J. Iran. Chem. Soc., Vol. 6, No. 2, June 2009, pp. 386-392.

JOURNAL OF THE
Iranian
Chemical Society

Preparation of Hydroxyapatite Nanoparticles: Comparison between Hydrothermal and Solvo-Treatment Processes and Colloidal Stability of Produced Nanoparticles in a Dilute Experimental Dental Adhesive

M. Sadat-Shojai*
Iran Polymer and Petrochemical Institute, P.O. Box 14965/115, Tehran, Iran

(Received 10 May 2008, Accepted 12 June 2008)

The main purpose of this study is to investigate the colloidal stability of hydroxyapatite (HAp) nanoparticles prepared by different methods. Nano-sized hydroxyapatite particles are synthesized by two different methods including hydrothermal and solvo-treatment processes. In hydrothermal process nanoparticles are synthesized at high temperature, while in solvo-treatment method nanoparticles are synthesized at room temperature by the use of surfactants and organic solvent. The samples are characterized by powder X-ray diffraction (XRD), fourier-transformed infrared spectroscopy (FT-IR), scanning electron microscopy coupled with energy dispersive X-ray analysis detector (SEM + EDXA), and phase separation analyzer. The produced nanoparticles are different in size, stoichiometric ratio, morphology, crystallinity and colloidal stability in a dilute dental adhesive. The results show that the nanoparticles synthesized by these two methods are quite dissimilar and particles prepared by hydrothermal method have a smaller size and higher colloidal stability.

Keywords: Hydroxyapatite, Hydrothermal, Solvo-treatment, Colloidal stability, Dental adhesives

INTRODUCTION

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) has widely been used in biomedical and dental applications due to its similarity to main mineral components of hard tissues of human body such as bone, dental enamel and dentin and also its biocompatibility, bioactivity and low solubility in moist medias [1-9]. In addition, hydroxyapatite can replace toxic ion in human body by its own ions [1]. However, there are several parameters that should be considered for hydroxyapatite nanoparticles such as size, morphology, appropriate stoichiometry, phase composition and crystallinity of nanoparticles [2,3].

There are different reported methods for producing HAp nanoparticles such as chemical precipitation [6,8], sol-gel process [6,8], hydrothermal method [3,6,9], biomimetic deposition [6], flame spray pyrolysis process [2] and solvothermal method [4]. The hydrothermal methods have usually been applied to prepare nanofibers or nanowires [3,9]. However, with hydrothermal treatment, the Ca/P ratio of the precipitates improves with the increase in hydrothermal pressure or temperature [4]. Therefore, this procedure can be employed to prepare HAp nanoparticles. The other process is solvo-treatment method in which, instead of pressure and temperature, the surfactants and organic solvent are used. This method, also known as solvothermal, is reported only for preparation of nanorods at high pressure [4,10,11].

Dental enamel that is the hardest tissue of human body consists of inorganic components, organic components and water [12]. The most important inorganic component is

*Corresponding author. M.Shojai@ippi.ac.ir

Archive of SID

www.SID.ir

hydroxyapatite that is the basic structural component of enamel. The use of adhesive materials in dental fields, particularly for restorative material such as composites, is increasing. During the process of the light curing of dental composites, shrinkage occurs and the component with the lowest elastic modulus, *i.e.* the adhesive layer, may fail because of standing the highest strain. To overcome this problem, it has been proposed that nanofillers can improve adhesion at the interface between the restorative material and the tooth structure through increasing mechanical strength of the adhesive layer and providing structural reinforcement [13]. It has been hypothesized that the nanofillers are stress-absorbing and have the role of elastic layer between dental composite and dentin [13]. In fact, reaching the highest adhesion and the least toxicity are the ultimate objects in dental adhesives and the use of hydroxyapatite is agreeable with these two objects. However the colloidal stability is a main problem for application of fillers in dilute systems. A common approach for increasing the colloidal stability is surface modification of fillers by various materials and subsequent surface grafting polymerization [14].

The present paper reports the successful synthesis of hydroxyapatite nanoparticles, *via* both the solvo-treatment method, using a mixture of ionic and nonionic surfactants, and the hydrothermal process. It was found that the produced hydroxyapatite nanoparticles by hydrothermal method without any surface modification have dramatically high colloidal stability in dental adhesive. It is probably due to the lower particles size, lower aggregation or higher surface charge of nanoparticles prepared by hydrothermal process than those synthesized by solvo-treatment method.

EXPERIMENTAL

Materials

Calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate were used as the starting materials in the synthesis of HAp nanoparticles. N-Cetyl-N,N,N-trimethylammonium bromide (CTAB), n-pentanol and n-hexane were of analytical grade and span 60 was of synthesis grade. All chemicals were purchased from Merck (Germany) and used as received.

The ingredients of the experimental dental adhesive were as follows: 2,2-bis[*p*-(2-hydroxy-3-methacryloxypropoxy) phenyl] propane (Bis-GMA) from Röhm (Degussa group, Germany), 2-hydroxyethyl methacrylate (HEMA) from Merck and trimethylolpropane trimethacrylate (TMPTMA) from Aldrich (Germany). Other solvents were obtained from Merck.

Procedures

Solvo-treatment method. First, 0.4 M $Ca(NO_3)_2$ and 0.2 M $(NH_4)_2HPO_4$ solutions were obtained by dissolving appropriate amount of $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$ in deionized water. 2.5 g CTAB, 3 ml n-pentanol and 5 ml Ca^{2+}-containing solution were added to 60 ml n-hexane. The suspension was stirred so that CTAB completely dissolved and then ultrasonicated to obtain a transparent solution. Then 0.25 g Span 60 was slowly added to the solution. When Span was dissolved completely, 6 ml PO_4^{3-}-containing solution was added to the mixture at a rate of 0.3 ml min^{-1} and under continuous stirring. The pH of the solution was kept at about 11 by adding ammonia solution. At the end of the reaction, the mixture was transferred into a sealed container and aged at room temperature for a week without stirring. Finally the synthesized powder was separated by centrifugation of microemulsion at 10000 rpm for 20 min and then washed three times by a mixture of distilled water and ethanol (volume ratio = 1:1) so that the pH had returned to 7. Final precipitate was washed with methanol to deter the agglomeration of the nanoparticles. The resulting powder was dried at about 50 °C for 24 h and then calcined at 600 °C for 2 h.

Hydrothermal process. The above stock solutions were diluted five times by deionized water to obtain the calcium and phosphate solutions with concentration of 0.08 and 0.04 M, respectively. The phosphate ion solution was added to the calcium ion solution at a rate of 0.3 ml min^{-1} and molar ratio of Ca/P was kept at stoichiometric amount according to its ratio in hydroxyapatite. During the reaction, the suspension was stirred and pH was adjusted to 11 by adding ammonia solution. Then the suspension was transferred into a sealed stainless steel reactor and stored at 200 °C for 60 h, followed by cooling naturally. At the end of the hydrothermal treatment, the precipitate was separated by centrifugation of suspension at 10000 rpm for 20 min and then washed and dried as well as before.

Addition of HAp Nanoparticles into Dental Adhesive

The experimental dental adhesive was used with the

Archive of SID

www.SID.ir

follwing formula: Bis-GMA + HEMA + acetone + ethanol + TMPTMA with 9, 29, 37, 18, 7 wt%. Then 1% (w/w) of the synthesized nanoparticles were added to the dental adhesive and subsequently sonicated for 2 min. The mixture was placed in the separation analyzer and sedimentation behavior was monitored for 12 h.

The HAp nanopowders were characterized by X-ray diffraction (D5000, SIEMENS Co.) with CuKα radiation (λ = 1.5418 Å) and fourier transform infrared spectroscopy (FTIR; EQUINOX55, BRUKER, Germany) using KBr pellet technique. The morphology and size of the nanoparticles were characterized by scanning electron microscopy (SEM: XL30, Philips, Netherlands). The chemical composition of the as-prepared powders was investigated using energy dispersive X-ray analysis (EDXA:QX2, RONTEC Co.) which was coupled by SEM. Monitoring of colloidal stability was performed by a separation analyzer (LUMiReader 416.1, LUM, Germany).

RESULT AND DISCUSSION

XRD patterns of produced HAp nanoparticles are shown in Fig. 1. They are identified as HAp according to ICDD (International Centre for Diffraction Data) standard (PDF Card no. 9-432) and all peaks correspond to hexagonal crystal system of HAp [5,15,16,17,18]. Meanwhile, the high purity of HA phase was confirmed by XRD patterns. However, Fig. 1 shows that the diffraction peaks of HAp in pattern (a) are much stronger than pattern (b) indicating that the crystallinity of the HAp nanoparticles prepared by hydrothermal process is much higher than that prepared by solvo-treatment method, which is attributed to the hydrothermal treatment in the synthesis process.

FTIR absorption spectra of the hydroxyapatite nanoparticles prepared by hydrothermal and solvo-treatment processes are shown in Fig. 2. As seen, the wave numbers for

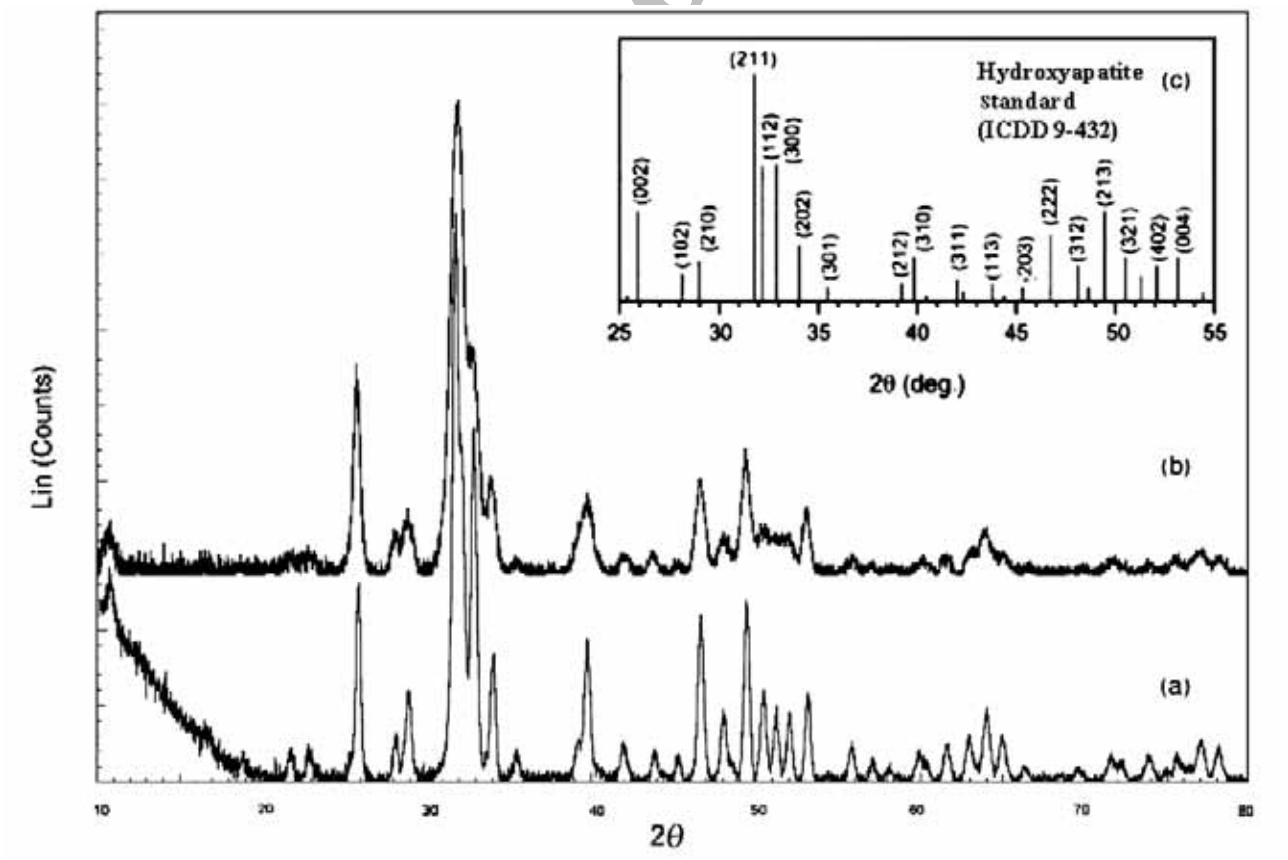

Fig. 1. XRD patterns of the HAp nanoparticles prepared by hydrothermal process (a) and solvo-treatment method (b). (c) ICDD standard (PDF Card no. 9-432) for hydroxyapatite [18].

www.SID.ir

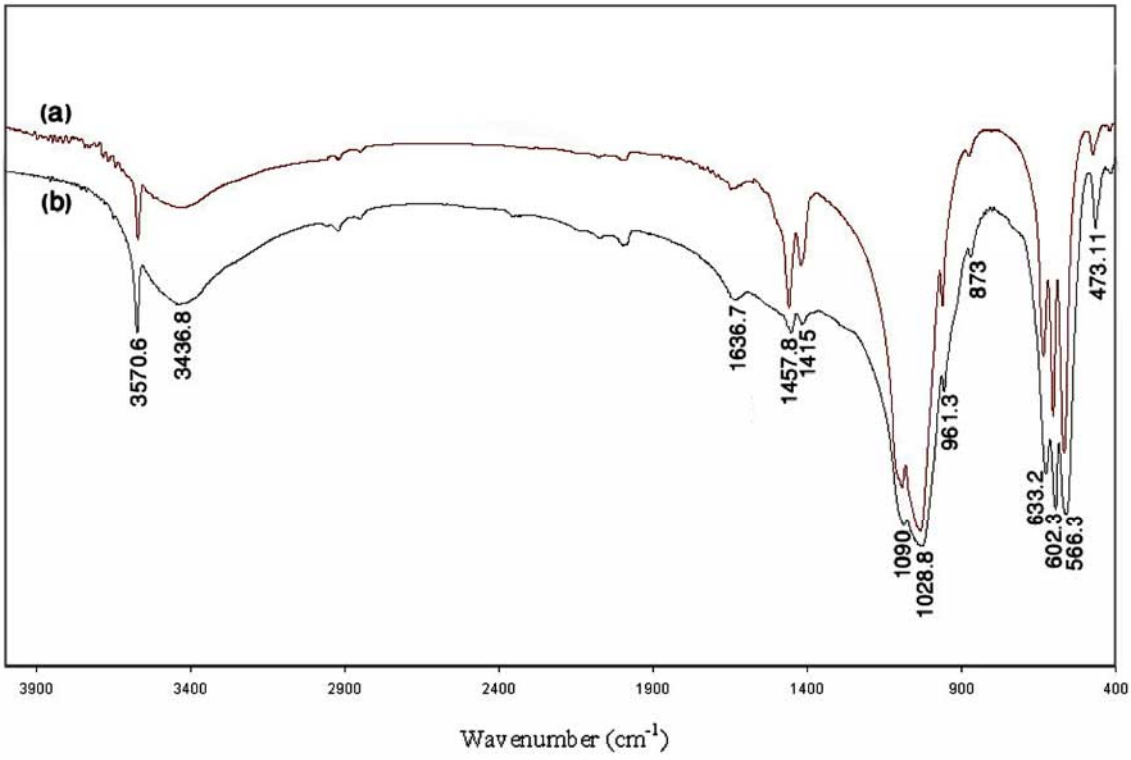

Fig. 2. FTIR absorption spectra of the HAp nanoparticles prepared by hydrothermal process (a) and solvo-treatment method (b).

Archive

characteristic peaks in both spectra are approximately identical. The characteristic peaks for PO_4^{3-} appear at about 1090, 1029, 602, 961, 566 and 473 cm^{-1} [4,10,11,15,17]. A duplex peak at wave numbers of about 1415 and 1458 cm^{-1} is attributed to carbonate impurities in the structure of hydrxyapatite. As it is seen, the intensity of this duplex peak in spectrum (a) is much higher than that in spectrum (b); this is rational because the possibility of absorption of carbon dioxide from the air during the reaction in watery media (hydrothermal process) is much higher than that in oily media (solvo-treatment method).

The peaks at 3437 and 1637 cm^{-1} are relevant to the bending modes of hydroxyl group in the adsorbed water, while the peaks at 3571 and 633 cm^{-1} are assigned to the stretching vibration of the hydroxyl group in crystal structure of hydroxyapatite [4,10,15,17]. Meanwhile the weak peak at about 873 cm^{-1} demonstrates the presence of low amounts of hydrogen phosphate in crystal structure of hydroxyapatite.

Figure 3 shows the size differences between two kinds of synthesized nanoparticles. According to Fig. 3a the size of hydroxyapatite nanoparticles prepared by hydrothermal method is 45-65 nm. Furthermore, all of nanoparticles have the same morphology with uniformity in size. Figure 3b shows the SEM image of hydroxyapatite nanoparticles prepared by solvo-treatment method. Here, the nanoparticles are approximately formless with an uniform particle-size of 75-98 nm.

In the present study, it was also tried to deter the formation of nanorods or nanowires. To approach this goal, we utilized the mixture of cationic and nonionic surfactants. Ying Jun Wang *et al*. have used CTAB as surfactant and n-pentanol as co-surfactant to form HAp nanowires and also they have elaborated the formation mechanism of HAp nanowires [19]. They have suggested that these nanowires are formed in the uni-directional fusion of reverse micelles and the electrostatic field is responsible to form HAp nanowires. They have also

www.SID.ir

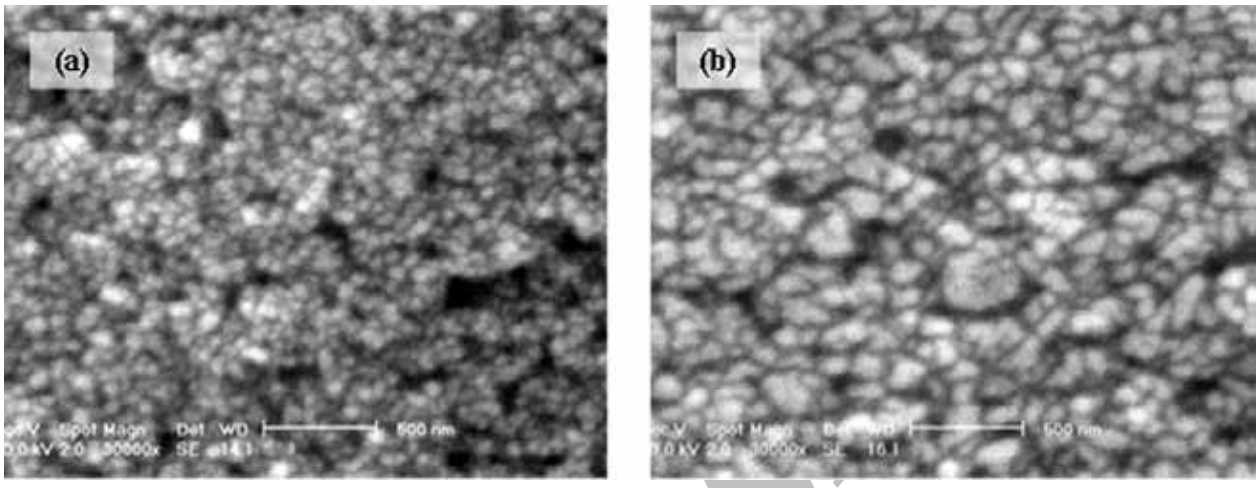

Fig. 3. SEM images of nano-sized hydroxyapaite prepared by hydrothermal process (a) and solvo-treatment method (b).

suggested that the electrostatic field should be tunable by the density of the charges on the surface of the reverse micelles as the intervention of the n-pentanol as non ionic co-surfactant in the reverse micelles should decrease the intensity of the electrostatic field and finally the CTAB molecule will lose the morphology control of nanoparticles. However, in our experiment, to deter the formation of nanowires we have utilized the Span 60 as a non ionic surfactant to decrease the electrostatic field. In this case the CTAB molecules do not have any significant role of morphology controlling and are used only for size controlling. It means that reverse micelles are formed but with the least electrostatic field and, as a result, they are very instable. In other words, the surfactant molecules are very mobile so that the formation of regular reverse micelles is impossible. In this case a series of irregular and nano-sized reverse micelles are formed and formation of HAp nanoparticles is performed in these irregular reverse micelles. As a result the morphology of nanoparticles is formless but their size is in nanometer scale.

Figure 4 shows the EDXA spectra of the produced HAp nanoparticles.

According to EDXA spectra, the presence of Ca and P is confirmed and the Ca/P peak intensity ratios were obtained as 1.69 and 1.78 for the synthesized nanoparticles by hydrothermal and solvo-treatment processes, respectively. These values are approximately close to the theoretical value in stochiometric HAp powder (1.67). The presence of carbon in the EDAX spectra provides further evidence of the presence of carbonate group in crystal structure of the samples. According to EDXA spectra, the ratios of carbon to phosphorus are 0.136 and 0.053 for synthesized nanoparticles by hydrothermal and solvo-treatment processes, respectively. These values confirm the relative intensities of characteristic peak of carbonate group in FTIR absorption spectra. However, the mineral component of bone is nonstoichiometric HAp with carbonate substitution [6,8,15,20,21].

Figure 5 shows the separation analyzer tests for colloidal suspensions of HAp nanoparticles in an experimental dental adhesive (weight ratio = 1:100). Figure 5a indicates that, even after 12 h, nanoparticles are not settled completely. However Fig. 5b shows that the nanoparticles prepared by solvo-treatment method are unstable as sedimentated completely after about 2 h. The separation analyzer tests suggest that the colloidal stability of hydroxyapatite nanoparticles, synthesized by hydrothermal process, in dental adhesive is dramatically higher than those synthesized by solvo-treatment method. It is probably due to the lower particles size, lower aggregation or higher surface charge of nanoparticles prepared by hydrothermal process.

www.SID.ir

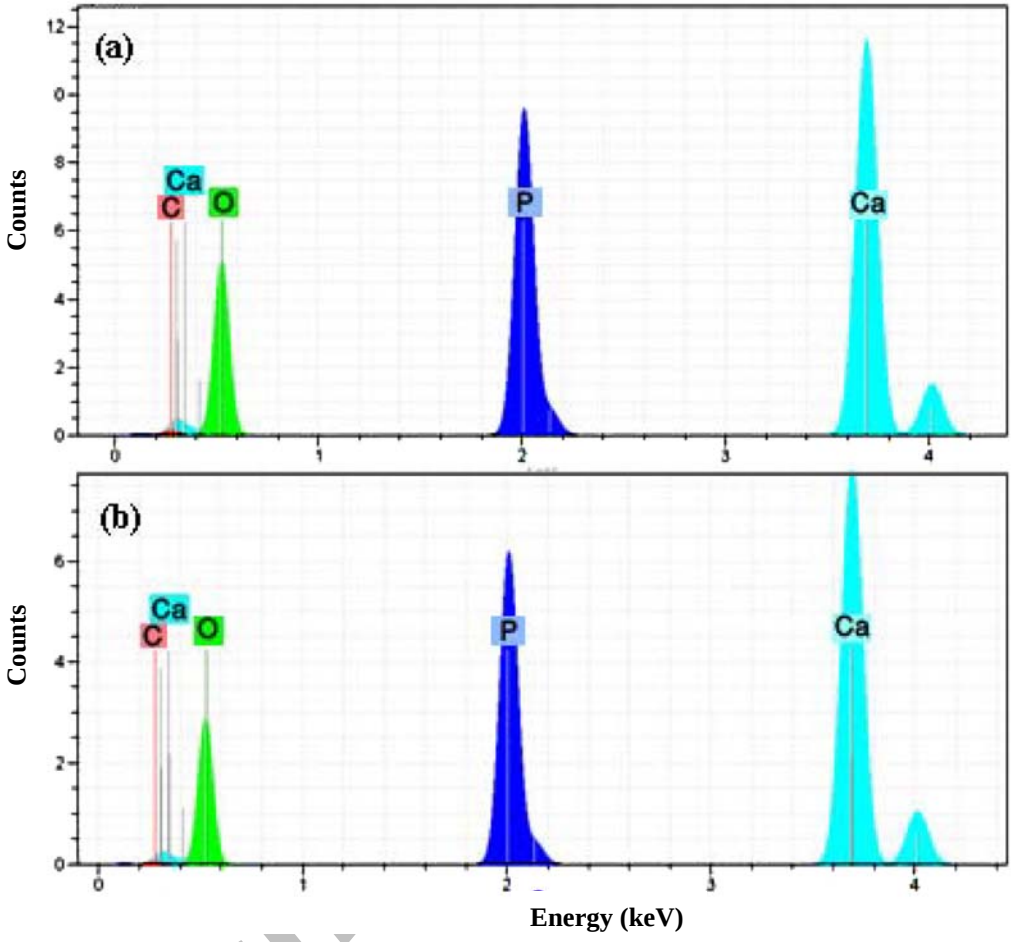

Fig. 4. EDXA spectra of the HAp nanoparticles prepared by hydrothermal process (a) and solvo-treatment method (b).

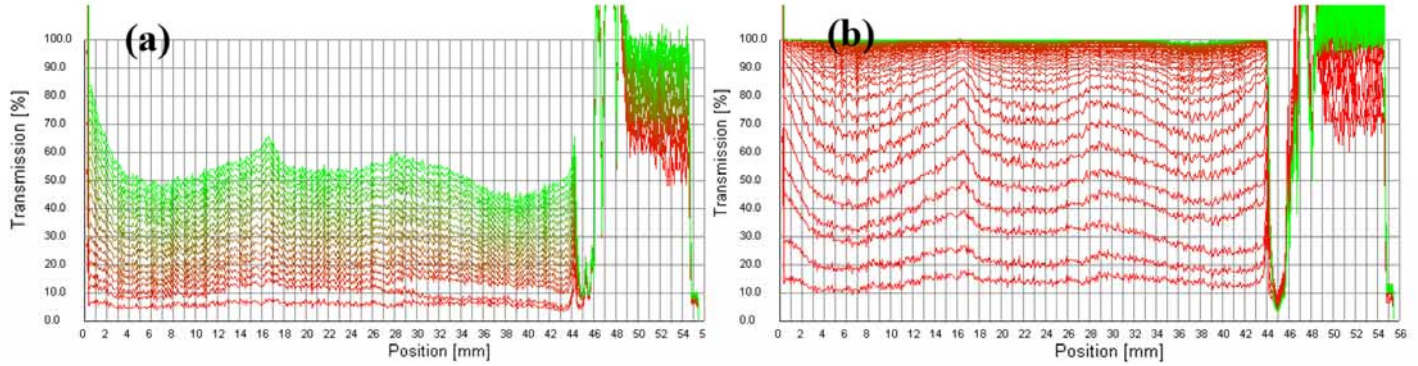

Fig. 5. Separation analyzer results for 1% w/w of hydroxyapatite nanoparticles added in an experimental dental adhesive in 12 h for nanoparticles prepared by hydrothermal process (a) and solvo-treatment method (b).

www.SID.ir

CONCLUSIONS

In summary, an attempt is made to synthesize hydroxyapatite nanoparticles by two different methods in the present paper. The HAp nanoparticles prepared by hydrothermal and solvo-treatment processes have diameters of 45-65 and 75-98 nm, respectively. The nanoparticles are approximately uniform in size and morphology. The HAp nanoparticles prepared by hydrothermal process have more degree of crystallinity as compared with those prepared by solvo-treatment method. FTIR and EDAX results confirm the chemical structure of the samples as they matched well with those reported in literature. The separation analyzer test for determining the colloidal stability reveal the much higher colloidal stability of HAp nanoparticles prepared by hydrothermal process than those prepared by solvo-treatment one. Thus, this study demonstrates the possibility of application of the hydroxyapatite nanoparticles prepared by hydrothermal process in dental adhesives without any surface modification. Due to their bioactivity, biocompatibility, colloidal stability and reinforcement properties, the application of hydroxyapatite nanoparticles in dental adhesives can potentially improve the mechanical properties of dental adhesives.

REFERENCES

[1] G.-H. An, H.-J. Wang, B.-H. Kim, Y.-G. Jeong, Y.-H. Choa, Mat. Sci. Eng. A 449-451 (2007) 821.

[2] J.S. Cho, Y.C. Kang, J. Alloy. Compd. 464 (2008) 282.

[3] J.S. Earl, D.J. Wood, S.J. Milne, J. Phys. Conf. Ser. 26 (2006) 268.

[4] K. Lin, J. Chang, R. Cheng, M. Ruan, Mater. Lett. 61 (2007) 1683.

[5] Y. Zhang, L. Zhou, D. Li, N. Xue, X. Xu, J. Li, Chem. Phys. Lett. 376 (2003) 493.

[6] M.P. Ferraz, F.J. Monteiro, C.M. Manuel, J. Appl. Biomater. Biomech. 2 (2004) 74.

[7] Q. Hu, B. Li, M. Wang, J. Shen, Biomaterials 25 (2004) 779.

[8] C.G. Vazquez, C.P. Barba, N. Munguia, Rev. Mex. Fis. 51 (2005) 284.

[9] F. Zhang, Z.-H. Zhou, S.-P. Yang, L.-H. Mao, H.-M. Chen, X.-B. Yu, Mater. Lett. 59 (2005) 1422.

[10] Y. Sun, G. Guo, Z. Wang, H. Guo, Ceram. Int. 32 (2006) 951.

[11] H. Chen, B.H. Clarkson, K. Sun, J.F. Mansfield, J. Colloid. Interf. Sci. 288 (2005) 97.

[12] M. Giannini, C.J. Soares, R.M. de Carvalho, Dent. Mater. 20 (2004) 322.

[13] J.-S. Kim, B.-H. Cho, I.-B. Lee, C.-M. Um, B.-S. Lim, M.-H. Oh, C.-G. Chang, H.-H. Son, J. Biomed. Mater. Res. B 72B (2004) 284.

[14] S.C. Lee, H.W. Choi, H.J. Lee, K.J. Kim, J.H. Chang, S.Y. Kim, J. Choi, K.-S. Oh, Y.-K. Jeong, J. Mater. Chem. 17 (2007) 174.

[15] G. Felício-Fernandes, M.C.M. Laranjeira, Quim. Nova. 23 (2000) 441.

[16] E.I. Suvorova, P.A. Buffat, Eur. Cell. Mater. 1 (2001) 27.

[17] H. Zhang, Y. Yan, Y. Wang, S. Li, Mater. Res. 6 (2002) 111.

[18] J.-S. Ha, J. Korean. Ceram. Soc. 40 (2003) 1154.

[19] Y.J. Wang, C. Lai1, K. Wei, X. Chen, Y. Ding, Z.L. Wang, Nanotechnology 17 (2006) 4405.

[20] C. Du, F.Z. Cui, Q.L. Feng, X.D. Zhu, K. de Groot, J. Biomed. Mater. Res. 42 (1998) 540.

[21] D.Z. Chen, C.Y. Tang, K.C. Chan, C.P. Tsui, P.H.F. Yu, M.C.P. Leung, P.S. Uskokovic, Compos. Sci. Technol. 67 (2007) 1617.

Archive of SID

www.SID.ir