Sol-gel synthesis and characterisation of nano-scale hydroxyapatite

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Abstract. Hydroxyapatite (HAp) forms the main mineral component of bone and teeth. This naturally occurring HAp is in the form of nano-metre sized crystallites of Ca_{10}(PO_{4})_{6}(OH)_{2} that contain a number of cation and anion impurities, for example CO$_3^{2-}$, F$^-$, Na$^+$, Mg$^{2+}$ and Sr$^{2+}$. Synthetic nano-sized HAp particles exhibit favourable biocompatibility and bioactivity and in order to better match the composition to natural HAp there is great interest in producing a range of chemically modified powders. In this study, two HAp powders have been synthesised via a water-based low-temperature sol-gel method and a third, commercial powder from Sigma-Aldrich have been analysed. Subsequent powder calcination has been carried out within the temperature range of 500-700 °C and the products characterised by bulk chemical analysis, X-ray diffraction and electron microscopy. Energy dispersive X-ray spectroscopy (EDX) in the TEM has been used to assess the composition of individual HAp particles. In order to do this accurately it is first necessary to account for the sensitivity of the HAp structure and composition to irradiation by the high energy electron beam of the TEM. This was done by monitoring the estimated Ca/P ratio derived from TEM-EDX of stoichiometric HAp under increasing levels of electron fluence. A fluence threshold (at a given beam energy) was established below which the measured Ca/P ratio can be considered to be stable. Subsequent elemental analysis at or below this threshold has enabled the variation in composition between particles both within and between synthesis batches to be accurately assessed. Compositional variability between particles is also evident, even in the commercial powder, but is far greater in the powders prepared by the sol-gel method.

1. Introduction
Hydroxyapatite (HAp) is a calcium phosphate ceramic. Naturally occurring HAp is the main mineral component of bone and teeth, in the form of nano-metre sized crystallites of Ca$_{10}$(PO$_{4}$)$_{6}$(OH)$_{2}$ containing a number of cation and anion impurities, for example CO$_3^{2-}$, F$^-$, Na$^+$, Mg$^{2+}$ and Sr$^{2+}$. Chemically pure, stoichiometric HAp has a composition with a Ca/P ratio of 2.151 by atomic weight, a Ca/P atomic ratio of 1.67, and within a pH range of 4.2-8.0 has a greater stability in aqueous media compared to alternative calcium phosphate ceramics [1]. HAp exhibits excellent biocompatibility and as such is utilised in many biomedical applications related to bone substitution, including prosthesis coatings, dentistry, and as matrices for controlled drug release. HAp is also used in many non-biomedical applications such as packing media for column chromatography, gas sensors, catalysis and as host materials for lasers [2, 3].

Many applications of synthetic HAp require careful control of the particle size, shape and phase. A commonly used approach to assess HAp stoichiometry is to identify the crystal structure by X-ray diffraction (XRD) and the average composition by X-ray Fluorescence (XRF). One can also use transmission electron microscopy (TEM) combined with elemental...
analysis such as energy dispersive X-ray (EDX) spectrometry to characterise the structure and composition of powders. The advantage of the microscopic technique is that individual particles can be characterised, enabling compositional variability, that may not be detected by the relatively bulk techniques of XRD and XRF, to be identified. However experiments investigating the impact of a progressively increasing electron fluence (the number of electrons applied per unit area) on the Ca/P atomic ratio in HAp irradiated in the TEM have shown that a fluence above $10^7$ electrons nm$^{-2}$ at 200 keV induces a radiolytic damage process involving phosphorus and oxygen loss, amorphisation and eventual re-crystallisation to calcium oxide (CaO) [4, 5]. In this study we will establish an electron fluence threshold below which the composition of HAp can be analysed in the TEM by EDX without significant elemental alteration. We will then assess the compositional variability between particles in two synthetic HApS and compare this to characterisation with XRD.

2. Experiment

Two powders, calcined at 500 °C and 700 °C respectively, have been synthesised via a sol-gel method outlined by Liu [2]. The method involves the dilutions of triethyl phosphite in anhydrous ethanol and a small amount of distilled water for hydrolysis. Once hydrolysis is complete (signified by a transformation of an opaque solution to transparent), a stoichiometric amount of calcium nitrate, dissolved in anhydrous ethanol, is added drop-wise with continuous stirring. Further stirring and subsequent oven drying results in a white gel which is ground with a mortar to a fine powder and heated in a furnace (for 2 hours) at various temperatures. For comparative purposes a third reagent grade HAp powder has been purchased from Sigma-Aldrich.

Samples were examined in a FEI CM200 field emission gun TEM operating at 197 kV and fitted 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, all HAp powders were ultrasonically dispersed in methanol and drop cast onto a holey carbon support film (Agar Scientific Ltd). Powder composition was monitored in the TEM with spot-EDX and specifically the Ca/P ratio (as determined from the ISIS processing software using virtual standards for the Ca and P Kα X-ray peaks), with a specimen tilt angle of 15° and take off angle of 20°. The beam current at a fixed condition (spot size 5-8 and largest (200 µm) condenser aperture) was estimated by using an FEI calibration curve based on the measured brightness (i.e. exposure time) on the (large) fluorescent TEM viewing screen: current (in nA) = 4.875/Exposure time for an emulsion setting of 2.0. Bulk compositional and phase analysis has also been performed using powder X-ray diffraction (XRD) with a Philips/Panalytical X’pert Diffractometer.

To determine a threshold-fluence, whereby accurate and representative data can be obtained without damage to the powders, experiments have been carried out using the Sigma Aldrich HAp powder. First, the irradiated area has been changed to provide approximate electron fluences across the range $\sim 10^5$-$10^9$ e nm$^{-2}$. At each fluence, spot-EDX measurements of 30 second total duration were taken over a particle cluster to obtain the Ca/P ratio. Secondly, 3 EDX measurements were taken across a cluster of particles, at a fixed irradiation area, to measure the effects of cumulative fluence over time. 30 second EDX measurements were taken after 10, 100 and 1000 seconds respectively to provide data across the fluence range $10^6$-$10^8$ e nm$^{-2}$. Upon establishing a threshold fluence, compositional data for the three powders has been sought. EDX data from 50 individual particles of each powder were measured to obtain Ca/P ratios, at a total fluence of $\sim 10^6$ e nm$^{-2}$ per particle.

3. Results & Discussion

In general all three samples consisted of particles 50-100 nm in length and 10-50 nm in diameter. Powder XRD patterns of all three samples can be indexed to a HAp phase. There are little or no impurities detectable in the Sigma Aldrich powder plots but there is a significant presence of CaO in the 700 °C and to a lesser extent the 500 °C sol-gel powders (figure 2 a, c and e).
3.1. Initial experiments: electron fluence induced damage

Exposure of aggregates of the Sigma-Aldrich HAp to the electron beam results in a dramatic increase in the estimated Ca/P ratio as the electron fluence exceeds \(10^7\) e nm\(^{-2}\) (figure 1a); consistent with reference [4]. Time-resolved variation in the fluence confirms that the Ca/P ratio is not completely stable below \(10^6\) e nm\(^{-2}\), the ratio changes from 1.42 to 1.53 between \(10^6\) e nm\(^{-2}\) and \(10^7\) e nm\(^{-2}\) (figure 1b). The signal to noise ratio (i.e. the intensity ratio of the P K\(\alpha\) peak/ peak noise) in these spectra changes from 8 to 10 respectively. To obtain reliable compositional statistics without significant alteration of the HAp subsequent measurements were made at \(10^6\) e nm\(^{-2}\).

![Figure 1](image1.png)

**Figure 1:** (a) EDX Ca/P ratio of the Sigma-Aldrich HAp as a function of electron fluence (b) EDX Ca/P ratio of the Sigma-Aldrich HAp as a function of cumulative fluence over time, measured at 10, 100 and 1000 seconds respectively.

3.2. Secondary experiments: compositional data across 50 individual particles

Using TEM-EDX and operating within the fluence threshold of \(10^6\) e nm\(^{-2}\), compositional data across the three HAp powders (Sigma Aldrich and sol-gel powders calcined at 500 and 700 °C) has been measured over 50 individual particles per sample. (figures 2b, d and f). All three powders display some variation in particle composition, with the Sigma-Aldrich powder having the least variability (all particles exhibiting Ca/P ratios < 2) and the 700 °C sol-gel HAp the most (with up to 40% of the particles exhibiting Ca/P ratios of > 2). This variability is reflected in the reported standard deviations of the Ca/P ratios, (figure 2). The mean Ca/P ratios suggest the production of a non-stoichiometric HAp phase in all cases. The Sigma-Aldrich powder displays a non-stoichiometric composition (below the desired 1.67) however this is in agreement with data from previous literature [6]. The mean Ca/P ratios for the powders calcined at 500 and 700 °C are above 1.67 and consequently suggest the presence of a Ca-rich phase; this is consistent with XRD findings however no discrete CaO particles were detected in the sample of 50. Bulk XRF is being undertaken to provide an independent measure of the average compositions. Although CaO was detected by XRD, no discrete CaO particles were found in the sol-gel powders by TEM-EDX; significant numbers of Ca-rich particles (Ca/P ratios of > 2) were present in the sol-gel powders but to be truly representative, more than 50 particles per powder should be measured.

XRD indicates that near stoichiometric HAp is the only crystalline phase in the sample, other than small amount of CaO, and the particles measured were generally single crystal. This may be explicable by the presence of an amorphous Ca-rich phase intimately mixed with near stoichiometric HAp particles. This amorphous phase may crystallize to CaO at increasing calcination temperature. More detailed TEM analysis will be undertaken to confirm this hypothesis alongside attempting removal of any amorphous material with a selective etch.
4. Conclusion
Radiolytic damage of hydroxyapatite induced by an electron beam has been investigated resulting in the confirmation of a critical threshold fluence [4]. Operating within this limit, TEM-EDX compositional data from individual particles has been recorded over three HAp powders, one purchased from Sigma Aldrich and two powders produced by a sol-gel method, calcined at 500 and 700 °C. The results highlight the processing variability in the sol-gel preparation. Refinement of the synthesis is underway to produce more stoichiometric HAp that will be characterised by the same robust method. This synthesis and characterisation route will then be utilised to develop chemically modified HAp for biomaterial application.

Acknowledgments
This work has been supported by a DTA scholarship courtesy of the EPSRC.

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