Hydroxyapatite, tricalcium phosphate and biphasic materials prepared by a liquid mix technique

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Abstract

The Pechini based liquid-mix technique has been applied to prepare either single phases of hydroxyapatite –$Ca_{10}(PO_4)_6OH_2$– (OHAp), α and β-tricalcium phosphate –$Ca_3(PO_4)_2$–, (α-TCP, β-TCP) or biphasic calcium phosphates (BCP). Compositions with a Ca/P molar ratio between 1.5 and 1.667 were synthesized and subjected to a thermal treatment up to 1400 °C. α and β-TCP were both prepared from a Ca/P ratio of 1.5, but while β-TCP is isolated at 900 °C and remains stable up to 1100 °C, it is necessary to anneal at 1400 °C for 72 h to obtain pure α-TCP. OHAp is obtained as a single phase from a 1.667 Ca/P ratio after annealing at 1000 °C for 24 h and starts to decompose at 1400 °C. Between these two extremes a whole range of biphasic calcium phosphates can be prepared by using this technique with an accurate control of the starting reactants. These materials have been characterized by FTIR, XRF, BET, XRD and, based on this technique, a phase quantification determination (QXRD). The solubility of these products was tested in a buffered solution at 37 °C and pH = 7.4.
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Keywords: Apatite; Biomedical applications; Powders-chemical preparation; X-ray methods

1. Introduction

There is an increasing attention being given to calcium phosphates, particularly apatites, an interest which seems to be driven mainly by the requirements for the development, understanding and manufacture of biomaterials. Several techniques have been utilized for the preparation of hydroxyapatite and other calcium phosphates. The synthetic routes employed can be divided into solid state reactions and wet methods, which includes precipitation, hydrothermal processing and hydrolysis of other calcium phosphates.[1–3]

Recently, there has been a growing interest in the preparation of mixtures of two or more calcium phosphates. These materials are commonly composed of hydroxyapatite and a more resorbable material like tricalcium phosphate (α or β) or calcium carbonate in different proportions depending on the characteristics required for the specific application. The synthetic routes employed commonly in the preparation of these mixtures include the blending of different calcium phosphates,[4] and precipitation.[5] Other techniques also employed are: solid state reaction,[6] treatment of natural bone,[7] spray pyrolysis [8] microwave [9] and combustion [10] processing, etc. Some authors have defended the superior properties of biphasic materials ''directly'' prepared over those obtained by mixing two single phases.[11]

Some of these synthetic routes, newly applied to prepare hydroxyapatite, β-tricalcium phosphate or biphasic materials, present considerable drawbacks such as expensive starting materials (e.g. metal alkoxides for sol-gel applications), expendable processing supplies (e.g. cryogenic agents for freeze-drying methods) and complicated procedures, which, although on a laboratory scale remain of great interest, hinder its application on a commercial level. Consequently, it is a matter of technical interest to develop a powder preparation technique that can avoid the above drawbacks.

In this work we propose the application of the Liquid Mix Technique, which is based on the Pechini patent.[12] This patent was originally developed for the preparation of multicomponent oxides, allowing the production of

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massive and reproducible quantities with a precise homogeneity in both composition and particle size. This method is based on the preparation of a liquid solution whose homogeneity is retained in the solid state. In such a way, not only is a precise control of the cation concentration possible but also the diffusion process is enormously favored by means of the liquid solution compared to other classical methods. The solution of metallic salts in polycarboxylic acid such as citrates is solidified by the addition of a diol which increases the solution viscosity due to the formation of ester-type three dimensional polymers. When the diol reacts with the citric solution a resin is formed, thus avoiding the partial segregation, which would modify the original homogeneity of the solution.[13]

In this work we will try to extend its application to the preparation of calcium phosphates. The main difficulty of this synthesis lies on the presence of PO_4^{3-} groups that cannot be complexed by citric acid, and may cause its segregation and the formation of separated phosphate phases. The success of this task would suggest the possibility, by modifying the synthesis conditions, of obtaining large amounts of single phases or biphasic mixtures with precise proportions of the calcium phosphates. This would yield enough material to prepare ceramic pieces for eventual utilization as biomaterials. Until now, autografts or allografts were the first choice when facing the problem of repairing damaged bones. However, due to the increasingly restrictive regulations and to the growing fear of infections such as AIDS or C-J disease, this approach has been called into question. This has caused an increase in the demand for artificial bioceramics as well as in the expectation for new products.

2. Materials and methods

2.1. Sample preparation

The Liquid Mix Technique process employed here to obtain phosphates has previously been described in connection with to the preparation of multicomponent oxides.[13] Briefly (depicted in Fig. 1), the metallic salts ($CaNO_3 \cdot 4H_2O$ and $H_2NH_4PO_4$) were dissolved in a 0.2 M citric acid (CA) solution in water previously heated on a stirring plate. The quantities employed to prepare the different compositions are detailed in Table 1. After

thorough mixing of the solution, the volume was reduced by slow heating on a hot plate with continuous stirring, at this moment ethylene glycol (EG) (at a 1:1 molar ratio with CA) is added to the solution. All reagents were Aldrich products (puriss.). The resulting resin was charred on the same stirring plate, ground and subsequently treated at 500 °C for 24 h in order to eliminate the organic components. Finally, the samples

NOMEN-CLATURE	1.50	1.52	1.54	1.56	1.58	1.60	1.62	1.64	1.66
Ca/P ratio [mol]	1.5000	1.5208	1.5416	1.5625	1.5833	1.6042	1.6250	1.6458	1.6667

Fig. 1. Flow chart of the Liquid Mix Technique and nomenclature and Ca/P molar ratio of the samples prepared.

Table 1
Quantities employed to prepare the samples

Ca/P ratio [mol]	1.5000	1.5208	1.5416	1.5625	1.5833	1.6042	1.6250	1.6458	1.6667
$Ca(NO_3)_2 \cdot 4H_2O$ [g]	23.615	23.615	23.615	23.615	23.615	23.615	23.615	23.615	23.615
$H_2NH_4PO_4$ [ml]	7.668	7.564	7.463	7.362	7.265	7.170	7.078	6.989	6.901
Citric acid (CA) [g]	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
Ethylene glycol (EG) [ml]	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0

were annealed accumulatively from 700 to 1400 °C for 24 h. The different compositions prepared and their denominations are collected in Fig. 1.

2.2. Powder characterization

N_2 adsorption was carried out on a Micromeritics ASAP 2000 instrument; the surface area was obtained by applying the BET method to the isotherm and the pore size distribution was determined by the BJH method from the desorption branch of the isotherm. Fourier transform infrared (FTIR) spectra were obtained in a Nicolet Nexus spectrometer over the 400–4000 cm^{-1} region using samples in the form of pellets formed with spectroscopic grade KBr. The chemical composition of these materials was determined on powder-pressed pellets by X-ray fluorescence on a S4 EXPLORER, Bruker AXS equipped with a Rh X-ray tube (1000 W, 50 mA). Firstly, a calibration curve was prepared by using seven calcium phosphate samples with different Ca/P ratio obtained by mixing the β-TCP and OHAp prepared by this method. In X-ray fluorescence analysis of homogeneous specimens, the correlation between the characteristic line intensity of an element and the concentration of that element is typically non linear over wide ranges of concentration, due to inter-element effects between the element and other elements in the specimen matrix. In order to compute the matrix term, the software employed uses the variable alphas method (alpha-coefficients) which depend on the concentrations and therefore is specific to each sample.

The crystallinity of the materials was analyzed by X-ray diffraction (XRD) in a Philips X-Pert MPD diffractometer (CuK_a radiation, 45 kV, 40 mA). The X'pert Plus software (Philips) was employed to determine the quantity of β-TCP and OHAp phases. These determinations were effectuated in measurements with a step size of 0.03 degrees and a time per step of 4.00 s.. Although this program or similar Rietveld based software have been previously utilized to quantify crystalline phases, in this work this method was tested on mixtures with known amounts of these phases prepared from the β-TCP and OHAp single phases obtained. The resulting curve could be linear fitted and a linear regression value of 0.99956 was obtained.

2.3. Degradation experiments

Degradation experiments of these materials in a $(NH_4)_2SO_4/NH_3$ buffer at 37 °C and a pH = 7.4 were carried out under constant stirring for 10 days. Calcium ion concentration in the buffer solution and the pH were measured by electrode ion selective measurement using an Ilyte Na$^+$, K$^+$, Ca^{2+}, and pH system. An X-Ray and IR characterization was effectuated on the resulting powder in order to study the possible transformations of these calcium phosphates after being immersed.

3. Results

Nine different samples have been prepared by modifying the Ca/P ratio as reflected in Fig. 1. Over the whole range of compositions, the following situations must be outlined: Firstly, when the Ca/P ratio is 1.667 (sample 1.66) a OHAp single phase is obtained after annealing at 1000 °C for 24 h. On the other extreme, a 1.5 Ca/P relation (sample 1.50) may lead to two different single phases depending on the thermal treatment, i.e. β-TCP can be found after annealing between 900 and 1100 °C, while α-TCP can be isolated after annealing at 1400 °C for 72 h. Intermediate ratios yield, in all cases. to biphasic mixtures with defined proportions of each of the calcium phosphates, that have been quantified, as detailed below, by means of a Rietveld based software. For the sake of clarity, only the results of the 1.58 sample will be fully represented.

Sample 1.66, which has the Ca/P ratio of a stoichiometric hydroxyapatite, shows an evolution, when characterized by X-ray or IR spectroscopy (Fig. 2), from a biphasic mixture of β-tricalcium phosphate-hydroxyapatite to a OHAp single phase (hydroxyapatite–Powder Diffraction File 9-432 JCPDS 2000). This transformation is completed after annealing at 1000 °C for 24 h, and the thermal stability of the products extends up to 1300 °C. At 1400 °C the first signs of a new phase (α-TCP) can be detected by the appearance of its highest intensity maximum (denoted as α in Fig. 2) (Powder Diffraction File 9-348 JCPDS 2000). Longer annealing times at this temperature (1400 °C) increase the quantity of α-TCP in the biphasic α-TCP-OHAp mixture. The FTIR spectra of the samples annealed up to 1000 °C show the characteristic bands of OHAp.[14] Annealing at 1400 °C shows a progressive broadening of the above-mentioned bands that makes it difficult to distinguish clearly the OHAp bands from the emerging α-TCP ones (Table 2).[14,15] The presence of carbonate in the samples treated below 1000 °C can be deduced from the appearance in their spectra of a broad band in the 1400 cm^{-1} region, corresponding to the ν_3 vibrational mode, and to a sharp band at 876 cm^{-1} attributable to the ν_2 vibrational mode.

At the other extreme of the compositions prepared, the 1.50 composition annealed at 900 °C turns into a β-TCP single phase (Powder Diffraction File 9-169 JCPDS 2000) and remains thermally stable up to 1100 °C (Fig. 3). This figure also shows the gradual transformation of the β-TCP into a α-TCP single phase with increasing annealing temperature and time of treatment. This transformation is not fully completed until an annealing at 1400 °C for 72 h is performed. The

FTIR spectra of Fig. 3 show both the characteristic bands ([14,15] Table 2) of β-TCP-bottom- and α-TCP-top-.

The thermal treatment applied to the samples prepared with compositional ratios leads to reaction between the constituting calcium phosphates, causing a modification in their proportions. A good example of this is sample 1.58 (Fig. 4), which, after being annealed at 900 °C for 24 h, yields a biphasic material (OHAp-β-TCP). Further treatment at 1000 °C provokes a slight increase in its crystallinity and in the OHAp/β-TCP ratio. This tendency is maintained after annealing at 1100 °C, but at 1200 °C the transformation of β-TCP into α-TCP is initiated, as described above, as can be deduced by the appearance of the most intense XRD maxima of α-TCP and by a modification of the definition of the β-TCP IR characteristic bands. A thermal treatment at 1400 °C for 72 h leads to a complete transformation of the β-TCP into α-TCP, as can be deduced from the corresponding X-ray pattern, which also shows OHAp maxima, but no evidence of other phases.

Once have been considered all the possible situations that have arisen depending on the Ca/P ratio or the
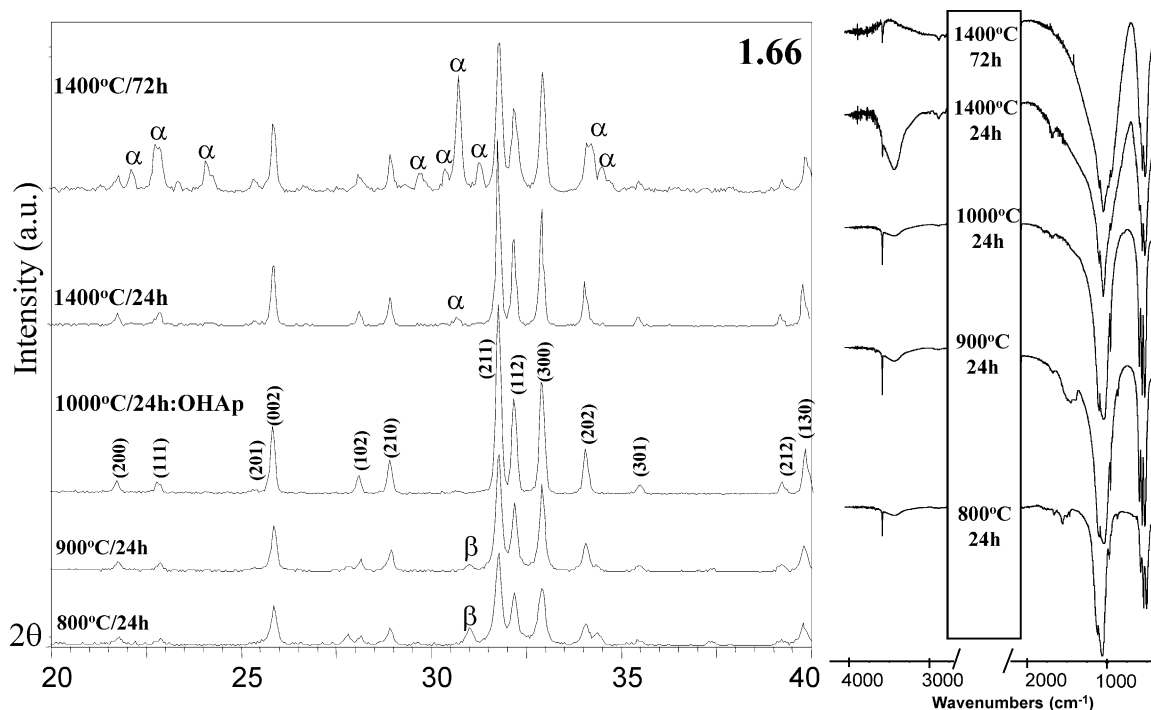
Fig. 2. X-ray diffraction patterns (left) and IR spectra (right) of sample 1.66 annealed at different temperatures. The OHAp phase is indexed in the (1000 °C/24 h) pattern. α and β corresponds to β-TCP and α-TCP maxima, respectively. The non-labeled patterns correspond to OHAp.

Table 2
Charateristics IR frequencies of OHAp (14), βTCP and α-TCP (15)

	OH⁻		PO₄³⁻					
	strech.	lib.	ν_3		ν_1		ν_4	
OHAp	3572	630	1087	1040	962		601	571
1.66	•	•	•	•	•		•	•
1.64	•	•	•	•	β • β		•	• β
1.58	•	•	•	•	β • β		•	• β
1.54	β •	β•	β	β	β β		β •	• β
1.50	β	β	β	β	β β		β β	β
β-TCP	1120	1042	1025		972	945	606 594	552
α-TCP		1055 1025	984		954		613, 597, 585, 563, 551	

● and β corresponds to the bands of OHAp and β-TCP, respectively in samples annealed at 1000 °C
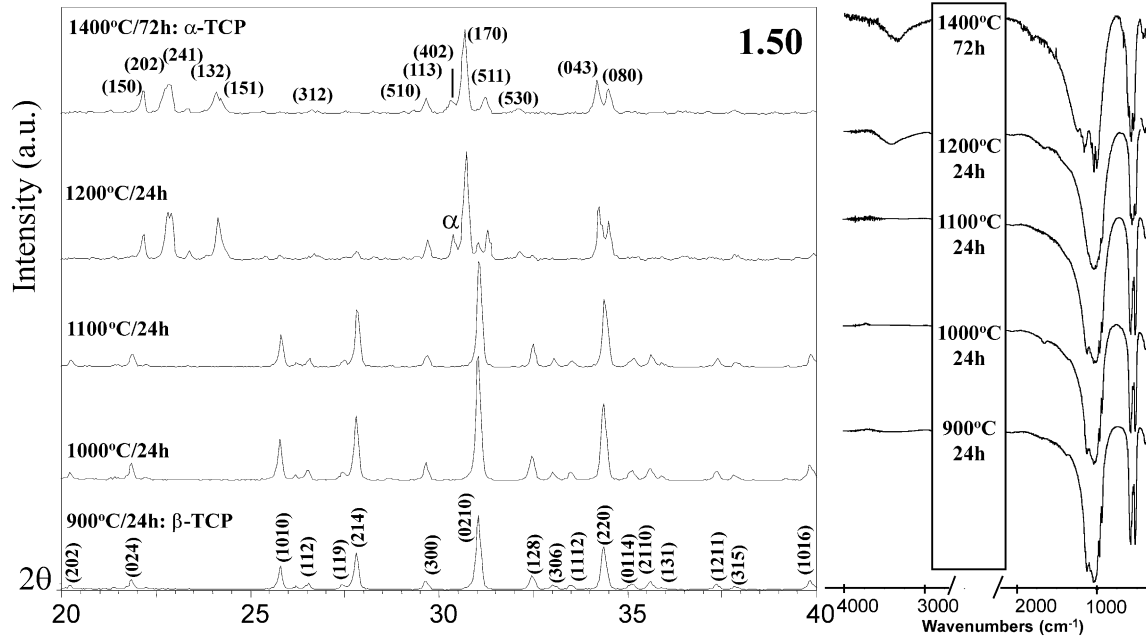
Fig. 3. X-ray diffraction patterns (left) and IR spectra (right) of sample 1.50 annealed at different temperatures. In the lowest pattern the β-TCP phase is indexed, while in the uppest one the hkl index correspond to α-TCP. α corresponds to α-TCP maxima, while the rest of the non-labeled maxima to β-TCP.
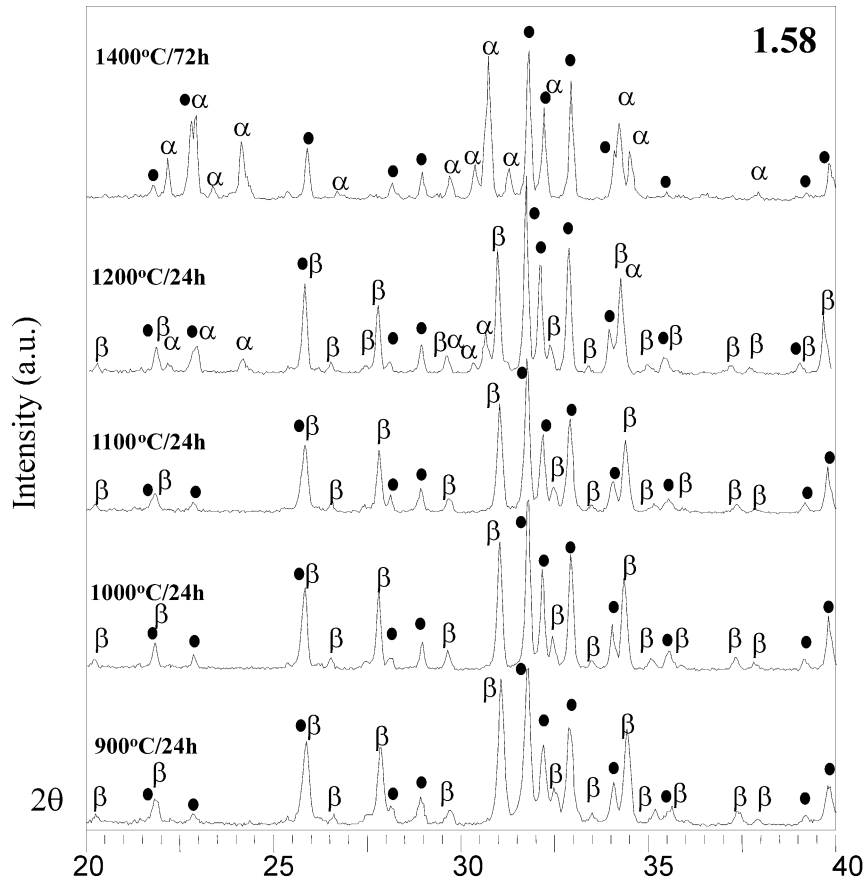
Fig. 4. X-ray diffraction patterns of sample 1.58 annealed at different temperatures. The maxima of α-TCP, β-TCP and OHAp are represented by α,β and ●, respectively.

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thermal treatment, it is of vital importance to determine the proportions of each of the calcium phosphates that compose the biphasic materials. For the clarity of the exposition of these results, we have focused on the samples annealed at 1000 °C for 24 h. The election of this temperature is due to the fact that it is the lowest temperature at which the hydroxyapatite phase can be found as a single phase, as already demonstrated.

Fig. 5 bring together the IR spectra and X-ray diffraction patterns of some of the compositions prepared and annealed at 1000 °C. Table 1 reflects the evolution of the characteristic IR bands of these samples and includes the theoretical bands of OHAp, β-TCP and α-TCP. Both techniques demonstrate a gradual variation in the β-TCP/OHAp ratio starting from β-TCP as a single phase and ending with a OHAp one. This progressive transformation was quantified by X-ray diffractometry and expressed in Fig. 6. As previously mentioned, these products were also characterized by X-ray fluorescence: Fig. 7 shows a comparison, expressed as the determined vs. the expected Ca/P molar ratio, between the results obtained by this technique and those deduced from the X-ray quantification. This X-ray quantitative method is based on the analysis of the full profile of the XRD patterns by the Rietveld method.[16] The structural parameters of OHAp [17] and β-TCP [18] found in the literature were used as starting parameters for the refinement. The agreement factors of the biphasic samples prepared are collected in the inset of Fig. 6 and an example of a full profile fit by the Rietveld program is shown in Fig. 8.

The degradation test was performed on this same samples, i.e. the ones annealed at 1000 °C. The BET surface area characterization shows very similar values that progressively decrease with the Ca/P ratio (from 1.66 = 1.55 m^2/g to 1.50 = 0.67 m^2/g). For this reason the powder to buffer solution ratio was based only on a weight to volume ratio (50 mg/ml). As shown in Fig. 9, the quantity of calcium in the solution increases with the proportion of β-TCP in the biphasic materials. Indeed, a considerable gap between sample 1.66, composed only of OHAp, and that with the lowest quantity of β-TCP, 1.64, is observed considering the successive differences among the compositions tested. The variation of the solution pH can be considered as negligible. A blank tube, containing the buffer solution, show calcium values within the apparatus error. The characterization by XRD and FTIR of the samples after being immersed in the buffer solution show no significant differences compared to the results obtained before soaking the samples.

4. Discussion

This synthetic method makes it possible to easily prepare not only single phase hydroxyapatite, β-TCP and even α-TCP, when the latter is calcined at 1400 °C, but also biphasic calcium phosphates over a wide range of compositions as has been schematized in Fig. 10. This figure represents the weight content of OHAp (z axis) for different initial Ca/P (x axis) annealed between 800
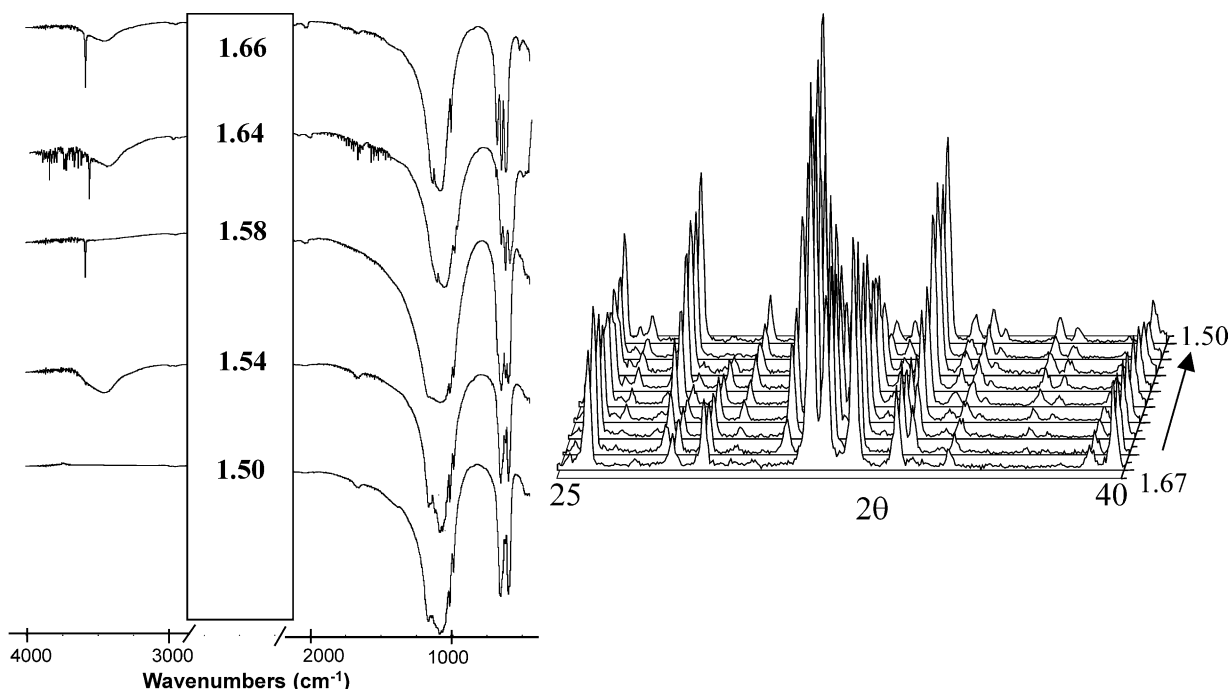

Fig. 5. IR spectra (left) and X-ray diffraction (right) patterns of samples annealed at 1000 °C for 24 h.

and 1100 °C (*y* axis). Focusing on this figure we discuss the following points:

1. The formation and stability domain of single phase OHAp within the annealing temperature range considered.
2. A study of a given intermediate composition, e.g. 1.58 at different annealing temperatures.
3. Quantification by XRD of the biphasic materials annealed at 1000 °C and comparison with the data obtained by XRF.

In addition, although not shown in this figure, the formation and thermal stability of β-TCP and the decomposition of all the samples prepared when annealed up to 1400 °C are considered.

Regarding the first point, Fig. 2 shows how the initial biphasic material obtained after annealing below 1000 °C turns into a hydroxyapatite single phase which remains stable up to 1300 °C. At 1400 °C the more intense maximum of the α-TCP appears; longer annealing times lead to an increase of the quantity of this phase as can be deduced from the presence of several

	1.52	1.54	1.56	1.58	1.60	1.62	1.64
OHAp % (err.)	5,9 (2)	24,4 (3)	35,1 (4)	47,8 (2)	62,0 (4)	74,9 (3)	87,4 (2)
β-TCP % (err.)	94,1 (6)	75,6 (5)	64,9 (5)	52,2 (4)	38,0 (2)	25,1 (2)	12,6 (2)
R_B(OHAp)	4,56	3,76	3,69	3,88	4,34	4,25	4,47
R_B(β-TCP)	4,60	4,05	2,75	4,18	4,48	6,89	4,99
R_p	7,93	7,09	6,10	6,64	6,94	7,62	7,31
R_{wp}	10,74	9,59	8,33	9,20	9,60	10,95	10,24
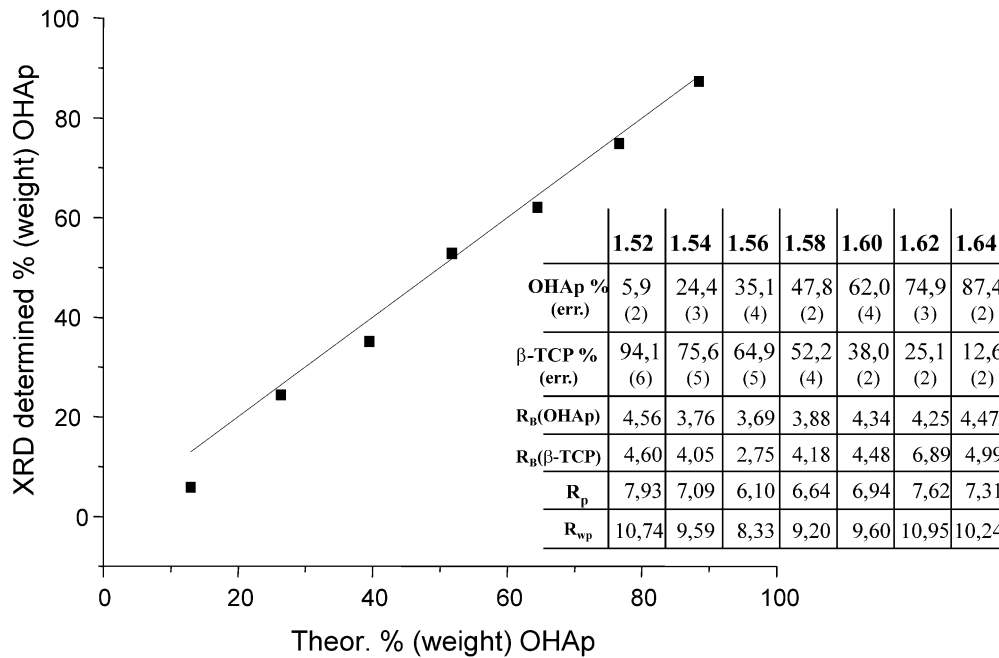
Fig. 6. Theoretical OHAp% weight vs. OHAp% weight determined by XRD and (inset) XRD determined weight percentages and agreement factors for biphasic samples.
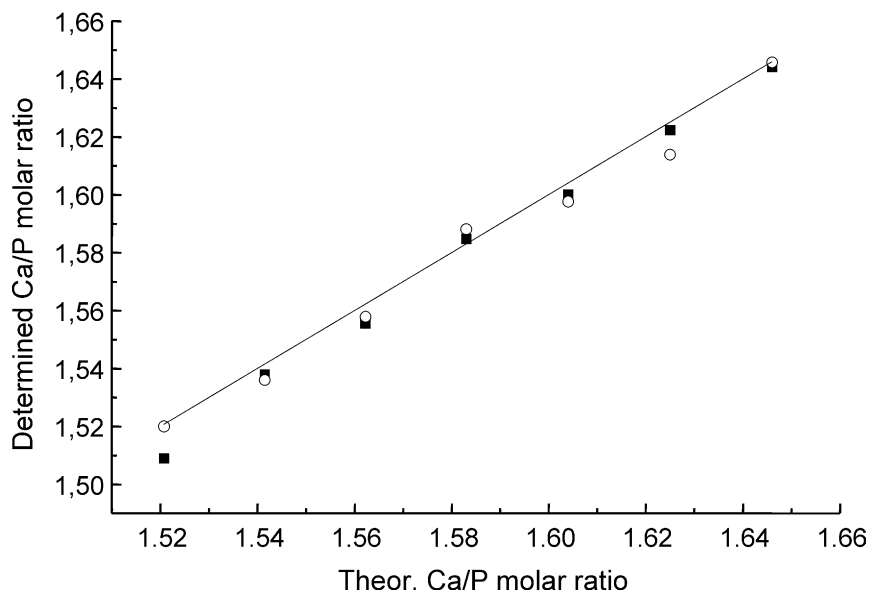
Fig. 7. Comparison between theoretical Ca/P ratio (line) with that obtained by QXRD (■) or XRF (○).

XRD maxima of α-TCP and the progressive transformation in the IR spectra. The presence of CaO (Powder Diffraction File 37-1497 JCPDS 2000) or $Ca_4(PO_4)_2O$ (TTCP, Powder Diffraction File 25-1137 JCPDS 2000) must be deduced from the maintenance of the Ca/P molar ratio, although this can be hardly distinguished on the X-Ray spectra owing to the low concentration and amorphous structure of these phases. The decomposition of OHAp at high temperatures has traditionally [2,3] been explained to yield α-TCP and tetracalcium phosphate $(Ca_4(PO_4)_2O)$.

At the other extreme, a Ca/P ratio of 1,5 leads to the formation of β-TCP at 900 °C which remains stable up to 1100 °C (Fig. 3). This transition has been described[2,3,19]
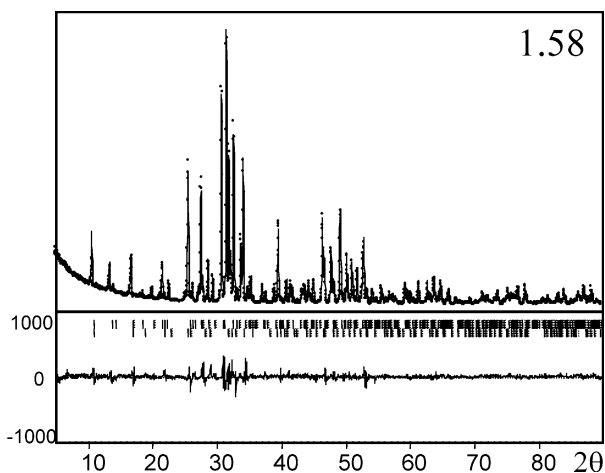
Fig. 8. Plot of (●) observed and (——) calculated and difference diffraction data resulting from the Rietveld analysis of sample 1.58).

to occur from an annealing temperature of 1100 °C. After annealing at 1200 °C a transformation into α-TCP starts which is not fully completed until a thermal treatment at 1400 °C for 72 h is applied.

Concerning the second point, Fig. 4 represents the transformation process of an intermediate composition 1.58, in which the biphasic material evolves towards a higher content on OHAp. Annealing temperatures over 1100 °C lead to the decomposition of β-TCP into α-TCP (starting at 1200 °C) and OHAp into α-TCP, CaO or $Ca_4(PO_4)_2O$ (from 1400 °C), although these last phases can be hardly, as already commented above, detected.

Fig. 5 collects the FTIR spectra (left) of some of the samples annealed at 1000 °C. The FTIR characterization shows the progressive disappearance of the stretching mode and vibrational mode of the hydroxyl group and the substitution of the characteristic bands of the PO_4^{3-} of hydroxyapatite for those attributable to β-TCP when decreasing the Ca/P ratio (Table 2). There is no clear evidence for CO_3^{2-} in these samples as could be detected for samples annealed at temperatures below 1000 °C (Fig. 2).

We have studied in more detail the X-ray spectra of these samples, depicted in Fig. 5 (right), in order to determine the feasibility of this synthetic method, i.e. the agreement of the initial Ca/P ratios in the precursor solutions with those deduced from the quantity of OHAp and β-TCP in these biphasic materials and their correspondence with those obtained by XRF. The determination of the amount of each phase required the use of a quantification tool—Rietveld based X-ray quantification— which has already been applied, and

Fig. 9. Calcium release of samples annealed at 1000 °C (24 h).

has proved to be, at least, as accurate as other traditional methods based on the comparison between the intensity or related parameters of the maxima of the phases implied.[20–23] Concerning the as prepared biphasic materials, this method shows a reasonable accordance between the Ca/P ratio in the solution, expressed as the theoretical quantity of hydroxyapatite in the mixture, and that obtained by this method (Fig. 6). In addition, the comparison of these results with those calculated from X-ray fluorescence validates this quantification method by showing a correspondence between the Ca/P ratios in the solution and the quantity of OHAp and β-TCP in the final products determined by both methods (Fig. 7). Consequently, these data validate this method as a way of obtaining Ca/P biphasic materials whose quantity of each phase can be previously determined. Fig. 8 collects the resulting plot of a Rietveld analysis effectuated on the 1.58 sample which yields a composition of 52.2% OHAp and 47.8% β-TCP, that does not differ greatly from the theoretical value (51.75% and 48.25%, respectively).

Concerning the possible segregation of the phosphate groups to yield separate phases, this seems not to happen at the temperatures considered. The slight alteration in the homogeneity is due to a physical entrapment of the PO_4^{3-} groups within the network of the polymer as has been already defended by some authors [24] for precursor solutions with higher amount of cations than they could chelate. The evaporation of water during the synthesis, which causes an increase in the viscosity, contributes to the entrapment of the PO_4^{3-} anions into the network due to a shrinkage of the polymer molecule.

From an application point of view the success of these biphasic products as bone substitutes lies in the combination of a higher solubility of β-tricalcium phosphate [25–27] and the superior biocompatibility of hydroxyapatite. Until now the 60/40 BCPs are the best known materials, however, different applications in diverse parts of the organism require different resorption times and this depends on the relation between OHAp and β-TCP in these mixtures. In this work we have tested the degradation of these mixtures in a buffer at pH = 7.4 and 37 °C. This study confirms the initial forecast of a progressive augmentation of the calcium presence in the solution with the increase of the β-TCP/OHAp ratio of the samples, although a considerable difference is
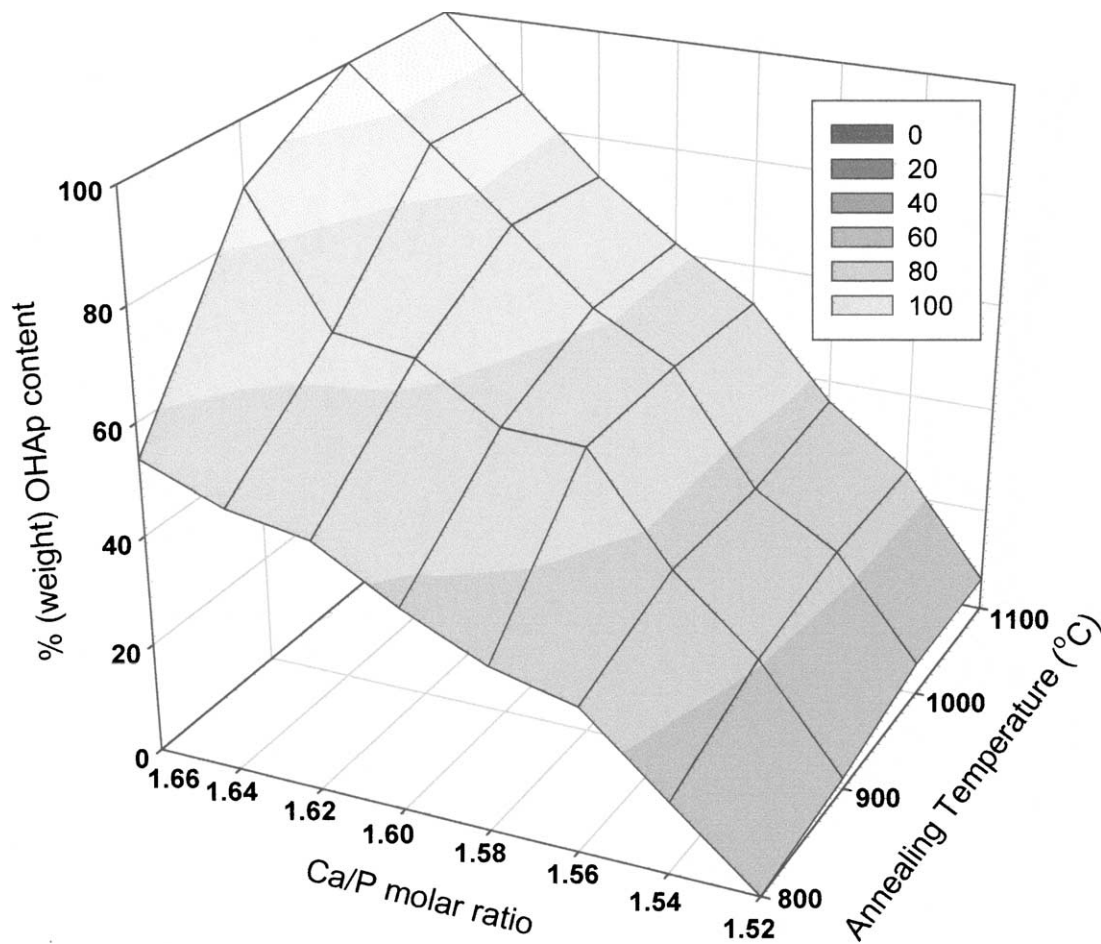

Fig. 10. Quantity (%) of OHAp, determined by QXRD, in the biphasic materials obtained from different Ca/P ratio annealed between 800 and 1100 °C.

observed between the single phase OHAp and the biphasic materials with the smallest quantity of β-TCP (sample 1.64).

5. Conclusions

In this work we propose the utilization of a Liquid Mix Technique for the preparation of different calcium phosphates. This method makes it possible to obtain single phase hydroxyapatite, β-TCP and α-TCP and also biphasic materials whose content in β-TCP and OHAp can be precisely predicted from the Ca/P ratio in the precursor solutions. Concerning the thermal stability of the single phases, the β-TCP is obtained by this method after annealing at 900 °C and remains stable up to 1100 °C, while in the case of OHAp these stability temperatures ranges between 1000 and 1300 °C. Thermal treatments over these temperatures lead to the formation of biphasic α-TCP-OHAp materials in different proportions. In the case of the sample 1,5 it is necessary to anneal at 1400 °C for 72 h to complete the transformation of β-TCP, previously isolated between 90 and –1100 °C, into pure α-TCP. The use of a Rietveld based software to determine the quantity of each phase in these biphasic materials has proved to be a powerful and feasible tool. Finally, the degradation studies show a progressive increase in the calcium dissolution in the buffer solution with the increase of the content of β-TCP, although a small gap is observed in this gradual dissolution of calcium between the single phase hydroxyapatite and that with the smallest quantity of β-tricalcium phosphate.

Acknowledgements

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