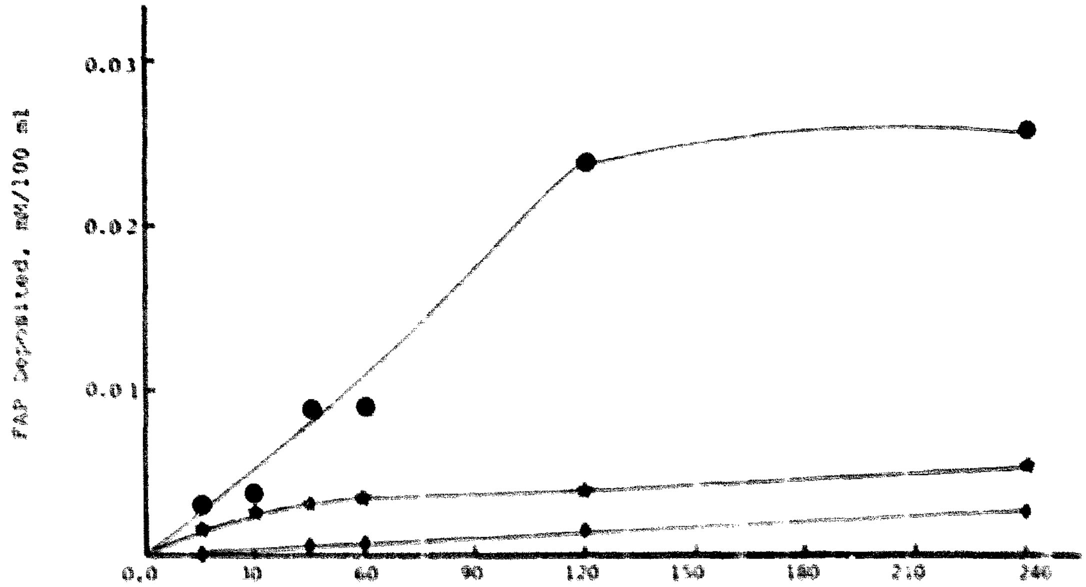

Fig. 9 The influence of the pK_{FAP} of the remineralizing solution on crystal growth of powder suspensions (15 mg/100 ml) as a function of time. Key ●, 108, •, 112, and ◆, 116.

levels at the surface were high when the pK_{FAP} of the remineralizing solution was 108 and decreased with increasing pK_{FAP}.

The investigation provided useful insights into relationships between the powder and pellet remineralization behavior. The pellet remineralization behavior in the surface region was well correlated with the rates of growth of hydroxyapatite crystal suspensions.

Acknowledgement

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