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# Inhibition of Octacalcium Phosphate Crystal Growth by Some Organic Phosphonates

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Phosphonate additives have important applications both in biomineralization processes and in industrial mineral scale formation. The constant composition method has been used to study the influence of some organic phosphonate additives: hydroxyl ethylene diphosphonic acid) (etidronate), alendronate (4-amino-1-hydroxybutylidene) *bis*phosphonic acid, risedronate (1-hydroxy-2-(3-pyridinyl) ethylidene) *bis*(phosphonic acid). On the kinetics of crystal growth of octacalcium phosphate (OCP) on octacalcium phosphate seed crystals at pH 6.50 and 37 ºC, ionic strength 0.15 mol $L^{-1}$ (maintained with NaCl) and an octacalcium phosphate relative supersaturation, σ, of 0.899. Depending upon the Ca/P ratio of the solution, that is equal 1.33. The results indicate that traces of some phosphonates (≤ $10^{-6}$ mol $L^{-1}$) are extremely effective in inhibiting crystal growth. Assuming that the adsorbed additives block discrete growth sites on the crystal surfaces, the kinetic results may be interpreted in terms of a Langmiur adsorption model yielding kinetic affinity constants. The order of inhibitory effectiveness, Alendronate > Risedronate > Etidronate, reflects the ability of the phosphonates to bind to the octacalcium phosphate.

**Key Words: Inhibition, Octacalcium phosphate, Crystal, Growth, Phosphonates, Alendronate, Risedronate.**

## INTRODUCTION

The precipitation of sparingly soluble alkaline earth metal phosphates from their supersaturated solutions has attracted the attention of several authors. Calcium phosphate precipitation is of particular interest because of its importance in industrial water systems where deposition of calcium phosphates on heat exchanger surfaces results in decreased efficiency of the systems. In addition, calcium phosphate phases are involved in waste water treatment processes, in agriculture as fertilizers and in biological calcification processes such as tooth and bone formation. During the precipitation process at ambient temperatures, different calcium phosphate phases, arranged in order of increased solubility, hydroxyapatite [$Ca_5(PO_4)_3OH$, HAP], tricalcium phosphate [$Ca_3(PO_4)_2$, TCP], octacalcuim phosphate [$Ca_8H_2(PO_4)\cdot 6.5H_2O$, OCP] and dicalcium phosphate dihydrate [$CaHPO_4.2H_2O$, DCPD] may be formed depending upon the level of super saturation, ionic medium and pH. Results of previous studies[1-4] on the spontaneous precipitation of calcium phosphates at physiological conditions indicate that the kinetically favoured precursor phases such as amorphous calcium phosphate, dicalcium phosphate dihydrate (DCPD), octacalcuim phosphate (OCP) and tricalcium phosphate (TCP) are formed prior to the formation of the thermodynamically stable hydroxyapatite. Moreover, it has been suggested that such precursor phases can be stabilized by the presence of certain additives[5,6]. Octacalcium phosphate is of biological importance, as well as hydroxyapatite, because OCP seems to be an important precursor in the formation of tooth and bone apatite[7,8]. Analysis of tooth enamel has given reliable evidence of the existence of OCP[9]. In the present work, the effectiveness of some different organic phosphonates (Table-1) on octacalcium phosphate (OCP) mineralization was studied by the use of the constant composition method[10,11]. In this procedure, supersaturation was maintained constant by the automated addition of calcium, phosphate, phosphonate and hydroxide ions, controlled by ion selective electrodes, following the addition of seed material to stable supersaturated calcium phosphate solutions. Using this method, mineralization reactions could be investigated over a range of constant thermodynamic driving forces and by choosing appropriately low supersaturation levels. Moreover, by employing the constant composition method, the rates of mineralization of OCP in the absence and presence of the phosphonate inhibitors could be measured with an accuracy and reproducibility unachievable by conventional free drift crystal growth methods. Data so obtained enable comparisons to be made of the phosphonates in terms of their effectiveness in inhibiting OCP crystal growth. The marked differences in the ability of various phosphonates to influence OCP crystallization may be related to their potential for forming complexes with calcium ions. Additional information about the interaction between OCP surfaces and

TABLE-1
PHOSPHONATES STUDIED

| Name | Structure | Abbreviation |
|---|---|---|
| Aminohydroxybutylidene diphosphoric acid | $H_2N(CH_2)_3C(OH)(PO_3H_2)_2$ | Ald. |
| Hydroxylpyridinyl ethylidene diphosphonic acid | $(H_4C_5H)CH_2C(OH)(PO_3H_2)_2$ | Rised. |
| Hydroxyethylene diphosphonic acid | $CH_3C(OH)(PO_3H_2)_2$ | Etid |

phosphonates was obtained from extensive ζ potential measurements as well as some selected equilibrium adsorption determinations.

## EXPERIMENTAL

Octacalcuim phosphate seed crystals, prepared as described earlier[12,13], had a specific surface area of 32.8 ± 0.6 $m^2 g^{-1}$ (BET nitrogen adsorption (30:70 $N_2$/He, quantasorb 11, quantachrome)[14]. Chemical analysis[15] showed a molar ratio of calcium to phosphate of 1.34 ± 0.02 with characteristic OCP morphology and infrared and X-ray diffraction spectra. Grade A glassware and reagent grade chemicals were used. Stock solutions (calcium chloride, potassium dihydrogen phosphate and sodium chloride), prepared using triply distilled water and reagent grade chemicals dried under vacuum, were filtered twice (0.22 μm Millipore filters) before use. Calcium chloride solutions were standardized by EDTA titration, the ion exchange method and atomic absorption spectroscopy phosphate stock solutions were made from potassium dihydrogen phosphate and were standardized potentiometrically by titration with standard potassium hydroxide. Phosphonate solutions were prepared from samples obtained from the Synthetic Organic Chemical Manufacturers Association. They were used as their sodium salts, after neutralization where necessary, with sodium hydroxide solution. Carbon dioxide-free potassium and sodium hydroxide solutions were prepared in a nitrogen atmosphere from washed pellets and standardized against potassium hydrogen phthalate. Crystal growth experiments were made in a double-walled Pyrex cell and magnetically stirred (*ca.* 450 rpm) at physiological temperature, 37.0 ± 0.1 ºC using the constant composition technique. The stable supersaturated solutions of calcium phosphate with a molar ratio of $T_{Ca} = T_P$ = 1.34 ± 0.02 were prepared by adjusting the pH of the premixed solution of calcium chloride and potassium dihydrogen phosphate to a value of 6.00 by slow addition of 0.10 M potassium hydroxide. A glass electrode (Orion No. 91-01, UK), coupled with a reference electrode (Orion 900100, UK) was used as a probe to trigger titrant addition by means of a potentiostat. The electrode pair was standardized before and after each experiment using NBS standard buffer solutions. The solutions were continuously stirred while nitrogen gas, presaturated with water at 37 ºC, was bubbled through the solution to exclude carbon dioxide.Following the addition of OCP seed crystals, the crystal growth reaction was monitored by the addition of titrant solutions from mechanically coupled automatic burettes mounted on a modified pH state (Model 600 series, Brinkman Instruments, Westbury, NY), one containing calcium and sodium chlorides and the other potassium dihydrogen phosphate, the phosphonate additive and potassium hydroxide. The concentrations of the titrant solutions were calculated using eqns. 1-5 to compensate for dilution effects caused by the use of multiple titrant burettes as well as the consumption of ions accompanying crystal growth.

$$[CaCl_2]_t = 2[CaCl_2]_{rs} + 4C_{eff} \quad (1)$$

$$[NaCl]_t = 2[NaCl]_{rs} - 8C_{eff} \quad (2)$$

$$[KH_2PO_4]_t = 2[KH_2PO_4]_{rs} + 3C_{eff} \quad (3)$$

$$[KOH]_t = 2[KOH]_{rs} + 5C_{eff} \quad (4)$$

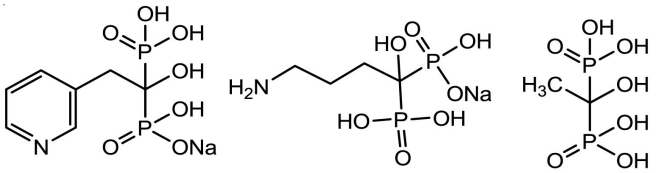
$$[phosphonate]_t = 2[phosphonate]_{rs} \quad (5)$$

In eqns.1-5, the subscripts t and rs reprensent titrant and reaction supersaturated solutions, respectively. $C_{eff}$, the effective OCP concentration, is the number of moles of growing phase per liter of added titrant solutions.

The rate of titrant addition, controlled by means of glass electrode, was used to calculate the rate of crystal growth normalized to initial seed surface area by eqn. 6

$$R = (dv/dt)\ (C_{eff}/A) \quad (6)$$

where A is the total surface area of the added seed crystals[16]. Kinetic studies and z potential measurements were performed for phosphonate concentrations ranging from $1.0 \times 10^{-8}$ mol $L^{-1}$ to$1.0 \times 10^{-6}$ mol $L^{-1}$ (Tables 2 and 3).




## RESULTS AND DISCUSSION

Constant composition growth experiments were first performed in pure supersaturated solutions of octacalcium phosphate (OCP) and then in the presence of the phosphonates as a function of additive concentration The results are presented in Tables 2 and 3 and are plotted in Figs. 1-3. The former shows typical plots of titrant volume required to maintain the supersaturation, as functions of time and of phosphonate concentration.

For comparison, a curve of titrant consumption for the growth of OCP in pure supersaturated solutions of the salt is included (std. curve). All the rate curves showed a rapid titrants addition immediately following the introduction of seed crystals. This frequently observed phenomenon, usually attributed to conditioning of the surface of the seed crystals in the supersaturated solution, may reflect ion exchange involving solution and surface cations and protons,or the removal of active growth sites on the seed crystals due to the rapid mineralization of high-energy sites[16,17].

Significant reductions of the initial surges observed in distance added of the phosphonates suggest their adsorption

TABLE-2
EFFECT OF SOME PHOSPHONATES ON THE RATE OF CRYSTAL GROWTH OF OCP, $T_{Ca} = 0.260 \times 10^{-2}$ mol $L^{-1}$, $TPO_4 = 0.195 \times 10^{-2}$ mol $L^{-1}$, NaCl = 0.139, KOH = $0.695 \times 10^{-3}$, pH = 6.5, $\sigma$ = 0.899 $C_{eff} = 3.0 \times 10^{-4}$

| Additive ($10^{-6}$ mol $L^{-1}$) | Rate ($10^{-6}$ mol $min^{-1}$ $m^{-2}$) | Inhibition (%) |
|---|---|---|
| – | 3.9340 | – |
| 0.098 Ald. | 2.3560 | 43.770 |
| 0.172 Ald. | 1.7510 | 56.330 |
| 0.320 Ald. | 0.9678 | 75.400 |
| 0.540 Ald. | 0.8250 | 79.010 |
| 0.760 Ald. | 0.6780 | 82.150 |
| 0.980 Ald. | 0.6337 | 83.900 |
| 9.800 Ald. | 0.6001 | 84.100 |
| 0.131 Rised. | 2.4210 | 38.460 |
| 0.304 Rised. | 1.3980 | 64.460 |
| 0.500 Rised. | 0.9239 | 76.515 |
| 0.653 Rised. | 0.7889 | 79.946 |
| 0.870 Rised. | 0.7506 | 80.920 |
| 1.090 Rised. | 0.7001 | 82.200 |
| 0.100 Etid. | 2.8020 | 28.830 |
| 0.300 Etid. | 1.7850 | 54.630 |
| 0.500 Etid. | 1.2920 | 67.160 |
| 0.651 Etid. | 1.0010 | 74.560 |
| 0.873 Etid. | 0.9780 | 75.350 |
| 1.000 Etid. | 0.9442 | 75.998 |

TABLE-3
VALUES OF $R_0(R_0-R_i)$-1AND FOR THE CRYSTAL GROWTH OF OCP at $\sigma$ = 0.899 AND $R_0 = 3.934 \times 10^{-6}$ mol $min^{-1}$ $m^{-2}$

| Additive ($10^{-6}$ mol $L^{-1}$) | $10^{-5}$ (Inhibitor)$^{-1}$ L $mol^{-1}$ | Rate ($10^{-6}$ mol $min^{-1}$ $m^{-2}$) | $R_0(R_0-R_i)^{-1}$ |
|---|---|---|---|
| 0.098 Ald. | 102.40 | 2.3560 | 2.490 |
| 0.172 Ald. | 58.14 | 1.7510 | 1.800 |
| 0.320 Ald. | 31.25 | 0.9678 | 1.330 |
| 0.540 Ald. | 18.52 | 0.8250 | 1.265 |
| 0.760 Ald. | 13.16 | 0.6780 | 1.208 |
| 0.980 Ald. | 10.20 | 0.6337 | 1.192 |
| 9.800 Ald. | 1.02 | 0.6001 | 1.180 |
| 0.131 Rised. | 76.34 | 2.4210 | 2.600 |
| 0.304 Rised. | 32.89 | 1.3980 | 1.550 |
| 0.500 Rised. | 20.00 | 0.9239 | 1.310 |
| 0.653 Rised. | 15.31 | 0.7889 | 1.250 |
| 0.870 Rised. | 11.49 | 0.7506 | 1.235 |
| 1.090 Rised. | 9.17 | 0.7001 | 1.216 |
| 0.100 Etid. | 100.00 | 2.8020 | 3.475 |
| 0.300 Etid. | 33.33 | 1.7850 | 1.830 |
| 0.500 Etid. | 20.00 | 1.2920 | 1.490 |
| 0.651 Etid. | 15.36 | 1.0010 | 1.340 |
| 0.873 Etid. | 11.45 | 0.9780 | 1.330 |
| 1.000 Etid. | 10.00 | 0.9442 | 1.315 |

at the higher energy sites on the OCP seed crystals, thus competing with other initial surface processes. It can also be seen in Fig. 1-3 that the linearity of the rate plots of OCP crystal growth (reflected by the constant slopes of volume versus time curves) was usually achieved 20-50 min after seed introduction to the supersaturated solution in experiments performed withor without phosphonate. It should be noted that at high concentrations of inhibitors OCP crystals appeared to grow only during the initial stage of the reaction.The growth rate reduction in the presence of phosphonate additives can be expressed as a per cent of inhibition (I), using eqn. 8:

$$I = 100[(R_0-R)/R_0] \quad (8)$$

where R and $R_0$ are the growth rates in the presence and absence (std.) of inhibitors, respectively. Values of I, given in Tables 2 and 3 may be used to compare the inhibition effectiveness of the phosphonate additives. They illustrate the strong growth inhibition by alendronate, risedronate and etidronate. It is quite well established that strong inhibitors of crystal growth, such as the phosphonates, act by blocking,through adsorption, active growth sites at the crystal surfaces[16,18]. Commonly, inhibition kinetics data are interpreted in terms of a simple Langmuir adsorption isotherm model. Assuming that the adsorbed phosphonate ions occupy a fraction (Ø) of the active growth sites, thereby preventing them from participating in the crystal growth reactions. The rates of crystal growth in their presence (R), can be written in terms of the unihibited rate $R_0$, as in eqn. 9:

$$R = R_0 (1-Ø) \quad (9)$$

Application of a simple Langmuir model leads to eqn. 10.

$$R_0/R = 1 + K [\text{phosphonate}] \quad (10)$$

In which K is the adsorption affinity constant with units of liters per mole. Linear plots of $R_0/R$ as a function of phosphonate concentration are shown in Fig. 3. In the proposed inhibition model, it is assumed that the phosphonates adsorb at growth sites on the OCP crystals. The OCP $\zeta$ potentials in the presence of all the phosphonates studied were markedly more negative than those in a pure saturated solution of OCP at the same pH. This clearly indicates an appreciable uptake of phosphonate anions at the positively charged OCP surfaces. As can be seen in Table-1, all the phosphonates,with the exception of etidronate, contained nitrogen atoms which would be positively charged at pH 6.0 and this might influence the net charge of the adsorbing phosphonate on the OCP crystal surfaces.

It is generally accepted that the inhibition of scale formation is influenced by both the location of the adsorbed inhibitor at the crystal surface and the extent of chemical bonding with the surface[6,16,18,19]. Three types of crystal surface sites, having different binding energies are available for the adsorbing inhibitor molecules.In order of decreasing bond strength they are kinks, steps and terraces[16]. In related studies[20-22], calculation of the effective area of coverage for the adsorption of phosphonates on gypsum and barite suggested that the maximum crystallization inhibition may be achieved when less than 5 % of the crystal surface is covered by adsorbate molecules. This suggests that the relatively small inhibitor molecules such as the phosphonates of the present work are preferentially adsorbed at the most active ($k_{ink}$) growth sites. The results of an attempted adsorption experiment, in which the phosphonate was equilibrated with the OCP crystals, seemed to support this hypothesis,since it indicated that a very small amount of the additive was adsorbed.The marked differences in the initial constant composition surges obtained in OCP growth studies in the prensence and absence of the phosphonate inhibitors also suggested that adsorption occurred at active kink sites. The adsorption of phosphonates at OCP crystal surfaces probably results from the binding of phosphonate anions to surface calcium ions.The initial bonding would be favoured if longer range electrostatic forces between the crystal surface and the approaching phosphonate molecule were possible. However,the interaction may be enhanced if hydrogen bonding involving partially protonated phosphonate groups also

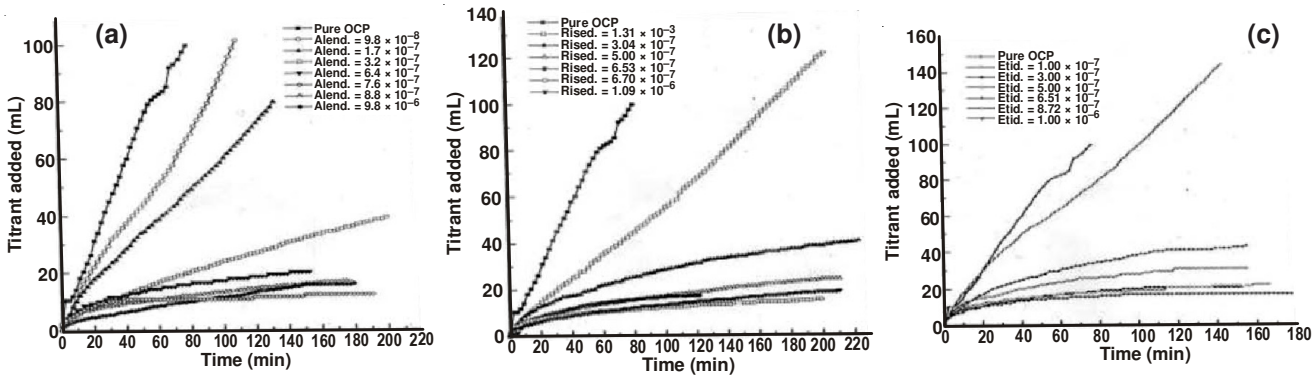



Fig. 1. Crystal Growth of OCP in presence of (a) Alendronate (b) Residronate and (c) Etidronate at $\sigma = 0.899$

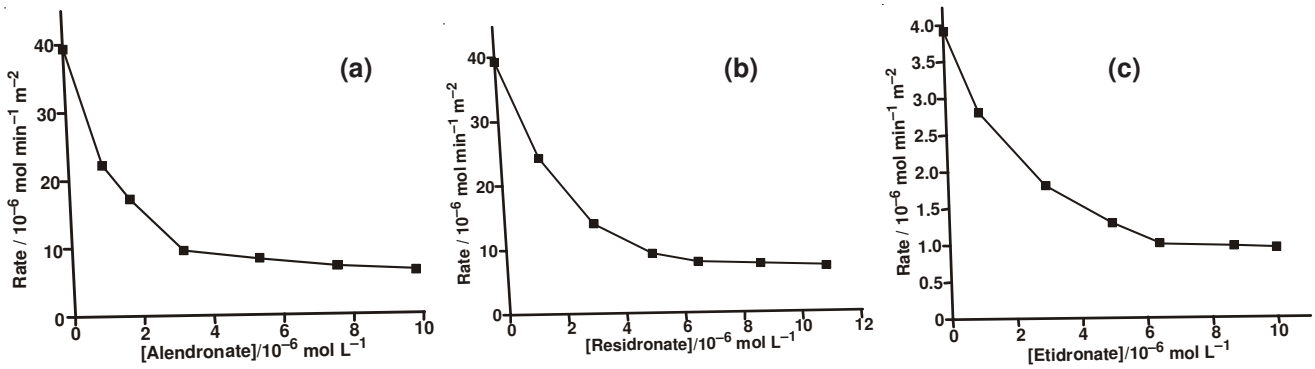



Fig. 2. Plot of rate of OCP growth as a function (a) [Alendronate] (b) [Residronate] and (c) [Etidronate] at $\sigma = 0.899$

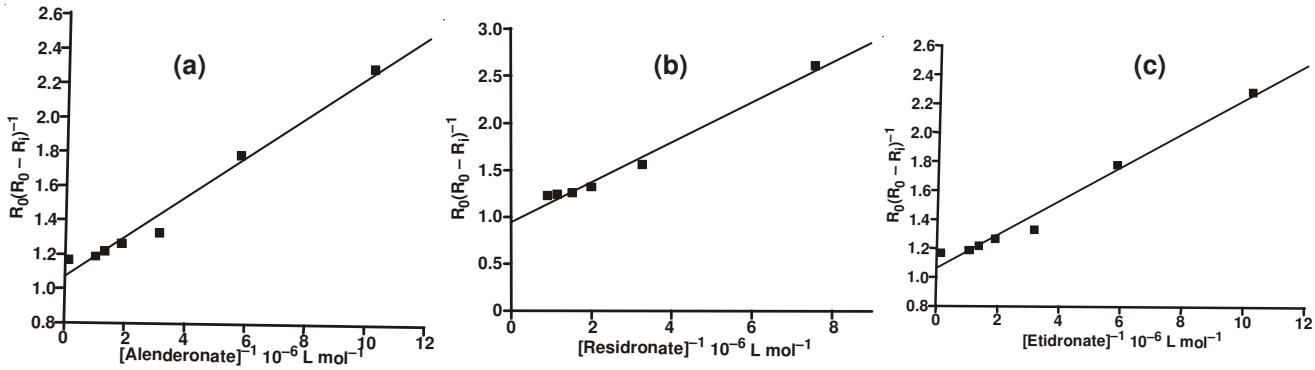



Fig. 3. Plot of $R_o(R_o - R_i)^{-1}$ as a function of (a) [Alendronate]-1 (b) [Residronate]-1 and (c) [Etidronate]-1 for the growth of OCP at $\sigma = 0.899$

participates in the surface binding[6,19,22,23]. It has been recognized, for poly carboxylic inhibitors, that highly deprotonated, relatively acidic molecules are less effective inhibitors of crystal growth than those which retain some degree of protonation. It has been suggested that the less acidic carboxylic acid groups form stronger complexes with alkaline earth cations typical of those at the crystal surface[19]. Studies of various phosphonic acids, over a wide pH range, have shown that the presenceof both deprotonated and protonated phosphonate groups leads to stronger interactions between adsorbing inhibitor molecules and crystal surfaces. Additionally, protentially electrodonating groups or atoms, such as hydroxyl, aminonitrogen and oxygen atoms, in the inhibitor molecule, can also participate in the coordination to surface cations, enhancing both adsorption and subsequently, crystal growth inhibition[1,2,6,19,23-25]. If these electrodonating species are not involved in calcium coordination,they may form hydrogen bonds with surface anions, thus shielding negatively charged growth sites[6,19]. In contrast, increasing the number of hydrophobic groups in the inhibitior molecule, even to the extent of a single methylene group, may result in adecrease in the inhibitor effectiveness as shown for both polyelectrolytes and phosphonate inhibitors[5,19]. Such group may also sterically hinder the interaction of active phosphonic and other functional electrodonating groups. It follows that several factors, such as the number of functional groups available for complexation with surface calcium ions, the degree of protonation of the phosphonic acid groups, hydrogen bond formation between phosphonate and the OCP surface and the presence of hydrophobic groups in the inhibitor molecules. Under the experimental conditions of the present work (phosphonate concentrations range from $1.0 \times 10^{-8}$ mol $L^{-1}$ to $1.0 \times 10^{-6}$ mol $L^{-1}$, temperature 25 ºC and pH 6) all the phosphonates reduced the rate of OCP crystal growth in the order alendronate >

risedronate > etidronate. The results indicate the importance of the number of active groups available for surface complexations. In this phosphonates, the charge localized on the nitrogen (resulting from the deprotonation of the phosphonic acid groups) can be reduced by the formation of hydrogen bonds between two phosphonate groups and the imino group[26]. The formation of hydrogen bonds involving etidronate hydroxyl groups and OCP surface anions may account for the relatively high activity of the phosphonate etidronate in reducing the crystallization rates.

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