



Synthesis and characterization of sodium-carbon apatite nano-crystals by chemical sedimentation method

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Abstract

In this paper, attempts have been made to prepare the nano-crystalline hydroxyapatite similar to the natural one. First, the pure hydroxyapatite solution was prepared via chemical sedimentation method. The pure hydroxyapatite was made in an aqueous media by adding calcium chloride (CaCl₂) to phosphoric acid (H₃PO₄) with a molar ratio of Ca/P=1.66 in the presence of 2M acetic acid (CH₃COOH). Then by inserting carbonate agent (CO₃⁻²) into the initial hydroxyapatite structure the carbon apatite was formed. This was done by adding different amounts of sodium carbonate (Na₂CO₃), and considering different molar ratios CO₃⁻²/PO₄⁻³. Sodium-carbonated apatite was precipitated in an alkaline solution. FTIR and XRD systems were utilized to evaluate the present of the agent groups and the desired phases. The morphology and microstructure of the samples were studied using a TEM apparatus. The results confirm the formation of sodium-carbon apatite nano-crystal. These were also pointed out that the increasing the carbon in hydroxyapatite structure causes the reduction of the crystallinity degree, increases the solubility rate and changes the morphology of nano-crystals.

Keywords: Hydroxyapatite (HA), sodium-carbonated apatite, crystal structure

Introduction

The biological hydroxyapatite is the most important component, which forms dental enamel, bone, and mineral tissues of body. From structural points of view, biological apatite is nano-size, with low crystallinity, which average dimensions, ranging from 25-150 nm in width and length (LeGeros, 1994). From a compositional point of view, the biological apatite is always carbonated. It means that The HA phase that is presented in natural bone, dentin, and enamel contains approximately 7.4, 5.6, and 3.5 wt % of carbonate respectively (LeGeros, 1994; Doi *et al.*, 1998; Roy *et al.*, 1974; Merry *et al.*, 1998; Fang *et al.*, 1992; Vaidhyanathan & Rao, 1996; Sivakumar *et al.*, 1996).

It appears that Na-CO₃HAP to be an excellent bio affinity and biocompatibility material for bio resorbable bone substitution (Hayakawa *et al.*, 2006). Regarding carbon as a sensitive and important factor in the solubility of biological apatite along with the continuity of producing osseous tissues, which is dependent on crystals solubility cycle, a lot of effort have been done in producing nano-crystal carbon apatite in the last two decades (Roy *et al.*, 1974; Merry *et al.*, 1998; Fang *et al.*, 1992; Vaidhyanathan & Rao, 1996; Sivakumar *et al.*, 1996; Suchanek *et al.*, 2002; Tadic *et al.*, 2002; Kim & Kumta, 2004; Vallet-Regi *et al.*, 1994; Barralet *et al.*, 1998; Okazaki *et al.*, 1981).

In another hand in vivo study, it was found that the quantity of inter medullar bone formed around CO₃HAP implants, increased with carbonated concentration. Also the uncertainly of crystal structures could be dependent on their chemical compositions such as Ca/P ratio and

contents of carbonate. Hence, attempts have been done to control morphology and the chemical compound of hydroxyapatite with the aim of providing a material that has the both function of bones resemble and low crystallization (LeGeros, 1965; LeGeros *et al.*, 1967; Okazaki *et al.*, 1981; Vallet-Regi *et al.*, 1994; Barralet *et al.*, 1998 De Maeyer *et al.*, 1993).

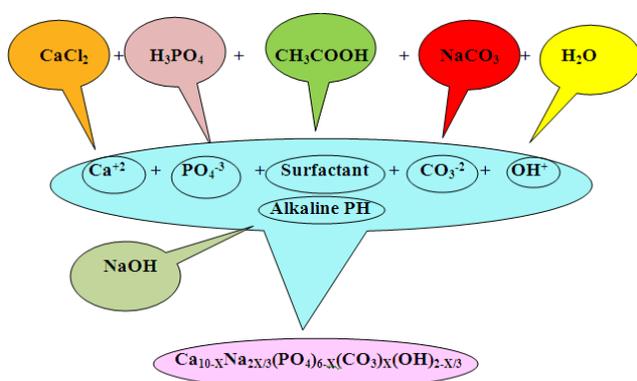
In general, the processes of producing hydroxyapatite can be categorized by wet and dry methods. The dry method or solid state processes consists of heat treated synthesis or mechanical alloying and the wet or chemical methods comprise sol gel, hydrothermal, hydrolyze, and direct sedimentation (Roy *et al.*, 1974; Merry *et al.*, 1998; Fang *et al.*, 1992; Vaidhyanathan & Rao, 1996; Sivakumar *et al.*, 1996; Sivakumar *et al.*, 1996; Suchanek *et al.*, 2002; Tadic *et al.*, 2002; Kim & Kumta, 2004; Vallet-Regi *et al.*, 1994; Barralet *et al.*, 1998; Okazaki *et al.*, 1981). One of the advantages of the wet method is that the side derivative product of the reaction is nearly water so that the possibility of entering impurity is low. In this study, the importance of the presence of carbonate in the biological hydroxyapatite compound has led to the production of a carbon apatite, which is very similar to the natural type. This has been done by following one of the wet chemical sedimentation methods, from the substitution of carbonate agent (CO₃⁻²) and sodium (Na⁺) in stoichiometric hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) (De Maeyer *et al.*, 1993). This particular type of hydroxyapatite, which consists of sodium along with carbonate, is called mimetic hydroxyapatite because it is similar to the biological type (De Maeyer *et al.*, 1993). This ionic substitution causes increase in solubility rate,

reduction of crystallinity, reduction of crystal size, reduction of a, b and c lattice coefficient, that consequently leads to chemical and thermal stability of the structure (Okazaki *et al.*, 1981; De Maeyer *et al.*, 1993). It should be mentioned that these characteristics have been constantly considered in various applications of hydroxyapatite such as coating implants, and other replaceable implants for bones defects, retrospective dosimetry (Ziaie *et al.*, 1999) and archeological estimations (Peak & Tumpane, 2007).

Materials and methods

Sample preparation

Fig. 1. A visual design of the way in which sodium-carbon apatite particles settle in alkaline solution



At first, a certain amount of calcium chloride was poured in seven balloons after scaling. Then by following the molar ratio of Ca/P=1.66 (just the same as biological ratio) phosphoric acid (H_3PO_4) was gradually added to each of the balloons. The stirrer under ventilator did the mixing of the transparent product dough, because it could produce toxic vapor during the process of adding phosphoric acid. After 30 minutes of mixing, 50 ml acetic acid (0.5 M) as an accelerating agent was added to each balloons within five minutes. Then the transparent product solution was added to 7 different amounts of sodium carbonate (Na_2CO_3) in which the ratios of PO_4^{3-}/CO_3^{2-} were 0, 1, 3, 5, 6.7, 8, and 10 respectively. This was done with the speed 1 g/min in a way that it does not lead to precipitation. While adding sodium carbonate to the obtained solution, it was steadily stirred by the use of a magnetic stirrer and finally all of the added material was solved in the solution. Then 2M, NaOH was added in drops to the solution via burette until suspended particles of carbon apatite appeared in an alkaline solution pH= 9. After an hour, the Na-CHA particles were settled by the use of centrifuge with cycle 5000 round per minute. Then they were immediately separated through funnel and kept in an oven at 100 °C for 24 hours and subsequently were sent for analysis and characterization. Figure 1 shows a visual design of the way in which sodium-carbon apatite particles settle in alkaline solution.

In order to prepare the samples for (Fourier transform infrared spectrometer) FTIR analysis, each of the

samples were mixed with potassium bromide powder (KBr). The proportion of the samples to KBr was 1 to 150 and then they were ground in a mortar. After that, by using a manual hydraulic press, some pellets (13mm in diameter) were produced. In order to prepare the samples for X-ray diffraction (XRD), each of the samples was milled into powder for 30 minutes. To prepare a good condition for transmitting electron microscopy (TEM), each of the samples was mixed with 0.5 ml ethanol and was floated in ultrasonic for 30 minutes. Then a drop of the existing suspension was placed on the grade of the microscope and dried.

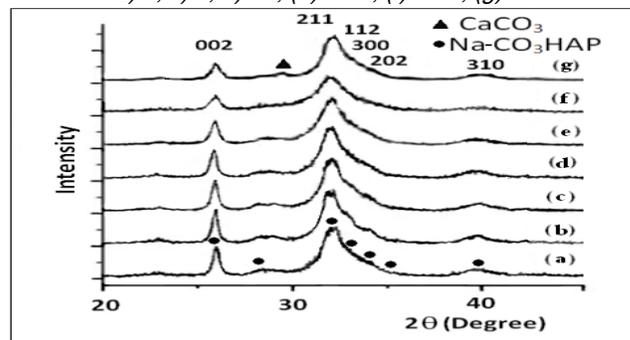
Characterization

For examining the presence of agent groups, each of the pellet shape samples was studied by infrared spectrometer (FTIR). In order to produce these spectrums, an infrared spectrometer (FTIR, model Spectrums, Series 100, Perkin Elmer Co) with a wave number between 400 cm^{-1} -4000 cm^{-1} was used. The evaluation of the existence of desirable phases in mixing the samples was done by X-ray diffraction (XRD, Philips Pw-1800) using Ni filtered Cu $K\alpha$ radiation (wave length = 1.5456 Å), in the 2 θ range of 10-45° at a scan rate of 2.4°/min, with a sampling interval of 0.02°. After obtaining the diffraction model, by comparing the angles and the intensity of diffraction peaks, each of the samples was characterized with the information presented in standard cards (JCPD). Then the altitude, position, intensity, and FWHM of the peaks were determined by X'pert high source software (the software of XRD unit itself) the results are shown in table 1. The average size of the crystals for each sample was calculated with Scherrer equation: $D = k\lambda/\beta\cos\theta$. In this equation, D is the average size of crystals in nano-meter, k is the fixed geometrical coefficient, which is equal to λ , 0.89 wide length of X ray in nanometer, β is the calculated width in radian for half of the separated body, and θ is the separation angle. The morphology of microstructures of the powder for each sample was studied by the use of an electron microscope, Philips 800, in 150keV.

Result and discussion

The X-ray diffraction patterns of all the samples

Fig. 2. XRD patterns of synthesized CHA samples with different sodium-carbonate content (CO_3^{2-}/PO_4^{3-} ratio) of, a) 0; b) 1; c) 3; d) =5; (e) =6.7; (f) =8.3; (g) =10



containing carbon and sodium can be observed in figure 2. In the appeared peaks, six peaks were more intense that had the highest degree of conformity with JCPD standard card of sodium-carbon apatite. It's number 00-019-0272 of. They were one at about 26° indexed to (002), one at 32° referred to broaden and overlapped peaks of (211), (112), (300), (202), one at 40° indexed (310) were showed. The extremely broader peak at 31° - 33° that was quite different with those apatites witch synthesized using other method. Because it consists of separated peaks of (211), (112), (300), and (002) that overlapped with each others just, in our pattern. Comparing the samples with different

carbonated contents in Fig. 1(a) to (g) showed that the increase of carbon amount causes decreasing [002] peak's altitude around 26° and regard to others researches showed: there were some impurity phases in the samples in 28° that refers to CaCO_3 (Suchanek *et al.*, 2002). We calculate the FWHM of this peak to evaluate the crystal size (Table1).

Table1. The average crystal-size of Na- CHA samples extracted from XRD pattern (002 peak).

Carbon ratio	FWHM (deg)	Average particles size
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=0$	0.188	502
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=1$	0.190	390
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=3$	0.207	173
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=5$	0.215	96
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=6.7$	0.382	64
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=8$	0.432	56
$\text{CO}_3^{2-}/\text{PO}_4^{3-}=10$	0.497	44

Increasing the amount of carbon, as well and the peak will widen. The more broaden peaks present, the higher the value of FWHM, indicating the lower crystal size of the CHA. In other words, the carbonated substitution in the HA crystal hindered crystal growth, increased solvability, reduced lattice parameter, which was concordant to previous reports (Sivakumar *et al.*, 1996; Suchanek *et al.*, 2002; Tadic *et al.*, 2002; Kim & Kumta, 2004; Vallet-Regi *et al.*, 1994; Barralet *et al.*, 1998; Okazaki *et al.*, 1981). The average size of the particles was calculated 44 to 502 according to Shearer

Fig. 3. FTIR spectrum of the synthesized samples with variant sodium carbonated contents: (a) $\text{CO}_3^{2-}/\text{PO}_4^{3-}=0$; (b) $\text{CO}_3^{2-}/\text{PO}_4^{3-}=1$; (c) $\text{CO}_3^{2-}/\text{PO}_4^{3-}=3$; (d) $\text{CO}_3^{2-}/\text{PO}_4^{3-}=5$; (e) $\text{CO}_3^{2-}/\text{PO}_4^{3-}=10$

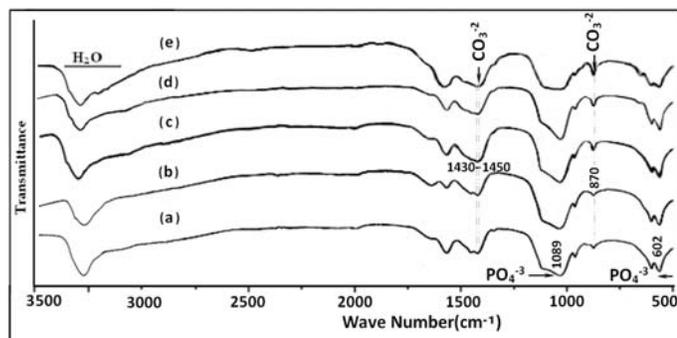
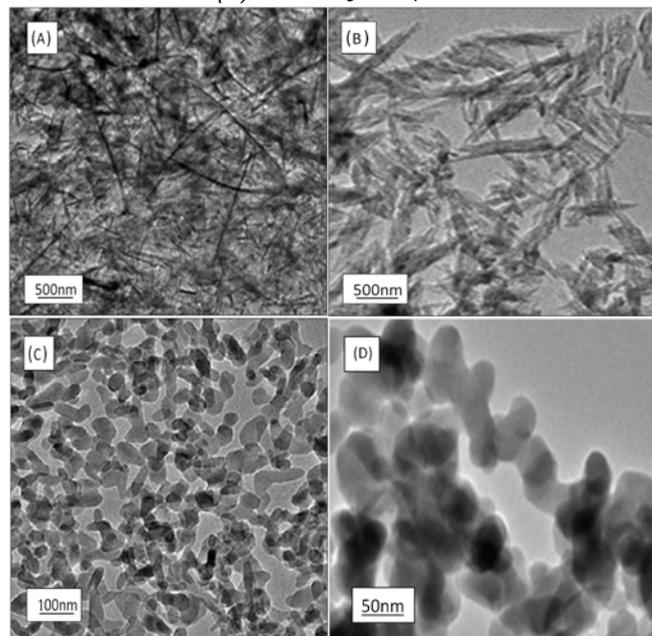


Fig. 4. TEM micrographs of CHA: (A) With $\text{CO}_3^{2-}/\text{PO}_4^{3-}=1$; (B) With $\text{CO}_3^{2-}/\text{PO}_4^{3-}=5$; (C) With $\text{CO}_3^{2-}/\text{PO}_4^{3-}=8.3$; (D) With $\text{CO}_3^{2-}/\text{PO}_4^{3-}=10$



law. According to table 1 the smallest size was obtained for 10 and the largest size for zero.

The FTIR analyses (Fig.3) Appearing of the peaks related to carbonate agent as a wide band in 1430cm^{-1} - 1450cm^{-1} , and a small peak in 870cm^{-1} , and the bands related to the phosphate agent in 1089cm^{-1} and 602cm^{-1} are representative of the Na-CHA (Hayakawa *et al.*, 2006). Detected more significant peaks at the wave numbers 870cm^{-1} , 1430cm^{-1} and 1450cm^{-1} bears a slight change by increasing of the amount of carbon concentration serving as evidence that the carbonate concentrations in the HA lattice could be controlled by varying the carbonate concentration in initial reacted solution. From The XRD and FTIR results, it could be observed that synthesized crystals were all Na-CHA ((LeGeros, 1994; Doi *et al.*, 1998; Roy *et al.*, 1974; Merry *et al.*, 1998; Fang *et al.*, 1992; Vaidhyathan & Rao, 1996; Sivakumar *et al.*, 1996; Suchanek *et al.*, 2002; Tadic *et al.*, 2002).

TEM observations have been shown for a number of the samples in Figures 4-A to 4-D. As it can be seen in the pictures, any change in the morphology of the samples can be identified. These changes according to the scale of pictures consist of the reduction size of the nano crystals with the increase of carbon, which is in accordance with the obtained results from XRD analysis (Scherrer equation) that predicted the crystallites reduction with increasing amount of carbon. It mentioned that the detailed morphology changed in accordance to the carbonated content. The chronological sequence of morphological change was observed from flat continuous platelet, needle- arrayed platelet, and particle-consisted plated, smaller platelet and finally spherical particle ((Kim



& Kumta, 2004; Vallet-Regi *et al.*, 1994; Barralet *et al.*, 1998; Okazaki *et al.*, 1981; LeGeros *et al.*, 1967; LeGeros, 1965; Fulmer *et al.*, 2002; Nelson, 1982; De Maeyer *et al.*, 1993; Hayakawa *et al.*, 2006; Ziaie *et al.*, 1999; Peak & Tumpane, 2007).

Conclusion

Nano-crystals of sodium-carbon-apatite was synthesized by introducing different amounts of carbon into the artificial type of hydroxyapatite in normal temperature and its production was confirmed by the results of TEM, FTIR, XRD analyses. The results also indicated that the synthesized particles have the size of nano. By the increase of carbon in the produced samples, the crystallization degree (particle size) reduces and consequently the rate of solvability will increase. In addition, the shape of nano-crystals, which was coherent before, becomes needle-like, rod-like, and finally spherical.

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