Synthesis and Characterisation of Hydroxyapatite Nanopowders by Hydrothermal Method

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1. INTRODUCTION

Hydroxyapatite (HAp), chemical formula Ca_{10}(PO_4)_6(OH)_2, is a bioactive material having the potential and opportunity for development as bone substitutes. It has been used extensively in medicine and dentistry for implant synthesis due to its biocompatibility with human bones and teeth (Earl et al. 2006, Hui et al. 2010, Verges et al. 1998, Padilla et al. 2005, Rajkumar et al. 2011). HAp has poor mechanical properties, due to in case of ceramics form cannot be used for high load bearing applications, nevertheless, popular applies includes drug delivery system, coating on metallic implants and bone graft (Earl et al. 2006, Rajkumar et al. 2011, Kumar et al. 2004).

The characteristics of HAp would be affected by control of particle size, morphology and chemical composition; several methods of chemical synthesis have been developed to prepare HAp, for instance the wet chemical route, where HAp is precipitated from either an acid-base reaction or a reaction between various salts, other techniques of HAp fabrication include the sol-gel technique,
biosynthesis methods and solid state reactions at elevated temperatures (Kumar et al. 2004). Hydrothermal method has been used to transform slurries, gels, or solutions into the desired crystalline phase through mild reaction circumstances typically under 350 °C (Earl et al. 2006, Koustsopoulos 2002). By this technique, typical powders have been synthesised which have been indicated of rod like particles between 20-40 nm in diameter and 100- 160 nm in length (Earl et al. 2006).

The importance of using hydrothermal fabrication method among all other methods is that it can achieve nanosized particles for infiltration of dental tubules for the alleviation of hypersensitivity, which is a common problem for millions of children and adults worldwide (Earl et al. 2006).

2. EXPERIMENTAL WORK

By dissolving di-ammonium hydrogen phosphate (NH4)2 HPO4) (6.603 g) and calcium nitrate tetrahydrate (Ca (NO3)2. 4 H2O) (11.81 g) in 50 ml pure water, stock solutions were prepared with amount of 1.0 M. After that, to get a more diluted solution from stock solutions, more distilled water was used till approach 0.10 M. Then, by dropwise addition of 16.7 ml of 0.10 M calcium nitrate tetrahydrate to 10 ml of 0.10 M di-ammonium hydrogen phosphate, due to well-mixed suspensions with (Ca/P) molar ratios of 1.67 was achieved. By using a glass electrode, the pH of the solution was measured about 5.1. After that, a Teflon lined hydrothermal reactor was used, model 4748, to transfer the solution into the laboratory oven for 24 hours when the temperature of the system was about 200 °C. Next step, leaving the process to cool down naturally at room temperature.

When the reacted powder cooled down, the process of washing sample was started by putting it in the distilled water in the ultrasonic bath for 5 min. Then the process of centrifugation applied to the sample, which was repeated 6 or more times, each time the amount of the water has replaced with the same amount of fresh pure water. This process was essential to get back the pH of the sample to 7. Sometimes, instead of using distilled water methanol was preferred to reduce the agglomeration of the Hap particles in the drying process. In laboratory oven, samples were dried for 4 hours at 50 °C for phase analysis.

In this work, XRD has been used to study the phase analysis of all samples, the scan speed of the machine was 0.05 S⁻¹ in the range of 5 to 70 ° (2θ), and the step of it was 0.025. SEM was used, LEO 1530. FEGSEM, to determine the size and morphology of the particles when the distance working was 3 to 4 mm at 3 KV. TEM (Philips CM 200 FEG TEM), and the elements such as DLS, SAED, and EDX have been used to study the characterization of the particles.

3. RESULTS AND DISCUSSION

The earliest and the best-known mechanism of Hydroxyapatite formation is going back to the work of Posner’s theory. According to X-ray diffraction patterns, Posner explained that the amorphous phase is formed at the early stage of the reaction. Then, the structure of the particles changes from amorphous to crystal gradually. The interpretation of radial distribution functions on X-ray diffraction patterns led to the model of this amorphous phase (Suvorova & Buffat 2001). Its structure includes of Ca 9(PO4) clusters, with Ca- and PO4- groups occupying approximately the same positions as in crystalline Hydroxyapatite (Suvorova & Buffat 2001). In figure (1) the XRD pattern of dried powders at pH 11 has been shown, the diffraction planes located at d= (3.08, 2.814, 2.778, 2.72, 2.631, 1.943, and 1.611) °A. The predominant phase was confirmed with JCPDS.
file number (09-0432) to be HAp. According to triangle symbols, the Hydroxyapatite includes the phase structure of powder (Kumar et al. 2004). The observed sharp peaks in the XRD patterns in all samples confirm that the samples are in the crystalline nature (Manafi et al. 2008). The crystallite size of the powder was evaluated from the peak broadening of XRD patterns based on Scherrer’s formula as, \( D = \frac{0.9 \lambda}{\beta \cdot \cos \theta} \). Whereas D is the crystallite size (nm), \( \lambda \) is the wavelength of the monochromatic X-ray beam \( (\lambda = 0.154056 \text{ nm for Cu K}\alpha \text{ radiation}) \), and \( \beta \) is FWHM which is the full width at half-maximum for the diffraction peak under consideration and \( \theta \) is the diffraction angle (Manafi et al. 2008).

In the majority of the reported approaches, phase-pure HAp products are commonly characterised as either rod-like (whiskers, needles, wires, fibres) or plate like. However, a few reports have been published where HAp powders show a hexagonal prism (Neira et al. 2009).

Scanning electron was used to investigate the morphology and particle size of the synthesized powders. When pH-5 has been used in solution, which is shown in figure (2) according to SEM the HAp had crystal structure long rod-like particles with different dimensions. Within rod like, in some places plate-like particles can be seen, owing to less pH. Therefore, they have small surface energy to large volume ratio. Enlarged picture in figure (2) demonstrates the HAp structure, noticeably that can be seen the particles agglomerated and stuck together in the form of long rod-like particles. However, when pH-9 and pH-11 have been used; the structure of the particles of the HAp has deformed, the dimension of particles have reduced, and within they agglomerated in the shape of cluster in different places with different dimensions. Effectiveness of both pH-9 and pH-11 have shown in figure (3).

Figure (1) shows the XRD method of the participated particles.
In this work, transmission electron microscope (TEM) has been used to study the structure and morphology of HAp powder. At the same case of pH-5, the crystal structure long rode like particles can be noticed with prismatic shape, and various dimension with agglomeration in several places. Whereas, for pH-11 showed that the crystal structure of particles was smaller rod-like particles, more agglomerated, with different dimension sizes and different diameters, as both cases of effective pH’s have been shown in figure (4).

Additionally, the Dynamic Light Scattering (DLS) has been used in this study to demonstrate the volume and size distribution of particles. In figure (5), it is shown two different amounts of HAp powder about 10% and 50% in solution, while both have the same amount of pH-11. Black line shows the 10% of powder, it can be seen that the black line has two peaks one is small and the other one is large. In general, they showed that the agglomeration happened of the particles in different groups. The small peak demonstrates the less fluctuation of particles in solution with diameter size about 220 nm, while the high peak illustrates the larger agglomeration of particles on the solution with larger diameter size approximately 295 nm. Furthermore, one reason for agglomeration of particles is concentration in the solution. On the other hand, blue line shows the various particles aggregated sizes, as mentioned before, the small peak explains the minor flocculation, which means the fewer particles were detected...
showed the major flocculation of particles in different sizes; such as: 1100 and 1700 nm left to right respectively.

Figure (4) demonstrates the TEM for HAp when pH; a) 5 and (b) 11.

Figure (5) DLS technique shows the size of particles in the same of pH- 11 but in different amount of powder in different solutions, black line (HAp- pH-11 10%) and blue line (HAp- pH- 11 50%).

The selected area electron diffraction (SAED) pattern taken from the synthetic HAp nanopowder, it includes a number of some distinct spots along with the ring contours, suggesting a hexagonal structure. The spots in an electron diffraction pattern arise owing to the diffracted electron beam from a set of lattice planes in the crystalline present in the sample satisfying the Bragg diffraction condition. That is, the ring is an envelope of all diffracted spots. Elastic scattering occurring from well-ordered arrangements of atoms as in a crystal results in coherent scattering and gives spot pattern (Manafi & Joughehdoust 2009, Venkateswarlu et al. 2010). If the crystals in the material were randomly oriented as in the case of a polycrystalline material, the elastic scattering results in a ring pattern as it has shown in figure (6). The plane distances have been found from figure (6), which are about 2.70, 3.37, and 4.06 Å. As a result, the SAED pattern confirms the crystalline structure of hexagonal HAp and is approximately in agreement with XRD results (Venkateswarlu et al. 2010).

Figure (6) SAED shows the HAp crystal structure pattern

According to EDX pattern, it can be observed in figure (7) that the Ca/P ratio stills the same in different pictures, which is indicated about 1.67 ratio of Ca/P. If this value would be compared with the standard value when used, then it would be the same amount of ratio, which means the HAp has formed from Ca and P.
4. CONCLUSIONS

Nanopowder of hydroxyapatite was synthesized by hydrothermal method, Ca(NO3)2.4H2O, (NH4)2 HPO4 and distilled water were used as a stock solution. Synthesised nanopowders were characterised, XRD showed that HAp has the crystalline structure. The pH had a significant impact on the size of HAp nanoparticles, when it was less about pH 5, the nanoparticles dimension size long rode like particles. While it was increased about 9 and 11 the dimension size short rode like particles. SEM and TEM showed that the HAp nanoparticles resemble nano rode like morphology. DLS technique explained the two peaks which are small and large, the small peak demonstrates the less fluctuation of particles in solution, while the large one illustrates the larger agglomeration of particles on the solution with larger diameter size. The SAED pattern confirms the crystalline structure of hexagonal HAp and is approximately in agreement with XRD results. Also, EDX explained the ratio of Ca/P is about 1.67, and the obtained HAp nanopowder can be used for biomedical applications and scientific research purpose.

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