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Characterizations of hydroxyapatite (HAp) nanoparticles produced by sol-gel method

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ABSTRACT

A pure ceramic powder of hydroxyapatite, HAp [$Ca_{10}(PO_4)_6(OH)_2$] can be produced by low temperature method namely sol-gel route. The sol-gel route is one of wet chemical synthesis techniques which produce the useful biomaterial particles. The production of HAp particles were carried out by varying the stirring speed from 100 to 500 rpm and sintering temperature from 600 ^oC to 800 ^oC. With further characterizations done, the HAp particles could be potentially used as immobiliser or solid support for microorganism attachment in fermentation process. The biomaterial possessed a good mechanical properties and excellent surface adsorption for microorganism by the electrostatic interation mechanism. The characterizations done were FT-IR for identifying functional groups, XRD for phase composition and crystallinity and SEM for surface morphology. Hence, the smaller primary particle size of HAp was produced with increased stirring rate and sintered temperature.

INTRODUCTION

Due to its inherent good biocompatibility and bioactivity, hydroxyapatite (HAp) has been studied extensively for various applications. The applications of HAp were mainly for biomedical purposes such as for bone implant and substitution for defective teeth because of its effectively biological performance [1]. This was due to its similarity in terms of structural and chemical characteristics with natural bone and teeth [2]. Nano hydroxyapatite (nHAp) had an ability to facilitate the new bone tissue in a short period of time and had proven that it could be bio-accepted [1].

There were many techniques to synthesize HAp that has been developed in recent years. The techniques include direct precipitation from aqueous solutions, hydrothermal synthesis, emulsion or micro-emulsion routes, electrochemical deposition and sol-gel method [3] . Among the various techniques, sol-gel method was preferably used in the study based on their advantages include low synthesis temperature, high product purity and homogenous molecular composition [2]. nHAp had also the capability to generate nanocrystalline powders, monolithic solids, bulk amorphous and thin films [4].

The common application of HAp was in medical field which it has similar characteristics to that of human bone [5]. One of the promising approaches to the application of HAp is as solid surface adsorption for microorganisms and in this research, it will focus on biofuel production. The HAp could potentially be used as immobilizer material due to the good mechanical properties and excellent surface adsorption for microorganisms by the electrostatic interation mechanism.

Immobilization of microbial cells for fermentation purposes was successfully investigated to improve economics and the process itself. The use of two-phase aqueous systems for the direct conversion of a substrate to desired product was another new technology development [6]. It was developed to enhance productivity and yield [7]. Microbial immobilization have a number of advantages such as reduced susceptibility of cells to contamination, increase cellular stability and reduce of procedural costs according to cell recovery and reutilization [8].

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The purpose of the research was to obtain HAp with specific characteristics via simple sol-gel process. The produced HAp would further be used as microbial immobiliser which could be applied specifically for fermentation purposes.

2.0 Methodology:

2.1 Materials:

The experiment initiated with the aqueous mixtures of calcium nitrate tetrahydrate [$Ca(NO_3)_2.4H_2O$] and phosphorus pentoxide (P_2O_5) in ethanol solution and the molar ratio of both chemicals were 10:6. The solution was vigorously stirred at 100 rpm, 300 rpm or 500 rpm. However, the concentrations of aqueous mixtures, temperature and mixing time were maintained constant, which were carried out at a molar ratio of Ca/P of 1.67, ambient temperature and 30 minutes respectively. After the mixing step, the solution was then allowed to undergo gelation and aging process at room temperature. The gel formed was kept in the oven at 80 ^{O}C to evaporate the excess ethanol which was used as solvent. The dried hydroxyapatite was then undergo the sintering process. The sintering temperatures were varied from 600 ^{O}C to 800 ^{O}C using a furnace. The HAp powder produced were characterized using FTIR for its functional groups identification, XRD to identify phase composition and crystallinity and SEM for surface morphology and particle size estimation.

2.2 Particle characterization:

2.2.1 Fourier transform infrared spectroscopy (FT-IR):

FT-IR analysis was conducted to identify the functional groups of the samples. Infrared spectra in the wavenumber range of 400-4000 cm^{-1} were recorded with Perkin Elmer Spectrum (version 10.02.00) using translucent pellets. The translucent pellets were prepared by mixing powder samples with potassium bromide, KBr. The analyses were carried out with 4 scans at 2 cm^{-1} resolution.

2.2.2 X-ray diffraction (XRD):

XRD analysis was carried out to identify crystallographic structural of the sample. The analysis was recorded over the 2θ range of 20-80^{O} at a scan rate of 0.02^{O}/min.

2.2.3 Scanning Electron Microscope (SEM):

SEM analysis was performed using a JEOL JSM 6460LA instrument and JFC 1600 instrument for platinum coating. The particles were coated with platinum for 3 min to get a thickness of 10 nm approximately.

RESULTS AND DISCUSSION

3.1 Fourier Transform Infra-Red (FTIR):

FT-IR patterns presented in Figure 3 confirmed the formation of HAp at 600 ^{O}C, 700 ^{O}C and 800 ^{O}C. The formation of HAp was denoted by the phosphate band symmetric stretching from about 1000 to 1100 cm^{-1}. The peaks with bands at 550 to 650 cm^{-1} and 900-950 cm^{-1} which also corresponded to the PO_4^{3-} ion. The major peaks of phosphate group were between 900 to 1100 cm^{-1} which was the most intensified peak among the phosphate vibration modes [3].

The broad peak was spread over the range between 3643 cm^{-1}, 632 cm^{-1}, 474 cm^{-1} was the stretching vibration of surface hydroxyl group (OH^-) whereas the bands assigned to the stretching modes of carbonyl group (CO^-) were recorded at about 1464 cm^{-1} and 874 cm^{-1}. There are two types of carbonyl stretching modes of carbonyl which were type A and B substitution that have been reported before. The bands which lied at 1550 cm^{-1}, 1457 cm^{-1} and 880 cm^{-1} are type A substitution while 1462 cm^{-1}, 1418 cm^{-1} and 876 cm^{-1} were the bands which belongs to type B substitution [9]. It was known that the hydroxyl and the phosphate sites in the HAp lattice were hospitable towards carbonate ions substitution that resulting in type A or type B apatites [10]. The presence of carbonate ions in apatite structure is believed to be taken place during the HAp preparation whereby the adventitious CO_2 from atmospheric reacted with hydroxyl ions and thus forming carbonate ions. The formation of carbonate ions as in the section below:

$$CO_2 + 2OH^- \longrightarrow CO_3^{2-} + H_2O \quad \text{(Eq. 1)}$$

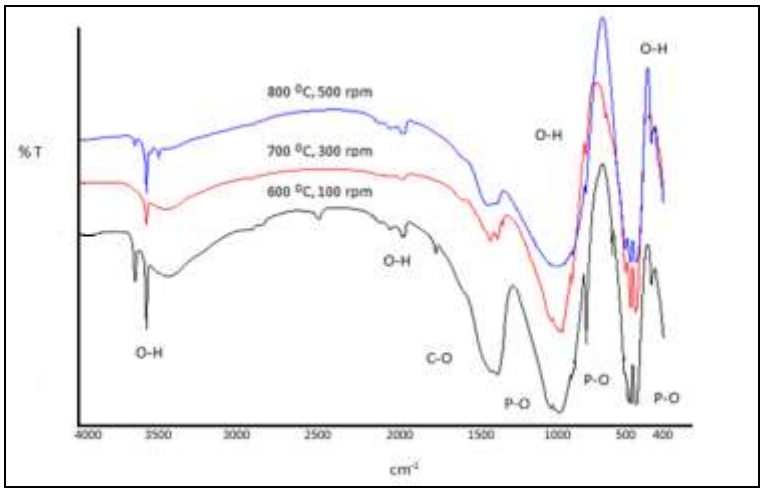


Fig. 1: FT-IR patterns for the HAp particles at different stirring rate and sintering temperature.

3.2 Phase and structural analysis (XRD):

HAp formation was identified through the intensed peak at 31.8^{O} which appeared on all of the HAp samples. The lattice parameters obtained for the pure HAp were in good agreement with standard (JCPDS file no. 09-0432). The narrow peaks of diffractogram indicated that the high crystallinity in HAp particles without any extraneous phases at sintering temperature of 600 ^{O}C. However, the HAp samples that were sintered at a higher sintering temperature than 600 ^{O}C, the extra phases formed were due to the decomposition of calcium nitrate that remain either partially reacted or unreacted in the gel during the sintering step. As the sintering temperature increased higher than 600 ^{O}C, the peaks seemed to be more distinct and it suggested an increased in the degree of crystallinity.

Calcium nitrate was believed to decompose into calcium oxide, CaO as the sintering temperature increased to 700 ^{O}C as shown in Figure 2. It could be suggested that by increasing the sintering temperature, the HAp crystallinity will also be increased [9]. Some of the XRD patterns for the HAp samples prepared are shown on Figure 5.

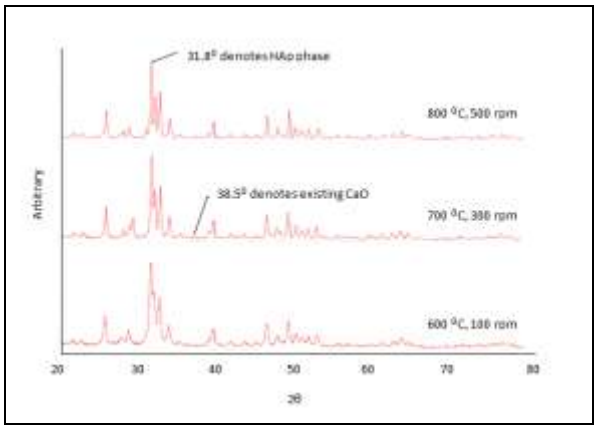
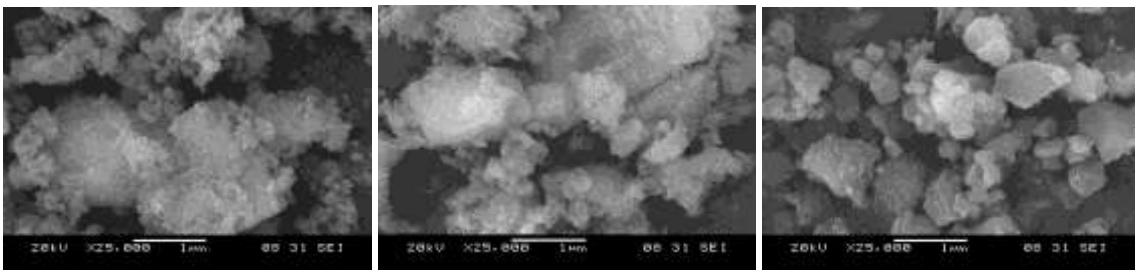


Fig. 2: Phase composition of the samples at different stirring rate and sintering temperature3.

3.3 Scanning electron microscopy (SEM):

Figure 3 shows the SEM micrographs of the HAp samples that were obtained after heat treatment at 600 ^{O}C, 700 ^{O}C and 800 ^{O}C. The powder tends to be highly agglomerated caused by the primary drying of gel formation. According to the micrographs analysis, the particle size of sintered powder increased as the sintering temperature increased. This suggest that the higher sintering temperature could contributed to powder particle growth and agglomeration. The particle size would increase because of the lack of any obstacle for particle growth when the higher sintering temperature was chosen.



(a) 600 ^{O}C, 100 rpm (b) 700 ^{O}C, 300 rpm (c) 800 ^{O}C, 500 rpm

Fig. 3: SEM images of HAp at different sintering temperature and stirring speed.

4.0 Conclusion:

A pure ceramic HAp was prepared *via* a simple sol-gel route with crystallites of nano size particles. The prepared HAp through appropriate sintering at 600 ^{O}C to 800 ^{O}C gave low crystallinity and carbonated apatitic structure. However, the crystallinity and crystallite size of HAp increased with increasing sintering temperature because of the particles agglomeration and growth.

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