Sol-gel synthesis and TEM-EDX characterisation of hydroxyapatite nanoscale powders modified by Mg, Sr or Ti

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Abstract. Chemically modified hydroxyapatite nanoparticles have been prepared by a sol-gel route. Analysis by TEM-EDX confirmed that the dopants, Mg, Sr or Ti had been incorporated into the product. However each sample displayed variability in the level of dopant incorporation in different particles within the same sample. Particle sizes were ≤ 100 nm for samples modified by Mg or Ti. Doping with Sr produced an elongated particle morphology, with dimensions ~ 200nm x 50 nm.

1. Introduction
Nanosized particles of synthetic hydroxyapatite (HAp), Ca₅(PO₄)₃OH, are of interest for a number of applications, including tissue scaffolds, drug delivery and the occlusion of dentinal tubules for the treatment of sensitive teeth [1,2].

Natural HAp, present as the main mineral component of bone and teeth, is a complex solid solution composition, in which Ca, P and OH ions are substituted by small amounts of a variety of different cations and anions. In order to enhance mineralisation reactions involving synthetic HAp it is desirable to devise preparation routes which enable ions present in natural HAp to be incorporated into the product. This would result in a material more closely matching the composition of natural HAp, leading to improved bioactivity.

Magnesium and strontium are two of the important divalent substituents readily taken up by bone. Strontium has been shown to increase bone formation and reduce resorption [3]. Doping synthetic HAp with Sr has been shown to change the phase content, increase the amount of tricalcium phosphate relative to HAp, and to promote the formation of an amorphous phase. Magnesium also affects phase content and osteointergration [4,5].

The substitution of Ti into HAp has been less widely studied. Titanium doped HAp biomaterials are of interest because of the possibility of an additional antibacterial function. A hydrothermal synthesis route has been used in the past to attempt Ti substitution on Ca lattice sites for photocatalytic applications [6]. There were uncertainties as to the amount of Ti incorporated into the needle-like HAp crystallites. Co-precipitation methods have also been used, and the product showed photocatalytic and bactericidal response [7].

The present paper reports results of TEM-EDX analysis of powders of HAp produced by a sol-gel route in which up to 5 % of the calcium starting reagent was replaced by magnesium, strontium or titanium reagents. The approach indicates that significant variations can arise in the amount of dopant incorporated into different particles within a sample.
2. Experiment
Sols were prepared from calcium nitrate and triethyl phosphite, according to procedures reported in reference 8. For Sr and Mg modifications, 5 mol % of the calcium reagent was substituted by Mg or Sr nitrate. In the case of Ti modified samples, 3 mol % of the calcium was substituted by titanium di-isopropoxide bis pentanedionate. The ratio of Ca:Ti substitution was 2:1 in order to maintain charge balance. After ageing the sols, followed by evaporation of solvent at 60 °C, the samples were dried to form a gel. This was then heat-treated at 650 °C for 2 h.
Powder characterisation was carried out using a FEI CM200 field emission gun TEM operating at 197 kV coupled with an Oxford Instruments ultra thin window ISIS energy dispersive X-ray (EDX) system and a Gatan imaging filter (GIF 200). For TEM sample preparation, powders were ultrasonically dispersed in methanol prior to being drop cast onto a holey carbon support film. Spot-EDX in the TEM allows for the detailed monitoring of powder composition as determined from the ISIS processing software using virtual standards for the Ca and P Kα X-ray peaks. EDX data were acquired with a probe current of ~ 1 nA over areas about the size of each measured particle such that the total electron fluence was ~ 10⁸ electrons nm⁻² (following the method and results described in Eddisford et al, 2007 [9] and Bilton et al. [10], these proceedings).

3. Results

3.1 Un-doped HAp
Particles were ≤ 100 nm in size (Figure 1). X-ray powder diffraction patterns showed only peaks due to hydroxyapatite. The individual nanoparticles were grouped into micron scale agglomerates.

![Figure 1: (a) Bright field TEM micrograph of unmodified hydroxyapatite powder; (b) Corresponding EDX spectrum](image)

3.2 Magnesium doping
The TEM micrograph of the Mg- modified sample shown in Figure 2a shows particle sizes up to 200-300 nm. Elemental analysis by TEM-EDX, (Fig 2b), confirmed the presence of Mg, along with Ca and P in the particles examined, but the ratio of Mg/Ca was higher in some of the smaller < 50 nm particles. There were also some particles in which Mg was not detected.
3.3 Strontium doping
Figure 3a shows that Sr modification produced particles that were more elongated than undoped or Mg doped HAp compositions. Dimensions of most particles were around 200 nm x 50 nm. Ten particles were examined by EDX; eight showed Ca, Sr and P, with Sr/Sr+Ca atomic ratios of around 4 (compared to a nominal ratio of 5 in the as-prepared starting sol), Fig 3b. One particle showed a very strong Sr signal with little evidence of Ca or P; another showed only Ca and P with a peak ratio of 1.7, consistent with hydroxyapatite (Ca/P 1.67). There was evidence from X-ray powder diffraction of tricalcium phosphate and calcium oxide secondary phases.

3.4 Ti doping
The Ti modified sample (Fig 4) displayed a greater variability in composition than for the Mg or Sr modified samples. Typical micrographs and EDX spectra are shown in Fig 4. TEM-EDX indicated: i) a Ti modified HAp composition; ii) a HAp phase with no Ti evident by EDX; iii) a Ti-rich phase also containing calcium; iv) a Ca–rich phase with little EDX evidence of other elements. Crystallites were mostly 50-100 nm in size, except for the Ca-rich phase, where the crystallites were much smaller, ~ 10 nm.
Figure 4: (a) TEM micrograph of Ti modified hydroxyapatite powder; (b) and (c) examples of TEM–EDX spectra illustrating variability in elemental content between different particles

4. Conclusions

TEM-EDX show that each doped sample is heterogeneous in terms of inter-particle composition. Each of the doped sol-gel compositions produced nanoparticles in which the dopant species was incorporated, but some particles indicated no evidence of dopant substitution. Titanium modification produced the greatest chemical heterogeneity. Sr doping led to elongated particles, around 200 x 50 nm, whereas other compositions produced equiaxed particle morphologies, this elongation associated with Sr doping is thought to be due to the increase in ionic radius from Ca to Sr. Refinements to process conditions, in particular the efficiency and duration of reagent mixing will be examined in future. This may allow for more intimate mixing. However the volatility of the P reagent triethyl phosphite at room temperature is a major concern for this specific sol-gel system in terms of achieving batch to batch reproducibility. The results highlight the importance of TEM-EDX analysis for the characterisation of doped synthetic hydroxyapatite powders.

5. References

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