

# Interaction of Pyrophosphate with Calcium Phosphates

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Interactions of pyrophosphate (PP) with hydroxyapatite (HAP), brushite, and calcium pyrophosphate (CaPP) have been studied using electrokinetic and ESCA measurements. PP adsorbs on HAP and brushite even when their surfaces are highly negatively charged. Importantly, the HAP zeta potential curves as a function of PP concentration show a precipitous drop in the low concentration range. Also, curves at different pH values show a progressive shift in the potential toward that of CaPP. These observations suggest the formation of CaPP on HAP surface and this has been further confirmed by ESCA studies. Thus, adsorption on HAP appears to involve a surface precipitation or chemisorption followed by surface precipitation or surface reaction phenomenon. It is also suggested that in the case of brushite the dissolved calcium from the mineral competes for the PP and in turn reduces its adsorption.

## Introduction

Regulation of calcium phosphate crystal growth is important in a wide variety of applications such as in the prevention of scale formation in industrial heat exchangers, in the removal of phosphates from polluted water, and, most importantly, in the biological mineralization of teeth and bones.<sup>1-5</sup> Pyrophosphates (PP) are known to inhibit formation and growth of calcium phosphate precipitates. Reported studies<sup>6-10</sup> show that the inhibitory activity of PP is due to its ability to adsorb on the growing nuclei and in turn block the crystal growth sites. In this regard, the adsorption of PP on hydroxyapatite (HAP) has been conducted and correlated with the crystal growth inhibition behavior in the past,<sup>6-10</sup> however, the mechanism of adsorption itself has not been fully established. Also, interaction of PP with brushite and other forms of calcium phosphate which exist as precursors to HAP during the formation of dental tartar<sup>11</sup> have not been examined. Significantly, while PP is an excellent inhibitor of HAP formation, it is only a moderate inhibitor of brushite crystal growth.<sup>12</sup>

In the present investigation, the interaction of PP with HAP and brushite has been studied using electrokinetic

and X-ray photoelectron spectroscopic (ESCA) techniques. These techniques measure the changes in the interfacial properties, namely of charge and surface chemical composition of the substrate and, therefore, can provide insight into the mechanism of adsorption of PP on HAP and brushite.

## Experimental Section

**Materials.** *Calcium Phosphates.* HAP and brushite powders were obtained from Albright and Wilson. The surface areas of these samples have been reported to be 26 and 3 m<sup>2</sup>/g, respectively, as measured by the BET nitrogen adsorption technique. Calcium pyrophosphate (CaPP) was purchased from Alfa Products and used without further purification.

*Pyrophosphate.* Reagent grade tetrapotassium pyrophosphate (TKPP) was purchased from Sigma Chemical Co.

*Other Reagents.* Sodium hydroxide and hydrochloric acid, used as pH modifiers, and sodium chloride, used for adjusting the ionic strength, were supplied by Fisher Scientific Co. Triply distilled water was used for preparing the solutions and the suspensions.

**Methods.** *Zeta Potential Measurements.* Samples of solids (0.05 g) were added to 50 mL of 2 × 10<sup>-3</sup> kmol/m<sup>3</sup> NaCl solution, of preadjusted pH, in a 150-mL beaker. The suspension was stirred for 2 h using a magnetic bar and the pH was measured at the end. Following this, 50 mL of 2 × 10<sup>-3</sup> kmol/m<sup>3</sup> NaCl solution containing the desired concentration of TKPP was added to the suspension. Prior to addition, the pH of the TKPP solution was adjusted to that of the suspension. The solids were conditioned for 1 h with TKPP. At the end of the conditioning period, the pH of the suspension was measured and about 50 mL of the suspension was transferred to the cell of the zeta meter (Zeta Meter, Zeta Meter Inc.). The mobilities of 10 to 15 particles were measured and zeta potential was calculated. A chart provided with the zeta meter manual was used for the conversion of mobility readings to zeta potential values.

*Calcium Binding Isotherms.* One-milliliter samples of 10 wt % TKPP solution were added to 99 mL of solutions of varying calcium chloride concentration. The pH of the resulting suspension was adjusted to the desired value (pH 7.5 or pH 11.0) and the calcium activity measured using a pH meter equipped with an Orion Model 93-20 calcium selective electrode.

*XPS Analysis.* X-ray photoelectron spectroscopic analysis of the HAP, brushite, and CaPP samples was carried out at Physical Electronics Laboratories, Edison, NJ, using a Perkin-Elmer Model 5000LS ESCA spectrophotometer. Samples for XPS analysis were prepared by treatment of HAP and brushite powders with 200, 500, and 1000 ppm of TKPP in aqueous

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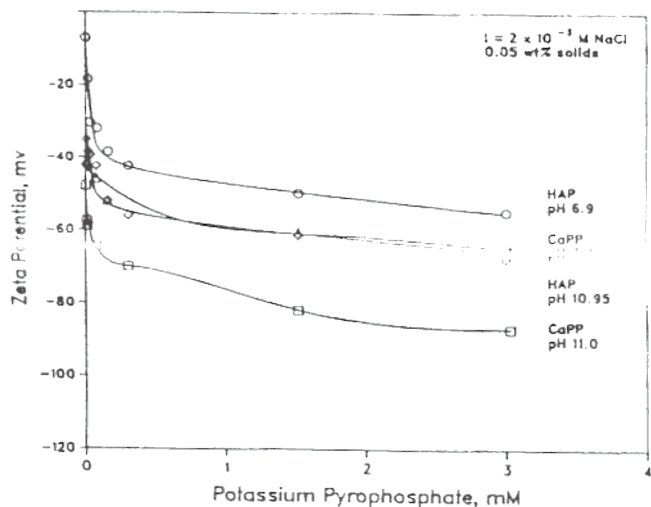


Figure 1. Zeta potential of HAP treated with pyrophosphate, effect of pH.

solution, followed by centrifugation and drying to yield fine powdered solids.

### Results and Discussion

**Interaction of Pyrophosphate with HAP. Zeta Potential.** The zeta potential behavior of HAP was determined as a function of PP concentration at two different pH values and the results are shown in Figure 1. For comparison, the zeta potential of CaPP is also plotted in the same figure. In the absence of PP, HAP under both the tested pH conditions exhibits a negative charge. Interestingly, the potential at low levels of PP exhibits a precipitous drop to values close to that of CaPP. Beyond this concentration, the dependence of HAP potential on PP concentration becomes similar to that of CaPP. Note that the behavior is essentially the same at both pH values (7 and 11). The sharp reduction in the potential of HAP at low levels of PP, even under conditions when the HAP surface is highly negatively charged as at pH 11, clearly shows the strong affinity of PP for the surface. This behavior is indicative of an adsorption process involving a phase change, possibly the formation of CaPP on HAP surface. The observation that the potential of CaPP itself depends on PP concentration in solution is not surprising since the latter is a potential determining ion for the surface.

Note that beyond the precipitous drop in zeta potential of HAP in PP solutions, values of the potential are not identical to those of CaPP but vary similarly to that of CaPP. Even if PP forms a CaPP layer on HAP surface, differences between the potentials of CaPP and PP-treated HAP are not unreasonable. For example, PP, being a potential determining ion for CaPP, can lower the CaPP potential. Therefore, identical values of zeta potential of CaPP and PP-treated HAP can be expected if and only if the residual levels of all ionic species are the same in both the systems. Inhomogeneities in the newly formed CaPP layer on HAP can also cause differences between the potentials of CaPP and PP-treated HAP.

The pH dependence of the zeta potential of HAP treated with solutions containing different levels of PP is shown in Figure 2. Again for comparison, the zeta potential of CaPP is included. It is evident from the zeta potential curves that the surface of HAP is becoming more negative with increase in PP concentration. Also, the curves appear to progressively shift toward that of CaPP. While this in itself cannot be taken as evidence of CaPP formation, this

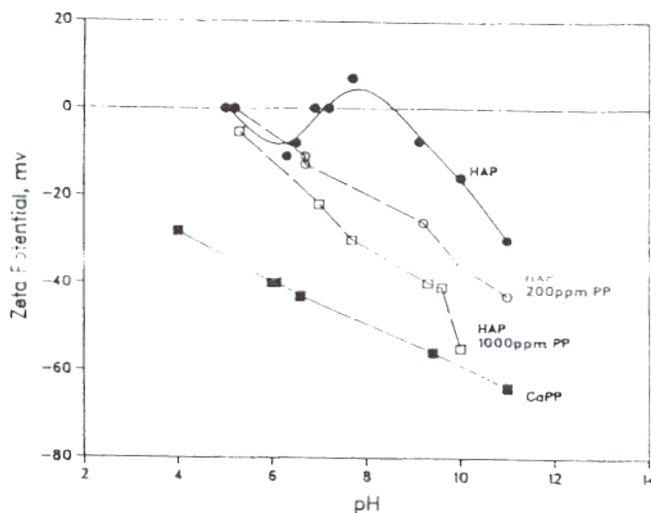


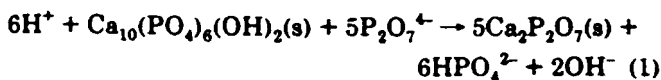
Figure 2. Effect of PP treatment on zeta potential of hydroxyapatite.

in combination with the data in Figure 1 suggests the formation of CaPP on HAP surface.

Formation of CaPP on HAP can occur by either a surface precipitation phenomenon or a surface reaction process. Surface reaction involves chemical interaction between the adsorbate species and the adsorbent, in this case pyrophosphate and the HAP surface. The reaction begins at the interface and proceeds inward. Surface precipitation, on the other hand, is the nucleation and growth of a stoichiometric precipitate of the adsorbate species by a complexing ion at the interface. In the present case, it will be the precipitation of CaPP on HAP surface. Note that often prior to surface precipitation, chemisorption of the adsorbate species can occur at the interface. Chemisorption can be distinguished from surface precipitation in the following manner. Chemisorption usually refers to adsorption resulting from the formation of a high-energy bond, such as a covalent bond, between the adsorbate species and site/sites on the solid surface and this will not result in the formation of a stoichiometric compound. Note that chemisorption can occur even if electrostatic factors are unfavorable for adsorption.

Adsorption has been shown to involve surface reaction or surface precipitation in a number of other mineral/inorganic systems in the past.<sup>13,14</sup> For example, formation of calcium carbonate and calcium fluoride on a HAP surface in the presence of dissolved carbonate<sup>13</sup> and fluoride, respectively,<sup>14</sup> and formation of calcium phosphate on calcite in the presence of phosphate<sup>13</sup> have been shown to involve a surface reaction or surface precipitation phenomenon.

The results from the current study suggest that in the presence of PP formation of CaPP can occur on the HAP surface. In order to test whether this observation is consistent with the expected thermodynamic behavior of the system, the following analysis was conducted. Essentially the feasibility of the reaction:



was examined using the literature values of free energy of formation of various species. The standard free energy

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Table 1. Free Energy of Formation for Reaction of HAP and PP

species	free energy of formation from elements, $G^\circ_f$ , kcal/mol	ref
H <sup>+</sup>	0	
Ca <sub>10</sub> (PO <sub>4</sub> ) <sub>6</sub> (OH) <sub>2</sub> (s)	-3030	15
HPO <sub>4</sub> <sup>2-</sup>	-260.3	15
P <sub>2</sub> O <sub>7</sub> <sup>4-</sup>	-458.7	15
OH <sup>-</sup>	-37.6	13
Ca <sup>2+</sup>	-132.3	15
CaPP(s)	-748.6	15
	-741.95	17

Table 2. Calculation of PP Levels Required To Form CaPP

free energy of formation of Ca <sub>2</sub> P <sub>2</sub> O <sub>7</sub> , kcal/mol	$\Delta G^\circ$ , kcal/mol	log $K_{eq}$	[P <sub>2</sub> O <sub>7</sub> <sup>4-</sup> ], mol/L	K <sub>4</sub> P <sub>2</sub> O <sub>7</sub> , ppm
-748.6				
-743.6				
-741.95				

change accompanying the reaction can be estimated from the free energies of formation of the reactants and products. The standard free energy change ( $\Delta G^\circ$ ) can be related to the equilibrium constant for the reaction,  $K$ , as

$$K = [\text{HPO}_4^{2-}]^6[\text{OH}^-]^2/[\text{H}^+]^6[\text{P}_2\text{O}_7^{4-}]^5 \quad (2)$$

Thus, if HAP is in equilibrium with the solution at a given pH (i.e. H<sup>+</sup>, OH<sup>-</sup>, and HPO<sub>4</sub><sup>2-</sup> are fixed), then the minimum amount of P<sub>2</sub>O<sub>7</sub><sup>4-</sup> required to force the above reaction to the right can be estimated.

The values of free energy of formation of various species and the source of the data are given in Table 1. As can be seen from the information in Table 1, there appears to be some uncertainty with regard to the value of free energy of formation of CaPP. The first value (-748.6 kcal/mol) was obtained from ref 15. The latter two values were obtained from unpublished work at Unilever Research.<sup>16,17</sup> The values -743.6 and -741.95 kcal/mol correspond to CaPP solubility product values of  $1.2 \times 10^{-15}$  and  $1.9 \times 10^{-14}$ , respectively. Since it was difficult to assess the accuracy of these estimates, each value was used to determine the corresponding three free energy and equilibrium constants for the above reaction. The results of this analysis are given in Table 2. The last two columns in the table show the concentrations of PP and TKPP for the present system at a pH of 6.9.

Note that because of pH-dependent hydrolysis reactions, only about 50% of the total PP will be P<sub>2</sub>O<sub>7</sub><sup>4-</sup> at pH 6.9. This has been accounted for in the calculation of the concentration of TKPP in equilibrium with both of the solid phases. It is clear from Table 2 that from the three possible estimates of PP levels, the worst case situation, i.e., the highest concentration of TKPP needed to cause the formation of CaPP on HAP, is just above 16.5 ppm. This simple analysis indicates that the formation of CaPP on HAP in the presence of soluble PP at relatively low levels is consistent with the expected behavior, i.e., PP can replace orthophosphate on the mineral surface.

**X-ray Photoelectron Spectroscopic Analysis (XPS/ESCA).** In order to further confirm the formation of CaPP on the HAP surface in the presence of dissolved PP, an ESCA study was conducted. The XPS spectra of HAP, CaPP, and PP-treated HAP are shown in Figure 3. Figure

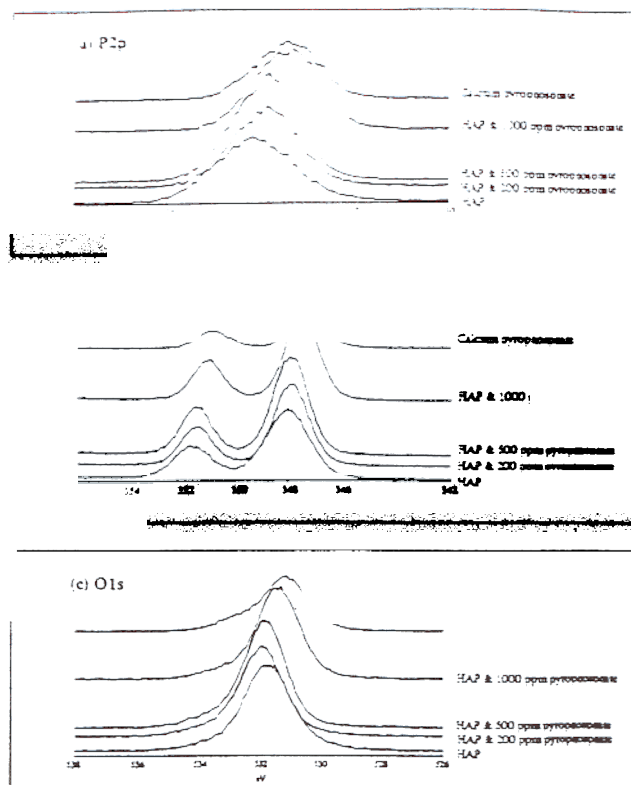


Figure 3. XPS analysis of hydroxyapatite treated with pyrophosphate.

3 is divided into parts a, b, and c which represent the binding energies corresponding to P2p (132–134 eV), Ca2p (347–348 eV), and O1s (531–532 eV), respectively. The XPS spectra for each element shows the spectra of untreated HAP, CaPP, and PP-treated HAP at PP concentrations of 200, 500, and 1000 ppm. For each element, the PP-treated HAP samples are between those of HAP and CaPP. The binding energy moves closer to CaPP as the concentration of TKPP increases. This indicates that the HAP surface, upon PP treatment, is becoming progressively similar to that of CaPP. Results of the XPS analysis thus support the inference, deduced from electrokinetic studies and thermodynamic analysis, of CaPP formation at the HAP surface.

**Interaction of Pyrophosphate with Brushite.** As in the case of HAP, the interaction of PP with brushite was also studied using zeta potential and ESCA techniques. The zeta potentials of brushite as a function of PP concentration are given in Figure 4. Here again, brushite is negatively charged under the test conditions and it shows a sharp reduction in potential after exposure to low levels of PP. Interestingly, the absolute values of the potential are lower than those observed for HAP. Also, beyond this initial drop there appears to be less dependence on PP concentration, in contrast to the behavior of the HAP/PP system described above. Thus, while zeta potential suggests some significant adsorption of PP on brushite, whether it involves surface conversion to a CaPP-type surface is not clear.

ESCA studies of brushite samples treated with PP were also conducted. Unlike the case of HAP, brushite and CaPP have very similar spectra and therefore it is not possible to use this technique to distinguish between the two phases.

One of the differences between HAP and brushite is their water solubility, with HAP having a relatively low solubility compared to brushite. For example, at a pH of

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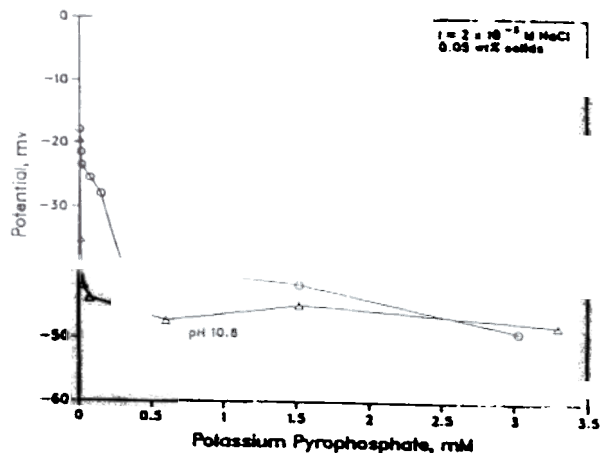


Figure 4. Zeta potential of brushite treated with pyrophosphate, effect of pH.

6.9, the concentration of calcium ions in equilibrium with HAP is about 2 ppm and that with brushite is about 28 ppm. When a reagent such as PP is introduced into a suspension of HAP or brushite, there exists a competition between complexation of bulk calcium vs adsorption. Because of the higher solution levels of calcium in the brushite suspensions, it is reasonable to expect that there will be more bulk complexation than in the case of HAP. This is supported by the observation that addition of 10 ppm of PP into supernatant solutions of HAP and brushite resulted in precipitation in the case of the latter and not in the case of the former. Thus, at a particular level of PP, there will be more PP available for surface adsorption/conversion on the HAP surface than on brushite. This may account for the differences in the dependence of zeta potential of HAP and brushite on PP concentration. This solubility difference appears to provide favorable conditions for bulk precipitation of CaPP in the brushite-PP system. In contrast to this, because of low calcium levels in solution, the interactions with HAP may be more like surface conversion or surface precipitation and this is in line with the ESCA results.

**Comparison of Affinities of PP to HAP, Brushite, and CaPP.** Zeta potential results presented in Figures 1 and 4 showed that addition of PP renders the surface of HAP and brushite negative. Therefore, variation of the charge density at the solid/liquid interface can be used as a measure of adsorption. Zeta potential values can be used as a measure of charge density at the solid/liquid interface and can be used for comparison provided the ionic strength of the medium is maintained constant since only the zeta potential, and not the charge density, depends on the ionic strength. As shown in Figures 5 and 6, addition of TKPP resulted in a significant increase of ionic strength, measured in terms of specific conductance. Therefore, the zeta potential results shown in Figures 1 and 2 were corrected for ionic strength variation using the procedure described by Hunter.<sup>18</sup>

Figures 7 and 8 show the zeta potential curves of TKPP-treated HAP and brushite corrected for a base ionic strength of  $2 \times 10^{-3}$  kmol/m<sup>3</sup> NaCl. Zeta potential curves obtained for the CaPP-TKPP system are presented in Figure 7 for the purpose of comparison. These corrected potentials can now be used to examine the dependence of zeta potential on pyrophosphate concentration. The approach involved the use of curve fitting software (Table Curve, Jandel Scientific, CA) to fit the experimental data

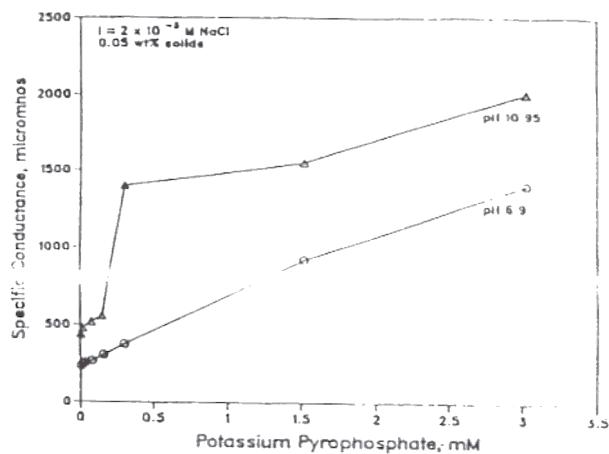


Figure 5. Specific conductance of HAP slurry in pyrophosphate solutions.

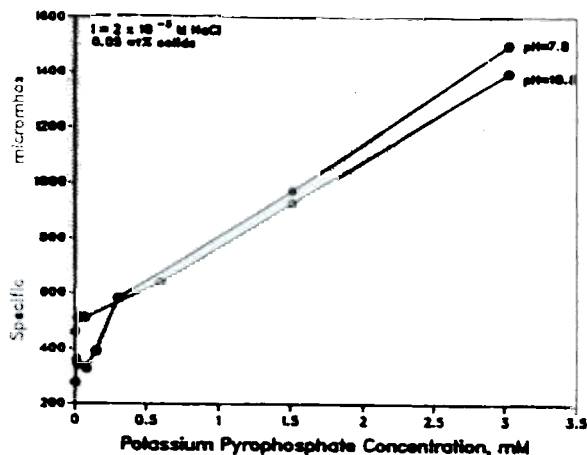


Figure 6. Specific conductance of brushite slurry in PP solutions.

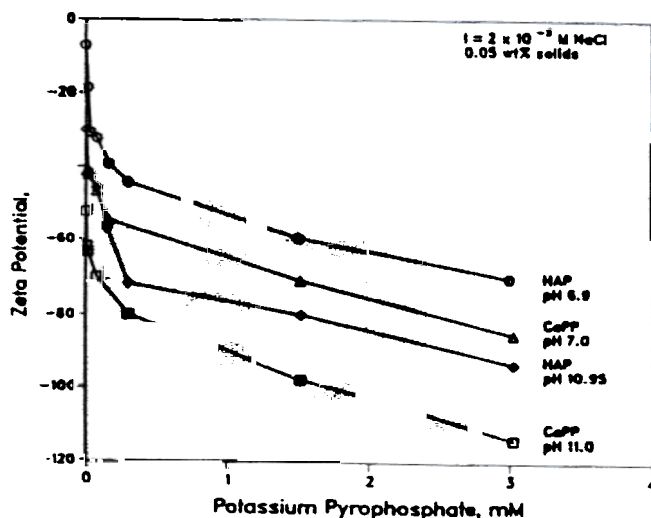


Figure 7. Zeta potential vs pyrophosphate concentration, zeta values corrected for ionic strength in PP solutions.

to different forms of empirical equations. The equation that showed best fit (highest correlation coefficient) of the experimental data was obtained and is shown in Table 3. This empirical equation shows that the negative zeta potential of all three surfaces increases as a function of the square root of the PP concentration. In the equations shown in Table 3, the constant  $A$  represents the zeta potential of the untreated calcium phosphate, while  $B$  can be taken as a measure of the affinity of TKPP to the calcium phosphate surface. Examination of Table 3 shows

(18) Hunter, R. J. *Zeta Potential in Colloid Science: Principles and Applications*; Academic Press: New York, 1981; Chapter 2, pp 11-58.

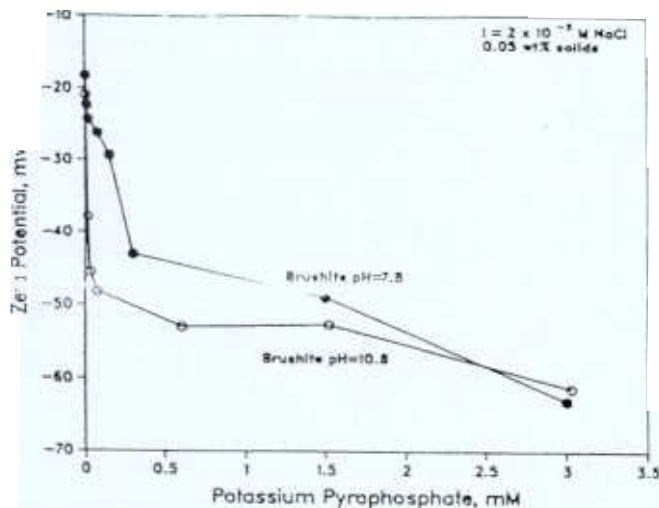


Figure 8. Zeta potential vs pyrophosphate concentration, zeta values corrected for ionic strength in PP solutions.

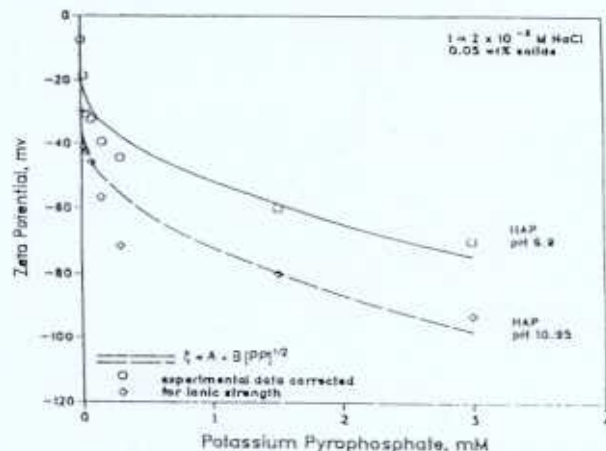


Figure 9. Zeta potential of HAP vs pyrophosphate concentration.

Table 3. Relationship Between Zeta Potential and Pyrophosphate Concentration: Empirical Models

	Equation: $\zeta = A + B [PP]^{1/2}$			
	pH	A	B	$\zeta_{avg}$
hydroxyapatite	6.90	-20.5	-31.4	-13.5
	10.95	-36.0	-36.0	-30.5
brushite	7.8	-20.6	-24.8	-18.3
	10.8	-31.9	-19.0	-21.0
calcium pyrophosphate	7.0	-41.0	-24.9	-41.4
	11.0	-57.4	-33.5	-52.3

that in all cases, except for the brushite-TKPP system at pH 11, the equation that best fitted the experimental data was of the same type in which the zeta potential showed a square root dependence on TKPP concentration. The quality of fit can be seen from Figures 9, 10, and 11 to be good for HAP and CaPP. Also, it can be seen from the "B" value that the affinity of TKPP is highest for HAP followed by CaPP and brushite. Further, the affinity of TKPP to the calcium phosphate surface is greater at higher pH. This is possibly due to differences in reactivity of  $P_2O_7^{4-}$  present at high pH values vs  $HP_2O_7^{3-}$  and other forms at lower pH.

**Dissolved Calcium in the Supernatant of Calcium Phosphate Suspensions.** Review of past results<sup>10</sup> and the results obtained in the present study suggest that the adsorption of PP on calcium phosphates involves chemical interactions and ultimately results from the formation of CaPP on HAP surface. Because of dissolution, the

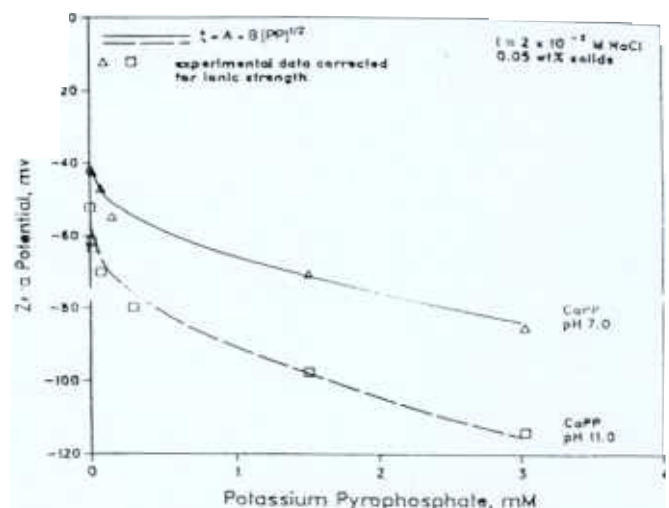


Figure 10. Zeta potential of CaPP vs pyrophosphate concentration.

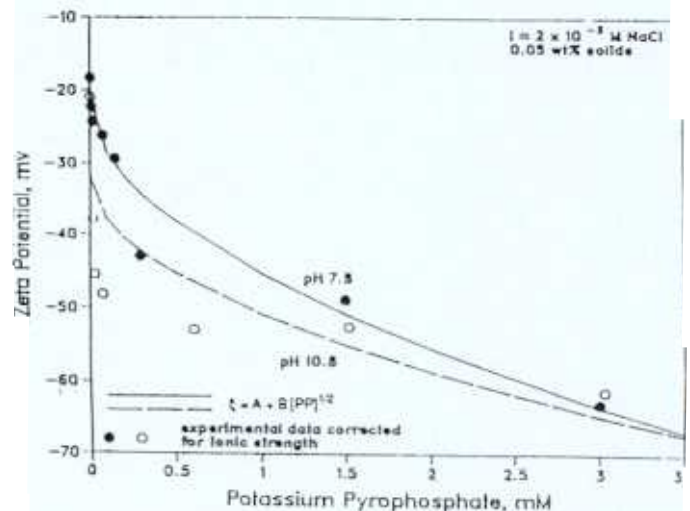


Figure 11. Zeta potential of brushite vs pyrophosphate concentration.

Table 4. Solubility of Calcium Phosphates

	pH	Ca <sup>2+</sup> , ppm
hydroxyapatite	7.50	0.93
	10.74	<0.10
brushite	7.50	24.00
	10.85	1.50
calcium pyrophosphate	7.55	0.82
	10.80	<0.10

supernatant of calcium phosphate suspensions would contain calcium ions which would compete with the surface for PP molecules. The affinity of PP to calcium phosphate surface would thus depend on both the concentration of dissolved calcium and its relative affinity to the surface.

Table 4 shows the dissolved calcium concentration in the supernatants of HAP, brushite, and CaPP at pH values of 7.5 and 11. It can be seen that the dissolved calcium concentration is much higher at the lower pH for all three minerals. The dissolved calcium is much higher in brushite supernatant. Presence of larger levels of dissolved calcium can account for the lower affinity of PP to calcium phosphate surface at lower pH and also its lower affinity to brushite surface compared to that for HAP and CaPP. The dissolved calcium concentration in CaPP supernatant is similar to that of HAP supernatant. However, the affinity of TKPP is higher for HAP than for CaPP (Table 3). This is explained by comparing the electrostatic

repulsion at the two surfaces, where the repulsion between the negatively charged phosphate species and the surface would be higher for CaPP than for HAP, as evidenced by the much higher negative charge density of the CaPP surface (ZP = -41 mV) compared to that of HAP (ZP = -13.5 mV).

### Conclusions

(1) Electrokinetic studies showed the negative zeta potential of HAP and brushite to increase sharply in the presence of very low concentrations of PP indicating strong affinity of the solute to the surface.

(2) Adsorption of negatively-charged PP species on highly negatively charged surfaces suggested chemisorption of solute on the surface. PP increased the negative

potential of CaPP at pH 6.9 and 10.95, indicating that it is a potential-determining species for the surface.

(3) Formation of CaPP on the HAP surface was supported by the results from XPS and electrokinetic studies.

(4) Affinities of PP for HAP are found to be higher than that for brushite. Lower affinity for brushite was attributed to the presence of dissolved calcium in the supernatant which competes with surface calcium for PP.

(5) An empirical fit of the zeta potential data shows that the negative potentials on HAP, CaPP, and brushite increase as a function of the square root of the PP concentration. Greater affinity between PP and the surface was observed for both HAP and CaPP at the higher pH (~11) than at the lower pH (~7).