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Development of calcium phosphate based apatite from hen's eggshell

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Abstract. **Stoichiometric hydroxyapatite with Ca/P molar ratio, 1·67, was synthesized using hen's eggshell as calcium source and phosphoric acid by precipitation method. Conventional EDTA titration and gravimetric methods were adopted to estimate the amount of calcium and phosphorous, respectively. Fourier-transform infrared (FT–IR) and X-ray diffraction (XRD) techniques were employed to investigate the formation of the HAP phase. Thermal analysis (TG–DTA) was carried out to investigate the thermal stability of HAP powder. FT–IR spectra show the characteristic peaks for phosphate and hydroxyl groups. XRD results reveal that the major characteristic peaks of HAP appear in the region of approximately 26°, 28°, 29°, 30–35°, 39°, 46°, 49° and 50° (2*q*) and also indicate that there are no occurrences of secondary phases during HAP formation. TG–DTA result depicts that the synthesized HAP was stable up to 1300°C.**

Keywords. **Hydroxyapatite (HAP); eggshell; phosphoric acid; precipitation.**

1. Introduction

It is well known that the composition of human bone is an inorganic/organic hybrid consisting of ~ 70% apatitic calcium phosphate and 30% organic (largely collagen) constituents, by weight (Cowin *et al* 1987). The apatitic calcium phosphate of bone mineral consists of carbonate, small amount of sodium, magnesium and other trace elements. The submicroscopic crystal of calcium phosphate in bone resembles the crystal structure of synthetic hydroxyapatite, [HAP, $Ca_{10}(PO_4)_6(OH)_2$] (Sinha *et al* 2001). Hence HAP has received great attention in the field of biomedical engineering.

Artificially prepared HAP, with the same structure as that of apatitic phosphate of natural bone has good biocompatibility with the human organism. It forms chemical bond with the host hard tissue and for this reason, it is widely used in medical applications as implants, as coating on prostheses or as bone filling material. HAP powder can be synthesized via numerous production routes, using a range of different reactants. Some of the processing techniques include dry process (Fowler 1974), wet chemical method (precipitation), hydrolyzation of calcium phosphate (Monma and Kamiyua 1987), hydrothermal synthesis (Takeo *et al* 1989; Sada *et al* 1991), spray pyrolysis (Senya and Akira 1987), freeze-drying (Takeo *et al* 1987), gel-diffusion, sol–gel technique (Yoshio *et al* 1990) and electrochemical deposition. Of these methods, precipitation scores over other processes by virtue of being simple, cheap and easy application in industrial production. Moreover, HAP prepared by precipitation also has the feature of small size, low crystallinity and high surfacial activation, which can meet different demands (Liu *et al* 2001).

Several methods of chemical synthesis have been developed to prepare HAP powder using various types of Ca and P sources (Asada *et al* 1987; Osaka *et al* 1991; Koumoulidis *et al* 2001; Milev *et al* 2003). In the present investigation, an attempt is made to synthesize pure and biocompatible HAP powder by using hen's eggshell as the Ca source. The eggshell consists of about 94–97% of $CaCO_3$ and the other 3% is organic matter and egg pigment. There are also as many as 8000 microscopic pores in the shell itself. The eggshell was found to be a waste material after egg's usage. Hence in this study, the eggshell, a waste material, has been used as a calcium precursor to synthesize pure HAP with Ca/P ratio, 1·67, through wet chemical method. The synthesized powder was characterized by FT–IR, TG–DTA and XRD techniques.

2. Experimental

2.1 *Synthesis of HAP*

Uncrushed and washed eggshell was calcined in an air atmosphere at 900°C for 1 h. The amount of calcium present in the calcined eggshell was estimated. A stoichiometric amount of calcined eggshell was dispersed in well-degassed distilled water. Under rigorous stirring reagent grade orthophosphoric acid solution (0·6 M) was added in drops at a controlled rate to the suspension at room temperature. Initially, the pH of the solution was

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*Author for correspondence (nivapraba@rediffmail.com) found to be 12, but at the end of addition of phosphoric acid, it decreased to 8·5. After completion of the addition, the precipitate formed was subjected to ripening (aging) treatment for 24 h followed by 1 h refluxing. It was then stirred for another 25 min without heating and left for over 10 h. The precipitate was filtered and thoroughly washed with double distilled water and filtered again. After drying at 80°C for 3 h, the precipitate was calcined at various temperatures, say, 400°C, 700°C and 900°C for 2 h.

2.2 *Characterization of powder*

2.2a *Chemical analysis*: The chemical analysis was performed to confirm the Ca/P molar ratio (1·67) of the synthesized powder. The Ca was analysed by EDTA titration and P by conventional gravimetric method. The concentration of Na and Mg present in the sample were analysed using atomic absorption spectrophotometer (AAS) by GBC Avanta system, Germany.

2.2b *FT-IR spectral analysis*: The formation of the HAP phase was tested by FT–IR spectral analysis. FT–IR transmittance spectra of the crushed samples were obtained in the 4000–400 cm^{-1} region on infrared spectrometer (SHIMADZU model 8300) by using KBr pellet technique (0·1 wt%).

2.2c *X-ray diffraction analysis*: The crystallographic phases of the synthesized HAP powders were identified by using X-ray diffractograms (XRD). X-ray analysis was performed by RICH SEIFERT model 3000 diffraction system, Germany, using CuK*a* (*l* = 1·5405 Å) radiation. The intensity data were collected in 0·02° steps in the 2*q* range, 10–70°. The full width at half maximum (FWHM) of the 002 and 003 reflections were taken as a measure of crystallinity.

2.2d *TG–DTA analysis*: The weight loss and thermal stability of the samples were also evaluated from the thermogravimetric analysis data. An HETZSCH-Geratebau GmbH thermal analyser having simultaneous thermal analyser (STA) 409 C microbalance with a heating rate of 10°C min^{-1} was employed.

3. Results and discussion

3.1 *Chemical analysis*

The phase constitution and chemical homogeneity of the sample were examined by quantitative chemical analysis via EDTA titration, gravimetry and AAS. The Ca/P molar ratio was found to be 1·67, which indicates the formation of pure hydroxyapatite. The percentage compositions of Ca, P, Na and Mg are presented in table 1.

3.2 *FT–IR spectral studies*

Figure 1 shows FT–IR spectra of hydroxyapatite heated at various temperatures, say, 80°C, 400°C, 700°C and 900°C. The infrared band positions and their assignments are summarized in table 2. The spectral data indicate that carbonate ion is present in the prepared HAP samples. The carbonate ion substitution is identified by characteristic doublet peak of the carbonate ions around 1420 and

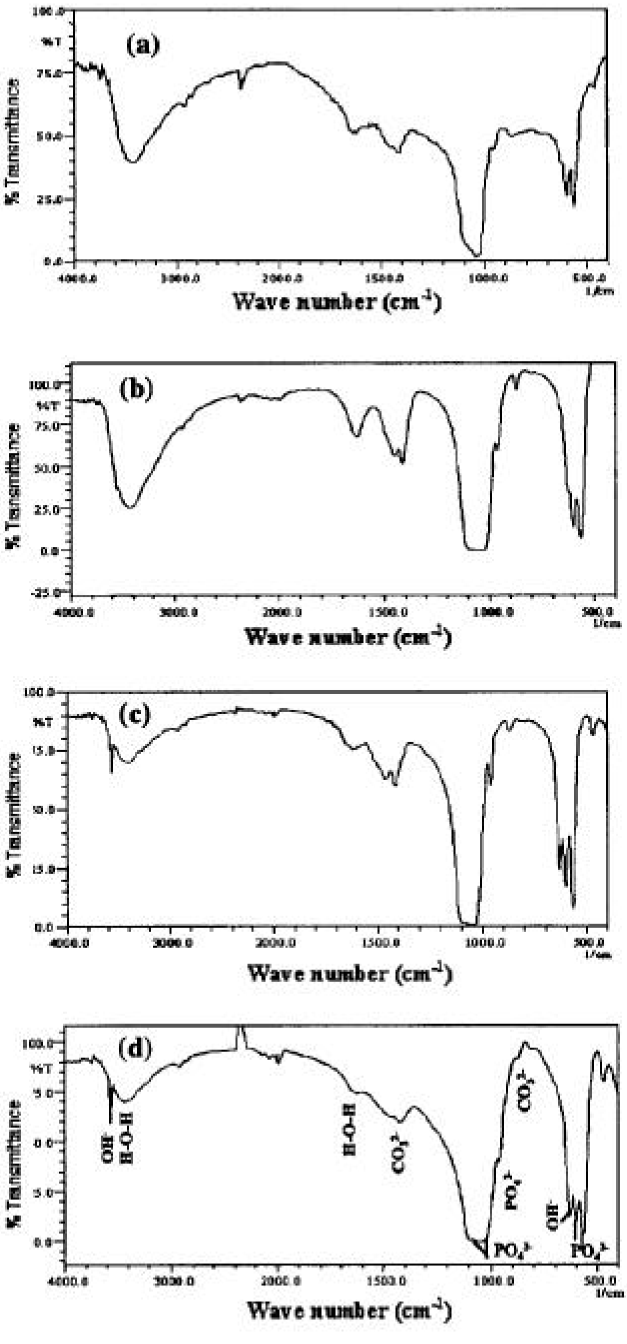

Figure 1. FT–IR spectra of HAP heated at (**a**) 80°C, (**b**) 400°C, (**c**) 700°C and (**d**) 900°C.

1480 cm^{-1} and a singlet peak at 875 cm^{-1} (Stoch *et al* 2000), which is attributed to the vibrational modes of the carbonate ions, substituted at the phosphate site. For samples heated at 80°C these peaks were rather broad, and it became well defined for those samples heated at 400°C and 700°C. However, peaks at 875 cm^{-1} and 1480 cm^{-1} became ill-defined when the samples were heated to 900°C. This confirms the elimination of CO_3^{2-} ion at higher temperature. The broad bands observed at around 1650 and 3440 cm^{-1} indicate adsorbed H_2O in the samples. The bands at around 3440 cm^{-1}, which is due to adsorbed water overlaps with the weak bands at around 3570 cm^{-1}, which is due to structural OH and these bands are not clearly visible for the samples heated at 80°C. The band (bending) due to structural OH in HAP also occurs at around 633 cm^{-1}. This peak for materials heated at 80°C, 400°C, 700°C appear as weak shoulders that gradually deepen and they merge to become a well-developed peak after heating at 900°C. This evolution also indicates good crystallinity for the material heated at higher temperature. The characteristic frequencies derived from PO_4^{3-} modes are seen at around 1090 cm^{-1}, 1040 cm^{-1}, 964 cm^{-1}, 605 cm^{-1} and 570 cm^{-1} and are better resolved with increase in heating temperature.

Table 1. The percentage composition of the constituents of synthesized HAP.

Constituents of HAP	Composition (wt%)
Calcium	39·298
Phosphorous	18·240
Sodium	0·117
Magnesium	0·209

3.3 *X-ray diffraction studies*

A typical XRD profile of HAP powder synthesized by wet chemical method has been shown in figure 2. The XRD phase analysis has been performed using JCPDS card number 09–0432. The samples heated at 80°C show broad peaks indicating the formation of microcrystalline phases, which increase with heating temperature. The crystallite sizes were calculated using Scherrer's relationship,

$$d = k l / b \cos q,$$

where *d* is the average diameter in Å, *k* the shape factor and *b* the broadening of the diffraction angle. The Bragg reflections at (002) and (003) planes of HAP were considered to calculate the crystallite size. The size of the crystallites for these planes were 34 nm and 10 nm for the samples heated at 80°C, 68 nm and 69 nm for those samples heated at 900°C. The expansion does indicate a structural change in the lattice. It should be noted that materials prepared via low temperature wet chemical processes are known to accommodate various ionic species, e.g. H_3O^+, HPO_4^{2-} etc and they may decompose in a variety of ways leading to structural changes (Simpson 1968).

3.4 *TG–DTA results*

Figure 3 illustrates the results of thermal analysis of HAP precursor. Two exothermic peaks at 147·5°C and 489°C and two endothermic peaks at 1335°C and 1363°C were observed in the DTA. In the investigated temperature range, there are three regions of interest in the TG curve: (i) up to 300°C, corresponding to the removal of adsorbed water, (ii) between 300 and 700°C, corresponding to HPO_4^{2-} decomposition according to the reaction: $2HPO_4^{2-}$

Table 2. Assignments of the observed vibrational frequencies of HAP heated at various temperatures.

	Observed vibrational frequencies (cm^{-1})			
Assignments	Heating temp. (80°C)	Heating temp. (400°C)	Heating temp. (700°C)	Heating temp. (900°C)
PO_4 bend ν_4	571	567	571	571
PO_4 bend ν_4	607	605	603	604
Structural OH	635	632	632	633
CO_3^- group	875	876	875	875
PO_4 stretch ν_1	961	964	961	964
PO_4 bend ν_3	1050	1039	1037	1035
PO_4 bend ν_3	1090	1097	1096	1099
CO_3^- group (ν_3)	1423	1416	1416	1423
CO_3^- group (ν_3)	1479	1464	1466	1470
H_2O adsorbed (ν_2)	1628	1636	1624	1630
H_2O adsorbed	3418	3448	3426	3441
Structural OH	–	3570	3572	3572
OH stretch	3740	3744	3744	3744

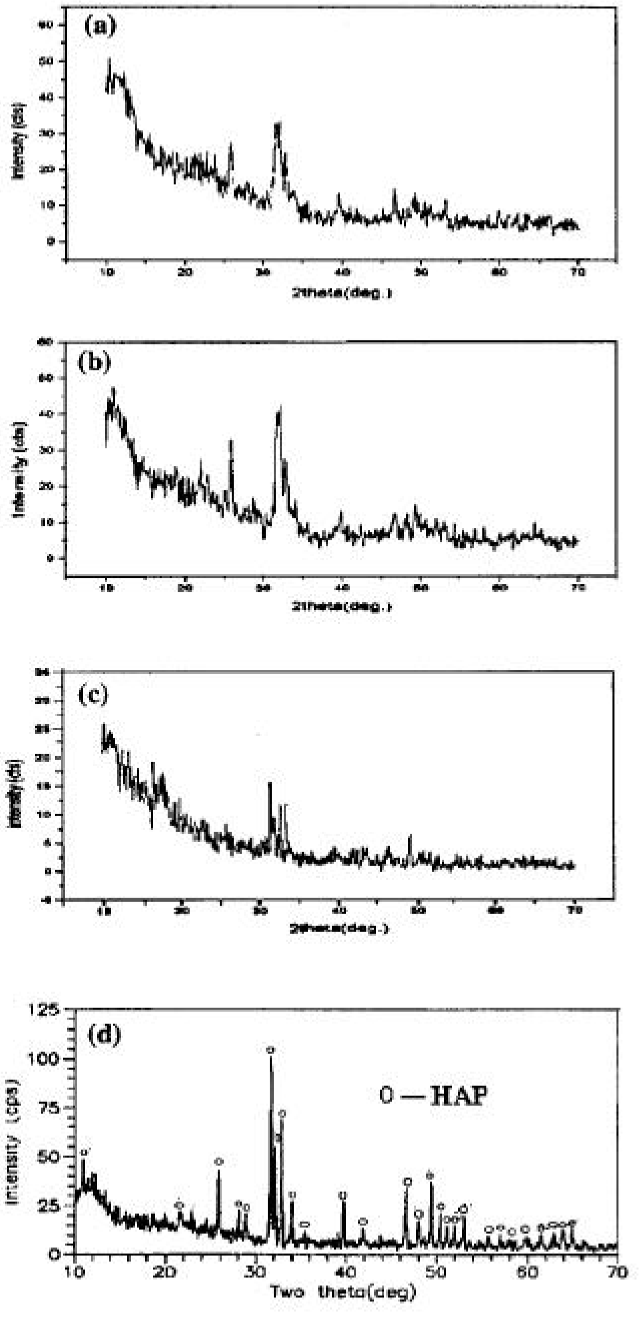

Figure 2. XRD pattern of HAP heated at (**a**) 80°C, (**b**) 400°C, (**c**) 700°C and (**d**) 900°C.

$\rightarrow P_2O_7^{4-} + H_2O$. Removal of interstitial water may also take place in this temperature range and (iii) in the range 700–800°C, reaction $P_2O_7^{4-} + 2OH^- \rightarrow 2PO_4^{3-} + H_2O$ may take place.

The total weight loss measured upon heating to 300°C was 5·87%, which is due to the elimination of adsorbed water. A weight loss in the range of 700–850°C was small compared with the loss measured at lower temperature and can be ascribed to the removal of interstitial water rather than to thermal decomposition of the samples. The

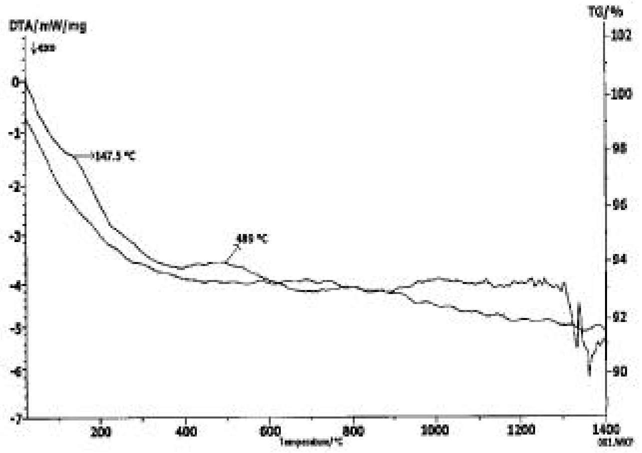

Figure 3. Thermal analysis of HAP.

endothermic peaks at 1335°C and 1363°C on the DTA curve was accompanied by a 8·56% weight loss on the TG curve, which was attributed to the decomposition of HAP to *b*-TCP or dihydroxylation.

4. Conclusions

Stoichiometric, pure and thermally stable HAP powder was synthesized using eggshell and phosphoric acid by precipitation method. FT–IR and XRD analyses indicated the phase purity and crystallinity of the HAP powder. TG–DTA results showed the thermal stability of HAP. The present study suggests the eggshell as a possible material-recycling technology for future waste management and ecology. Also, eggshell-originated HAP is a potential ceramic, which could be useful as an inexpensive ceramic for biomedical applications.

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