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Chemical synthesis of bone-like carbonate hydroxyapatite from hen eggshells and its characterization

J.L. ACEVEDO-DÁVILA[1], J. LÓPEZ-CUEVAS[2,*], G. VARGAS-GUTIÉRREZ[2], J.C. RENDÓN-ANGELES[2] AND J. MÉNDEZ-NONELL[2,3].

[1]COMINSA, Apartado Postal 491, C.P. 25290, Saltillo, Coahuila, México.
[2]CINVESTAV-IPN Unidad Saltillo, Apartado Postal 663, C.P. 25000, Saltillo, Coahuila, México.
[3]CIQA, Blvd. Enrique Reyna nº 140, C.P. 25253, Saltillo, Coahuila, México.

Carbonate hydroxyapatite (CHAp) was synthesized from domestic hen eggshells by using three alternative wet chemical methods at room temperature. In the first method, the powdered eggshells were reacted directly with H_3PO_4. In the other two methods, calcium acetate was obtained in a first step by dissolving the eggshells in acetic acid. Then, calcium acetate was reacted with $Na_3PO_4{\cdot}12H_2O$ and $(NH_4)_2HPO_4$ in the second and third methods, respectively. The synthesized CHAp was characterized by X-Ray Diffraction (XRD), Fourier Transformed Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Fine and poorly crystallized CHAp was obtained under all experimental conditions employed. Among all synthesized materials, the one produced by the first method showed the closest resemblance to bovine bone, which was related to similar carbonate contents in both materials. In general, acicular CHAp crystals with a size ranging from 10 to 100 nm were obtained, which had an aspect ratio of ~1/4. The morphology of the synthesized CHAp crystals was consistent with their estimated carbonate content.

Keywords: Carbonate hydroxyapatite; Eggshells; Calcium acetate; Chemical Synthesis.

Síntesis química de carbonato-hidroxiapatita similar al hueso a partir de cascarón de huevo de gallina y su caracterización.

Se sintetizó carbonato-hidroxiapatita (CHAp) a partir de cascarón de huevo de gallina, usando tres métodos químicos alternativos vía húmeda a temperatura ambiente. En el primer método, el cascarón pulverizado fue hecho reaccionar directamente con H_3PO_4. En los otros dos métodos, el primer paso fue la obtención de acetato de calcio mediante la disolución del cascarón en ácido acético. Luego, el acetato de calcio fue hecho reaccionar con $Na_3PO_4{\cdot}12H_2O$ y $(NH_4)_2HPO_4$ en el segundo y tercer método, respectivamente. El CHAp sintetizado fue caracterizado por difracción de rayos X (DRX), espectroscopia infrarroja por transformada de Fourier (FT-IR), microscopía electrónica de barrido (MEB) y microscopía electrónica de transmisión (MET). Bajo todas las condiciones experimentales empleadas se obtuvo CHAp fina y poco cristalina. De entre todos los materiales sintetizados, el producido empleando el primer método mostró la mayor similitud con respecto al hueso de bovino, lo cual fue relacionado con un contenido similar de carbonato en ambos materiales. En general, se obtuvieron cristales aciculares de CHAp con un tamaño en el rango de 10 a 100 nm, los cuales presentaron una relación de aspecto de ~1/4. La morfología de los cristales de CHAp sintetizados fue consistente con su contenido estimado de carbonato.

Palabras clave: Carbonato-hidroxiapatita; cascarón de huevo; acetato de calcio; síntesis química.

1. INTRODUCTION

Human and animal hard tissues contain from 70 to 90wt% of an inorganic phase known as "biological apatite", whose chemical composition corresponds approximately to the general formula $(Ca,M)_{10}(PO_4,CO_3,Y)_6(OH,F,Cl)_2$, where M represents minor elements such as Mg, Na and K, etc., as well as trace elements such as Sr, Pb and Ba, while Y represents acid phosphate, HPO_4^{2-}, sulfates, borates, vanadates, etc. (1). Biological apatite is commonly calcium deficient and it is always carbonate substituted. For this reason, it is more appropriate to refer to it as "carbonate apatite" (CA) (1). The carbonate ions substitute primarily for the phosphate groups of biological apatite, which is designated as Type B substitution. However, the hydroxyl (OH) groups can also be substituted, which is in turn designated as Type A substitution (1). The carbonate content present in human bones, dentine and enamel, fluctuates from 3 to 8wt% (1,2).

Hydroxypatite (HAp), whose stoichiometric chemical composition corresponds to the formula $Ca_{10}(PO_4)_6(OH)_2$, is a synthetic material which is very attractive for biomedical applications, mainly for the replacement of hard tissues (3). Pure HAp differs from biological apatite in its stoichiometry, chemical composition and crystallinity, as well as in other physical, chemical and mechanical properties (1). The occurrence of Types A and B carbonate substitutions is also very frequent in HAp, altering its structure and properties (1). When such substitutions take place, HAp is commonly referred to as "carbonate hydroxyapatite" (CHAp). The incorporation of CO_3^{2-} into the HAp structure results in an

increased reactivity, which leads to a faster dissolution in acidic media (4). Additionally, CHAp is less stable thermally than stoichiometric HAp. It has been pointed out that in order to obtain high density and high strength HAp sintered bodies, it is necessary to start by using stoichiometric HAp (1,3). In spite of all that, it is considered (3) that the incorporation of carbonate ions into the HAp structure constitutes a potential way to increase the chemical similarity of this material with respect to biological apatite. This would result in HAp having an increased bioactivity and biocompatibility. It has been suggested (5) that synthetic carbonated apatites may be promising in dental and medical applications as bioactive materials due to enhanced dissolution and increased osteoclast mediated resorption. This is supported in part by the observation that the dissolution in a physiological environment of so-called "reabsorbable materials", such as β tricalcium phosphate [β-TCP, β-$Ca_3(PO_4)_2$], leads in time to the in-situ precipitation of CHAp of low crystallinity, which gradually achieves a Ca/P ratio of ~1.67, which is very similar to that of stoichiometric HAp, resulting in the growth of new bone (6).

A large variety of chemical methods has been reported for the synthesis of HAp, many of which are characterized by an inherent simplicity, easy controllability and low cost. One of the characteristics of these synthesis methods is the relatively easy incorporation of CO_3^{2-} into the HAp structure from the reactor's atmosphere and/or from the raw materials. This depends very strongly on the synthesis method and conditions employed. For instance, the incorporation of CO_2 from the atmosphere into the reacting suspensions, and from there into the HAp structure, is promoted by chemical synthesis under ambient air at alkaline pH and at relatively low reaction temperatures (4). Two of the more commonly employed HAp chemical synthesis methods were originally reported by Rathje (7) and by Hayek and Newesely (8). Rathje's method (7) consists in the dropwise addition of H_3PO_4 to a stirring aqueous suspension of $Ca(OH)_2$. The method of Hayek and Newesely (8) consists in the reaction of $Ca(NO_3)_2$ with $(NH_4)_2HPO_4$, under a basic pH adjusted by the addition of NH_4OH. Since the appearance of these reports, many other chemical synthesis methods have been developed which employ a large variety of raw materials, either synthesized or naturally occurring, one of which is calcium carbonate ($CaCO_3$) (9-15).

CHAp has been chemically synthesized by using $CaCO_3$ and H_3PO_4 as initial reactants (16), as well as by employing the traditional Rathje's method (7). An example of the latter is the work of Suwa et al. (3). A variety of other techniques have also been employed for the synthesis of HAp and CHAp from natural or reagent-grade $CaCO_3$. For instance, CA plus a small amount of Mg-substituted β−$Ca_3(PO_4)_2$ (β−TCMP) have been obtained by hydrothermal exchange from coral skeletal carbonate, constituted mainly by aragonite ($CaCO_3$). This conversion is carried out in the presence of ammonium phosphate solution (17). Legeros et al. (18) have pointed out that in apatites precipitated from aqueous solutions the carbonate substitutes mainly for the phosphate groups and the presence of carbonate disturbs the crystallization of the apatite. When HAp is precipitated at 37-60°C, it is poorly crystallized, and this material more closely resembles the natural apatites in comparison with the synthetic apatite prepared at 900-1000°C. Precipitates with low carbonate content tend to have needle-like crystals.

The eggshells, which constitute from 9 to 12% of the total weight of the domestic hen eggs, are an abundant, under exploited and cheap raw material which is a natural potential source of $CaCO_3$ that could be used for the chemical synthesis of CHAp. The eggshells are generally considered to be a waste, in spite of being useful as a fertilizer and for the production of some nutritional complements for humans and animals, due to their high calcium and nitrogen content (19). Chemically, the eggshells are formed by calcite (94wt%), magnesium carbonate (1wt%), calcium phosphate (1wt%), traces of other minerals and approximately 4wt% of organic matter (20,21). Structurally, the major mineralized region of the eggshells is constituted by the palisades, which are columns of a mineralized porous matrix having an approximate thickness of 250 μm. The palisade layer contains a polysaccharide (dermatan sulfate) that influences the calcification process as well as the morphology of the calcium carbonate crystals during the eggshell formation. A thin cuticle covering the entire eggshell surface is located at the external side of the latter layer, while the innermost surface of the eggshell comprises two fibrous meshworks, known as the inner and the outer shell membranes, which contain keratan sulfate proteoglycans as well as type I and X collagen. Lastly, a layer of mineralized matrix foci, known as calcium reserve assemblies, can be found between the outer membrane and the palisade layer (22). Recently, pulverized hen eggshells have been tested in the biomedical area as a filler material for cranial defects in rats (23).

Rivera et al. (14) reported the synthesis of HAp from hen eggshells. In their method, previously to the synthesis of HAp, the eggshells were heat-treated initially for 2h at 450°C to eliminate the organic matter, and then for further 2h at 900°C to transform the $CaCO_3$ into CaO. After allowing the CaO to cool down to room temperature, it was mixed with water and with a phosphate solution and subsequently heat-treated inside a reactor for 3h at 1050ºC. HAp was produced as a result of the occurrence of a chemical reaction between CaO, $Ca_3(PO_4)_2$ and water. The main disadvantage of this method is the employment of several heat-treatment steps which contribute significantly to increase the total cost of the HAp synthesis process.

The purpose of the present work was to synthesize CHAp from domestic hen eggshells by using three alternative wet chemical methods at room temperature. The main difference between our work and that of Rivera et al. (14) is that we intended to obtain a carbonated material which was very similar to bone with regard to its composition and structural characteristics, while the mentioned authors obtained carbonate-free well-crystallized hydroxiapatite, which are not necessarily the best characteristics in terms of bioactivity and biocompatibility (5).

2. MATERIALS AND METHODS

2.1 Synthesis and processing

In order to eliminate as much organic matter as possible, the eggshells were first boiled several times in an aqueous solution containing 30 vol% of reagent grade H_2O_2, rinsing in deionized water in between. After cleaning and drying the eggshells, these were crushed and ground in order to obtain a fine powder with a particle size smaller than 100μm. Subsequently, CHAp was chemically synthesized by using

three different wet chemical methods. In Method 1, an aqueous solution of phosphoric acid (1M) was added dropwise to a vigorously and continuously stirred aqueous suspension prepared with the powdered eggshells. In Methods 2 and 3, the first step involved the reaction of a similar aqueous suspension with reagent grade acetic acid, while boiling. The objective of the latter step was to obtain an aqueous solution of calcium acetate, which was then separated by centrifuging and filtration from any residual insoluble organic matter that could be still present in the eggshells, as well as from any unreacted eggshell material. Then, the calcium acetate was precipitated and the residual acetic acid was eliminated by evaporation. After this, the calcium acetate was redissolved in boiling deionized water and allowed to cool down to room temperature. This was followed by reaction with an aqueous solution of tribasic dodecahydrate sodium phosphate [$Na_3PO_4 \bullet 12H_2O$] in Method 2, and with an aqueous solution of dibasic ammonium phosphate [$(NH_4)_2HPO_4$] in Method 3. In both cases, the chemical reaction was carried out by adding the phosphate aqueous solution dropwise, under a vigorous and continuos stirring, to the calcium acetate aqueous solution. Fine CHAp particles plus a residual aqueous solution were obtained in all three synthesis methods employed. The synthesized CHAp was separated from the liquid by vacuum filtering and subsequently washed several times with deionized water, until a neutral pH was obtained, which was followed by drying at 100°C for 12h. Hereafter, the CHAp obtained is referred to as CHAp-1, CHAp-2 and CHAp-3, for Methods 1, 2 and 3, respectively. In all cases, the required relative volumes of the chemical reactants were determined, according to the volume capacity of the chemical reactor (one liter), aiming for a final Ca/P molar ratio of 1.67 in the resulting aqueous suspensions. Also in all cases the CHAp synthesis was carried out under a still air atmosphere at room temperature, except for a small heat release due to the exothermic nature of the chemical reaction. The solution pH was continuously monitored but not controlled.

2.2 Characterization

Phase analyses were carried out by X-Ray Diffraction (XRD), using nickel-filtered Cu-Kα radiation in a Philips X-Pert MPD apparatus operated at 40 kV and 30 mA, in the 2θ range of 10-80º. A Nicolet Avatar 320 Fourier Transformed Infrared Spectrometer (FT-IR) was used for the determination of functional groups in the synthesized CHAp. The B-type carbonate content of the latter materials was estimated from the FT-IR spectra based on the work of Featherstone et al. (24). The FT-IR and XRD results obtained for the starting egshells, as well as for the synthesized calcium acetate and CHAp, were compared with those corresponding to reagent grade calcium carbonate and calcium acetate, for the case of the former two materials, and with those corresponding to bovine cortical bone [BCB], for the case of CHAp. It is worth mentioning that since it is known (25) that the use of aqueous solvents and high temperatures to remove the organic constituents of bone results in partial dissolution and transformation of the mineral phase, with a significant alteration in its crystal structure, chemical composition or internal short-range order and organization (including the local environments of labile carbonate and phosphate ions), essentially untreated BCB was employed as a reference material, except for the partial removal of its organic mater by mechanical means (ultrasonication in deionized water). The morphology and size of the synthesized CHAp crystals were analysed by Scanning Electron Microscopy (Philips XL30 ESEM) as well as by Transmission Electron Microscopy (Philips CM200 TEM).

3. RESULTS AND DISCUSSION

3.1 Characterization of raw materials

Figure 1 shows the XRD patterns for powdered eggshells pre-treated in a H_2O_2 aqueous solution (Fig. 1a) and for reagent grade $CaCO_3$ (Fig. 1b). Figure 2 gives the FT-IR spectra for powdered eggshells with (Fig. 2b) and without (Fig. 2a) previous treatment in H_2O_2, as well as for reagent grade $CaCO_3$ (Fig. 2c). It can be seen that all three FT-IR spectra are very similar to each other. Thus, the XRD and FT-IR results confirm the expected chemical composition for the eggshells. It can also be noticed that the treatment with H_2O_2 did not cause any significant change in the chemical composition of the latter. Figure 3 compares the FT-IR spectrum of calcium

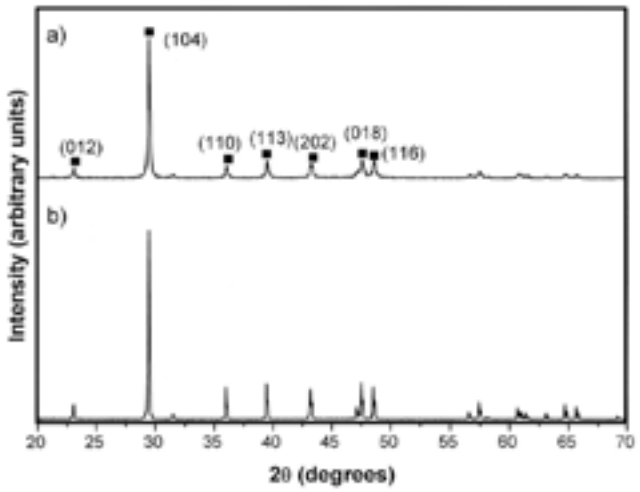

Fig. 1- XRD patterns corresponding to: a) powdered eggshells pre-treated in a H_2O_2 aqueous solution and b) reagent grade $CaCO_3$ (JCPDS diffraction card number 05-0586).

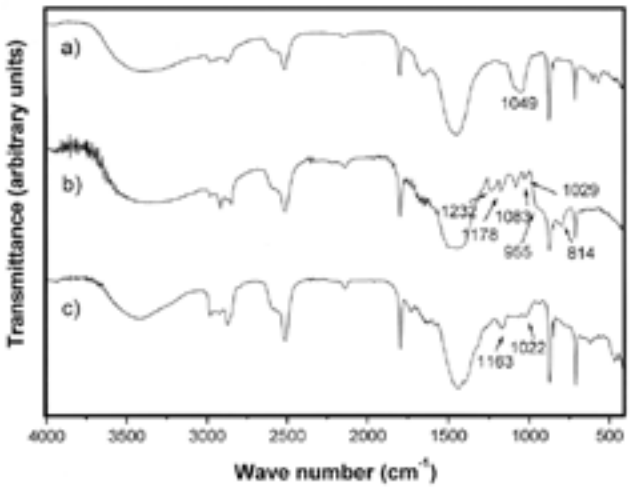

Fig. 2- FT-IR spectra for: a) untreated powdered eggshells, b) powdered eggshells pre-treated in a H_2O_2 aqueous solution and c) reagent grade $CaCO_3$.

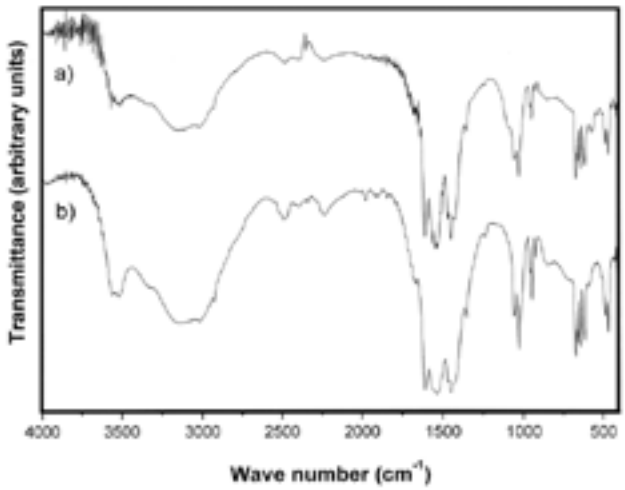

Fig. 3- FT-IR spectra for: a) synthesized calcium acetate and b) reagent grade calcium acetate.

acetate obtained by dissolving the eggshells in acetic acid (Fig. 3a) with that corresponding to reagent grade calcium acetate (Fig. 3b). Again, both espectra are very similar to each other, and thus, the chemical composition of the obtained product is confirmed.

One point that deserves to be emphasized is that although a significant amount of residual organic matter was not detected in the eggshells after their cleaning up with H_2O_2, in order to ensure that any remaining traces of organic matter are completely eliminated from the material, this processing step could be followed by a thermal treatment at 450°C/2h (14), minimizing in this way any future risks of causing an immunoresponse in-vivo. This temperature is well below the ~ 870°C required for the thermal decomposition of non-compacted powdered $CaCO_3$ under an air atmosphere (26), thus, the chemical composition of the main inorganic component of the eggshells will not be altered by the recommended treatment.

Lastly, reagent grade calcium carbonate and calcium acetate were used only as comparison standards in Figs. 2 and 3, and since no significant differences were observed between the FT-IR spectra corresponding to the latter compounds and those corresponding to the eggshells and the synthesized calcium acetate, a detailed indication of the observed bands was not given in these particular Figures.

3.2 Characterization of synthesized CHAp

3.2.1 Carbonate content

The estimated carbonate content of the synthesized CHAp and the final pH of the reacting suspensions are given in Table I. As can be seen, the carbonate content increases in the order CHAp-3 < CHAp-2 < CHAp-1 < BCB. Thus, synthesis Method 1 produced the material that was more similar to bone, in terms of its carbonate content. This can be explained based on the fact that CHAp-1 was produced directly from the powdered eggshells, constituted mainly by $CaCO_3$, as already mentioned. Thus, in this case it can be assumed that most of the carbonate contained in the CHAp came from the raw materials rather than from the reactor's atmosphere. This is further supported by the fact that in this case the final pH

TABLE I: ESTIMATED CARBONATE CONTENT IN THE PRECIPITATED CHAP AND FINAL PH OF THE REACTING SUSPENSIONS.

Sample	CO_3* (Wt%)	Final pH
BCB	5.8	---
CHAp-1	4.5	6.49
CHAp-2	3.2	11.5
CHAp-3	1.5	5.47

*Estimated error of ±5%.

of the reacting suspension was nearly neutral, which is not particularly favorable for the dissolution of CO_2 from the ambient air. In contrast, when the eggshells were transformed into calcium acetate, and the latter into CHAp, the carbonate content was lower, with a tendency to increase with increasing final pH in the reacting solution. In this case it can be assumed that most of the carbonate contained in the CHAp came from the reactor's atmosphere rather than from the raw materials, with the highest carbonate content corresponding to the CHAp produced by using an aqueos solution with the strongest tendency to pick up CO_2 from the ambient atmosphere.

It is worth mentioning that the solubility behaviour of CO_2 in pure water is somewhat different to that observed in aqueous solutions, under ambient air at atmospheric pressure, with regard to the effect of three main factors: a) pH, b) presence of other ions in solution, and c) temperature. While the dissolution of CO_2 in pure water results in a decrease in pH due to the formation of carbonic acid (27), it is known (28) that the solubility of CO_2 in aqueous solutions increases with increasing pH, probably due to the formation of ion complexes such as $Ca(CO_3)_4^{6-}$. The fact that most carbonates are very soluble at low pH values could cause some confusion at this respect, but we must take into account that the dissolution of carbonates in acidic aqueous media is always accompanied by a release of CO_2, which basically does not remain dissolved in the solution. On the other hand, it is known (29) that the presence of sodium ions in the aqueous solution promotes the absorption of CO_2 from the ambient air. This is related, for the case of the synthesis of HAp, to the exchange of Na^+ for Ca^{2+} ions. It has been pointed out (1) that the carbonate in biological apatites substitutes mainly for the phosphate groups in a coupled manner, i.e., Na-for-Ca and CO_3-for-PO_4, in order to balance charges for the substitution of PO_4^{3-} by a CO_3^{2-} ion. Finally, CO_2 shows a "retrograde solubility" in pure water, i.e. as the temperature increases, the solubility of CO_2 in water at a given pressure decreases, resulting in a higher pH value (27). This is the reason why water can be decarbonated by boiling it, especially in the presence of calcium ions, since calcium carbonate shows also a retrograde solubility in water. This situation can also be observed for the case of many carbonates and phosphates. However, the solubility of CO_2 in alkaline aqueous solutions is not retrograde, which means that it increases with increasing temperature.

Thus, according to the latter discussion, the fact that Method 2 produced the CHAp with the highest carbonate content, when compared with that produced by using Method 3, was related in part to the employment of a very alkaline pH in Method 2. Besides, the acidic final pH of the aqueos solution employed in Method 3 made the absorption of CO_2 more difficult. The presence of sodium ions in the $Na_3PO_4 \bullet 12H_2O$ aqueous solution employed in Method 2 was another contributory factor to the higher carbonate content of CHAp-2 with respect to CHAp-3. Lastly, while CHAp-2 can

contain a small amount of sodium due to the occurrence of the phenomenon just described, CHAp-3 is expected to be more pure, since it has been pointed out (30) that the NH_4^+ ions are practically not incorporated into the HAp structure.

3.2.2 XRD studies

Figure 4 shows the XRD patterns corresponding to BCB as well as to CHAp produced by using the three synthesis routes considered in the present work. It can be seen that the spectra obtained for the synthesized CHAp (Figs. 4a to 4c) had a shape that is typical of fine and poorly crystallized HAp, which is very common for the case of wet chemical methods (1). It can also be noticed that all of these XRD spectra differ somewhat from that of bone mineral (Fig. 4d) because they all show a slightly higher crystallinity degree when compared to the latter, as evidenced by the narrowing and differenciation of the main peaks. Among the three synthesized materials, CHAp-1 (Fig. 4a) showed the closest resemblance to bone. This can be attributed to similarities in the carbonate content of both materials, as mentioned in the previous section.

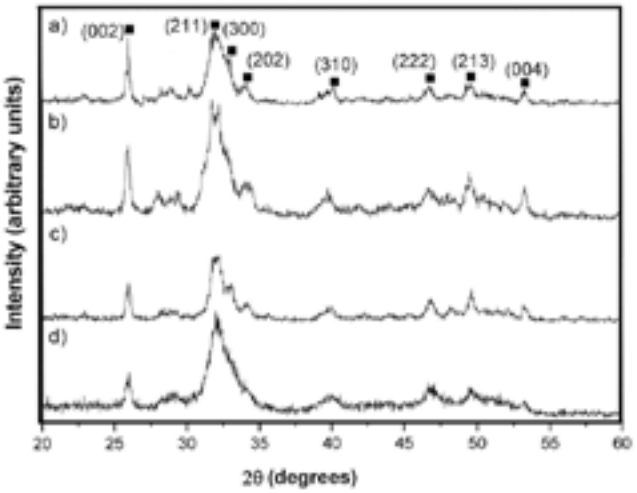

Fig. 4- XRD patterns corresponding to: a) CHAp-1, b) CHAp-2, c) CHAp-3 and d) BCB (JCPDS diffraction card number 9-432).

3.2.3 FT-IR studies

Figure 5 gives the FT-IR spectra corresponding to BCB (Fig. 5a) as well as to CHAp-1 (Fig. 5b), CHAp-2 (Fig. 5c) and CHAp-3 (Fig. 5d). The spectra of Figs. 5b to 5d are typical of CHAp, showing the presence of ν_2 and ν_3 CO_3 bands characteristic of the presence of CO_3^{-2} ions occupying the B-sites of HAp. Table II shows the functional groups corresponding to the bands observed in the FT-IR spectra. It is known (18) that a broadening and an increase in the intensity of the ν_2 and ν_3 CO_3 bands are associated with an increased concentration of CO_3^{2-} ions into the HAp structure. Another indicator confirming this trend is the behavior of the ν_1 and ν_3 PO_4 bands, with the former becoming broader and less intense, and the latter becoming progressively less resolved, with increasing concentration of CO_3^{2-} (18,31). Although all these changes in the FT-IR spectra of the synthesized CHAp are not clearly appreciated in Figure 5, the incorporation of CO_3^{2-} ions into the HAp structure can be confirmed by an analysis of the changes occurring in the triplet located around 600 cm^{-1}, which correspond to the ν_4 band of PO_4 (Table II). It can be clearly seen that this triplet becomes less resolved with increasing carbonate content in the order CHAp-3 < CHAp-2 < CHAp-1 < BCB, see Section 3.2.1 (Table I), which can be attributed to a corresponding decrease in the degree of crystallinity in the CHAp (32), due to the structural distortion caused by the substitution of PO_4^{3-} by the larger CO_3^{2-} ions. This tendency agrees well with that shown by the XRD patterns of Fig. 4.

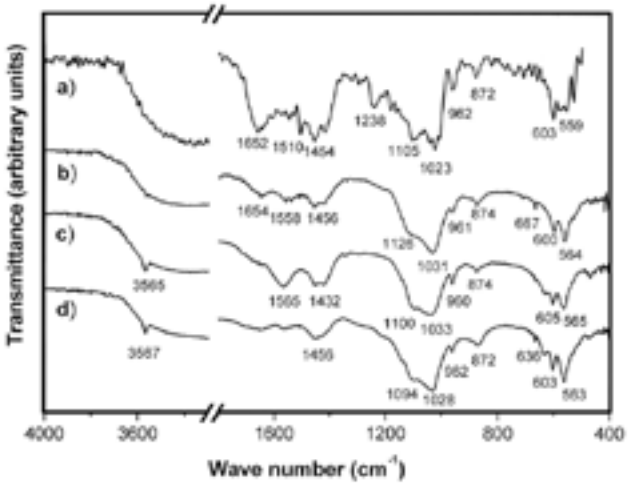

Fig. 5- FT-IR spectra corresponding to: a) BCB, b) CHAp-1, c) CHAp-2 and d) CHAp-3.

TABLE II: BANDS OBSERVED IN THE FT-IR SPECTRA (FIG. 5), ACCORDING TO REFS. (3, 29, 30).

Sample	OH		PO^{-3}_4			CO^{-2}_3		
			ν_1	ν_3	ν_4	ν_3		ν_2
BCB		1652	962	1023	603,559	1454	1510	872
CHAp-1		1654	961	1105, 1031	667,600,564	1456	1558	874
CHAp-2	3565		960	1100,1033	636,605,565	1432	1565	874
CHAp-3	3567		962	1094,1028	636,603,563	1456		872

The absence of the acetate functional group in Figs. 5c and 5d constitutes an advantage of synthesis Methods 2 and 3, since this indicates that when calcium acetate is employed as the calcium source, the acetate ions are not incorporated into the HAp structure, unlike ions such as nitrate or chloride, which may, when chemical reagents such as calcium chloride or nitrate are employed (1).

3.2.4 SEM and TEM studies

The micrographs shown in Figures 6 and 7 reveal the morphology of the synthesized CHAp-1 crystals, which was similar to that shown by CHAp-2 and CHAp-3 materials. The SEM micrograph of Figure 6 shows the presence of agglomerates of very fine CHAp particles, which can be seen in more detail in the bright field TEM micrograph of Fig. 7b. The poor resolution of Fig. 6 could be attributed to the small CHAp particle size. In general, acicular CHAp crystals with a size ranging from 10 to 100 nm were obtained, which had an aspect ratio (width/length) approximately equal to 1/4 (Fig. 7c). The acicular crystals observed in the present work are consistent with the carbonate contents mentioned in Section

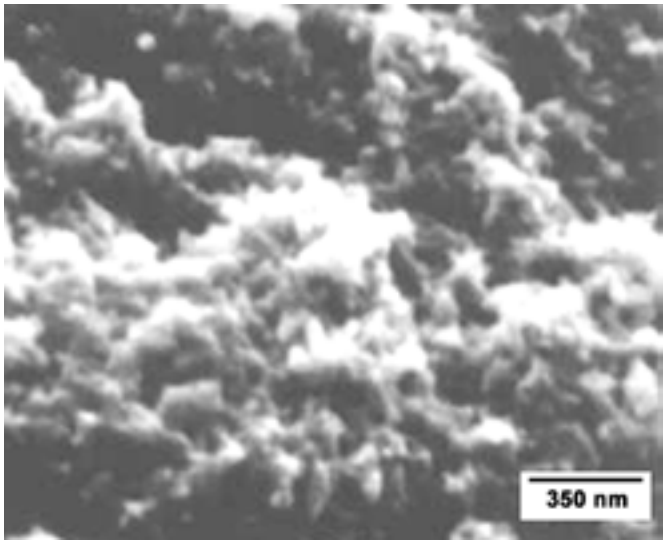

Fig. 6- SEM micrograph of CHAp-1.

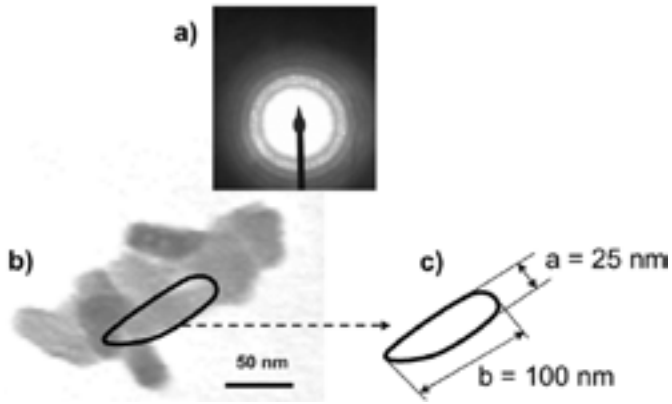

Fig. 7- a) TEM electron diffraction pattern, b) bright field micrograph and c) aspect ratio of CHAp-1 needle-like crystals.

3.2.1 (Table I), according to the data and micrographs shown by R.Z. LeGeros and J.P. LeGeros (1). Fig. 7a corresponds to an electron diffraction pattern for CHAp-1 crystals in which a series of diffused rings is observed. This electron diffraction pattern is characteristic of poorly crystallized polycrystalline materials, and it is compatible with the HAp structure (30,33). These results are in accord with those obtained from the XRD and FT-IR analyses carried out.

4. CONCLUSIONS

CHAp was produced from domestic hen eggshells by using three alternative wet chemical methods at room temperature. Among all synthesized materials, CHAp-1 showed the closest resemblance to bone, which was related to similar carbonate contents in both materials. This was evidenced by the XRD and FT-IR spectra. It was assumed that in this case most of the carbonate contained in the CHAp came from the raw materials. In contrast, when the eggshells were transformed into calcium acetate, and this one into CHAp, the carbonate content was lower, with a tendency to increase with increasing final pH in the reacting solution. In this case, the carbonate contained in the CHAp was introduced mainly from the reactor's atmosphere. CHAp-2 showed a higher carbonate content when compared to CHAp-3 due to the strongly alkaline pH of the aqueous solution employed in Method 2, as well as due to the presence of sodium ions in the latter. In all cases, although the XRD spectra obtained for the synthesized CHAp were typical of fine and poorly crystallized HAp, all of them showed a slightly higher degree of crystallinity with respect to BCB. The incorporation of CO_3^{2-} ions into the HAp structure could be more clearly confirmed by the changes occurring in the ν_4 triplet of PO_4 in the FT-IR spectra, which were attributed to a decrease in the degree of crystallinity of CHAp with increasing carbonate content. In general, acicular CHAp crystals with a size ranging from 10 to 100 nm were obtained, which had a width/length aspect ratio of ~1/4. The morphology of the synthesized CHAp crystals was consistent with their estimated carbonate content.

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