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Synthesis of Ca-hydroxyapatite Bioceramic from Egg Shell and its Characterization

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Abstract

Ca-hydroxyapatite (HA) was synthesised from egg shell under different conditions and characterised by using AAS, FT-IR, XRD, SEM and EDS techniques. The results of these analyses combined together indicate that sintered HA sample1 resembles the feature of pure and single apatite phase having hexagonal structure. A Ca/P molar ratio of 1.69 was achieved in this case. On the other hand HA obtained from calcined egg shell is a combination of both amorphous and crystalline phase and revealed a sub-stoichiometric Ca-deficient apatite form with a Ca/P ratio of 1.50.

Keywords: Ca-hydroxyapatite, Biomaterials, Hexagonal, stoichiometric apatite, Ca-deficient apatite

Introduction

Recently, applications of bioceramic materials in biomedical fields has received considerable attention (Feng *et al.* 2005, Xu *et al.* 2001, Balázsi *et al.* 2007, Sasikumar *et al.* 2006) and researchers are now actively engaged in developing many routes to synthesis Ca-phosphate based bioceramic materials using different sources of calcium (Ca) and phosphate (P) (Feng *et al.* 2005, Xu *et al.* 2001, Balázsi *et al.* 2007, Sasikumar *et al.* 2006). Bioceramics are required to alleviate pain and restore function of diseased and damaged body parts. Ca-phosphate based

synthetic ceramic materials, particularly, Ca-hydroxyapatite (HA), β -tricalcium phosphate (β -TCP) and their composites are extensively used for medical purposes that includes tissue engineering, implants, coating on prostheses and bone filling materials (Prabakaran *et al.* 2005). However, among these bioceramics, HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_4$, is an important inorganic material in both biology and chemistry (Markovic *et al.* 2004) and due to its excellent bioactive, biocompatible nature and bone bonding ability with surrounding tissues it is widely used in tissue

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engineering and bone replacement (Balázsi *et al.* 2007). It is well known that human bone is a hybrid composition of inorganic (~ 70% apatitic calcium phosphate with a Ca/P ratio 1.66) and organic (~ 30% collagen) materials. The demands for synthetic HA as a substitute material for bone and teeth in orthopedic and dentistry fields are increasing day by day. Considering the numerous applications of HA in biomedical fields, development of various synthesis methods is a major issue now and obviously, a simple cost-effective procedure is highly desirable to the researchers. A number of synthesis techniques using various sources of Ca and P have been developed which includes wet chemical method (precipitation), sol-gel method, hydrothermal synthesis procedure, continuous precipitation, thermal deposition and solid state reaction method (Feng *et al.* 2005, Balázsi *et al.* 2007, Thamaraiselvi *et al.* 2006). However, among these methods, wet chemical precipitation route is popular because of its simple, cheap and easy application in industrial production.

Although the demand of wet precipitation method scores high, the choice of raw materials to use as Ca and P precursors is the key factor in any method to be developed. From this point of view, in this work we have adapted wet chemical method to synthesize pure HA bioceramics with Ca/P ratio 1.66, using egg shell as Ca source. Depending on feeding egg shell contains about (94 - 97) %

CaCO₃ while rest of the percentage is organic matter and egg pigment. Egg shell is a waste material after the usage of egg. Hence, utilisation of egg shell will benefit in two ways: (i) egg shell derivated HA will be cost-effective bioceramic material for biomedical applications, and (ii) utilisation of egg shell will be an effective material-recycling technology for waste management.

Materials and Methods

Synthesis of HA

At first the raw egg shells were cleaned thoroughly with water followed by boiling for ½ hr. It was then dried overnight at 110°C in an oven and then crushed and powdered using an agate mortar. This egg shell powder was then divided into two portions: one portion remained uncalcined and percentage of CaCO₃ (~ 94%) was determined via titration method, while the 2nd portion was calcined at 950°C to burn out the organic and other substances. Both the uncalcined and calcined egg shell powder was used to synthesize HA according to the following procedure.

A stoichiometric amount of egg shell powder (both uncalcined and calcined) was dissolved in conc. HNO₃ and 15 mL of distilled water was added to the acidic egg shell solution and filtered to get clear solution. Final volume was made up to 50 mL with distilled water and the pH of the solution was increased to ~ 10.0 with aqueous ammonia. A requisite amount of 0.10 M Na₂HPO₄ · 12H₂O in ammonia solution (pH ~ 10.0) was

added drop wise to the egg shell solution with continuous stirring condition. White gelatinous precipitate of HA was formed which was stirred for overnight in the mother solution for ripening. The precipitate was filtered through a Buchner funnel and thoroughly washed with plenty of distilled water. At this stage, the filtered precipitate was dried at 110°C to remove any trace of water. The HA (sample 1) synthesised from uncal-

cined egg shell was then subjected to calcination at 900°C for $\frac{1}{2}$ hr, while the HA (sample 2) obtained from calcined egg shell remained as received, i.e. no further calcination was done, but crushed to achieve fine powder. A flow diagram of the synthetic procedure is given in Figure 1.

All the chemicals used for this study were Analar grade either from E. Merck or BDH

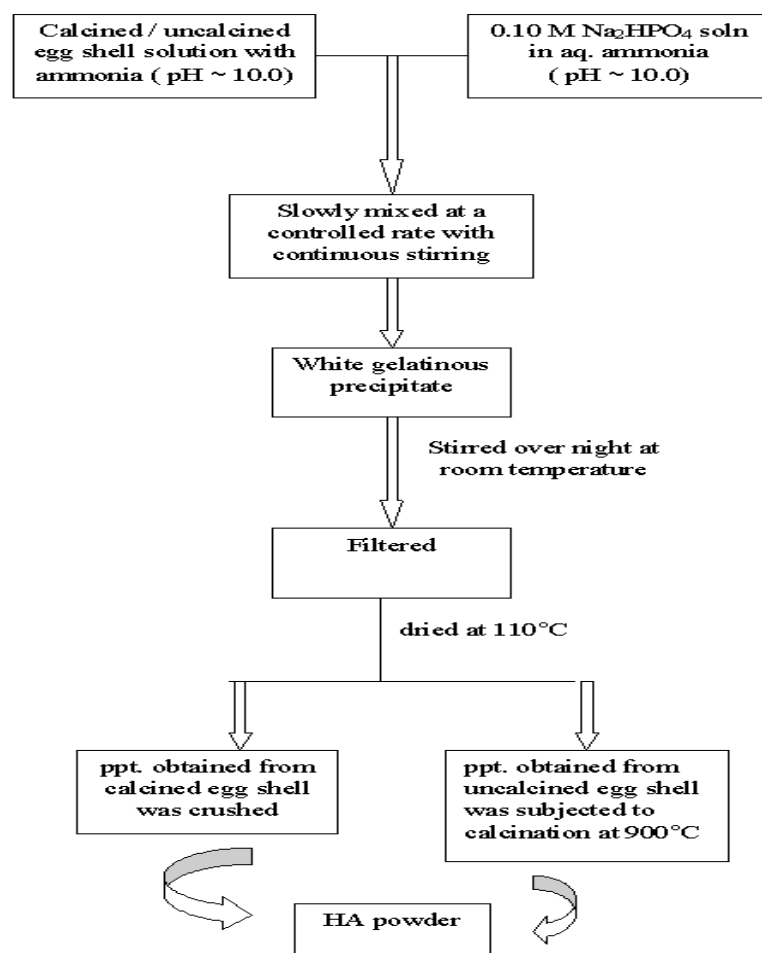


Fig. 1. Flow diagram of hydroxyapatite synthesis procedure from egg shell

and the solutions were prepared using double distilled water.

Characterisation of synthesised HA powders

The semi quantitative elemental information of the samples was achieved by energy dispersive spectroscopy (EDS) as a preliminary technique. Then chemical analysis was done to determine the percentage of Ca and P by atomic absorption (AAS) and UV spectrophotometric methods respectively. Fourier transform infrared spectroscopy (FT-IR, Model no. FT-IR - 8900, SHIMADZU) was used to identify the functional groups. Experimental spectra were obtained by using KBr disks with a 1:100 "samples-to-KBr" ratio and the samples were scanned in the wave number range of 4000 cm^{-1} - 400 cm^{-1} with an average of 30 scans. The resolution of the spectrometer was 4 cm^{-1} . Phase purity of the synthesised samples was examined by using PANalytical (X'Pert PRO XRD PW 3040). The intensity data were collected in 0.02° steps following the scanning range of $2\theta = 20^\circ - 80^\circ$ using CuK α ($\gamma = 1.54178^\circ\text{A}$) radiation. The phases developed in both the samples were compared and confirmed using standard JCPDS files (card no. 89-6439). The surface morphology and microstructural features of the samples were observed by Hitachi, S-3400N scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS).

Results and Discussion

The EDS spectra of HA samples are shown in Figures 2a and 2b respectively. The EDS spectrum of HA (sample 2) clearly shows the peaks of Ca, P, C and O as expected. The presence of C is quite usual in this case as the sample is only oven dried at 110°C , whereas in case of HA sample 1 which was calcined at 900°C , there is no trace of C. This observation was further validated by FT-IR and XRD results. The Ca/P molar ratio (1.74 and 1.499 for sample 1 and 2 respectively) was qualitatively calculated using EDS data. However, the chemical assessment provided quantitative information regarding the Ca/P molar ratio of the HA samples. A ratio of 1.69 confirmed the stoichiometric nature of HA sample 1 whereas Ca/P molar ratio of 1.50 showed the sample 2 as sub-stoichiometric calcium deficient HA.

The FT-IR spectra of the HA samples are shown in Figures 3a and 3b. The band positions and their respective assignments are given in Table I. The IR spectra of both the HA samples (1 and 2) exhibited well-defined absorption bands in the range of $962 \sim 1040\text{ cm}^{-1}$ and $565 \sim 635\text{ cm}^{-1}$, characteristic of a well-crystallized apatite phase (Xu *et al.* 2001). The peaks appeared at 1039.6 cm^{-1} (HA sample 1) and 1035.7 cm^{-1} (HA sample 2) are assigned to asymmetric stretching vibration (ν_3) of PO_4^{3-} groups, while the peaks at 962.4 cm^{-1} corresponds to the sym-

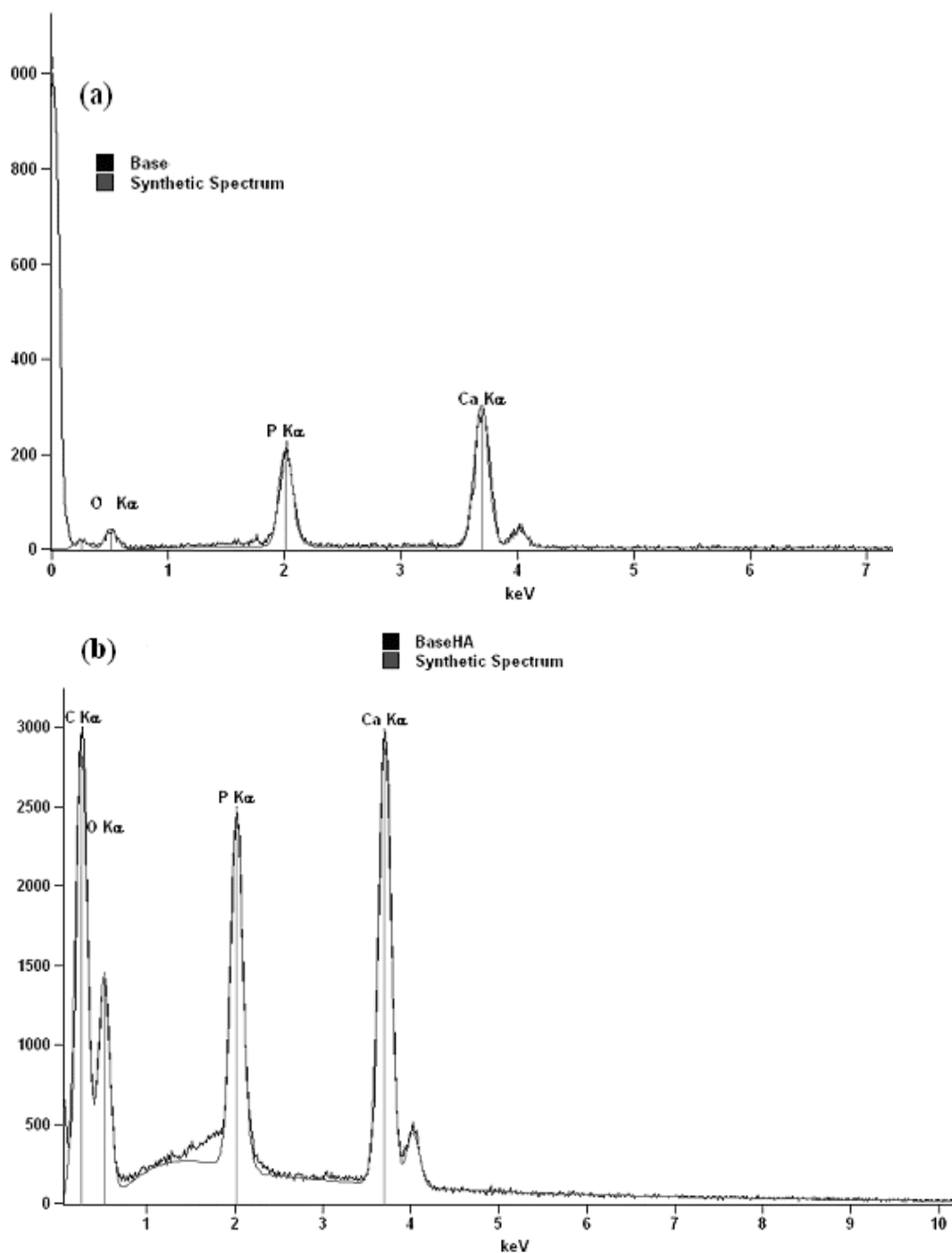


Fig. 2. EDS spectra of synthesised HA (a) HA sample 1 (b) HA sample 2.

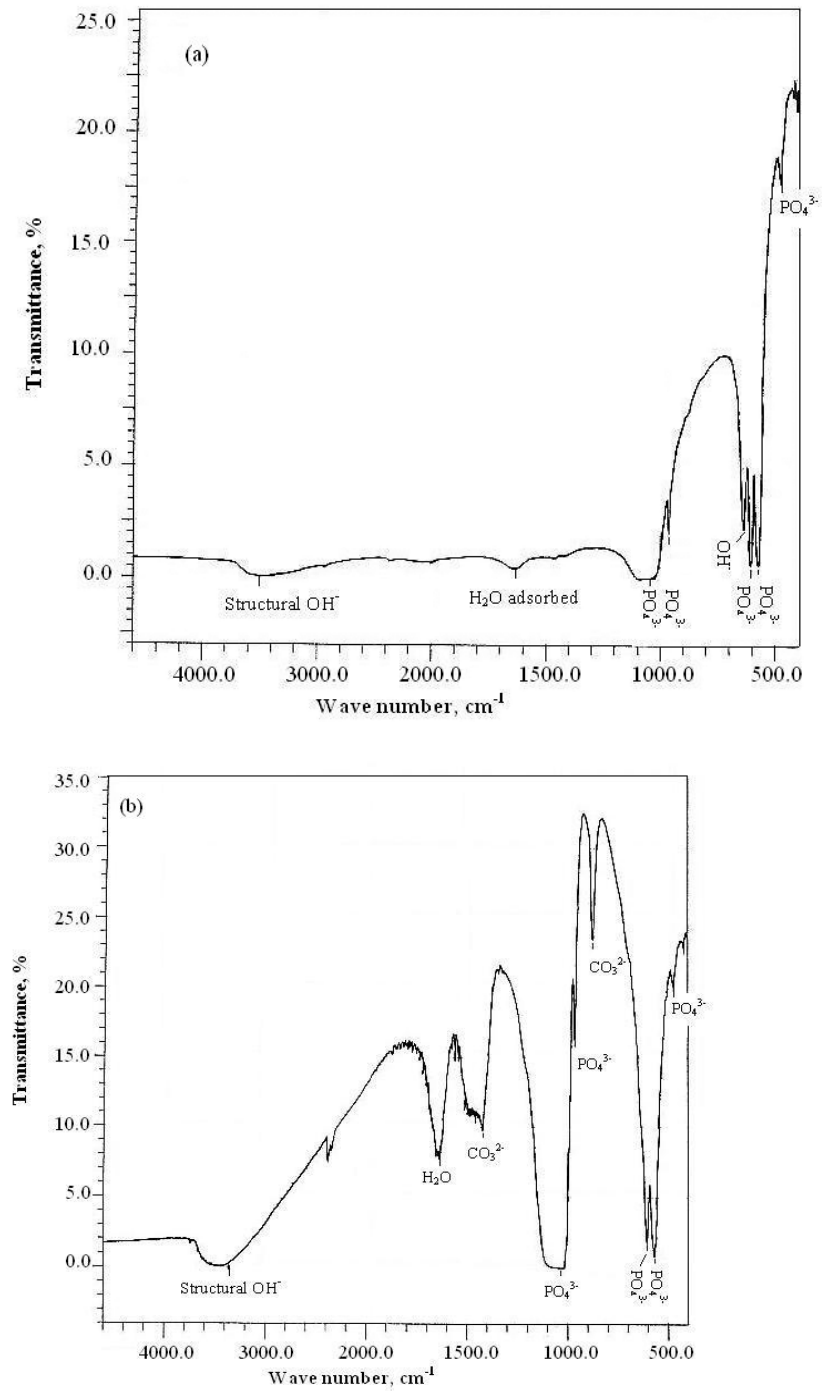


Fig. 3. FT-IR spectra of synthesised HA (a) HA sample 1 (b) HA sample 2.

Table I : FT-IR band positions and their corresponding assignments

Observed band positions (cm ⁻¹)		Corresponding assignments
HA sample 1	HA sample 2	
474.5	472.5	PO ₄ ³⁻ bending (ν ²)
570.9	565.1	PO ₄ ³⁻ bending (ν ⁴)
601.7	603.7	PO ₄ ³⁻ bending (ν ⁴)
632.6	----	OH liberation (Structural OH-)
----	873.7	CO ₃ ²⁻ group
962.4	962.4	PO ₄ ³⁻ stretching (ν ¹)
1039.6	1035.7	PO ₄ ³⁻ bending (ν ³)
---	1419.5	CO ₃ ²⁻ group (ν ³)
1650.0	1635.5	H ₂ O adsorbed (ν ²)
3500.0	3361.7	Structural OH-

metric stretching (ν₁) mode of PO₄³⁻. Bending (ν₃) mode of PO₄³⁻ group is observed at positions 474.5 cm⁻¹ and 472.5 cm⁻¹ for samples **1** and **2** respectively (Tas 2000). The sharp and peaks at 601.7 and 570.9 cm⁻¹ for sample **1** and 603.7 and 565.1 cm⁻¹ for sample **2** are assigned to the bending mode of PO₄³⁻. The noticeable large separation of these two bands further suggests the presence of crystallized apatitic phase (LeGeros 1991, Tas 2000). The IR spectra of the HA sample **2** (Fig. 3b) resembles the nature of hydrated carbonated apatite. The absorption bands observed in the region 1419.5 cm⁻¹ and 873.7 cm⁻¹ are due to the vibrational mode of CO₃²⁻ group associated in amorphous phase (Xu *et al.* 2001). This observation is consistent with the XRD results. However, in case of calcined HA (Sample **1**, calcined at 900°C), there is no noticeable bands for CO₃²⁻ group (Fig. 3a)

indicating the elimination of CO₃²⁻ due to the release of volatile gas. This sample also shows a sharp band at 632.6 cm⁻¹, which is indicative of the presence of structural OH- (OH liberation mode), whereas in the IR spectrum of HA sample **2**, the same band is not visible. This result attributed to the formation of good crystallinity for the HA sample **1** after calcination at 900°C which is also evidenced from the corresponding XRD data (Fig. 4a). The bands observed at 1635.5 cm⁻¹ and 3361.7 cm⁻¹ in HA sample **2** confirms the presence of adsorbed H₂O which is quite usual since this sample remains in uncalcined state. On the other hand in case of sample **1**, these bands are very weak indicating the loss of H₂O molecules during calcination, but the additional band at 3500 cm⁻¹ clearly represents the hydroxyl stretching mode associated with surface P-OH groups (Mahabole *et al.* 2005).

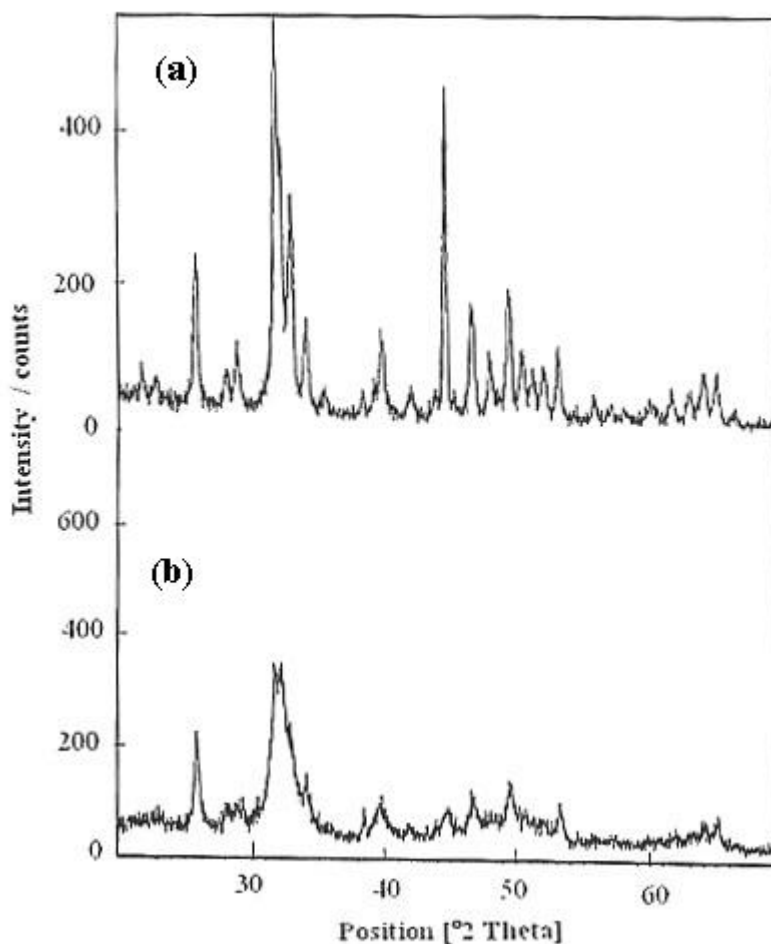


Figure 4. XRD spectra of synthesised HA (a) HA sample 1 (b) HA sample 2.

A typical XRD diffraction of sample **1** is presented in Figure 4a. The major d spacing values are compared with the JCPDS standard data (Ref. code: 89-6439) for HAP and are summarized in Table II. A good agreement between the experimental data (achieved for sample **1**) and JCPDS standard for calcium hydroxyapatite (Ref. code: 89-6439) both in terms of intensity and d -spacings (Table II) was obtained. Particularly the strong diffrac-

tion peaks corresponding for hydroxyapatite at 2θ position 31.7858° (211) plane together with other two peaks at 32.2665° and 32.9515° of almost equal intensities confirmed the formation of pure hydroxyapatite in crystallite form [19]. However no peak was detected at 29.399° for calcium carbonate with HA phase (Vijayalakshmi *et al.* 2006) which also satisfies the IR spectral data for HA sample **1** where the elimination of CO_3^{2-} peak has already been observed.

Table II. Indexed *d*-spacings (in hexagonal unit cell) of XRD data obtained for synthesised HA sample 1

<i>d</i> -spacings (°A)		Intensity (%)		(h k l)
Experimental	JCPDS	Experimental	JCPDS	
4.0748	4.0777	6.38	6.40	2 0 0
3.8999	3.8885	4.45	5.80	1 1 1
3.4395	3.4399	38.68	35.50	0 0 2
3.1673	3.1695	8.36	8.50	1 0 2
3.0861	3.0825	14.60	14.15	2 1 0
2.8152	2.8131	100.00	100.00	2 1 1
2.7744	2.7776	59.40	47.60	1 1 2
2.7183	2.7185	55.42	64.20	3 0 0
2.6296	2.6293	23.44	21.90	2 0 2
2.5271	2.5282	5.30	4.00	3 0 1
2.2619	2.2619	19.31	22.20	1 3 0
2.1482	2.1487	5.28	5.50	1 3 1
2.0610	2.0617	5.54	5.20	1 1 3
1.9443	1.9428	28.19	27.60	2 2 2
1.8914	1.8899	14.58	11.70	1 3 2
1.8413	1.8399	32.19	30.30	2 1 3
1.8060	1.8054	15.67	16.00	3 2 1
1.7806	1.7797	11.83	11.00	1 4 0
1.7538	1.7539	12.30	11.90	4 0 2
1.7212	1.7199	15.73	13.70	0 0 4
1.6447	1.6436	4.41	5.60	3 2 2
1.4742	1.4738	6.99	7.40	5 0 2

The crystallite sizes for HA sample **1** were calculated from Scherrer's relationship $D = 79.5/\Delta \cos \theta$, where D = crystal size (°A), Δ = FWHM in degree.

The Bragg reflection at (211), (112) and (300) planes of this sample were considered to calculate the crystallite size. The sizes of the crystallites for these phases are 41 nm,

65 nm and 34 nm respectively. The lattice parameters of this HA sample **1** were measured as $a = b = 9.4164$ °A and $c = 6.8789$ °A which are very close to those values obtained from the JCPDS (ref. code: 89-6439) standard data ($a = b = 9.4172$ °A and $c = 6.8799$ °A) and the lattice parameters of human bones ($a = b = 9.419$ °A and $c = 6.88$ °A)

(LeGeros *et al.* 1993). This good agreement between the experimental data and standard values reflects that the synthesized HAP sample resembles the feature of pure and single apatite phase having hexagonal structure. The formation of apatite of single phase also confirmed the Ca/P molar ratio 1.66 which is very close to that of crystalline apatite (Elliot *et al.* 1994, Xu *et al.* 2001).

Since sintering temperature or calcination effect plays an important role in the formation of crystallite phase (Feng *et al.* 2005), the XRD profile of the as received HA

sample (i.e. after oven dried at 110°C) was recorded and displayed in Figure 4b. Although the formation of hydroxyapatite was confirmed by comparing the experimental data with the JCPDS standard file (ref. code: 89-6439), but it is cleared from the XRD pattern that the HA sample under investigation has formed in poorly crystalline phase together with amorphous phase supporting the IR informations. This behaviour can be attributed to the temperature effect, since this sample 2 remained in only oven dried state. It is well established that the increasing of sintering temperature

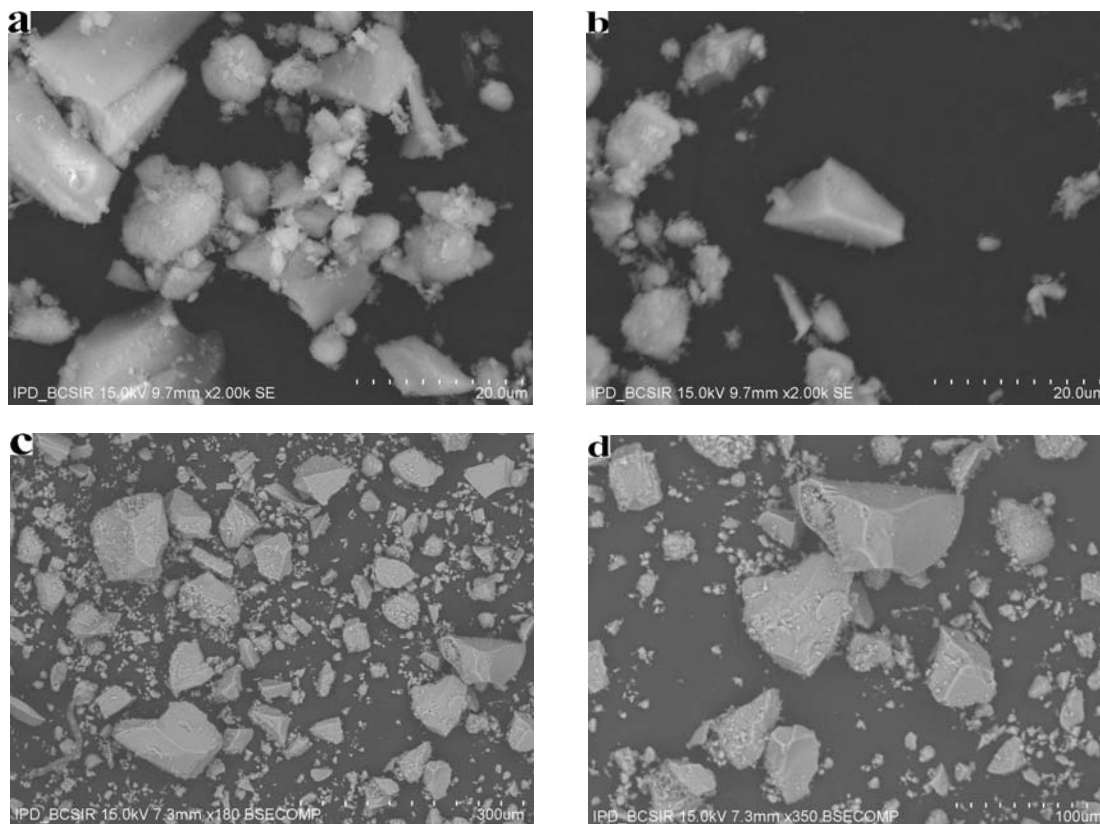


Figure 5. SEM micrographs of synthesised HA sample 1 (a and b) magnification 2000 (c) magnification 180 and (d) magnification 350.

increases the crystallite degree resulting several distinct peaks for HA (Feng *et al.* 2005). So the low crystallinity and amorphous nature can be upgraded or improved to a solid, crystalline phase via the thermal treatment (Santos *et al.* 2004).

The morphology and micro structural features of the HA samples (both 1 and 2) recorded at different magnifications are shown in SEM micrographs. The micrographs (Fig. 5a - 5d) representing the

micro structural features of HA sample 1, exhibit a combination of spherical, hexagonal and cylindrical shapes having crystal size of ~30 - 60 nm. On the other hand, the different magnified micrographs of HA sample 2 (Fig. 6a - 6b) does not shows clear crystallite phases since the sample was a combination of amorphous and crystallite phase.

Conclusions

Ca-hydroxyapatite nano powders have been successfully synthesised using calcined and uncalcined egg shell as the prime source of Ca. The results showed that HA sample 1 consists of single crystallite apatite phase and sample 2 contains both amorphous and crystalline phase. The crystallinity of this later sample can be improved by further sintering of the product. However, the egg shell derivated HA will no doubt be a cost-effective bioceramic material for biomedical applications. Additionally, utilisation of egg shell will be an effective material-recycling technology for waste management.

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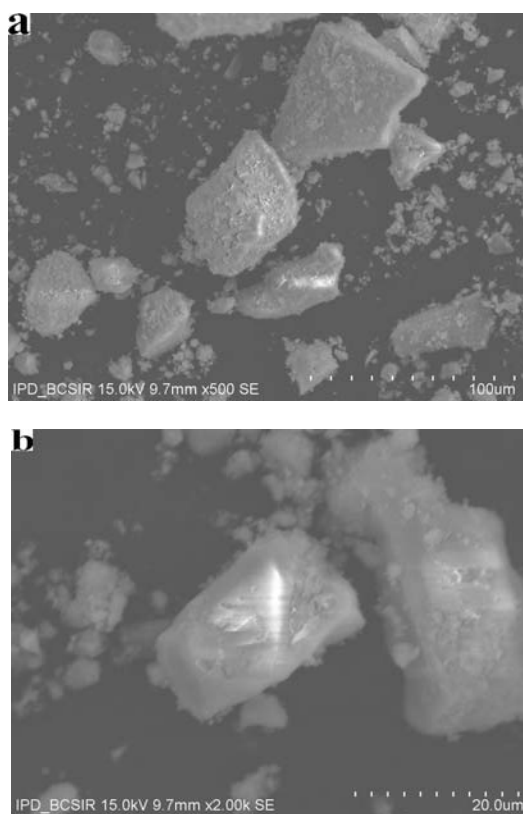


Fig.6. SEM micrographs of synthesised HA sample 2 (a) magnification 500 (b) magnification 2000.

References

- Balázs, C. Wéber, F. Kövér, Z. Horváth, E. Némth, C. (2007) Preparation of calcium-phosphate bioceramics from natural resources, *J. Euro. Ceram. Soc.* **27**: 1601.
- Elliot, J.C. (1994) Structure and Chemistry of The Apatites and The Other Calcium Orthophosphates (Elsevier Science, Amsterdam).
- Feng, W. Mu-sen, L. Yu-peng, L. Yong-xin, Q. (2005) A simple sol-gel technique for preparing hydroxyapatite nanopowders, *Mater. Lett.* **59**: 916.
- Mahabole, M.P. Aiyer, R.C. Ramakrishna, CV. Sreedhar B., Khairnar RS., (2005) Synthesis, characterization and gas sensing property of hydroxyapatite ceramic, *Bull. Mater. Sci.*, **28(6)**: 535.
- LeGeros, R.Z. Calcium Phosphate in Oral Biology and Medicine, Karger: Basel, Switzerland, 1991.
- LeGeros, R.Z., LeGeros, J.P. (1993) Dense Hydroxyapatite, In: Hench LL, Wilson J, editors. An introduction to bioceramics. London: World Scientific Publishing Co, 144.
- Markovic, M. Fowler, B.O. Tung, M.S. (2004) Preparation and Comprehensive Characterization of a Calcium Hydroxy - apatite Reference Material, *J. Res. Natl. Inst. Stand. Technol.*, **109(6)**: 553.
- Prabakaran, K. Balamurugan, A. Rajeswari, S. (2005) Development of calcium phosphate based apatite from hen's eggshell *Bull. Mater. Sci.*, **28(2)**: 115.
- Santos, M.H. Oliveira, M de. Souza LPdeF., Mansur, H.S. Vasconcelos. W.L.(2004) Synthesis Control and Characterization of Hydroxyapatite prepared by wet precipitation process, *Materials Research*, **7(4)**: 625.
- Sasikumar, S. Vijayaraghavan, R. (2006) Low Temperature Synthesis of Nanocrystalline Hydroxyapatite from Egg Shells by Combustion Method, *Trends Biomater. Artif. Organs*, **19(2)**: 70.
- Thamaraiselvi, T.V. Prabakaran, K. Rajeswari, S. (2006) Synthesis of Hydroxyapatite that Mimic Bone Mineralogy, *Trends Biomater. Artif. Organs*, **19(2)**: 81.
- Tas, A.C. (2000) Synthesis of biomimetic Ca-hydroxyapatite powders at 37°C in synthetic body fluids, *Biomaterials*, **21**: 1429.
- Vijayalakshmi, U. Rajeswari, S. (2006) Preparation and Characterization of Microcrystalline Hydroxyapatite Using Sol Gel Method, *Trends Biomater. Artif. Organs*, **19(2)**: 57.
- Xu, G. Aksay, I.A. Groves, J.T. (2001) Continuous Crystalline Carbonate Apatite Thin Films. A Biomimetic Approach, *J. Am. Chem. Soc.*, **123**: 2196.

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