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Synthesis of nano-hydroxyapatite from silica suspensions through chemical compensation

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A novel method to produce pure nano-hydroxyapatite from silica suspension was devised. Its main advantage consists in allowing the control of the reaction rate of calcium and phosphate ions through the use of a chelate, namely EDTA. Further control of acidic conditions allows both the particle size and the shape to vary.

Keywords: hydroxyapatite; silica; chemical compensation

1. Introduction

The synthesis of calcium phosphates has attracted a great deal of attention in the last few decades, particularly hydroxyapatite (Hap), the main mineral component of human bones, its chemical formula being $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Its relevance as a material arises from the possibilities of using it in surgical implants and minimising the risk of rejection since it is a natural constituent of the human body. Additionally, Hap implants have the proven ability to stimulate the formation of natural bone [1] and to permit the invasion of both capillaries and muscular tissue into their porous structure [1,2].

Generally speaking, the reported methods for producing Hap [1–4] can be classified as follows:

- (a) Solution procedures [5,6]. Solutions of various sources for phosphate and calcium chemicals are employed and Hap crystallites are produced by precipitation. The method is very simple and involves inexpensive equipment. However, the control of crystallite size represents a difficulty and the presence of metastable phases in the final product of the reaction is perhaps the main disadvantage. In general, solution methods are among the most important methods for producing Hap as biomaterial at an industrial or semi-industrial scale.
- (b) Solid state methods [7–10]. These methods have the convenience of avoiding undesirable calcium phosphate phases and producing good quality Hap from very inexpensive raw materials. The main disadvantage in this case is the energy

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- consumption involved in the procedure. The possibility of producing composites by *in situ* reactions also shows an interesting potential in the area of biomaterials.
- (c) Hydrothermal systems [4,11,12]. These procedures have been employed for producing industrial ceramics and materials, other than phosphate-based biocompatible compounds. Poor control of the variables and the production of only relatively large particles are the main disadvantages. However, the current technology allows the precise control of the thermodynamical parameters involved, and recently the possibility of producing nanometre-sized calcium phosphates has been reported. This field is expected to attract more attention in the near future.
 - (d) Low temperature methods [13–15]. Low temperature methods for the production of calcium phosphates (including Hap) particulates at near human body temperature, in a simulated body fluid on top of an inorganic (silica) gel have been reported. This route opens exciting possibilities for discovering how ‘nature’ itself manages to produce ceramic materials at nearly room temperature.
 - (e) The sol–gel method. This is a technique which has received little attention. Reports using calcium salts and phosphoric acid esters have been published recently. However, Hap is obtained only at temperatures above 500°C [16,17]. This stresses the need for more work in this area.

Other methods used to obtain Hap coatings include electrochemical deposition and plasma sprayed [18–25].

A common disadvantage of the aforementioned methods is the difficulty of obtaining Hap particles homogeneous in size, since both the structure and the composition of Hap are very dependent on the reaction conditions. Thus, low crystallinity is typical and the presence of other calcium phosphate phases is common. Besides, most of these methods require either high temperatures or long reaction times [23–27].

The technique described in this work allows the controlled precipitation of pure Hap from a starting homogeneous solution of Ca^{+2} and PO_4^{-3} ions at low temperature and low pressure conditions, and in short periods of time. It also allows the formation of nanometre-sized composites of Hap and silica.

2. Experimental

2.1. Synthesis route

2.1.1. Preparation of complexed solutions

Solutions were prepared with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Riedel-de Haën, analytical grade), $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (Aldrich Chemical Co., analytical grade) and K_2HPO_4 (Riedel-de Haën, analytical grade). One litre of the solution was prepared in order to obtain CaCl_2 0.25 M, Na_2EDTA 0.25 M and K_2HPO_4 0.15 M at different pH values. About 200 mL of each solution were kept in a pressure reactor (Parr, model 4841) at 160°C and 100 PSI during 4 h. Samples with pH 6 and 9 are discussed.

2.1.2. Suspensions preparation

The starting solution for the sol–gel route is prepared as follows: 3.5 mL of tetraethyl orthosilicate (TEOS) (Aldrich Chemical Co., analytical grade), 1 mL of distilled water,

5 mL ethanol (Baker Analytical grade) and 0.5 mL of hydrofluoric acid (Baker, 40% wt.). After 2 min the sol was formed. This sol was put into 100 mL of the solution described in Section 2.1.1 and refluxed under stirring for 4 h. The white precipitate obtained was filtered and dried for 3 h at 110°C.

2.2. Characterisation techniques

Morphology and constituent elements of the samples were determined through a Low Vacuum Scanning Electron Microscope, JEOL-5900LV (LV-SEM), equipped with an energy dispersive X-ray analysis probe, Oxford (EDS). Samples were fixed to the aluminium holder via adhesive carbon foil.

X-ray diffraction analysis was performed with a powder diffractometer Siemens D5000, at 30 keV, 25 μ A, scanning from 5° to 70° with 0.1° steps. Samples were previously ground until a very fine powder was obtained.

3. Results and discussion

Under standard conditions, calcium phosphate aqueous solutions are at the verge of supersaturation, due to their extreme insolubility. Thus, in very short time, precipitates such as Hap, tricalcium phosphate (TCP), octacalcium phosphate (OCP), dicalcium phosphate anhydrous (DCPA) and dicalcium phosphate dihydrate (DCPD) are produced. This is due to the poor control of the reactivity conditions of Ca ions during the reactions involved in all the synthesis methods described above. In fact, this is reflected in the corresponding phase diagrams, which show a rather narrow region, particularly in terms of pH, in which Hap can be produced without the presence of other phosphate phases. Thus, a way to control chemically the availability of reacting Ca ions during the changing conditions, in terms of pH and supersaturation, is necessary if pure Hap with specific crystallite sizes is to be produced.

Accordingly, a reservoir of calcium ion could be made available in the aqueous medium, as a Ca^{2+} /EDTA soluble complex. As a matter of fact, with EDTA one is able to control the equilibrium of the reaction, the precipitation rate and even the size of the particles obtained, in addition to obtaining the desired phase.

By mixing Ca^{2+} /EDTA/ PO_4^{3-} in the proportion 5:5:3 one can obtain the Hap phase alone, because it is thermodynamically more stable. In order to precipitate it, the equilibrium of the reaction can be shifted conveniently by heating to temperatures $\geq 130^\circ\text{C}$. Varying the pH from acidic to basic solutions produces changes in particle size and the corresponding equilibrium shapes. Figure 1 shows a SEM micrograph of the particles obtained by this procedure at pH = 6; one can appreciate the very small needle-like structures, which grow radically from a nucleation centre. The size of these needles is about 1 μm long and less than 0.1 μm in thickness. Figure 2 corresponds to a XRD pattern taken from this sample. It shows very clearly the presence and high degree of crystallinity of the Hap produced.

Another sample prepared by the same procedure but at pH = 9 is shown in Figure 3; needles are also formed but their size is of 10–15 μm in length and some up to 1 μm in thickness, which are definitively larger than those of pH = 6. Figure 4 corresponds

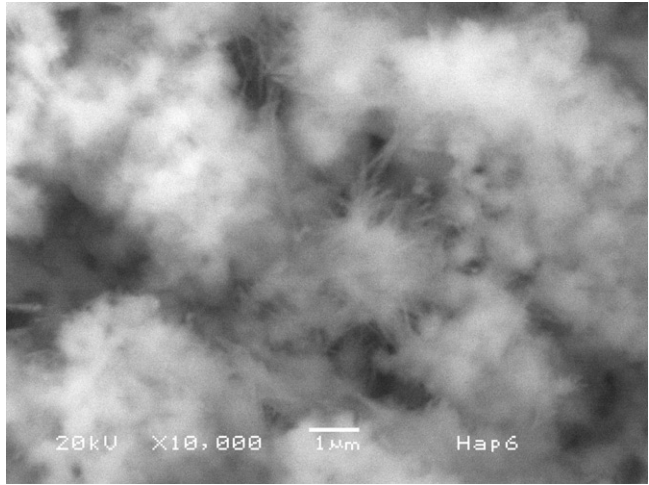


Figure 1. SEM micrograph of sample prepared as described in the text at pH = 6. Hap needles about 1 µm long and 0.1 µm thick form bundles from a common origin. These bundles join one another and fill the space (marker = 1 µm).

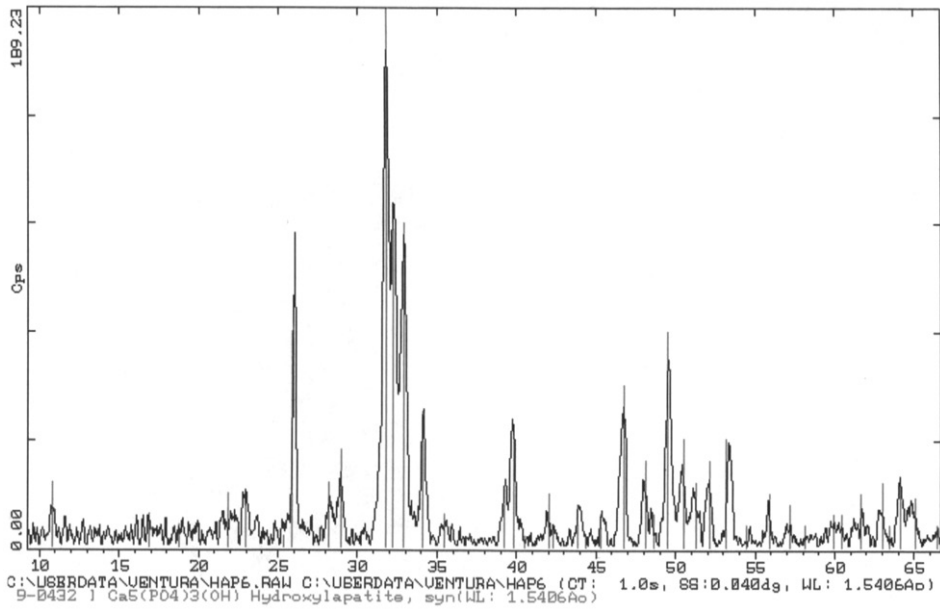


Figure 2. XRD from sample in Figure 1 shows the presence of Hap with very high crystallinity. Peaks for other calcium phosphates are not present.

to a X-ray diffractogram of the sample shown in Figure 3; again one can appreciate the high degree of crystallinity obtained and the purity of the phase.

In general the higher the pH, the bigger the particles formed but the size distribution would also be broader. This effect is under further study and the details of the growth

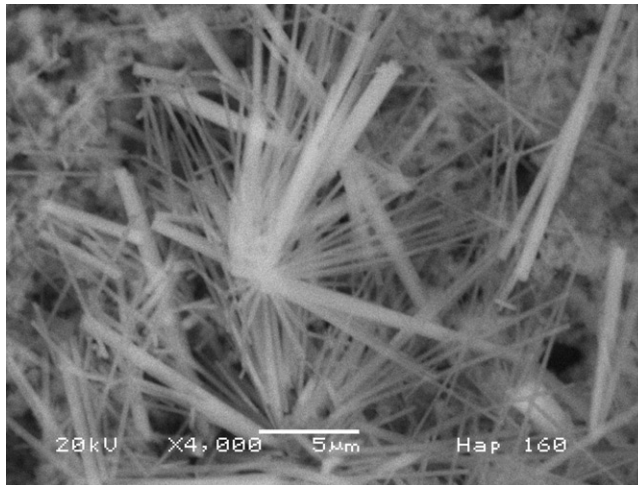


Figure 3. SEM micrograph of sample prepared at pH=9. Hap needles 12 μm long are observed, some as thick as 1 μm are present. Some form bundles from a common origin.

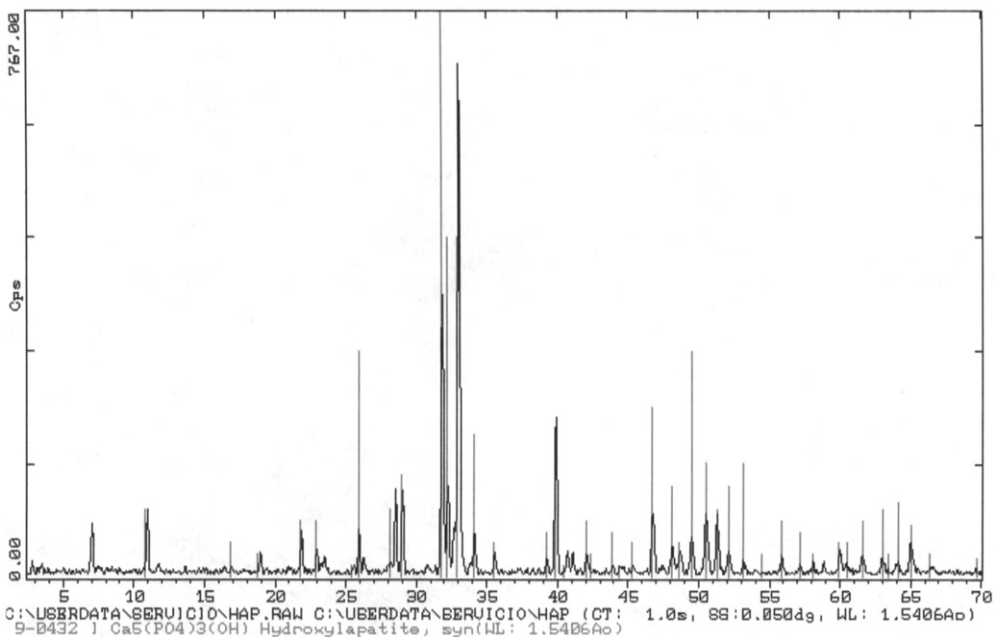


Figure 4. XRD of sample of Figure 3 shows the presence of Hap with very high crystallinity. No peaks for other calcium phosphates are present.

kinetics and its relationship to the controlled pH conditions, through a modified Avrami approach, are currently under way and will be reported separately.

Based on the results described in Section 2, and the reported growth of Hap on silica blocks produced by the sol-gel route [14,15], a variation was devised as explained

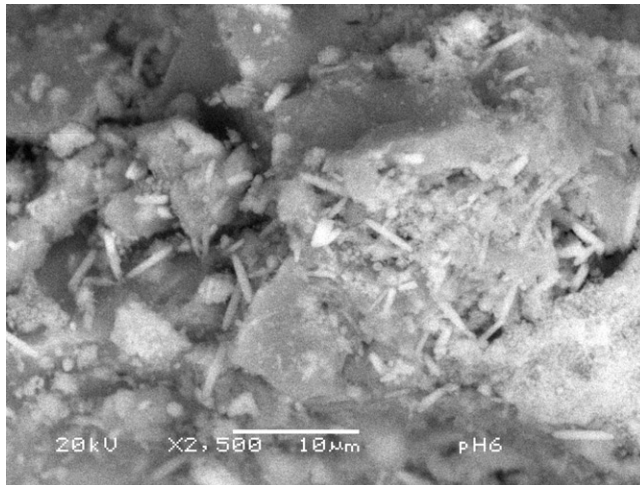


Figure 5. Backscattered electrons micrograph from sample prepared at pH=6 shows elongated crystallites about 5µm long and 1µm thick embedded in a SiO₂ matrix. Hap crystallites appear isolated, not in bundles.

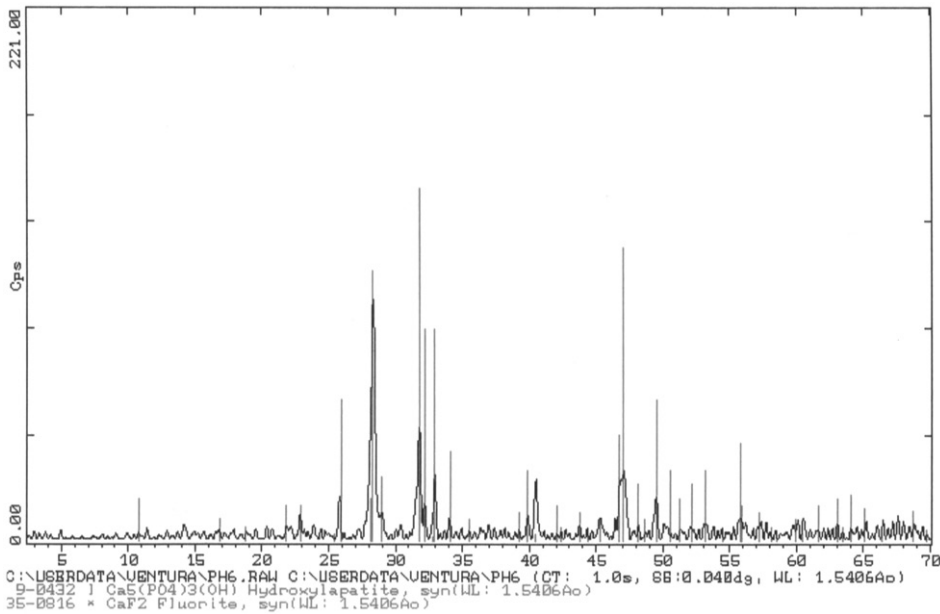


Figure 6. XRD from sample in Figure 5. It shows that the needles are crystalline Hap, although a couple of extra peaks are present. These could be due to CaF₂ or KCl₂.

in Section 2. The standard sol-gel procedure was followed as usual, but while the materials are still in the sol state, a Hap solution prepared as explained was added under reflux. A composite is then obtained, consisting in amorphous silica particles with particles of crystalline Hap. The method allows for the variation of the pH. Figure 5 shows

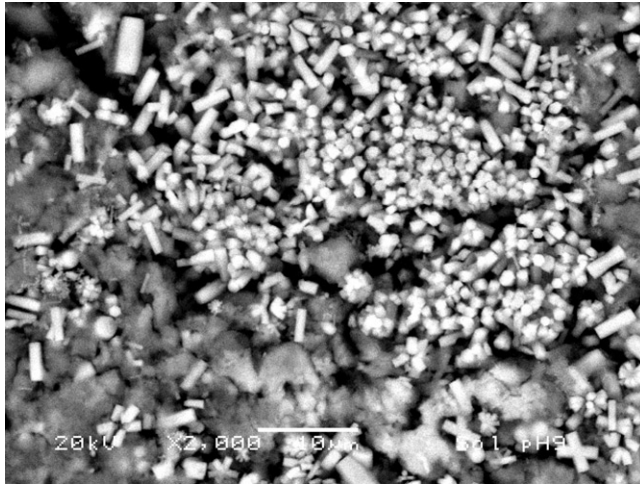


Figure 7. SEM micrograph of sample prepared as in Section 2.2.2 at pH=9. Hap bars are observed as growing on top of the SiO₂ particles. Bars are about 8 μm × 1 μm and SiO₂ particles show dimensions in the order of 5 μm. The hexagonal cross section is clearly observed.

a backscattered SEM picture of sample prepared at pH=6. The SiO₂ particles are present and Hap elongated crystallites are embedded in the SiO₂ matrix. The crystallites are about 5 μm long and 1 μm thick, much larger than the sample in Figure 1, which also corresponds to pH=6. Figure 6 is a X-ray diffractogram of the sample in Figure 5. The presence of Hap and its high degree of crystallinity are very clear. However, the peaks at 28° and 47° could be explained by the presence of KCl or CaF₂. In this diffractogram, the broad reflection corresponding to SiO₂ was suppressed with the background.

Figure 7 shows a SEM micrograph of the sample prepared with the procedure similar to the sample in Figure 5, but at pH=9. Bars are about 8 μm × 1 μm and SiO₂ particles show dimensions which vary from 1 to 5 μm. One can also observe that some crystallites form groups and interesting twins are observed. Again, the higher the pH, the bigger the particles formed with this process as with the former one. The hexagonal cross section of the crystallites evidences the equilibrium shape, which reflects, in turn, the thermodynamical conditions of the reaction. Figure 8 shows an X-ray diffractogram of the sample in Figure 7. Hap is present in a very crystalline phase, but spurious peaks at 28° and 47° appear again. The broad reflexion corresponding to SiO₂ was suppressed.

It is thought that the *in situ* production of silica promotes the formation of Hap at lower temperatures and with a much larger crystallites size. It seems that the presence of the SiO₂ has a nucleation effect, which can be controlled through the chemistry of the silica gel.

As for the mechanical properties of these materials, the resulting powder is very stable and homogeneous and it should be sintered to produce bodies of this nanocomposite [28]. However, since Hap itself is a rather weak material, the presence of a silica glass component will quite likely add mechanical strength to the final composite, in particular for powder coating technologies, as it has been reported elsewhere [29].

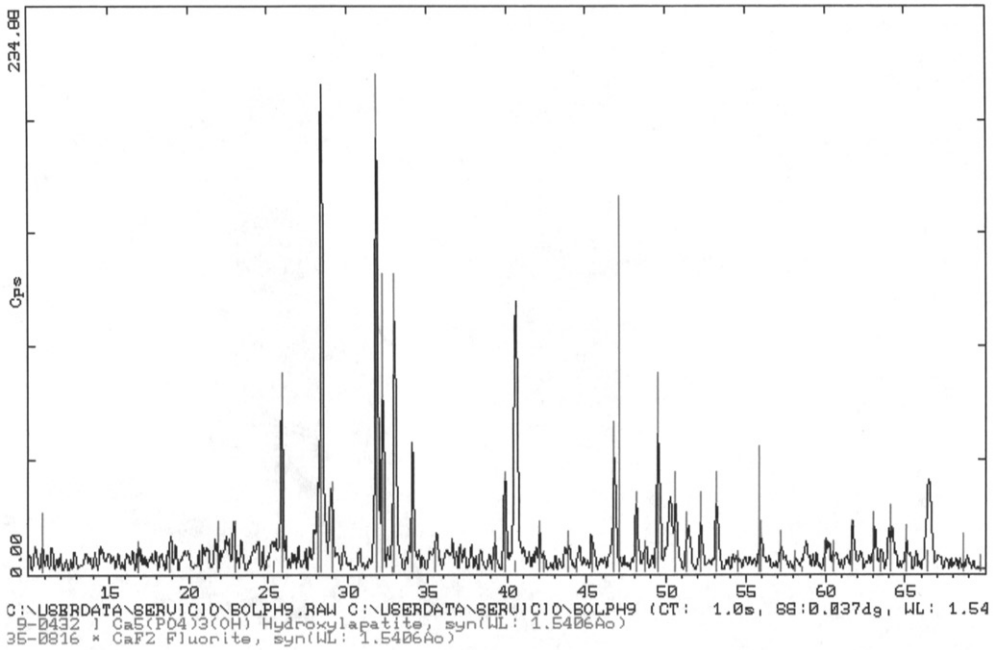


Figure 8. XRD from sample in Figure 7. It shows that the bars correspond to crystalline Hap, although other peaks are present. These could be due to CaF_2 or KCl_2 .

4. Concluding remarks

The use of a convenient complexing agent allows us to control properly the reaction conditions of extremely reactive ions, such as those involved in the synthesis of Hap, which represents a particularly cumbersome system, since the corresponding phase diagram shows a very narrow zone where Hap can be produced free of other phosphate compounds. This zone is highly pH-dependent, and pH itself changes drastically as the reaction proceeds. In addition to the practical application of this methodology to the present case, the use of multifunctional complexing agents opens interesting possibilities for synthesising metastable inorganic compounds.

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