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Studies on the precipitation behavior of calcium phosphate solutions

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The chemical equilibrium of phosphate aqueous solutions is discussed. To obtain the concentration of the species in solution as a function of pH, we used an acid-base equilibria system, complex formation equilibria, and balance mass equations; and to resolve the non-linear equation system we used a computer program PV-WAVE with ZEROSYS subroutine function. It will be demonstrated that the chemical composition of the calcium phosphate precipitate depends decisively on the species concentration in solution. Also, the pH and the reaction conditions for the selective precipitation of hydroxyapatite are critical. A small variation of these conditions allows either the coprecipitation of other phosphates or no precipitation at all. The solubility variation of the phosphates as a function of the solution ionic force is also discussed. Finally, proper reaction conditions are proposed for hydroxyapatite growth starting from saturated solutions of calcium phosphates.

Key words: Calcium phosphates, Hydroxyapatite, Precipitation, Computer simulation, Phase diagrams.

Introduccion

The precipitation of hydroxyapatite (HAp) and other calcium phosphates is of considerable biological significance because vertebrate bone tissues contain them as their main mineral constituents. Indeed, hydroxyapatite is the main inorganic bone constituent and it is an interesting natural apatite with many potencial applications which have not yet been explored in detail [1-10]. One of the most relevant properties of HAp is its high resistance to dissolve in water, which, along with the cellular structure of natural bone [2, 9, 10-20], produces an excellent biomaterial, as well as appropiate components for the design and synthesis of alternative materials such as composites prepared by the growth of minerals on different substrates, like silica. In all the above examples, a detailed knowledge of the chemical solution precipitation behavior is mandatory [20-25].

From the standpoint of basic research, the study of the precipitation conditions in solutions containing calcium phosphate ions provides a challenge to the chemist because of the numerous calcium and phosphate phases that may be involved in the crystallization reactions. In fact, at least 5 soluble crystaline phases have been characterized, including HydroxyApatite (HAp), tribasic calcium phosphate (TCP), octacalcium phosphate (OCP), dibasic calcium phosphate anhydrous (DCPA) and dibasic calcium phosphate dihydrated (DCPD) [11].

The composition of these phases appears to depend upon the precipitation conditions: thus, the purpose of this paper is to study the precipitation of calcium phosphates as a function of the concentration of the calcium ions, phosphate ions and the pH at constant ionic force, aiming to obtain the best reaction conditions for the Hydroxyapatite precipitation and also to establish a diagram of the species in solution as a function of the pH.

The methods commonly used to calculate the species in an homogeneous solution are through equilibrium constants and free energy minimization. The first one uses an approach in which a base is selected with the species that has the higher concentration in equilibrium; the other species concentrations in solution are obtained starting from the choosen base using the equilibrium constants. The result that is obtained is a series of non-linear simultaneous equations that can be solved by conventional numerical methods. The free energy minimization method only uses approaches of free energy for the chemical equilibria. It does not make distinctions among the species that constitute it, and it is basically a problem of constrictions of non linear minimization. One of the broadly used methods is the Newton-Raphson scheme, which was employed in the development of EQUIL, to carry out quadratic conversions quickly for the calculation from the concentrations to the equilibria of electrolyte mixtures [15-17].

Experimental

In order to obtain the best reaction conditions for the

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hydroxyapatite precipitation, plots of the solubility products from the five calcium phosphate phases involved, i.e.; HAp, TCP, OCP, DCPA and DCPD; as a function of the concentration of calcium ions, phosphate ions and pH, were obtained. The solubility products were also obtained as a function of the ionic force at constant pH. The diagram of the species in solution was obtained as a function of pH from a solution of calcium chloride 0.0015 mol/l, potassium chloride 0.143 mol/l and potassium phosphate 0.009 mol/l. To obtain it we used the equilibrium thermodynamic constants acid-base as well as the complex formation equilibria, and the mass balance equations. The system of non linear equations resultants were solved using the computation program PV-WAVE with the ZEROSYS subroutine function.

Results and Discussion

Calcium phosphates precipitation

Table 1 contains the solubility constants at 25 ºC of HAp, TCP, OCP, DCPA and DCPD reported in the literature. Expressions in the square brackets indicate the molar concentrations of the suitable species, and y_z the species activity coefficient with valence z. The species ionic activity coefficients can be calculated by the Debye-Huckel extended equation, as proposed by Davies[12] which is the following:

$$-\log y_z = Az^2[I^{1/2}/(1 + I^{1/2}) - 0.3I] \quad (1).$$

With this equation the ion activity coefficients of multiple charge can be calculated up to an ionic force of 0.2 mol/l, with an error of 1%. In this equation y_z is the activity coefficient, A stands for the Debye-Hucke constant, that includes factors such as the solvent dielectric constant, T absolute temperature, the Boltzman constant, the atmosphere ionic radius and the factor of conversion of the common natural logarithms; for water at T 25 ºC, A is similar to 0.50. The ionic force I is defined by means of the following equation:

$$I = 1/2\sum Z_i^2 C_i \quad (2)$$

where Z is the ionic charge and C_i is the molar concentration of each one of the ions in solution. It should be observed that the ion activity coefficient by the Debye-Huckel Model is determined by its charge and total ionic force in solution and not in principle, by its own solution concentration [13].

If we fix the ionic force experimentally for a solution to a constant value, we can obtain the activity coefficients of the species using the two previous equations (1 and 2) and therefore, obtain the solubility products to this new condition; in Table 2 are seen the results in the value of Kps to different ionic forces, it can be appreciated that an increase of solubility due to the effect of a diverse ion or salt effect on the solution, which is due to the influence of the solution ionic forces in the activity coefficients of not very soluble ions.

To better appreciate the difference among calcium phosphates solubilities the figure of $-\log[PO_4^{3-}]$ ($p[PO_4^{3-}]$) will be plotted as a function of $-\log[Ca^{2+}]$ ($p[Ca^{2+}]$), to the pH value and a constant ionic force of 0.134 for each calcium phosphate. We used the ecuation of the solubility products shown in Table 2 and the respective equilibrium constants described in Table 3. In order to obtain the following ecuations:

For HAp we have:

$$p[PO_4^{3-}] = 12.924 + 1/3\ pH - 5/3p[Ca^{2+}] \quad (3)$$

For the case of the TCP we have:

$$p[PO_4^{3-}] = 12.724 - 3/2p[Ca^{2+}].. . \quad (4)$$

For the case of OCP we have:

$$p[PO_4^{3-}] = 15.6334 + 1/3pH - 4/3p[Ca^{2+}]. \quad (5)$$

For the case of DCPA we have:

$$p[PO_4^{3-}] = 18.342\text{-}pH - p[Ca^{2+}] \quad (6)$$

For the case of DCPD we have:

Table 1. Solubility constants at 25 ºC for HAp, TCP, OCP, DCPA and DCPD[16]

Hydroxyapatite (Hap); $Ca_5(PO_4)_3$ OH
$[Ca^{2+}]^5[PO_4^{3-}]^3$ $[OH^-]$ $y_2^5y_3^3y_1 = 4.7 \times 10^{-59}$
Tribasic calcium phosphate (TCP); $Ca_3(PO_4)_2$
$[Ca^{2+}]^3[PO_4^{3-}]^2$ $y_2^3y_3^2 = 1.2 \times 10^{-29}$
Octacalcium phosphate (OCP); $Ca_4H(PO_4)_3$ $2.5H_2O$
$[Ca^{2+}]^4[PO_4^{3-}]^3[H^+]$ $y_2^4y_3^3y_1 = 1.25 \times 10^{-47}$
Dibasic anhydrous calcium phosphate (DCPA); $CaHPO_4$
$[Ca^{2+}][HPO_4^{2-}]$ $y_2^2 = 1.26 \times 10^{-7}$
Dibasic dihydrated calcium phosphate (DCPD); $CaHPO_4$ $2H_2O$
$[Ca^{2+}][HPO_4^{2-}]$ $y_2^2 = 2.49 \times 10^{-7}$

Table 2. Solubility products at different ionic forces [16]

Compound	Kps	Kps I = 0.143	Kps I = 0.2
HAp	4.7×10^{-59}	1.69×10^{-53}	4.44×10^{-53}
TCP	1.2×10^{-29}	3.56×10^{-26}	6.51×10^{-26}
OCP	1.25×10^{-47}	1.55×10^{-42}	3.60×10^{-42}
DCPA	1.26×10^{-7}	1.06×10^{-6}	1.24×10^{-6}
DCPD	2.49×10^{-7}	2.10×10^{-6}	2.47×10^{-6}

Table 3. Constants of thermodynamic association to 25 ºC

$H^+ + H_2PO_4^- = H_3PO_4$	$K_a = 164.1$
$H^+ + HPO_4^{2-} = H_2PO_4^-$	$K_b = 1.58 \times 10^7$
$H^+ + PO_4^{3-} = HPO_4^{-2}$	$K_c = 2.33 \times 10^{12}$
$K^+ + HPO_4^{2-} = KHPO_4^-$	$K_d = 3.98$
$Ca^{2+} + H_2PO_4^- = CaH_2PO_4^+$	$K_1 = 31.9$ l
$Ca^{2+} + HPO_4^- = CaHPO_4$	$K_2 = 681$
$Ca^{2+} + PO_4^{3-} = CaPO_4^-$	$K_3 = 2.9 \times 10^6$
$Ca^{2+} + OH^- = CaOH^+$	$K_4 = 32.4$
$H^+ + OH^- = H_2O$	$K_w = 1 \times 10^{14}$

$$p[PO_4^{3-}] = 18.045\text{-}pH - p[Ca^{2+}]. \quad (7)$$

The information that we can obtain from this type of graph is the minimum concentrations that are required of PO_4^{3-} and of Ca^{2+} ions, under determined conditions to begin the precipitation of the different phosphates types; which is of utility to determine the best precipitation conditions of some specific phase.

To study the influence of pH on the equilibrium concentrations of the solid phases with species in solution, the series of Figs. 1-6, at an ionic force of 0.143 were obtained. There, we can observe the variation of equilibrium concentration as a function of the pH for the different calcium phosphates.

Figs. 1 and 2 show that at low pH values, the first phosphate precipitating is the DCPD until an approximate pH of 6. Under low concentrations of PO_4^{3-} and high concentration of Ca^{2+} ions, the first phosphate precipitating is HAp, later on DCPD. As the quantity of PO_4^{3-} ions increases and concentration of Ca^{2+} ions diminish the first DCPD is followed by the HAp precipitate.

At a pH of 7 (Fig. 3) we observe that with $p[Ca^{2+}]$ concentrations smaller than approximately 5.5, HAp is the first phase that precipitates. At a $p[Ca^{2+}]$ value similar to 6 there can precipitate as much HAp as DCPA and DCPD. At higher values, the first precipitates are DCPA and DCPD. If we have a $p[Ca^{2+}]$ similar to 3 we shall have

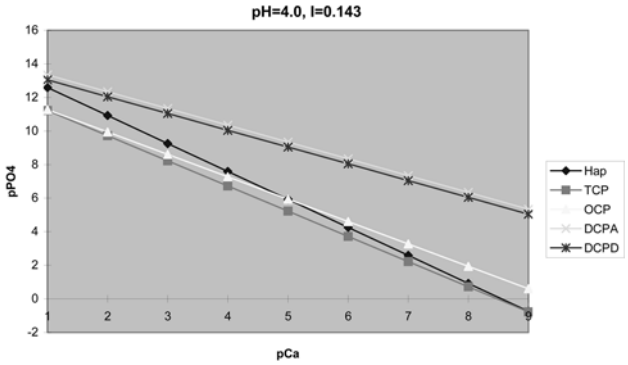

Fig. 1. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 4, I = 0.143.

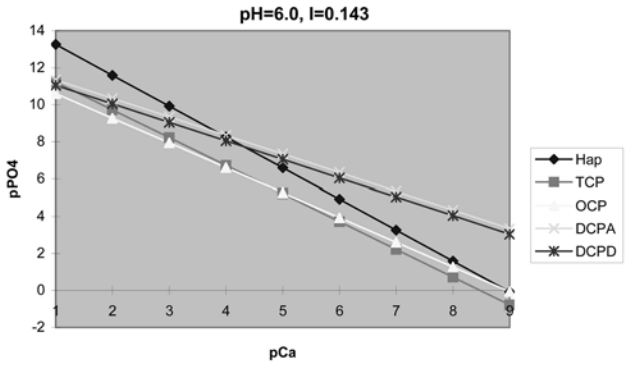

Fig. 2. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 6, I = 0.143.

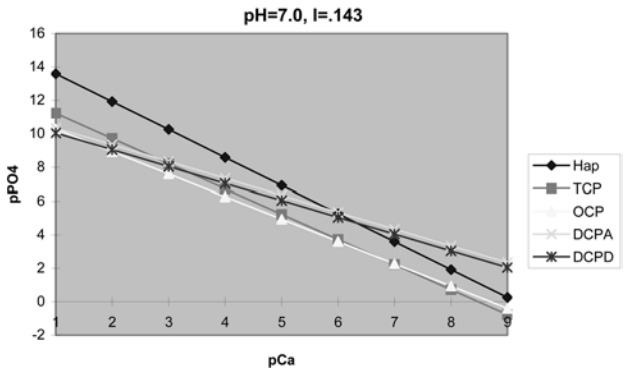

Fig. 3. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 7, I = 0.143.

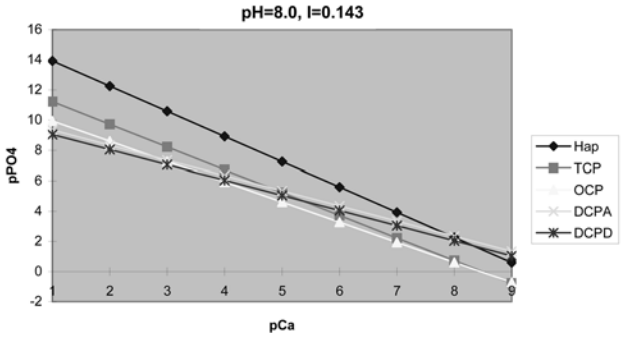

Fig. 4. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 8, I = 0.143.

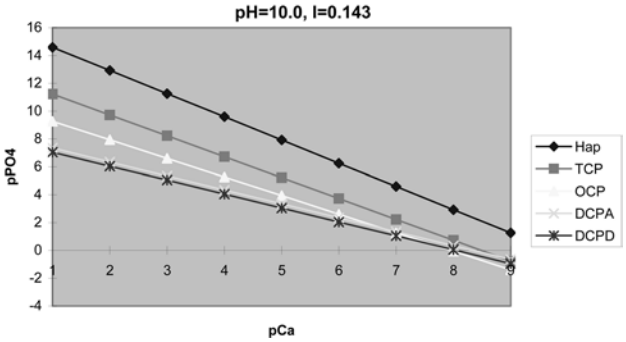

Fig. 5. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 10, I = 0.143.

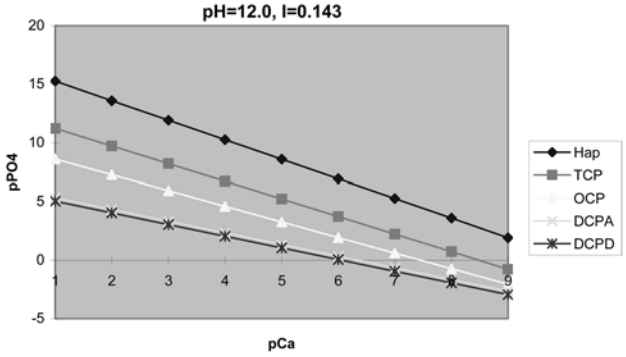

Fig. 6. Variation of $-\log[PO_4^{3-}]$ as a function of $-\log[Ca^{2+}]$ to pH = 12, I = 0.143.

to have in solution an approximate concentration of 10 p $[PO_4^{3-}]$, so that HAp precipitates and it should not be higher than 9 p$[PO_4^{3-}]$, so that TCP does not precipitate.

Starting from a pH similar to 8 (Fig. 4) we observe that the first precipitates being formed are the series HAp TCP, OCP and DCPA. At values higher than pH 8, the phosphate phase that precipitates first is the Hap (Figs. 5 and 6). It has been also observed that when the pH increases with a fixed concentration of calcium, a smaller quantity of phosphate is needed to begin the HAp precipitation. At high pH values it is possible to precipitate HAp, with high concentrations of PO_4^{3-} ions and get rid of Ca^{2+} ions.

It can be observed in Fig. 3 that at same reaction conditions, but at a pH of 6, we can obtain the coprecipitation of HAp and DCPA, as well as at a lower pH values the four phosphates precipitate. On the other hand, an increase in pH for example to 8 (Fig. 4) has been observed to give only HAp precipitates.

As can be observed in the graphs of Figs. 1-6, the variation of the pH is critical; since with small variations we will have the possibility to precipitate more than one calcium phosphate phase. It is also observed in these figures that there are concentrations at which it is feasible to precipitate two phases. For example, at pH = 7, I = 0.143, p[Ca2+] = 6 p[PO43-] = 5; where DCPA, DCPD and HAp can precipitate; or there are concentrations thermodynamically when it is feasible to precipitate the five phases for example: $p[Ca^{2+}] = 3$ and $p[PO_4^{3-}]^- = 4$, pH = 7, I = 0.143 (Fig. 3). At pH = 8, I = 0.143, $p[Ca^{2+}] = 8$ and $p[PO_4^{3-}] = 2.3$ is feasible to precipitate the phases HAp, DCPA and DCPD (Fig. 4).

Species in Solution

To calculate the ionic solution concentrations as a function of the pH we have to express the mass balance ecuations for the species present in solution:

$$[PO_4^{3-}]_T = PO_4^{3-} + HPO_4^{2-} + H_2PO_4^- + H_3PO_4 + KHPO_4 + CaPO_4^- + Ca\,HPO_4 + CaHPO_4^{++}. \quad (8)$$

$$[Ca^{2+}]_T = Ca^{2+} + CaOH^+ + CaPO_4^- + CaHPO_4 + CaH_2PO_4^+ \quad (9)$$

$$[K^+]_T = K^+ + KHPO_4^- \quad (10)$$

To reduce the number of variables (concentrations of the species in solution) we used complex equilibrium constants formation equations and the acidity depicted in Table 2 obtaining the following ecuations as a function of H^+, PO_4^{3-} and Ca^{2+} concentrations:

$$[PO_4^{3-}]_T = [PO_4^{3-}][1+[H^+][Kc] + [H^+]^2[Kc][Kb] + [H^+]^3[Kc][Kb][Ka]] + [Ca^{2+}][PO_4^{3-}][[K_3] + [H^+][K_2][Kc] + [H^+]^2[K_1][Kc][Kb]] + [K^+][PO_4^{3-}][H^+][Kd][Kc] \quad (11)$$

$$[Ca]_T = [Ca^{2+}][1+[K_4][K_w]/[H^+]] + [Ca^{2+}][PO_4^{3-}][[K_3] + [H^+][K_2][Kc] + [H^+]^2[K_1][Kc][Kb]] \quad (12)$$

$$[K]_T = [K^+] + [K^+][PO_4^{3-}][H^+][Kd][Kc] \quad (13)$$

This non-linear system was solved as a function of pH for the following fixed values of total concentration: total phosphate 0.0009 mol/l, total calcium 0.0015 mol/l and total potassium 0.0018 mol/l; the values of the constants are given in Table 3. To solve this system of non linear equations the computation program PV-WAVE was used with the ZEROSYS subroutine function; this system was used to solve the Powel algorithm hybrid system equations. This algorithm is a Newtonian method variation that takes precautions to avoid long stages.

The results obtained are shown in Figs. 7, 8 in wich the fraction of phosphate species was traced as a function of the pH (Fig. 7) as well as the fractional concentration of the calcium species as a function of the total calcium concentration (Fig. 8).

What can be observed is that the phosphates concentration in solution is very low even at a basic pH; this is due to the formation of $CaPO_4^-$ and $Ca(OH)^+$ ions in the solution. It is observed in Fig. 7 that at low pH values the acidic species: H_3PO_4, $H_2PO_4^-$ prevail and in a lower quantity smaller than 1% CaH_2PO_4; at pH between 7 and 9 where are several predominant species: HPO_4^{2-}, $CaHPO_4$, $H_2PO_4^-$, $CaHPO_4$ and $CaPO_4$; the fraction of the PO_4^{3-} species is so low, that it can not be observed, at high pH values the $CaPO_4^-$ species prevails, the $KHPO_4^-$ species is lower than 1% in the whole pH interval.

For the case of the calcium species as a function of the calcium total concentration, we observe that at acidic pH values the species that prevails is Ca^{2+} , up to pH 6, when also the species $CaHPO_4$ and $CaPO_4^-$ begin to prevail.

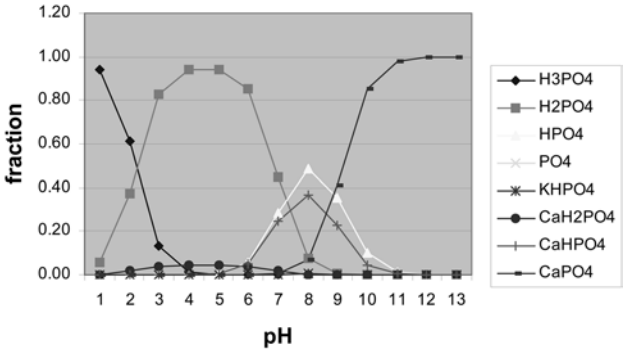

Fig. 7. Fraction of phosphate species as a function of pH.

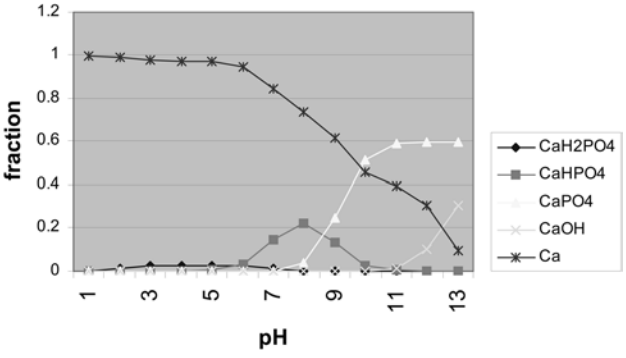

Fig. 8. Fraction of calcium species as a function of pH.

At pH of 8 an increase in the fraction of $CaPO_4^-$ begins and $CaHPO_4$ falls. Starting from pH 11, the value of $Ca(OH)^+$ is increased; it is necessary to mention that calcium phosphate species not only depends on the total calcium phosphate concentration. The concentrations of PO_4^{3-} and of Ca^{2+} ions at pH = 4 are respectively of 2.3×10^{-15} mol/l and 0.00146 mol/l; if one obtains the –log of both to be able to compare them with the concentrations in Fig. 1, they are $p[PO_4^{3-}]$ of 14.64 and of $p[Ca^{2+}]$ of 2.836.

From the plot in Fig. 1 we observe that this value is up to the solubility product equations of all phases; this means that it is not feasible to precipitate them under these conditions; this being the same case at pH of 6. At a pH of 7 (Fig. 3), the concentrations of $p[PO_4^{3-}] = 8.96$ and $p[Ca^{2+}] = 2.85$ are among the two solubility product equations below HAp and slightly superior to TCP; this indicates that it is possible to precipitate HAp only under these conditions.

At a pH of 8 the concentrations are $p[PO_4^{3-}]$ of 7.73, and $p[Ca^{2+}]$ of 2.96; these concentration values observed in the graph of Fig. 5 are below most of the equations, which means that most of the phosphate phases precipitate. At values higher than pH 8 will have an increase in the concentration of PO_4^{3-} ions and a decrease of Ca^{2+} ions, therefore more than one phase will precipitate. It is necessary to trace the $p[PO_4^{3-}]$ graphs as a function of $p[Ca^{2+}]$ to values close to 7 in order to obtain the interval of low pH, where it is feasible to precipitate only HAp under these total calcium concentrations and choosen phosphate values.

Conclusions

The species diagrams were obtained and the concentrations of a solution of calcium phosphate as a function of pH was obtained; as well as the concentrations of PO_4^{3-} and of Ca^{2+} ions in equilibria with the calcium phosphate solid phases as a function of the pH. To carry out the selective precipitation of HAp it is convenient to use a pH higher than six and low concentrations of PO_4^{3-} and Ca^{2+} ions. The concentration intervals when HAp precipitates are limited and it is necessary to have good control these, to avoid the precipitation of other phases. At a pH of seven it is possible to precipitate HAp with low concentrations of PO_4^{3-} and high of Ca^{2+}, ions for example 1×10^{-10} mol/l and 3.2×10^{-2} mol/l repectively.

By increasing the ionic force from the solution at a constant pH, an inrease of solubility is observed, the reason why it is suggested to maintain it constant is to avoid changes in the required concentrations of PO_4^{3-} and Ca^{2+} ions, in the solution of the phases before phosphate precipitation. It is observed that for a complex formation in the solution, the quantity of PO_4^{3-} ions is very low; but it is carried out even in this way since the phases of calcium phosphate which precipitate are very insoluble, which means that at low concentrations the solution reaches saturation.

The right pH to carry out the precipitation of HAp from a solution of total phosphate content of 0.0009 mol/l and total calcium content of 0.0015 mol/l, I = 0.143 is around pH 6 and 7; at pH = 6 there is no precipitation, and at a pH higher than 7.4 it is feasible that HAp and TCP precipitate. If we want to precipitate HAp at a higher pH it is necessary to diminish the concentration of PO_4^{3-} ions in solution.

In the case of the precipitation of HAp the presence of ionic and cationic species different to Ca^{2+} and PO_4^{3-} in the solution would modified the final composition of the HAp crystals obtained.

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