

## Preparation of Copper-Doped Hydroxyapatite with Varying x in the Composition $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$

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### Abstract

The pro-angiogenic potential of copper ions could increase the viability of bone forming cells within a bone substitute scaffold, and so hasten healing, by stimulating infiltration of blood vessels into the scaffold. Copper-doped hydroxyapatite with  $x = 0, 0.5, 0.75$  and  $1$  in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$  was prepared by solid state synthesis. Lattice parameters increased as  $x$  was increased, consistent with formation of Cu-O species on hydroxyl sites. In FT-IR spectra, the OH stretch ( $3572\text{ cm}^{-1}$ ) and the OH libration ( $631\text{ cm}^{-1}$ ) decreased in intensity as  $x$  was increased, and a band at  $771\text{--}778\text{ cm}^{-1}$  was assignable to a Cu-O stretch, characteristic of  $\text{Cu}^+$  bands between  $3140$  and  $3450\text{ cm}^{-1}$  related to copper-disturbed OH stretching vibrations. In UV visible spectra, bands between  $400$  and  $800\text{ nm}$  were assignable to d-d transitions of  $\text{Cu}^{2+}$ . Therefore we propose that Cu is present in these materials both as  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ .

**Keywords:** Hydroxyapatite; Copper; Solid state synthesis; Powder X-ray diffraction; Infrared spectroscopy; UV-visible spectroscopy

### Introduction

Copper (Cu) ions are of interest with respect to materials for bone regeneration because of their pro-angiogenic potential. One of the problems with synthetic bone scaffolds is the fast decrease in viability of cells with increasing distance from the surface of the scaffold material, which results from the inability of nutrients and oxygen to diffuse further than  $150\text{--}200\text{ }\mu\text{m}$  from the surface without a blood supply [1]. Stimulating infiltration of blood vessels into a bone substitute scaffold, therefore, could increase the viability of bone forming cells within the scaffold and so hasten the healing process. Recently, the preparation of  $\text{CuSO}_4$ -loaded calcium phosphate scaffolds and of Cu-doped sol-gel bioactive glasses for this purpose has been reported [2,3]. Here we report the preparation of hydroxyapatite structurally doped with Cu ions, with the aim of developing a bio-instructive scaffold. In order to tune the biological response to the material, the rate and amount of ion release needs to be controlled: the first step in this task is to prepare materials with varying dopant levels. We have prepared Cu-doped hydroxyapatite with  $x=0, 0.5, 0.75$  and  $1$  in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$  by solid state synthesis.

### Materials and Methods

#### Preparation of materials

Calcium hydrogen phosphate ( $\text{CaHPO}_4$ , Acros, 97%), calcium carbonate ( $\text{CaCO}_3$ , Sigma-Aldrich, ACS reagent) and copper (II) oxide ( $\text{CuO}$ , Aldrich, 99.99% trace metals basis) were thoroughly homogenised with a pestle and mortar. Powders were pressed into discs and heated in a furnace at  $1100^\circ\text{C}$ . Copper-containing mixtures were heated for 24 h, quenched in air, ground up and re-pelletized, and heated again for 2 h before quenching in air to give the final product. The copper-free composition was heated for two periods of 24 h to give a pure phase product, slow-cooling to room temperature at  $2.5^\circ\text{C}/\text{min}$  each time and grinding and re-pelletizing in-between.

#### Characterisation of materials

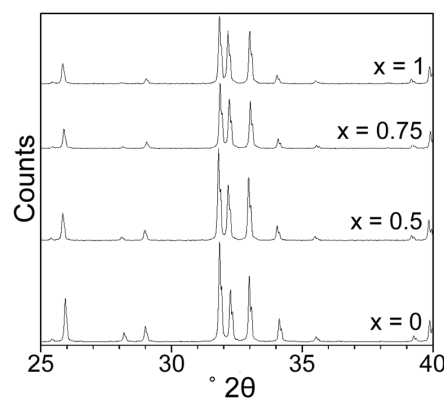
Powder X-ray diffraction patterns were collected from  $5\text{--}100^\circ 2\theta$  with a step size of  $0.02^\circ$  and time per step of 10 s, using  $\text{Cu K}\alpha_{\beta}$  radiation in a Siemens D5000 X-ray diffractometer operating at 40 kV and 25 mA. Rietveld analysis of the collected data was performed using

TOPAS-Academic software. Fourier-transform infrared spectra of the product powders as KBr discs (0.002 g of sample diluted with 0.198 g spectroscopic grade KBr) were recorded using a Nicolet Nexus FT-IR spectrometer equipped with a DTGS detector, resolution  $4.0\text{ cm}^{-1}$ , over the range  $4000\text{--}400\text{ cm}^{-1}$ , with 256 scans. Diffuse reflectance UV-visible spectra of the powders were recorded with respect to  $\text{BaSO}_4$  as a reference in a Varian Cary 50 UV-visible spectrometer.

### Results and Discussion

#### Powder X-ray diffraction (PXRD)

The synthesised materials were pure-phase apatite by PXRD



**Figure 1:** Powder X-ray diffraction patterns of synthesised materials ( $25\text{--}40^\circ 2\theta$  range) with  $x = 0, 0.5, 0.75$  and  $1$  in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$ .

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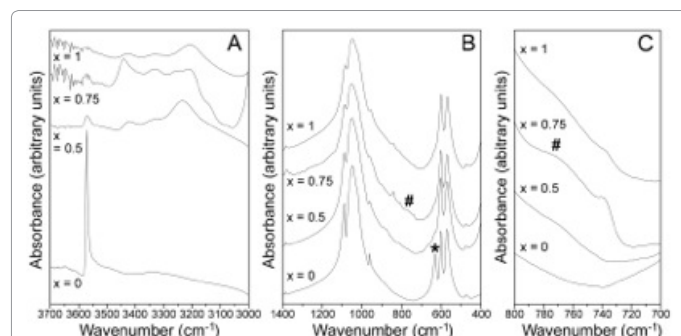
(Figure 1). As the doped amount of Cu was increased, both *a* and *c* lattice parameters (Table 1) increased. This confirmed the substitution of Cu for a (much smaller) proton on the OH lattice site. The structural expansion was in agreement with results in the literature for similarly doped calcium, barium and strontium apatite materials [4-8].

### Fourier-transform infrared (FT-IR) spectroscopy

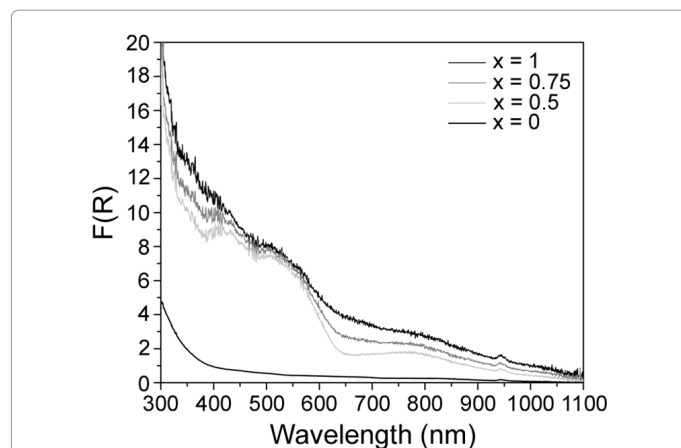
FT-IR spectra (Figure 2) showed phosphate bands typical of hydroxyapatite. The presence of Cu-O species was confirmed by a band at 771-778  $\text{cm}^{-1}$ , most obvious in the spectrum of the *x* = 0.75 material, which was assigned to a Cu-O stretch (characteristic of  $\text{Cu}^+$ ) in O-Cu-O units [4]. The appearance of this feature was concurrent with greatly decreased intensity of the OH stretch ( $3572 \text{ cm}^{-1}$ ) and the OH libration ( $631 \text{ cm}^{-1}$ ) bands upon Cu doping. Bands related to OH stretches disturbed by nearby Cu [5] were observed between 3100 and 3500  $\text{cm}^{-1}$ .

<i>x</i>	<i>a=b</i> (Å)	<i>c</i> (Å)	<i>V</i> (Å <sup>3</sup> )
0	9.423(2)	6.885(2)	529.4(3)
0.5	9.427(3)	6.907(3)	531.6(4)
0.75	9.439(3)	6.924(3)	534.2(4)
1	9.448(6)	6.938(5)	536.4(8)

**Table 1:** Lattice parameters of synthesized materials with *x* = 0, 0.5, 0.75 and 1 in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$ .



**Figure 2:** FT-IR spectra of synthesised materials with *x* = 0, 0.5, 0.75 and 1 in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y(\text{H})_z$ , showing the OH stretching region (A), the main phosphate bands (B) and the  $\text{Cu}^+$ -O stretch (C). For clarity, peaks in A and C are magnified by ca. 10 times relative to B. \* = OH libration band; # =  $\text{Cu}^+$ -O stretch.



**Figure 3:** UV-visible spectra of synthesized materials with *x* = 0, 0.5, 0.75 and 1 in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$ .

### UV-visible diffuse reflectance spectroscopy

The presence of  $\text{Cu}^{2+}$  in the Cu-doped samples was confirmed by UV-visible spectra (Figure 3), which showed three bands at 419, 515 and 774 nm. The bands at 515 and 774 nm are in good agreement with those attributed by Kazin [6] to d-d transitions in linearly coordinated  $\text{Cu}^{2+}$ . The band at 419 nm could be a charge transition due to  $\text{Cu}^{2+}$  in another site, as proposed by Kazin with regard to a band observed at 450 nm [6].

### Conclusions

Cu-doped hydroxyapatites with *x* = 0, 0.5, 0.75 and 1 in the nominal formula  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$  were successfully prepared by a solid state method. PXRD confirmed the phase purity of the materials. Rietveld analysis showed an increase in unit cell volume with increasing Cu content, which confirmed the substitution of Cu onto the OH sites in the hexagonal channels of the apatite structure. FT-IR spectra confirmed the presence of Cu-O species on the OH sites, with a band at 771-778  $\text{cm}^{-1}$  assigned to a Cu-O stretch characteristic of  $\text{Cu}^+$  and with decreasing intensity of the OH stretch ( $3572 \text{ cm}^{-1}$ ) and the OH libration ( $631 \text{ cm}^{-1}$ ) bands as *x* was increased. Bands related to OH stretches disturbed by nearby Cu were observed between 3140 and 3500  $\text{cm}^{-1}$ . UV-Vis spectra showed bands assignable to d-d transitions of  $\text{Cu}^{2+}$ . Therefore we propose that Cu is present in these materials as both  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ , which could have implications for the bioactivity of the materials due to the general insolubility of  $\text{Cu}^+$  salts. The obvious presence of Cu ions in two valence states, but lack of quantitative information as to the proportions of these, meant that the proportions of O and H ions (and possibly vacancies) necessary for charge balance were not determined. Thus the non-specific formula of  $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$ , as proposed by Baikie et al. for these materials [8], was used throughout this work.

### Acknowledgement

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