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Characterization of hydroxyapatite and carbonated apatite by photo acoustic FTIR spectroscopy

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An understanding of the interfacial relationship between a bioceramic implant and the adjacent bone tissue is facilitated by precise characterization of the associated structures. The structure of different commercial synthetic hydroxyapatite powders and a novel carbonated apatite have been studied with photo-acoustic (PAS) Fourier transform infrared (FTIR) spectroscopy. The PAS technique is an ideal method for analysing biomaterials, as materials can be analysed without the need to reduce the particle size or to dilute with KBr. Spectra from carbonated apatite appear to be different from those of commercial hydroxyapatite powders, with the main difference lying in the carbonate and phosphate ratio. Commercial hydroxyapatite powders from different sources have also been analysed and compared.

1. Introduction

Fourier transform infrared spectroscopy (FTIR) is a widely used analytical technique that is routinely applied to the characterization of biomaterials. However, preparing samples of biomaterials for infrared spectroscopy is often a tedious process. The main sampling problem in FTIR characterization of biomaterials is that nearly all solid materials are too opaque in their normal forms for direct transmission analysis in the mid-infrared region. This problem can be solved by reducing the optical density of samples to a suitable level by employing various sampling techniques [1, 2]. These procedures, however, can alter the nature of the sample and are time consuming.

Various alternative techniques, such as diffuse reflectance (DRIFT) and attenuated total reflectance (ATR) can be employed, but are limited in applications due to stringent surface requirements. Another approach is to avoid totally the opacity of the mid-infrared spectral region and work within the near-infrared region by using overtone and combination absorbance bands for analysis, where absorption coefficients are relatively lower and samples are less opaque [3]. Unfortunately, a limited amount of information is available within the near-infrared spectral region, whereas, the mid-infrared region provides most spectral bands for the required characterization.

Photo-acoustic sampling (PAS) provides a solution to these problems. A photo-acoustic signal is generated when infrared radiation absorbed by a sample is converted into heat within the sample. This heat diffuses to the sample surface and into the adjacent atmosphere. Thermal expansion of this gas produces the PAS signal. The signal generation process isolates a layer extending beneath the sample's surface, which has a suitable optical density for analysis, without altering the sample. The technique directly measures the absorbance spectrum of this layer.

In this study, the PAS technique was employed to characterize a novel carbonated apatite developed in the IRC, and various commercial hydroxyapatites.

The aim of such studies is to identify differences between the carbonated and standard hydroxy apatites and allow comparison of commercial hydroxyapatite powders from different sources. The contributions of the PAS-FTIR technique in the spectrochemical analysis of biomaterials is also accessed.

2. Materials and methods

Commercial hydroxyapatite (HA) powders (P88, P120, P141, P149 types, different batches) as received from Plasma Biotal, HA powder from Merck, and novel carbonated apatite synthesized within IRC in biomedical materials in powder form were analysed.

2.1. Spectroscopic analysis

FTIR spectra were obtained using a Nicolet 800 spectrometer in conjunction with a MTech PAS cell. Spectra were obtained at 4 cm^{-1} resolution averaging 128 scans. The sample chamber of the PAS cell was purged with helium gas and dried by employing magnesium perchlorate as drying agent.

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3. Results and discussion

The spectra of hydroxyapatite and carbonated apatite powders provide a number of spectral details indicating some similarities with each other. Spectra of the powders are given in Figs 1 to 6 and their peak positions are tabulated in Table I.

3.1. Hydroxyl bands

Hydroxyl stretch is observed at 3569 cm^{-1} in the spectra of synthetic commercial hydroxyapatite and carbonated apatite powders. The hydroxyl band at 3569 cm^{-1}, in the spectrum of carbonated apatite has a lower intensity compared to that from commercial samples, which is also masked by broad H_2O absorptions (Fig. 7). The large decrease in the hydroxyl band intensities of the carbonated apatite powder may be attributed to the increase in the carbonate substitution. This effect is measured by calculating the peak area of the carbonate and hydroxyl groups (Table II). The peak area of hydroxyl stretch in the case of carbonated and P120 hydroxyapatite powders is 1.92 and 7.4, respectively, indicating that the hydroxyl group decreases with an increase in the carbonate substitution. The results obtained agree with the data reported by Elliot et al., who suggested the replacement of carbonate ions with hydroxyl ions [4].

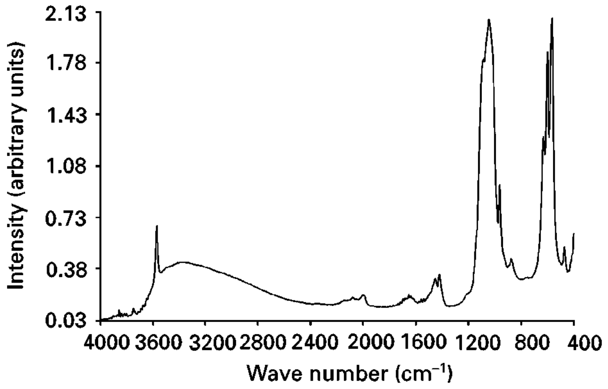

Figure 1 FTIR spectrum of hydroxyapatite (P88) powder.

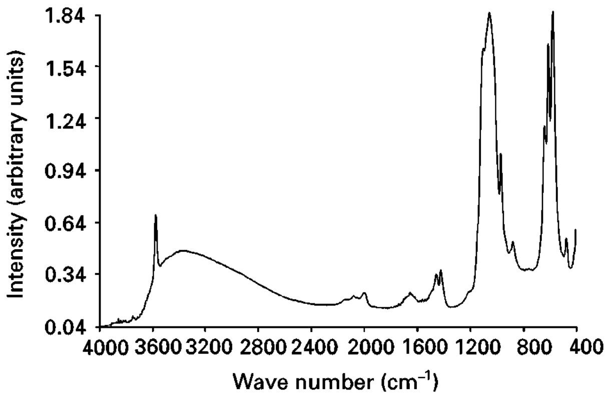

Figure 4 FTIR spectrum of synthetic hydroxyapatite (P149) powder.

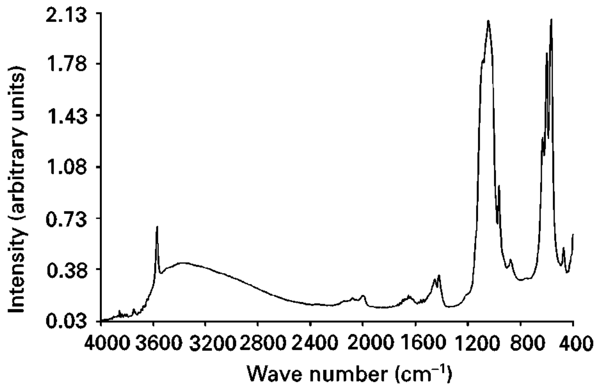

Figure 2 FTIR spectrum of synthetic hydroxyapatite (P120) powder.

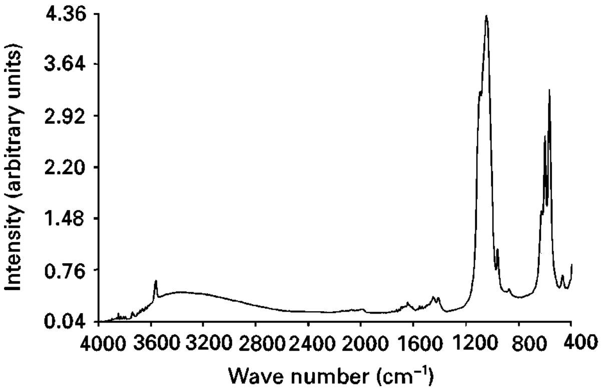

Figure 5 FTIR spectrum of hydroxyapatite (Merck) powder.

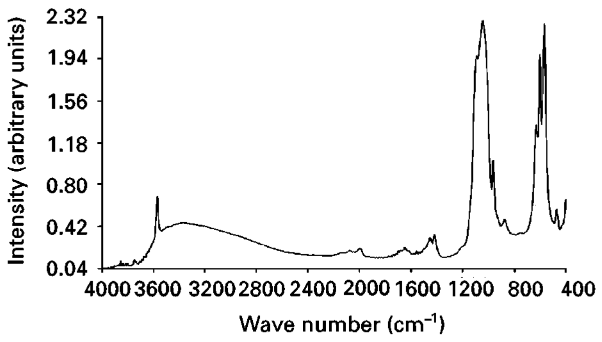

Figure 3 FTIR spectrum of synthetic hydroxyapatite (P141) powder.

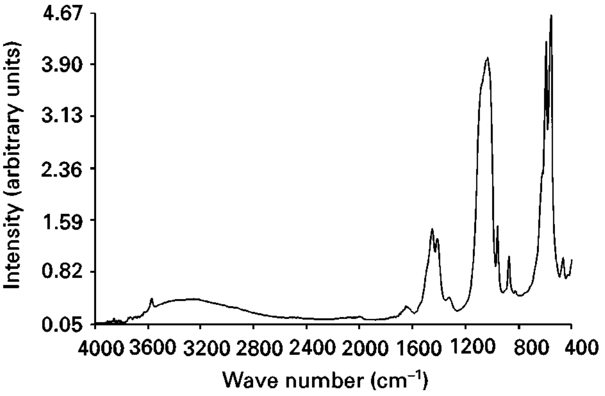

Figure 6 FTIR spectrum of synthetic carbonated apatite powder.

TABLE I Observed infrared band positions for carbonated and hydroxy apatite powders

Peak assignments	Carbonated apatite	Commercial hydroxyapatite powders				
HA cm^{-1}	IRC (CA)	Merck HA	Plasma Biotal (P88)	(P120)	(P141)	(P149)
Hydroxyl stretch	3571	3570	3570	3568	3570	3569
Carbonate ν_3	1650–1300	1650–1300	1650–1300	1650–1300	1650–1300	1650–1300
— (m)	1650	1648	1648	1648	1650	1651
— (m)	1454	1455	1454	1454	1454	1454
— (m)	1417	1417	1419	1419	1419	1419
— (w)	1321	–	–	–	–	–
Phosphate ν_3	1190–976	1190–976	1190–976	1190–976	1190–976	1190–976
— (vs)	–	1091	1088	1092	1089	1089
— (vs)	1041	1042	1045	1042	1043	1043
Phosphate ν_1 (m)	961	962	962	962	961	961
Carbonate ν_2 (ms)	873	877	875	874	873	875
Phosphate ν_4	660–520	660–520	660–520	660–520	660–520	660–520
— (m)	629	632	633	633	633	633
— (vs)	603	602	602	602	602	602
— (vs)	567	566	567	566	566	565
Phosphate ν_2 (w)	469	472	473	472	472	472

HA = Hydroxyapatite; CA = Carbonated apatite; cm^{-1} = Wave number unit (spectroscopy).

TABLE II Peak area calculation for hydroxyl, carbonate and phosphate bands of carbonated and hydroxy apatite spectra

Peak assignments	Carbonated apatite	Commercial hydroxyapatite powders				
HA cm^{-1}	IRC (CA)	Merck HA	Plasma Biotal (P88)	(P120)	(P141)	(P149)
Hydroxyl stretch	1.92	5.06	6.78	6.62	6.59	6.37
Carbonate (ν_3)	164.8	11.2	11.2	12.7	11.1	12.1
Phosphate (ν_3)	314.5	351.6	165.7	225.8	182.0	138.1
PO_4^{3-}/CO_3^{2-}	1.9/1	1/31.4	1/14.8	1/17.8	1/16.4	1/11.4

HA = Hydroxyapatite; CA = Carbonated apatite; cm^{-1} = Wave number unit (spectroscopy).

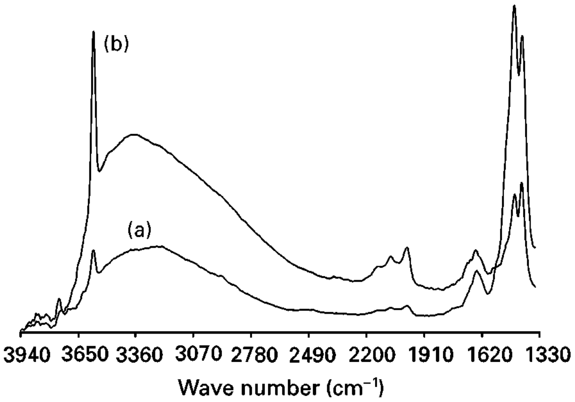

Figure 7 Hydroxyl and carbonate bands of the (a) carbonated and (b) hydroxyapatite powders.

3.2. Carbonate bands

Theoretically, carbonate ions have four vibrational modes, three of which are observed in the infrared spectrum and two of which are observed in the Raman spectrum [5]. The carbonate ν_4 bands have very low intensity and are seldom seen in the infrared spectrum [6]. Usually ν_1 and ν_4 have strong vibrational bands in the Raman spectra of bone and carbonated apatite [5, 7], and ν_2 and ν_3 vibrational modes are observed in the infrared spectra. Carbonate ions occupy two different sites in carbonated apatite: peaks in the region of 1650 to 1300 cm^{-1} are due to ν_3 vibrational mode carbonate ion and the peak at 873 cm^{-1} is due to the ν_2 vibrational mode [8]. These carbonate bands in the region of 1650 to 1300 cm^{-1} are assigned to surface carbonate ions, rather than to carbonate ions in the lattice of phosphate ions. The ν_3 has peak split in two peaks centred at 1649 and 1470 cm^{-1}, respectively, with the distribution of the carbonate ν_3 sites depending on the maturation and formation of apatite crystals. Occupancy of the ν_2 sites is considered to occur competitively between the OH^- and carbonate groups at the interface of growing crystal, whereas occupancy of the ν_3 sites depends on competition between the phosphate and carbonate ions [4, 8]. This presence of ν_2 and ν_3 vibrational modes of carbonates in carbonated apatite may contribute to the decrease of hydroxyl ions in the spectrum, as the hydroxyl band present in the spectrum of carbonated apatite is weaker in intensity than that in commercial HA powders.

The spectra of synthetic hydroxyapatites also have peaks in the region of 1650 and 1300 cm^{-1}. Carbonated apatite has two well-defined peaks for the ν_3 sites centred at 1649 and 1470 cm^{-1}, whereas synthetic commercial hydroxyapatite has three sites for ν_3 vibrational mode centred at 1648, 1454 and 1419 cm^{-1}. Results obtained by peak area calculation of the carbonate ν_3 band indicate that carbonated apatite has more carbonate moiety than hydroxyapatite. The peak areas of carbonated apatite and hydroxyapatite

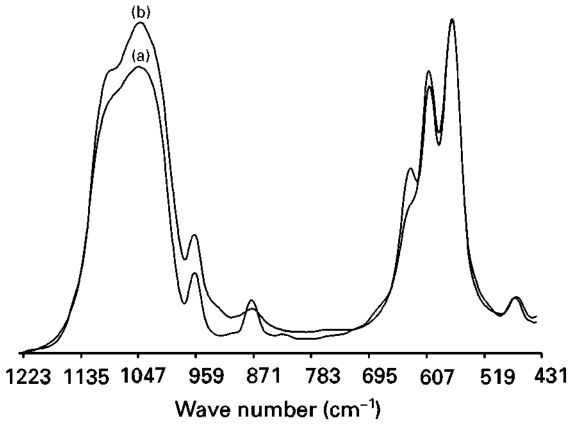

Figure 8 Phosphate (ν_3) and (ν_4) bands of the (a) carbonated and (b) hydroxyapatite powders.

powders are 165 and 13 respectively. The spectra of the carbonated bands of carbonated apatite and hydroxyapatite powders are compared in Fig. 7.

3.3. Phosphate bands

Theoretically, there are four vibrational modes present for phosphate ions, ν_1, ν_2, ν_3 and ν_4. All these modes are Raman and infrared active and are observed for all the spectra of carbonated apatite and hydroxyapatite powders.

In the carbonated spectrum, a single intense ν_3 band is present at 1046 cm^{-1}, whereas, in the hydroxyapatite spectra, the ν_3 band has three different sites present at 1096, 1085 and 1056 cm^{-1} (Fig. 8 and Table I). The intense ν_3 band is thought to be responsible for totally obscuring the ν_1 carbonate bands [5]. The peak area of the ν_3 band was calculated and used to determine phosphate/carbonate ions ratio. The peak areas of the ν_3 band of carbonated and hydroxy apatite powders are given in Table II. This ratio increases with an increase in the phosphate content.

Phosphate ν_1 band is present at 961–2 cm^{-1} and can be observed in all the spectra of hydroxyapatite and carbonated apatites (Figs 1 to 8 and Tables I and II).

Phosphate ν_4 band is present in the region of 660 and 520 cm^{-1} and is a well-defined and sharp band, observed in the carbonated and hydroxy apatites. It has two sites in the case of carbonated apatite, centred at 603 and 567 cm^{-1}, and hydroxyapatite spectra have three sites observed at 633, 602 and 566 cm^{-1} (see Fig. 8 and Table I). This splitting of the ν_4 vibrational band indicates the low site symmetry of molecules, as two and three observed bands confirm the presence of more than one distinction site for the phosphate group [9].

Phosphate ν_1 band is observed in the region of 475 and 440 cm^{-1} and has two sites. These are weak bands, not as strong as the ν_3 and ν_4 bands. In carbonated apatite, these sites are at 467 and 450 cm^{-1}, whereas in commercial hydroxyapatite, it has only one site, at 472 cm^{-1}.

FTIR spectra of synthetic commercial hydroxyapatite and synthetic carbonated apatite powder indicate that there are a number of differences between the two samples. The most obvious change in the spectrum of carbonated apatite is the large decrease of the hydroxyl peak centred at 3568 cm^{-1}, compared to the commercial sample's spectra, which have a well-defined, sharp peak at the same position (see Fig. 7 and Table II). Infrared spectra of commercial hydroxyapatites have a hydroxyl band at 624 cm^{-1}, which is absent in carbonated apatite.

4. Conclusion

The quality of the structural and compositional detail which can be revealed by this technique, combined with minimal sample preparation makes PAS-FTIR spectroscopy a very attractive technique for the analysis of biomaterials. Materials can be easily analysed by PAS-FTIR without the need to reduce the particle size or dilute with KBr, allowing the analysis of biomaterial in a physiological condition. Quantitative analysis of the apatite powders confirm the presence of more carbonate moiety in the carbonated apatite, compared to commercial hydroxyapatite samples. The carbonate moiety amount varies within the commercial samples, and from batch to batch.

Acknowledgement

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