

Full Length Research Paper

Synthesis and characterization of nano-hydroxyapatite (n-HAP) using the wet chemical technique

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Hydroxyapatite nanoparticles were synthesized, using a wet chemical technique with diammonium hydrogen phosphate and calcium nitrate tetrahydrate precursors, respectively. The pH of the system was maintained at 10.8 throughout the stirring process, by using 0.1 M sodium hydroxide. The mixture was allowed to remain stirred overnight and a white precipitate was formed. The precipitate was vacuum dried and cleaned with distilled water and ethanol simultaneously three or four times. The prepared powder was used for further characterization. The prepared nano-hydroxyapatite powder was characterized for phase composition, using X-ray diffractometry; elemental dispersive X-ray and Fourier transform infrared spectroscopy. The elemental compositions of the nano-hydroxyapatite were analyzed and confirmed by elemental dispersive X-ray (EDX). The particle size and morphology were studied using the scanning electron microscope (SEM) and transmission electron microscopy (TEM). The particle size of the nano-hydroxyapatite was also analyzed, using the dynamic light scattering (DLS) experiment.

Key words: Hydroxyapatite, wet chemical, elemental dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), transmission electron microscopy (TEM).

INTRODUCTION

Tissue engineering is a multidisciplinary science, encompassing diverse fields like materials engineering and molecular biology, in the effort to develop biological substitutes for failing tissues and organs. Tissue engineering thus seeks to replace diseased and damaged tissues of the body. An important factor for the success of tissue engineering is the ability of developing materials, which can interface with tissues structurally, mechanically, and bio functionally (Yang et al., 2001). Many biomaterials lack the desired functional properties to interface with biological systems, in spite of numerous uses of materials in tissue engineering. Thus, developing new materials to reach these issues is necessary. Composites of hydrophilic polymers and inorganic minerals like hydroxyapatite can be good materials for biomedical applications. Bone apposition and differentiation of mesenchymal cells to osteoblasts can

be promoted by the attachment of hydroxyapatite nanoparticles to a polymer surface (Sinha and Guha, 2008). Hydroxyapatite is a significant biomaterial in the health care industry. Its chemical and mineral phases are analogous to those of natural bone and hence, its usage in the field of dentistry and orthopedics has been explored (Zhao and Ma, 2005; Chen et al., 2002; Hornez et al., 2007). Properties like osteoconductivity and osteoinductivity enhance bone regeneration and make hydroxyapatite an important material in tissue engineering (Burg et al., 2000), and its biocompatibility leads to its use as bioactive coating over implants (Wang et al., 2006; Ye et al., 2007). Bone is considered as a nanocomposite of minerals and proteins, and recently nano-level hydroxyapatite has been investigated and demonstrated as having a good impact on cell biomaterial interaction (Elliot, 1994; Webster et al., 2000).

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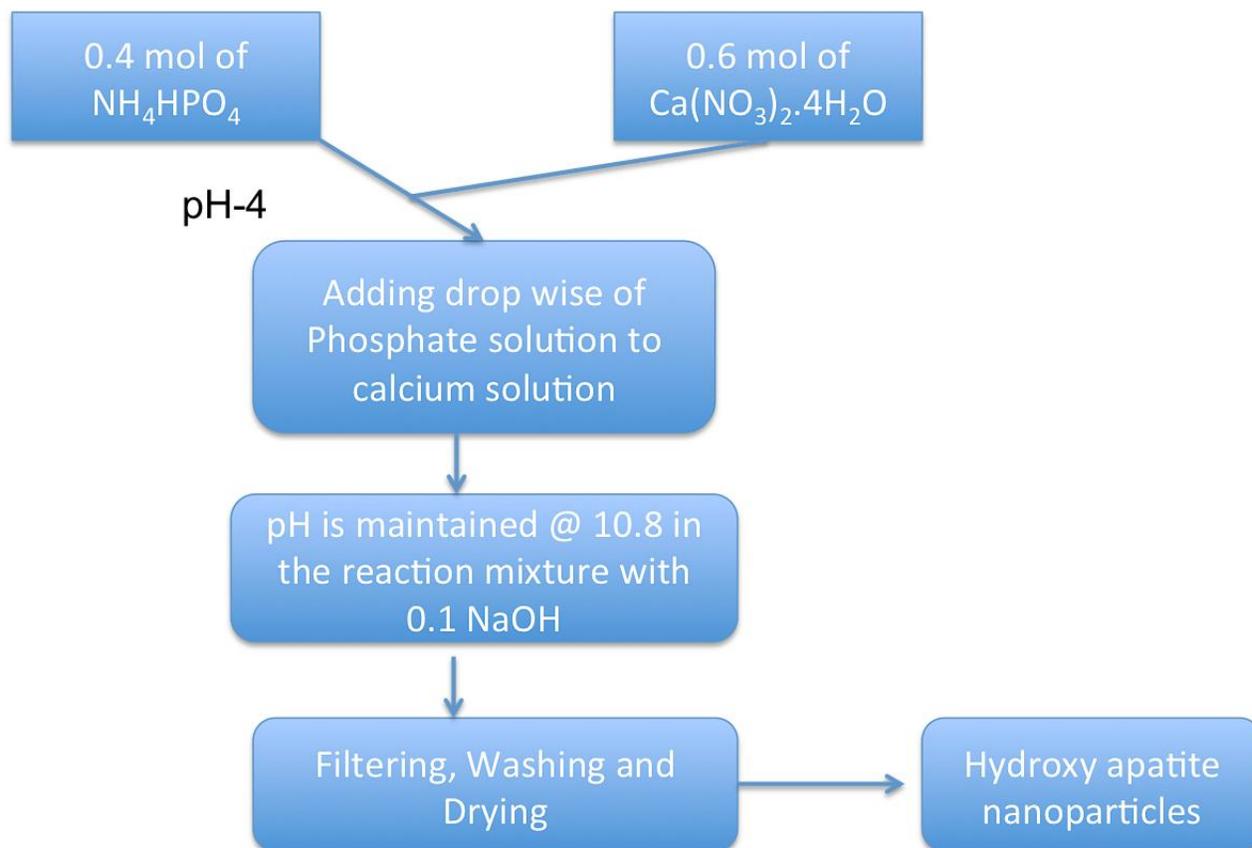


Figure 1. Flow chart of hydroxyapatite nanoparticles preparation by wet chemical method.

However, the migration of the nano- hydroxyapatite particles from the implanted site into the surrounding tissues might cause damage to healthy tissue (Miyamoto et al., 1998). To find a solution, composites of nano-hydroxyapatite and polymers were researched to find a material that retained the good properties of nano-hydroxyapatite and prevented the nano- hydroxyapatite particles from migrating. In the present investigation, we report the synthesis and characterization of nano-hydroxyapatite by a wet chemical synthesis technique. The as prepared hydroxyapatite nanoparticles are characterized by X- ray diffraction, Fourier transform infrared spectroscopy (FTIR), elemental dispersive X-ray (EDX) analysis, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) analysis.

EXPERIMENTAL PROCEDURES

Synthesis of hydroxyapatite nanoparticle

Hydroxyapatite nanoparticles were synthesized by the wet chemical method. 500 ml of 0.4 mol of diammonium hydrogen phosphate with pH-4.0 was vigorously stirred in 2 L beaker at room temperature and 500 mL of 0.6 mol calcium nitrate tetrahydrate with

pH=7.4 was added drop-wise over 4 h. The pH of the system was maintained at 10.8 throughout the stirring process, by using 0.1 M sodium hydroxide. The mixture was allowed to remain stirred overnight. A white precipitate was formed. The precipitate was vacuum dried and cleaned with distilled water and ethanol simultaneously for three or four times. The prepared powder was used for further characterization. The schematic presentation of the procedure is given in Figure 1. This precipitation reaction for synthesis of hydroxyapatite nanoparticles was first proposed by Yagai and Aoki, as indicated by Bouyer et al. (2000).

RESULTS AND DISCUSSION

XRD analysis

The structural analysis of sample was done by the powder X-ray diffraction. The XRD patterns of the synthesized nano hydroxyapatite are shown in Figure 2. The XRD pattern of nano hydroxyapatite shows sharper peaks which indicate better crystallinity. The peak positions are in good agreement with the JCPDS (896438). As can be seen, hydroxyapatite XRD patterns, with the diffraction peaks, obtained with d-spacing values of 2.82\AA , 2.79\AA and 2.72\AA and the other d-spacing values match exactly with the hexagonal system with

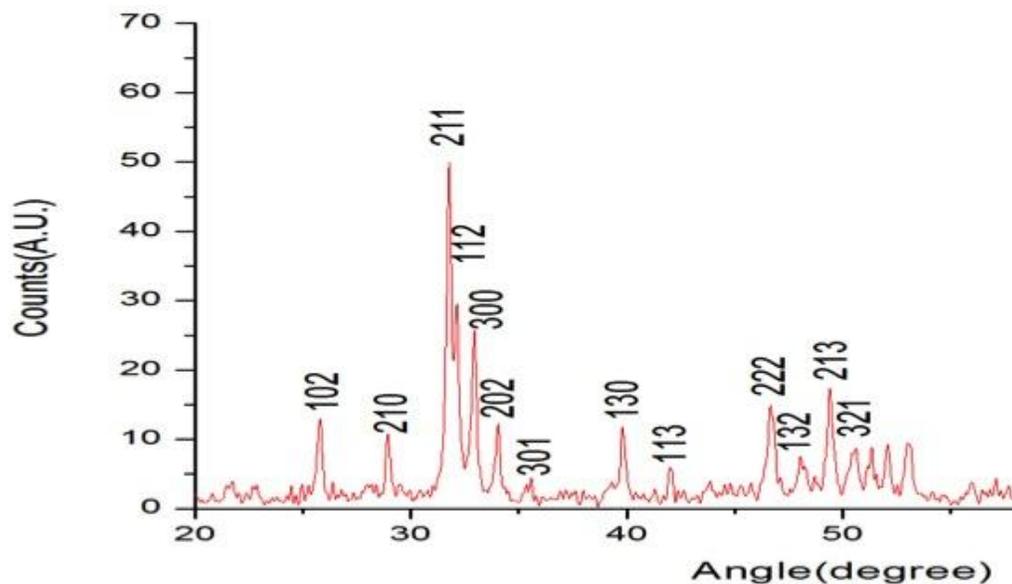


Figure 2. XRD spectra of hydroxyapatite nanoparticles.

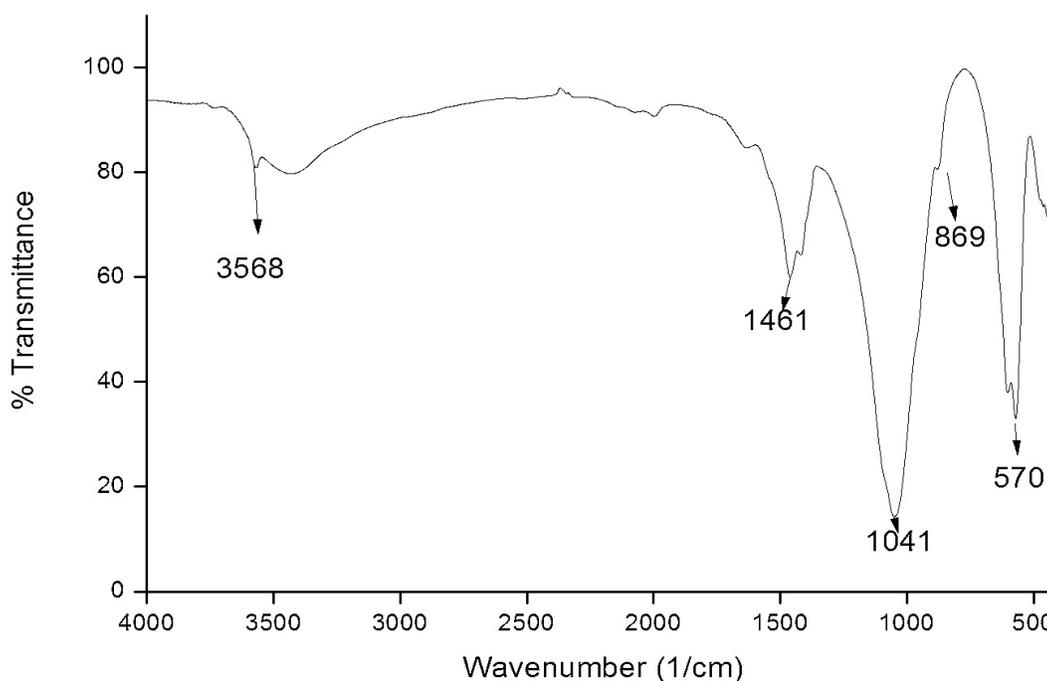


Figure 3. FTIR spectrum of nano- hydroxyapatite.

primitive lattice. The results of XRD analysis obtained in the present investigation are in good agreement with the reported results (Bouyer et al., 2000).

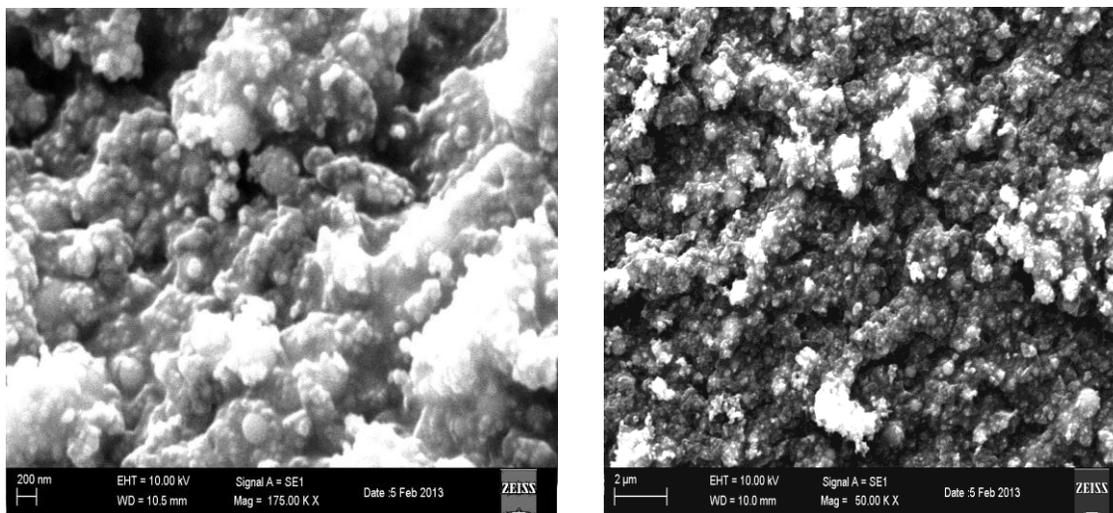
FTIR analysis

Functional groups associated with hydroxyapatite were

identified by FTIR spectroscopy. The FTIR spectra of the prepared samples are given in Figure 3. The ion stretching vibration around 3568 cm^{-1} confirms the presence of a hydroxyl group. Likewise, the other stretching vibrations for carbonyl and phosphate groups were also observed as reported earlier (Cengiz et al., 2008). The observed functional groups and their corresponding assignments are presented in Table 1.

Table 1. Some important functional group assignments of hydroxyapatite nanoparticles.

Wavenumber cm^{-1}	Stretching mode	Functional group
3568	Ion Stretching	OH^-
1461	Asymmetric stretching	CO_3^{2-}
1041	Asymmetric stretching	PO_4^{3-}
869	Out of plane bending mode	CO_3^{2-}
570	Asymmetric bending vibration	PO_4^{3-}

**Figure 4.** SEM Image of the hydroxyapatite nanoparticle.

The functional groups of the hydroxyapatite powder predicted from FTIR spectra analysis are compared with the results of Choi et al. (2004) for the confirmation.

SEM analysis

The scanning electron microscope (SEM) was used for the morphological study of nanoparticles of hydroxyapatite. Figure 4 shows the SEM images of the as-prepared hydroxyapatite nanoparticles. The hydroxyapatite nanoparticles formed were highly agglomerated. The agglomeration of the nanoparticles might be because of Ostwald ripening. The spherical shaped particles with clumped distributions are visible from the SEM analysis. The SEM images show the spherical shaped particles as confirmed by Ferraz et al. (2004) for reported results of hydroxyapatite nanoparticles.

EDX analysis

In Figure 5a and b, and Table 2, the standard EDX spectra recorded on the examined nano-hydroxyapatite

are shown. The presented spectrum shows the Ca/P value of the synthesized nano-hydroxyapatite to be 1.68, which is quite close to the Ca/P ratio of the human bone (Trommer et al., 2007).

TEM analysis

The structure and morphology of the samples were further confirmed by the TEM and TEM images of the prepared nano-hydroxyapatite, as shown in Figure 6. The transmission electron microscopic analysis confirms the presence of the spherical shape morphology of the prepared hydroxyapatite nanoparticle with the particle size of around 60 to 70 nm (Figure 7). The particle size is also found to be in agreement with the report results of Ferraz et al. (2004).

DLS studies

Dynamic light scattering (DLS) is an important tool for characterizing the size of nanoparticles in a solution. The DLS measures the light scattered from a laser that passes through a colloidal solution and by analyzing the

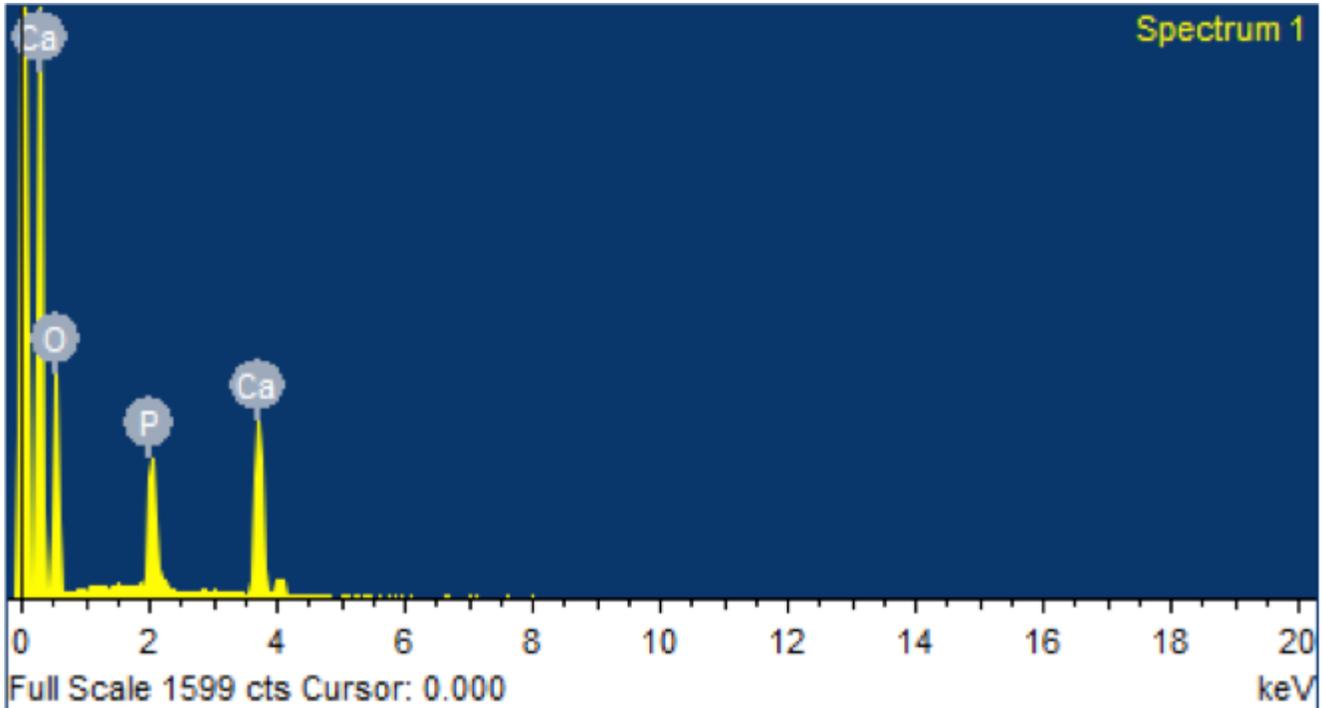


Figure 5a. EDX spectrum of nano-hydroxyapatite.

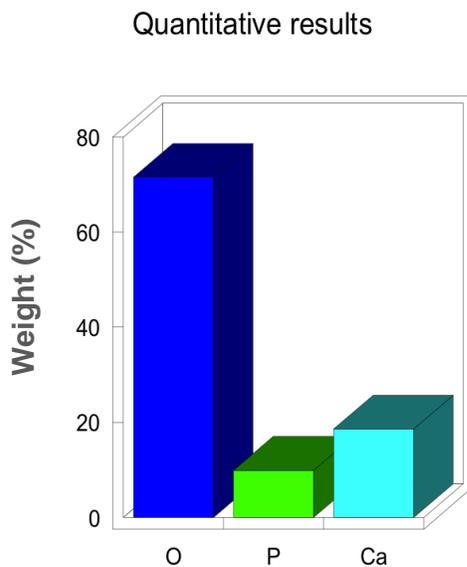


Figure 5b. Chart showing the weight % and quantitative results of EDX spectrum of nano-hydroxyapatite.

modulation of the scattered light intensity as a function of time, the hydrodynamic size of the particles and particle agglomerates can be determined. Larger particles will diffuse slower than smaller particles, and the DLS instrument measures the time dependence of the

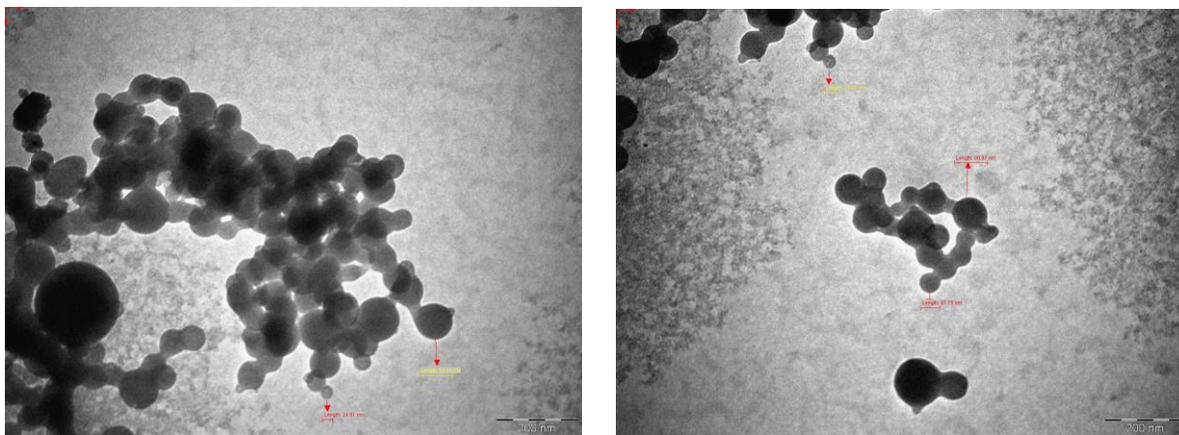
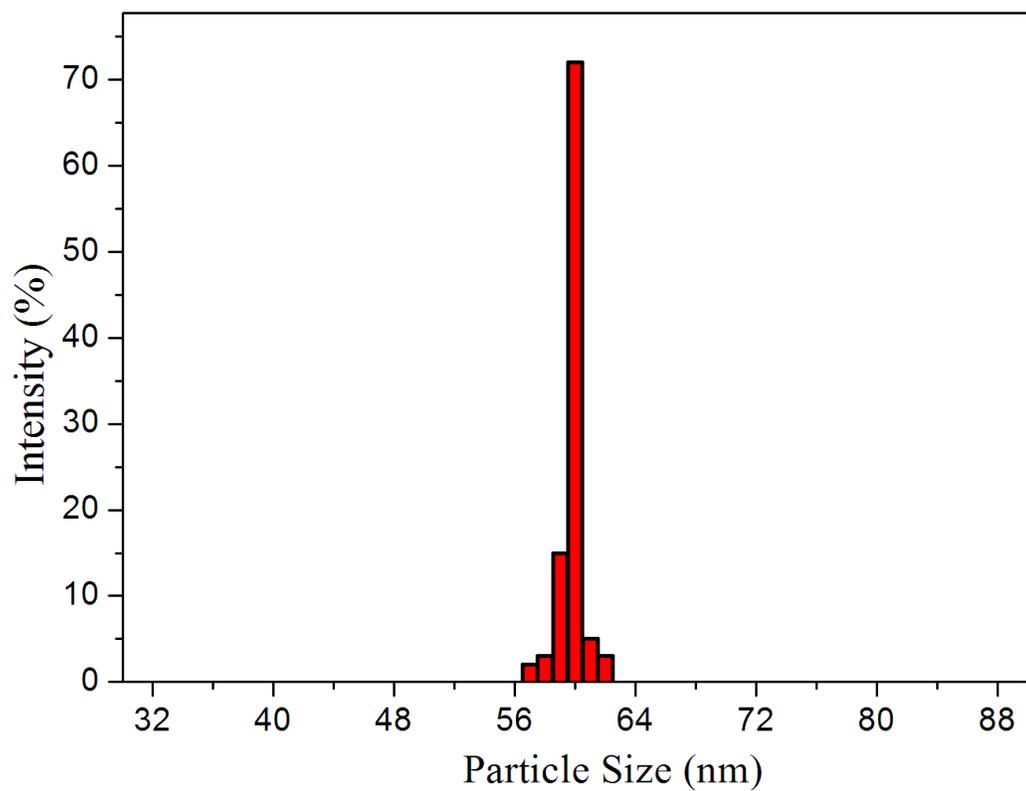
scattered light, to generate a correlation function that can be mathematically linked to the particle size. The DLS is a valuable tool for determining and measuring the agglomeration state of the nanoparticles as a function of time or suspending solution. When the DLS sizing data is compared to the transmission electron microscopy images, the aggregation state of the particles can be determined. In an unagglomerated suspension, the DLS measured diameter will be similar or slightly larger than the TEM size. If the particles are agglomerated, the DLS measurement is often much larger than the TEM size, and can have a high polydispersity index (large variability in the particle size). The dynamic light scattering experiment shows that the particle size distribution is in the range of 50 to 70 nm, which is well supported by the TEM analysis. Dynamic light scattering is used to monitor the size of the precipitating particles and to provide information about their concentration (De Bruyn et al., 2013).

Conclusion

Nano-hydroxyapatite has been successfully synthesized using the wet chemical technique. The formation of hydroxyapatite nano particles was confirmed by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). The elemental compositions were examined using the EDX analysis. The size and morphology of the samples were characterized using

Table 2. Elemental composition of nano-hydroxyapatite.

Element	App	Intensity	Weight (%)	Weight (%)	Atomic (%)
	Conc.	Conc.		Sigma	
OK	2.26	0.7087	73.52	0.96	85.09
PK	0.56	1.2858	9.88	0.55	6.07
Ca K	0.82	0.9935	16.60	0.70	8.84
Total			100.00		

**Figure 6.** TEM images of hydroxyapatite nanoparticles.**Figure 7.** Particle size of hydroxyapatite nanoparticles.

scanning and transmission electron microscopy (SEM and TEM). The spherical shaped particles were confirmed through the SEM analysis. The transmission electron microscopic analysis confirms the prepared hydroxyapatite nanoparticles with the particle size of around 60 to 70 nm. The particle size of the nano-hydroxyapatite range of 50 to 70 nm was determined using the dynamic light scattering (DLS) experiment which is in good agreement with the TEM analysis.

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REFERENCES

- Bouyer E, Gitzhofer F, Boulos MI (2000). Morphological study of hydroxyapatite nanocrystal suspension. *J. Mater. Sci. Mater. Med.* 11: 523-531.
- Burg KJL, Porter S, Kellam JF (2000). Biomaterial developments in bone tissue engineering. *Biomaterials.* 21:2347-2359.
- Cengiz B, Gokce Y, Yildiz N, Aktas Z, Calimli A (2008). Synthesis and characterization of hydroxyapatite nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 322(1-3):29-33.
- Chen F, Wang ZC, Lin CJ (2002). Preparation and characterization of nano-sized hydroxyapatite particles and hydroxyapatite/chitosan nano-composite for use in biomedical materials. *Mater. Lett.* 57(4):858-861.
- Choi D, Marra K, Kumta PN (2004). Chemical synthesis of hydroxyapatite/poly (caprolactone) composite. *Materials Research Bulletin.* 39:417-432.
- De Bruyn JR, Goiko M, Mozaffari M, Bator D, Dauphinee RL (2013). Dynamic light scattering study of inhibition of nucleation and growth of hydroxyapatite crystals by osteopontin. *Plus One.* 8(2):e 56764.
- Elliot JC (1994). Amsterdam: Structure and Chemistry of the Apatites and Other Calcium Orthophosphates. Elsevier Science. 111.
- Ferraz MP, Monteiro FJ, Manuel CM (2004). Hydroxyapatite nanoparticles: A review of preparation methodologies. *J. Appl. Biomater. Biomech.* 2:74-80.
- Hornez JC, Chai F, Monchau F, Blanchemain N, Descamps M, Hildebrand HF (2007). Biological and physico-chemical assessment of hydroxyapatite (HA) with different porosity. *Biomol. Eng.* 24:505-509.
- Miyamoto Y, Ishikawa KI, Takechi M, Toh T, Yuasa T, Nagayama M, Suzuki K (1998). Basic properties of calcium phosphate cement containing atelocollagen in its liquid or powder phases. *Biomaterials.* 19:707-715.
- Sinha A, Guha A (2008). Biomimetic patterning of polymer hydrogels with hydroxyapatite nanoparticles. *Mater. Sci. Eng. C.* 29:1330-1333.
- Trommer RM, Santos LA, Bergmann CP (2007). Alternative technique for hydroxyapatite coatings. *Surface Coatings Technol.* 201(24):9587-9593.
- Wang YJ, Chen JD, Wei K, Zhang SH, Wang XD (2006). Surfactant-assisted synthesis of hydroxyapatite particles. *Mater. Lett.* 60:3227-3231.
- Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R (2000). Enhanced functions of osteoblasts on nanophase ceramics. *Biomaterials.* 21:1803-1810.
- Yang S, Leong KF, Du Z, Chua CK (2001). The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Engineering.* 7:679-689.
- Ye W, Wang XX (2007). Ribbon-like and rod-like hydroxyapatite crystals deposited on titanium surface with electrochemical method. *Mater. Lett.* 61:4062-4065.
- Zhao YF, Ma J (2005). Triblock co-polymer templating synthesis of mesostructured hydroxyapatite. *Micro. Meso. Mater.* 87:110-117.