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Measurement of fluoride substitution in precipitated fluorhydroxyapatite nanoparticles

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ABSTRACT

Fluoride substitution can improve the chemical stability and biocompatibility of precipitated hydroxyapatite nanoparticles, leading to advantages such as improved cell adhesion in osteo applications and reduction of tooth enamel erosion in dental applications. To achieve the desired improvement and minimise the risk of excess fluoride release, the concentration of fluoride and the mechanism of incorporation must be known. This is difficult due to the small effect fluoride substitution has on the hydroxyapatite structure, the low concentration of fluoride in fluorhydroxyapatite (0–3.77 wt%), and the interference of impurities as a result of the precipitation process. Several techniques for measuring fluoride substitution in precipitated fluorhydroxyapatite nanoparticles have therefore been investigated. Fluorhydroxyapatite nanoparticles with fluoride substitution levels ranging from 0 to ~92% have been synthesised by wet precipitation and characterised using XRD, FTIRS, XPS, TEM, and chemical analysis. XRD and FTIRS measured fluoride substitution more accurately than bulk concentration methods, as they detected apatite fluoride separately from other fluoride in the sample.

1. Introduction

The mineral phase of bones and teeth is essentially impure hydroxyapatite ($Ca_5(PO_4)_3OH$, HA) [1,2] and makes up approximately 50% of dentine and bone [3], and 98% of tooth enamel [4] by weight. In each case the HA is found in a complex hierarchical structure of HA nanoparticles and organic components [4–7]. As a result, synthetic nanoscale HA has great potential in various biomedical applications [3,4,8–12].

HA is rarely found in pure form as its structure allows for easy substitution of any of the calcium, phosphate, or hydroxyl ions. Biologically important substitutions in HA include: magnesium, sodium or strontium substituted for calcium [7,13]; carbonate substituted for phosphate or hydroxyl ions [5,14,15]; and fluoride or chloride substituted for hydroxyl ions [16–19]. In addition, biological HA is calcium deficient (i.e., it has a Ca/P ratio of approximately 1.61–1.63; less than the stoichiometric value of 1.67) [4].

The amount of fluoride substitution can be anywhere from 0% (HA) to 100% ($Ca_5(PO_4)_3F$, fluorapatite, FA), and intermediate concentrations are known as fluorhydroxyapatite (FHA). The smaller, symmetric fluoride ions fit more neatly into the crystal structure than the hydroxyl ion [2,19,20], leading to higher stability and lower solubility [16,21]. This allows the fluoride concentration to be adjusted to produce tailored biochemical and mechanical properties combining those of HA and FA. Fluoride substitution has been shown to increase bone cell activity [22–25] and inhibit tooth erosion [26–29], opening up a wide range of orthopaedic and dental applications, and encouraging significant interest in the synthesis of FHA nanoparticles.

Direct synthesis of FHA nanoparticles receives a lot of attention, and the most commonly used method is wet precipitation [16,18,30–36]. This method is fast, simple and flexible; substitutions can be made simply by changing the concentration of reactants, and the reaction conditions can be used to control particle morphology [31,33,37–40]. These particles can then be used in sintered or composite materials to improve mechanical or biochemical properties [11,16,41–46]. Another approach involves apatite-mullite glass ceramics, which are initially amorphous and form FA and mullite ($Al_6Si_2O_{13}$) upon crystallisation. These are being considered for applications from dental implants to orthopedic implant coatings due to their formability in the glass phase and the bioactivity afforded by the nanoscale FA in the crystallised phase [47–49]. Other studies have included the precipitation of FHA directly onto a surface as a repair mechanism for enamel [50], or as a bioactive coating for implants [51,52].

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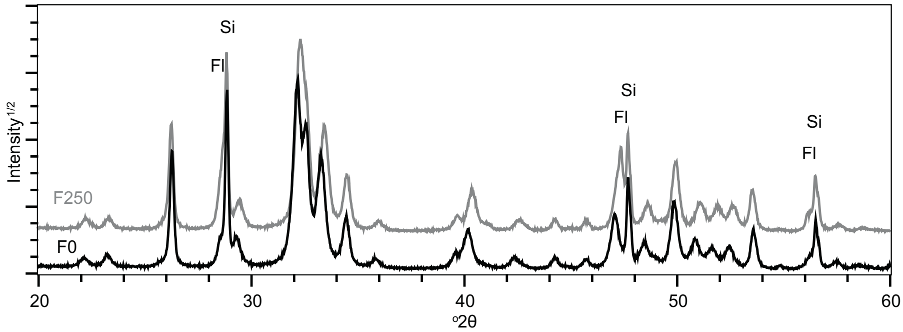

Fig. 1. XRD patterns for F0 and F250. Silicon (Si) and fluorite (Fl) peaks are marked and all other peaks belong to HA or FHA. Only slight changes in peak position and intensity are visible and all other XRD patterns were similar.

Whatever the application, the performance of the material and the rate of fluoride release depend on both the total fluoride concentration in the sample and the form in which it is included. Fluoride addition during the wet precipitation of HA may produce a solid solution of FHA, separate HA and FA phases, additional phases such as calcium fluoride (CaF_2), or a combination of these [21,53]. This depends on several factors, such as pH, reactant concentrations, and mixing rate, as local variations within the mixture potentially lead to different precipitation mechanisms in the same batch. FHA can precipitate directly from solution or via intermediate phases including amorphous calcium phosphate and octacalcium phosphate [54,55], and the resulting FHAs exhibit differences in structure and chemistry [55]. The wet precipitation process tends to produce calcium deficient apatite with some carbonate impurity [10,56]—these impurities affect the stability of the final crystals [56,57], and fluoride can alter the level to which they occur. This inherent complexity and variability in the process means that the fluoride substitution cannot easily be calculated from the starting reactant concentrations; careful analysis is required to determine the actual fluoride content of the product for a given set of starting conditions.

Several techniques have previously been used to characterise FHA nanoparticles, each with their own strengths and limitations. The F-selective electrode technique is commonly used to measure the total fluoride concentration [16,21,58], but it requires solid samples to be dissolved and so provides no information about structure. Inductively coupled plasma mass spectrometry, atomic emission spectrometry and atomic absorption spectroscopy (ICP-MS, ICP-AES and AAS) are useful for calcium and phosphate measurement, but not fluoride [16,17]. Fourier transform infrared spectroscopy (FTIRS) gives excellent qualitative data for the level of fluoride in FHA, but is difficult to use quantitatively and some relevant phases such as CaF_2 contain no IR-active bonds [59,60]. X-ray diffraction (XRD) can detect very small changes in crystal structure or phase composition, but linking structural changes to chemistry requires careful analysis alongside complimentary techniques. In the case of FHA nanoparticles, XRD is further complicated by the effects of small crystals and by the very small effect fluoride substitution has on the crystal structure. For detailed chemical structure analysis, magic angle spinning nuclear magnetic resonance (MAS-NMR) may be used. MAS-NMR can detect all of the fluoride in a solid sample and can differentiate different chemical environments [61]; this ability has previously been used to measure fluoride substitution in FHAs and other materials [62,63]. However, the equipment required is highly specialised and not widely available, making the technique impractical for routine measurements.

These difficulties mean that fully characterising FHA nanoparticles remains a challenge even after fairly significant research [16–18,21,30,33,36]. The present study presents a practical combination of techniques that can be used to fully characterise FHA nanoparticles, and compares the effectiveness of several techniques for measuring fluoride substitution in fluorhydroxyapatite.

2. Results

2.1. XRD

XRD patterns for F0 and F250 are shown in Fig. 1. The patterns indicate primarily HA or FHA, although increasing amounts of fluorite (CaF_2) also formed with increased concentration of fluoride in the batch; from 0 wt% in F0 to 4.0 wt% in F250 (Table 1). No other crystalline phases were detected. No amorphous phases were visible and quantitative Rietveld analysis showed that all samples were at least 98% crystalline.

The unit cell a-parameter was used as an indirect measure of fluoride substitution. HA and FA structures are very similar, but a decreases by approximately 0.0509 Åfrom HA to FA due to the smaller F ion fitting more easily within the OH/F channels [64,65]. A linear relationship between percentage fluoride substitution (F_s) and a is expected, so a can be used to estimate F_s:

$$F_s = \frac{a_{\mathrm{F0}} - a_{\mathrm{F}x}}{a_{\mathrm{HA}} - a_{\mathrm{FA}}} \times 100 \tag{1}$$

where F_s is the percentage fluoride substitution, a_{F0} is the unit cell a parameter for F0, $a_{\mathrm{F}x}$ is the unit cell a parameter for any batch, and a_{HA} and a_{FA} are taken from the standard PDF cards for HA and FA [64,65].

Table 1
Unit cell parameters, crystallite size, and fluorite concentration determined by Rietveld analysis of XRD data. The values in () are the estimated standard deviation of the last digit in the value calculated from the refinement.

Test	F0	F25	F50	F75	F100	F150	F200	F250
a (Å)	9.4320(2)	9.4196(3)	9.4079(3)	9.4027(4)	9.3954(4)	9.3876(3)	9.3869(4)	9.3854(3)
c (Å)	6.8838(2)	6.8849(2)	6.8858(2)	6.8864(2)	6.8875(2)	6.8889(2)	6.8887(2)	6.8892(2)
[h00] (nm)	23.1(7)	24.9(9)	20.9(6)	16.2(5)	17.7(4)	24.6(5)	25.2(7)	26.4(11)
[00l] (nm)	67(2)	67(2)	56(2)	47(1)	49(2)	56(2)	55.8(2)	62.3(3)
CaF_2 (wt%)	0	0.3(3)	0.4(5)	0.6(4)	1.1(4)	1.8(5)	3.5(5)	4.0(6)