Research Journal of Pharmaceutical, Biological and Chemical Sciences

Investigation of Structural, Crystallochemical and Surface Electrical Properties of Synthetic Nano-Sized Calcium-Deficient Carbonated Hydroxyapatite.

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ABSTRACT

CHAP (carbonated hydroxyapatite) with various degrees of calcium-deficiency were synthesized. Characterization of the samples using X-ray diffraction, Fourier transform infrared spectroscopy (FT-IR) and Brunauer–Emmett–Teller (BET) methods showed that the synthesized samples are single phase, nano-sized carbonated hydroxyapatites, possessing high specific surface areas (up to 184 m^2/g). The sign and magnitude of zeta potential of HAP (hydroxyapatite) nanoparticles are influenced by the molar ratio Ca/(P+CO_3^{2-}). The results suggest that synthetic CHAP (carbonated hydroxyapatite) can become a promising biomaterial as orthopedic and dental implants.

Keywords: hydroxyapatite, calcium deficient hydroxyapatite, carbonated, substitution coefficient, zeta potential, crystalline morphology, specific surface area.

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INTRODUCTION

Materials based on HAP (hydroxyapatite) are widely used in dentistry [1], reconstructive bone surgery [2], orthopedics [3, 4]. In recent years much attention is drawn to investigating the possibility of using nano-sized HAP in gene and drug delivery because of its high biocompatibility, great affinity to DNA (deoxyribonucleic acid) and drug release control ability [5-8].

It is known that in living organisms HAP does not exist in pure form but always contains impurities in its structure, mainly carbonate ions [9, 10], due to its ability to adapt to the constantly changing conditions of the organism's internal environment. In general, biological apatite can be seen as calcium-deficient carbonated hydroxyapatite (d-CHAP), whose $Ca/(P+CO_3^{2-})$ molar ratio is lower than 1,67 [9]. The aim of this work is to synthesis d-CHAP with different $Ca/(P+CO_3^{2-})$ molar ratios and study their properties.

MATERIALS AND METHODS

The starting materials were calcium hydroxide, orthophosphoric acid and ammonium carbonate. Four samples of CHAP (C150, C156, C162 and C167) were obtained using wet synthesis method. The samples were named corresponding to their $Ca/(P + CO_3^{2-})$ molar ratios (1.50, 1.56. 1.62 and 1.67, respectively). The chemical equation of the reaction can be described as follow:

$$(10-d)Ca(OH)_2 + (6-x)H_3PO_4 + x(NH_4)_2CO_3 \rightarrow Ca_{10-d}(PO_4)_{6-x}(CO_3)_x(OH)_{2+x-2d}\downarrow + 2xNH_3 + (18-x)H_2O$$
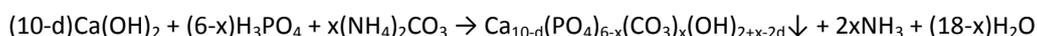
where x equals 1, which corresponds to a mass fraction of about 6% of carbonate ions. The precipitate was allowed to stand at room temperature for 24 hours, then was separated from the mother liquor by filtration, washed with distilled water to remove reaction byproducts and dried at 105^oC. The obtained dry products were pulverized using mortar and pestle to fine powder.

Measurement of zeta potential of the CHAP particles was carried out on a Zetasizer Nano ZS (Malvern Instruments). Microscopic morphological examination of the samples in hydrogel form was performed on a JEM 2100 transmission electron microscope (JEOL Ltd., Japan) with a resolution of 0.2 nm. Data on X-ray diffraction pattern were collected on a Rigaku Ultima IV diffractometer (Japan) equipped with a D/teX Ultra detector in reflection mode (Bragg-Brentano geometry) using Cu Kα radiation. The spectra were recorded in the range of $2\theta = 5^o - 85^o$ with an interval of 0.02^o and speed of 3° per minute. Qualitative phase analysis and determination of the lattice parameters and crystal size were done using the PDXL Qualitative Analysis program package and the database of ICDD (PDF 2008). Specific surface area, pore volume and average pore size of the samples were determined from low temperature nitrogen adsorption isotherm by BET method on a TriStar II 3020 analyzer (Micromeritics, USA). Qualitative analysis on the presence of carbonate ions in various positions of the crystal structure of CHAP was carried out on an FTIR Nicolet 6700 spectrometer (Thermo Electron Corporation, USA), equipped with an MCT-A detector (50 microns). Thermogravimetric (TG) and differential thermal analysis (DTA) of the powder samples (140 mg) were conducted on a Q-1500 derivatograph in the temperature range of $20 - 1020^o$C at a heating rate of 10^oC/min.

RESULTS AND DISCUSSION

Table 1: Zeta potential of CHAP samples

Sample	pH after synthesis	pH after maturing	Zeta potential, mV
C150	11.60	11.10	-27,8
C156	11.62	11.13	-15,7
C162	11.65	11.24	-12,5
C164	11.69	11.28	+2,3
C167	11.76	11.33	+12,3

Since delivery of genes and intracellular transport of drugs using nanoparticles HAP significantly depends on cellular adsorption [15], the understanding of the mechanisms of interaction of HAP nanoparticles with cells is crucial in material synthesis. Surface charge of HAP nanoparticles is an important characteristic, which plays a determining role in the process of cellular adsorption. In this aspect the negatively charged HAP

has a distinct advantage, as the cell surface is also slightly negatively charged [15, 16]. Table 1 shows the zeta potential of CHAP particles in hydrogel form, measured after 24 hours of aging in the mother liquor.

It is clear from the table that the sign and the magnitude of the zeta potential of CHAP particles vary systematically with $Ca/(P + CO_3^{2-})$ molar ratio. The surface of CHAP particles with $Ca/(P+CO_3^{2-})$ molar ratio close to stoichiometry (1.64-1.67) is positively charged. With the increase of the calcium-deficiency, the sign of zeta potential changes from positive to negative and its magnitude also increases.

The results of Transmission electron microscopy (TEM) examination of the samples in hydrogel form show that CHAP particles appear as rod-like crystals with a width of 10-20 nm and a length of 75-150 nm (Fig. 1).
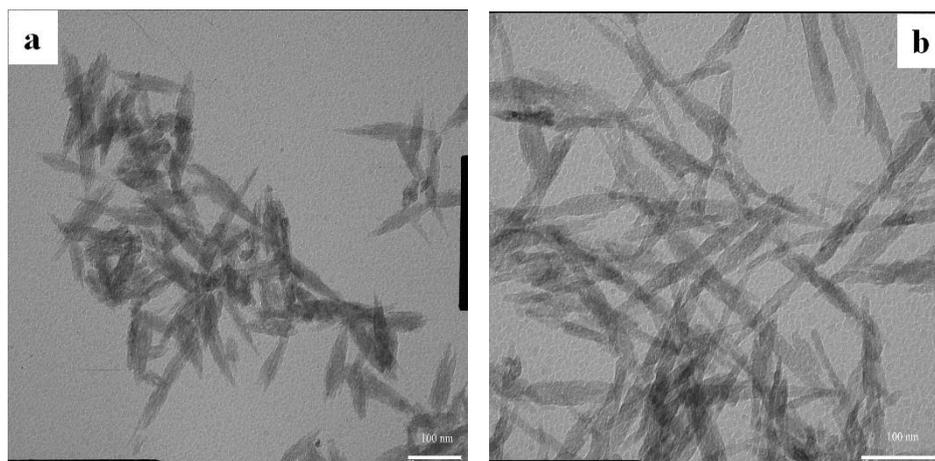
Figure 1: TEM images of CHAP nanoparticles in hydrogel; a) C150 and b) C167.

The dried white powders of CHAP are particles of 1-10 microns in size, which are insoluble in alkalis and partially stable in weakly acidic medium. Scanning Electron Microscope (SEM) micrographs of the powders are shown in Fig. 2.
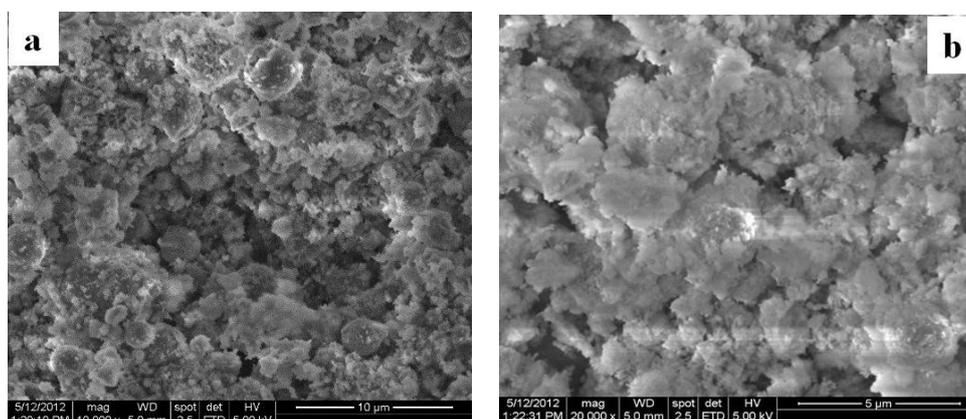
Figure 2: SEM micrographs of CHAP powders: a) C150 and b) C167

Table 2: Crystallochemical characteristics of CHAP samples

Sample	Number of phases	Parameters of the crystal unit cell, Å		Crystal size, nm	Crystallinity, %
		a=b	c		
C150	1	9.424	6.905	35.7	94.7
C156	1	9.434	6.895	73.9	87.8
C162	1	9.467	6.902	32.0	92.4
C167	1	9.380	6.881	17.7	92.0

Crystallochemical characteristics of the samples are presented in Table 2. X-ray diffraction analysis of the CHAP powders showed that all the samples possess apatite crystal structure and are single-phase, i.e. possible impurities which can arise in the synthesis process ($CaCO_3$, $CaHPO_4$ etc.) are not present in the detection limit of the XRD method.

The Fourier transform infrared spectroscopy (FTIR) spectra of modified CHAP was compared to that of unmodified HAP (Fig. 3). CHAP has two peaks originating from v_2 vibrational mode of CO_3^{2-} ions at 1481 cm^{-1} and 1416 cm^{-1} and one peak from v_3 vibrational mode at 876 cm^{-1}. HPO_4 groups also give a vibrational band at around 875 cm^{-1}, which can be identified if the sample do not contain carbonates. However, in this case the HPO_4 band overlaps with the v_3 band of CO_3^{2-} ions so they become indistinguishable.
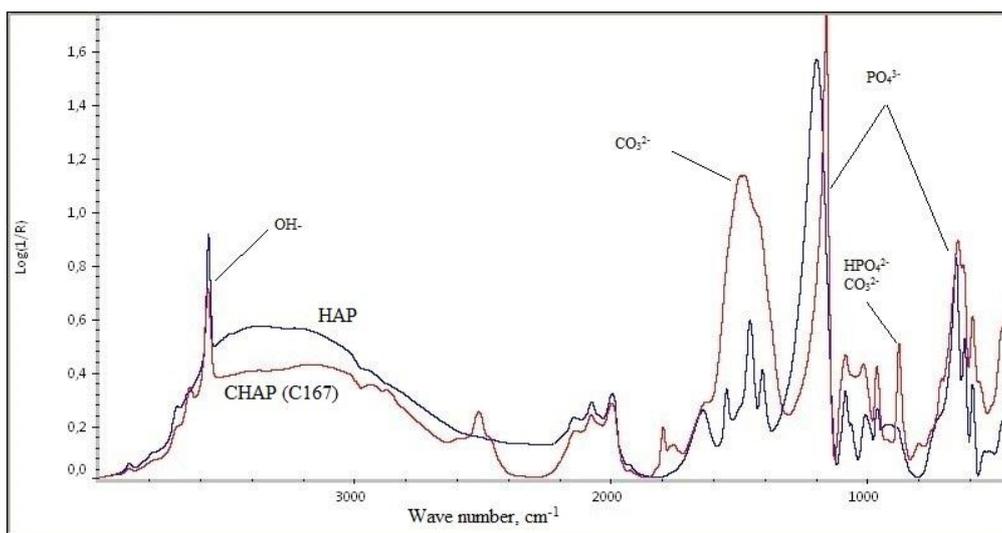
Figure 3: FTIR spectra of CHAP and HAP

In general, synthetic calcium-deficient HAP usually contains adsorbed water, which can be easily removed at relatively low temperature of heating (around 100°C), and structural water, which incorporates into the crystal structure of the material and is much more difficult to eliminate. According to DTA data, structural water loss in the sample C167 starts at 517.8^{o}C and carbonate loss starts at 611.8^{o}C (Fig. 4).
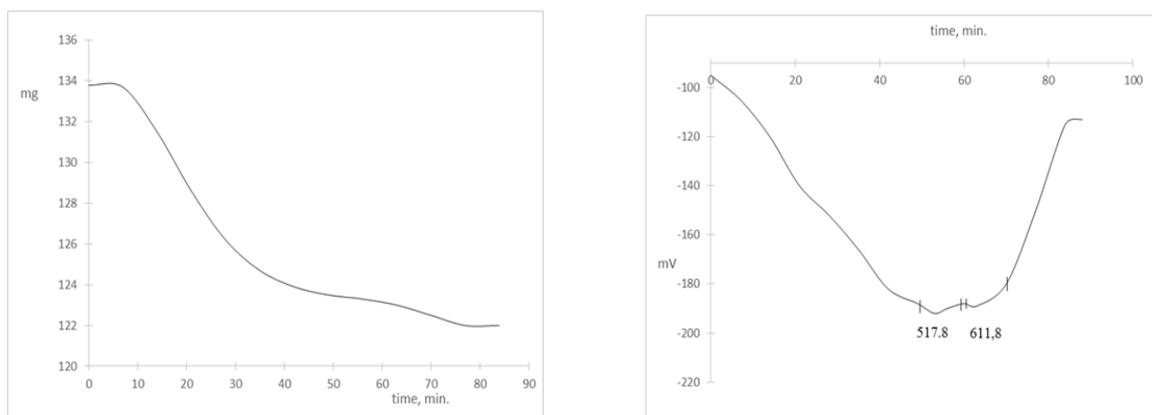
Figure 4: TG and DTA diagrams of sample C167

Results of specific surface area, pore volume and pore size determined by BET method are given in Table 3. The values of specific surface area are relatively high compared to data in literature (40-60 m^2/g) [17-19]. There is a tendency of increasing specific surface area and decreasing pore volume and pore size as the calcium deficiency increases.

Table 3: Specific surface area, pore volume and pore size of CHAP samples by BET method

Sample	Specific surface area, m^2/g	Pore volume, cm^3/g	Pore size, nm
C150	184.3	0.41	8.7
C156	174.0	0.45	10.4
C162	164.0	0.44	10.8
C167	91.0	0.50	21.8

CONCLUSION

Single-phase nano-sized carbonated hydroxyapatite with various degrees of calcium deficiency were synthesized using wet method. The samples were characterized using XRD, FTIR, TEM, SEM, TG-DTA and BET methods. The zeta potential of the samples ranges from -27.8 mV to 12.3 mV. In samples with high degree of calcium deficiency the zeta potential values are negative with increasing magnitude, which is preferable in application in living organisms. On the basis of these facts we can conclude that synthetic calcium-deficient carbonated hydroxyapatite can become a promising biomaterial as orthopedic and dental implants.

ACKNOWLEDGEMENTS

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