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Characterizations of Biocompatible And Bioactive Hydroxyapatite Particles

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Abstract

The most common gene vectors used are virus based (viral gene vectors). These possess high immunological risk, so a non-viral gene vector maybe preferable. Nanocrystalline hydroxyapatite, HAp [$Ca_{10}(PO_4)_6(OH)_2$] is an example of a possible non-viral gene vector. This is due to its good biocompatibility, bioactivity and proven results as a non-viral gene vector. The HAp particles produced in this study was by the sol-gel method and the processing conditions were varied in terms of the processing temperatures (20 ^oC, 30 ^oC or 40 ^oC) and the stirring rates (200 rpm, 400 rpm or 600 rpm). The particles formed from all of the processing conditions were systematically characterized and compared to each other. The characterizations performed were FTIR, for identifying functional groups, XRD for phase composition, crystallinity and particle size estimation (by applying Scherrer's formula) and SEM for surface morphology. The characterizations data obtained showed that the functional groups, phase composition, crystallinity and surface morphology were similar for all of the samples, the only difference being on the calculated particle size. It also showed that, at a lower processing temperature and higher stirring rate, smaller particle sizes were formed.

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Keywords: Particle characterizations; Hydroxyapatite;XRD

1. Introduction

Calcium phosphates are of interest for many biomedical applications due to their good boicompability and bioactivity (Figure 1). Hydroxyapatite (HAp) has been used as implant coating [1] and substitutes [2]. Also, calcium phosphate nanoparticles have a range of applications in a number of fields, such as drug delivery, gene therapy (Figure 2), bone cements, dental applications, chromatography and waste water remediation. Each application has a need for the nanoparticles to be of a particular size range. There are various techniques reported in the literature for the production of nanosized calcium phosphate particles. These include wet chemical precipitation [3], sol-gel synthesis [4], hydrothermal synthesis [5], mechano-chemical synthesis [6] and several other methods by which nanoparticle of various shapes and sizes can be obtained.

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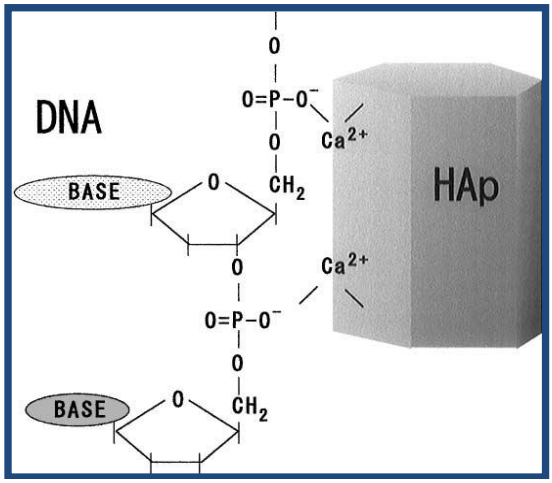


Fig. 1. Schematic model of an affinity binding between a nHAp crystal and DNA [1].

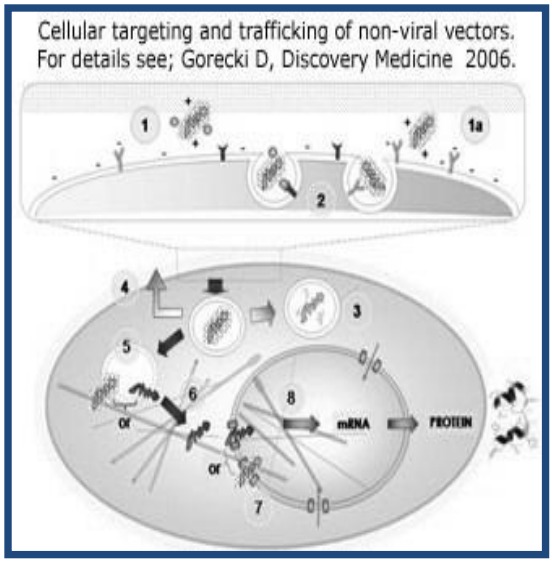


Fig. 2. Mechanism of cellular uptake of non-viral gene vectors (via endocytosis).

Specific characteristics of particles (size, shape, surface, crystal structure and morphology) are among the important factors needed to control technological and biopharmaceutical properties of drug products. In general, morphology (crystal habit) can influence the physical and chemistry stability of solid dosage forms, a narrow size distribution is important to obtain content uniformity, while spherical particles allow good flowability and tablettability. Furthermore, micronisation increases the surface area with a consequent increase of dissolution rate and bioavailability of the drug, thus promoting the formulation of active principle ingredients which may be insoluble or slightly soluble in aqueous media.

2. Experimental Method

A stoichiometric amount of aqueous solution of calcium nitrate tetrahydrate [$Ca(NO_3)_2.4H_2O$] was used and mixed with aqueous solution of ammonium phosphate $(NH_4)_3PO_4$ with the molar ratio of 10:6 by using a peristaltic pump into a stirred beaker. This Ca/P molar ratio is the desired Ca/P ratio as observed in HAp. In mixing step, the processing conditions were varied in terms of the processing temperatures (20 ^oC, 30 ^oC or 40 ^oC) and the stirring rates (200 rpm, 400 rpm or 600 rpm). The concentrations of both precursors, which were the calcium nitrate tetrahydrate and ammonium phosphate and also stirring duration were constant. Then, in filtration step, HAp particles were filtered using a 0.22 μm filters. The filtered samples were then dried at a temperature of 100 ^oC and the drying step was carried out for 24 hour. Lastly, the dried samples were crushed using a pastle and mortar apparatus for 5 minutes. The procedure for the production of nanoparticles is outlined in Figure 3.

The particles formed from all of the processing conditions were systematically characterized and compared to each other. The characterizations performed were FTIR, for identifying functional groups, XRD for phase composition, crystallinity and particle size estimation (by applying Scherrer's formula) and SEM for surface morphology.

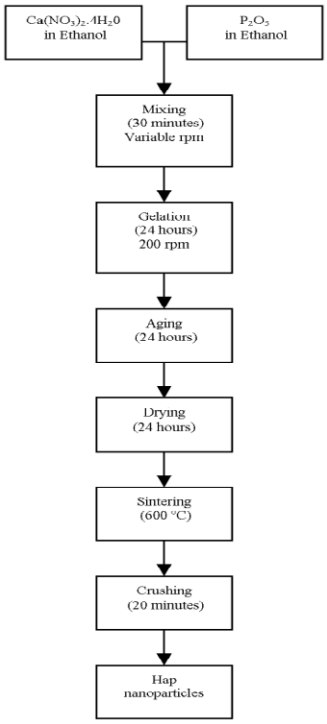


Fig. 3. Flow chart of the sol-gel synthesis of hydroxyapatite powder

3. Results and Discussion

Phosphate and hydroxide absorption peaks for HAp were seen in the FTIR spectra (Figure 4). The formation of HAp was indicated by the characteristic peak occurring at $2\theta = 31.8^{o}$, which appeared on all of the HAp samples (Figure 5).

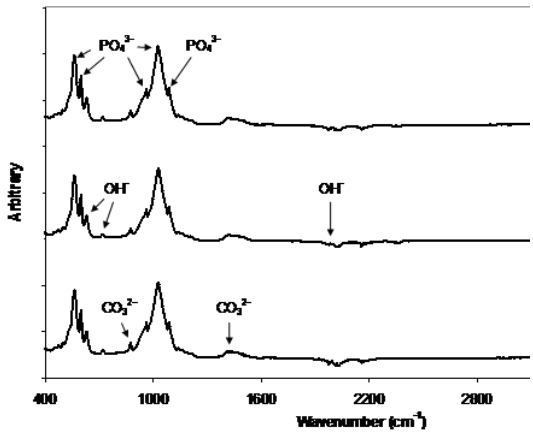


Fig. 4. The FTIR spectra for hydroxyapatite samples prepared at 200, 400, 600 rpm and 30 °C

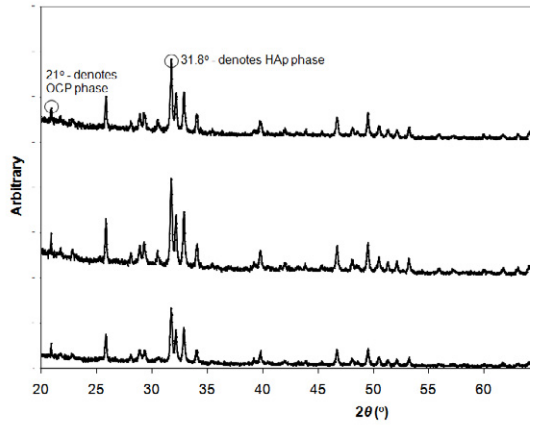
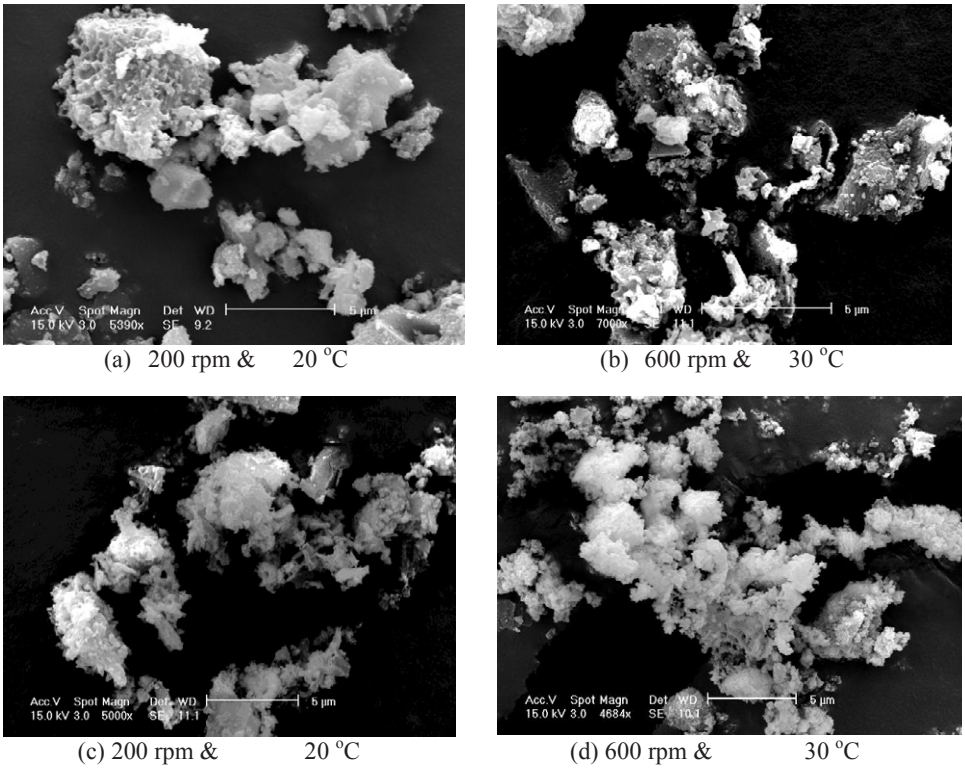


Fig. 5. The XRD patterns for hydroxyapatite samples prepared at 200, 400, 600 rpm and 30 °C

Particle size estimations by applying Scherrer's formula were shown to be in the nanosized range. Finally, SEM images of HAp nanoparticles showed that the particles formed agglomerates of approximately < 1 to 5 μm (Figure 6).



(a) 200 rpm & 20 °C (b) 600 rpm & 30 °C

(c) 200 rpm & 20 °C (d) 600 rpm & 30 °C

Fig. 6. The SEM images for hydroxyapatite samples prepared at 200 & 600 rpm and 20 & 30 °C

4. Conclusion

The characterizations data obtained showed that the functional groups, phase composition, crystallinity and surface morphology were similar for all of the samples, the only difference being on the calculated particle size. It also showed that, at a lower processing temperature and higher stirring rate, smaller particle sizes were formed.

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