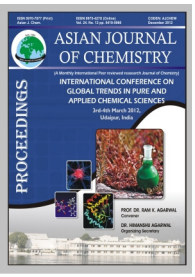
Asian Journal of Chemistry; Vol. 24, No. 12 (2012), 5497-5499

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



In vitro Antiinflammatory Study of Poly(vinyl alcohol)/Hydroxyapatite Nanocomposites: Synthesis and Characterization†

C.P. DHANALAKSHMI[1], L. VIJAYALAKSHMI[2] and V. NARAYANAN[1,*]

[1]Department of Inorganic Chemistry, University of Madras, Guindy Maraimalai Campus, Chennai-600 025, India
[2]Department of Chemistry, S.D.N.B. Vaishnav College for Women, Chrompet, Chennai-600 044, India

*Corresponding author: E-mail: vnnara@yahoo.co.in; cpdhanlakshmi@yahoo.com

AJC-11677

Poly(vinyl alcohol)/hydroxyapatite nanocomposites of varying composition for biomaterial applications have been synthesized and characterized by XRD, FTIR, ^{31}P NMR, TGA, DTA and FESEM. Hydroxyapatite nano rod embedded composite was prepared using poly(vinyl alcohol) as a matrix with different weight percentages. The results indicated that the size and crystallinity of hydroxyapatite nano particles decreases with increase in poly(vinyl alcohol) concentration in the composite. SEM confirms the presence of hydroxyapatite nano rod crystals in poly(vinyl alcohol) matrix. Poly(vinyl alcohol)/hydroxyapatite nano composites were screened for antimicrobial activity.

Key Words: Hydroxyapatite, Nanocrystalline, Nanocomposite, Poly(vinyl alcohol).

INTRODUCTION

The calcium phosphate based bioceramics particularly hydroxyapatite play an excellent role in biomedical applications owing to their excellent biocompatible, osteoconductive and bioactive properties and its close chemical and physical resemblance to mineral component of bone tissue, enamel and dentin[1]. The major mineral phase of bone is hydroxyapatite (HAp) with a ratio of calcium-to-phosphate is 1.67, which is embedded as nano crystalline form in collagen triple helix structure[2]. Currently, researchers are trying to mimic this natural nano composite system for tissue engineering applications. However, the brittleness and poor performance of mechanical stability of pure hydroxyapatite limit its use for the regeneration of non-load-bearing bone defects and tissue engineering applications[3]. Composite biomaterials like metal and polymer matrix are used to improve the mechanical compatibility of nano hydroxyapatite. Generally, the composite biomaterials are prepared by using biocompatible/biodegradable and synthetic/natural polymers[4,5]. The inorganic minerals such as hydroxyapatite[6], bioactive glasses[7], metal oxides and carbon nanotube are incorporated into polymer matrixes to impart bioactivity. This enables us to develop the composite with desired properties.

The addition of nano sized particles is desirable to develop the composite with a good mechanical strength since the natural bone contains mineral crystals which are at the nanometer scale and embedded in the collagen matrix. The polymer composites are designed to meet the specific requirement of biomedical applications like tissue engineering and drug delivery system. The right choice of the composition of both filler and polymer matrix are essential in addition to the process method to obtain suitable biopolymer composites. Recently, attempts have been made to develop nano composites, wherein nano hydroxyapatite particles are embedded in poly(vinyl alcohol) polymeric matrices. In this paper poly(vinyl alcohol)/hydroxyapatite nano composite is prepared. This biomaterial will be easy to adhere to tissue and fix in site for a long-term. This composite is a promising material for use in artificial articular cartilage.

EXPERIMENTAL

Analytical grade calcium hydroxide [$Ca(OH)_2$] and ammonium dihydrogen phosphate [$(NH_4)H_2PO_4$], were obtained from Merck. Poly(vinyl alcohol) (M.W. 1,25,000) was purchased from Loba and used. Doubly distilled water was used as the solvent.

Synthesis of nano hydroxyapatite: The nano hydroxyapatite was synthesized by following a modified wet chemical method. At 25 ºC, 7.48 g of $Ca(OH)_2$ was first dissolved in a 100 mL volume of an ethanol-water mixture (50:50 %, v/v) and stirred for 3 h. A solution of 6.7 g $(NH_4)H_2PO_4$ was

†Presented at International Conference on Global Trends in Pure and Applied Chemical Sciences, 3-4 March, 2012; Udaipur, India

dissolved in 100 mL volume of water and then added to the $Ca(OH)_2$ solution over a period of 24 h.

Synthesis of poly(vinyl alcohol)/hydroxyapatite nano composites: The Poly(vinyl alcohol) (1,25,000)/hydroxyapatite nano composites were coded as PVAH 10 to PVAH 100 where poly(vinyl alcohol) denoted poly(vinyl alcohol), H denoted hydroxyapatite and the numbers denoted poly(vinyl alcohol) wt %. Water was used as the solvent to prepare polymer solution. Hydroxyapatite in water was mixed with polymer solution under agitation. The homogeneously mixed solution is immediately taken to deep freeze at -18 ºC. After 48 h freezing the samples were freeze dried.

Physical-chemical characterization: The prepared samples were studied by Fourier transform infrared spectroscopy (FTIR). The structure of the samples were analyzed by X-ray diffraction (XRD). The morphology of the materials was analyzed by Field-emission scanning eletron microscopy (FE-SEM) Thermo gravimetric analysis (TGA) coupled with differential thermal analysis (DTA) of the material was performed ^{31}P-MAS-NMR spectra were recorded on a Bruker MSL 300 spectrometer (HORIBA Scientific USA) equipped with an Andrew type rotor rotating at a frequency of 10 KHz.

Antiinflammatory activity test by HRBC membrane stabilization method: The human red blood cells (HRBC) membrane stabilization has been used as method to study the antiinflammatory activity. After approbation of human research ethics committee and signed consent form, blood samples collected from healthy volunteer were used in this test.

$$\text{Protection}(\%) = \frac{100 - \text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

RESULTS AND DISCUSSION

XRD analysis: The reflection planes corresponding to the characteristic XRD spectral peaks of pure nanohydroxyapatite and poly(vinyl alcohol)/hydroxyapatite nanocomposites are shown in Fig. 1. The observed diffraction peaks are identified by standard JCPDS (File no. 09-0432) file and are assigned as crystalline hydroxyapatite. The XRD patterns show diffraction peaks with line broadening and high intensities, which confirms the nanosize with crystalline nature. Fig. 1, reveals that the crystallite size decreases with increase in the composition of poly(vinyl alcohol)[8].

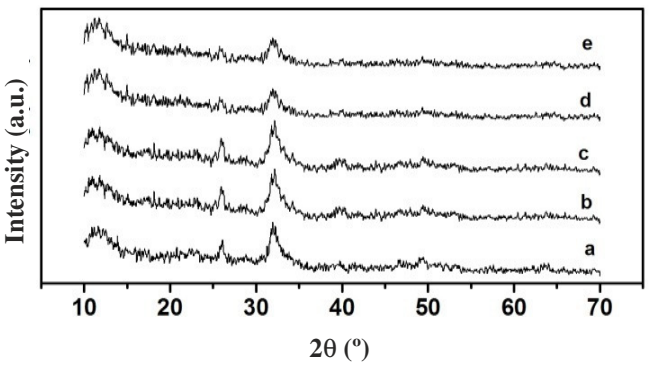


Fig. 1. XRD spectra of (a) nano hydroxyapatite, b) nano hydroxyapatite/poly(vinyl alcohol) 20, c) nano hydroxyapatite/poly(vinyl alcohol) 40,d) nano hydroxyapatite/poly(vinyl alcohol) 60, e) nano hydroxyapatite/poly(vinyl alcohol)80

FTIR analysis: The FTIR spectra of pure nano hydroxyapatite and nano hydroxyapatite/poly(vinyl alcohol) composites are shown in Fig. 2. The ν_2 phosphate stretching mode is appeared at 472-471 cm^{-1} corresponds to PO_4^{3-} group in hydroxyapatite[9]. The observed band at 602 cm^{-1} corresponds to O-P-O bending and ν_1 symmetric P-O stretching modes. The observed bands at 1384 cm^{-1} is due to the stretching mode of carbonate, which may be due to the acquisition of air during mineral precipitation[10]. Similarly, the observed bands at 1416 and 874-857 cm^{-1} are assigned to carbonate ions. The lattice H_2O exists in the range of 1603-1608 cm^{-1}, while the bands observed at 3569-3400 cm^{-1} overlap the -OH group. The band observed between 2944-2942 cm^{-1} corresponds to C-H stretching band of poly(vinyl alcohol)[11]. A new peak of stretching band is observed at 2944 cm^{-1}, when the poly(vinyl alcohol) is added. This indicates the chemical bond interactions between hydroxyapatite and poly(vinyl alcohol)[12].

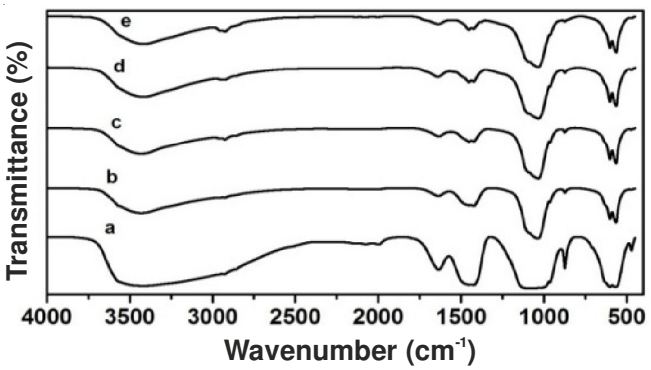


Fig. 2. FTIR spectra of (a) nano hydroxyapatite, b) nano hydroxyapatite/poly(vinyl alcohol) 20, c) nano hydroxyapatite/poly(vinyl alcohol) 40, d) nano hydroxyapatite/poly(vinyl alcohol) 60, e) nano hydroxyapatite/poly(vinyl alcohol) 80

Field emission-scanning electron microscopy: SEM images of pure nano hydroxyapatite and different weight percentages of poly (vinyl alcohol) compositions are illustrated in Fig. 3. The SEM picture shows that particles exhibit nano rod morphology. The particles size of pure hydroxyapatite is 27 nm. In case of composites, when the composition of poly(vinyl alcohol) is added to hydroxyapatite, the rod-like morphology starts to disappear. The increase in the poly(vinyl alcohol) compositions *i.e.*, 0, 20, 40, 60 wt. % leads to a corresponding change from rod-like to an irregular morphology. Further, it is evident that the particle size decreases with increase in poly(vinyl alcohol) composition.

Thermo gravimetric analysis: The thermogram and its differential thermo gravimetric plots were shown in Fig. 4. In Fig. 4a decomposition behaviour of poly(vinyl alcohol)/hydroxyapatite nano composite is shown. The nano hydroxyapatite content is calculated from the residual weight in TGA curves at 600 ºC. However, since it is very difficult to control adsorbed water content in the composites, this nano hydroxyapatite content is only an approximate value. The fact that the second step is initiated at slightly higher temperature and the third step occurs at slightly lower temperature than in pure poly(vinyl alcohol) is suggestive of the presence of chemical interaction between poly(vinyl alcohol) and the nano hydroxyapatite.

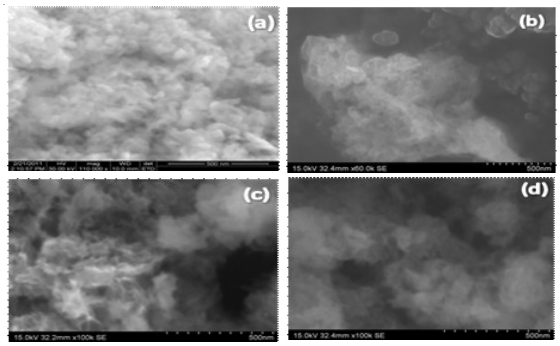


Fig. 3. FE-SEM images of (a) nano hydroxyapatite, (b) nano hydroxyapatite /poly(vinyl alcohol) 20, (c) nano hydroxyapatite/poly(vinyl alcohol) 40 and (d) nano hydroxyapatite/poly(vinyl alcohol) 60

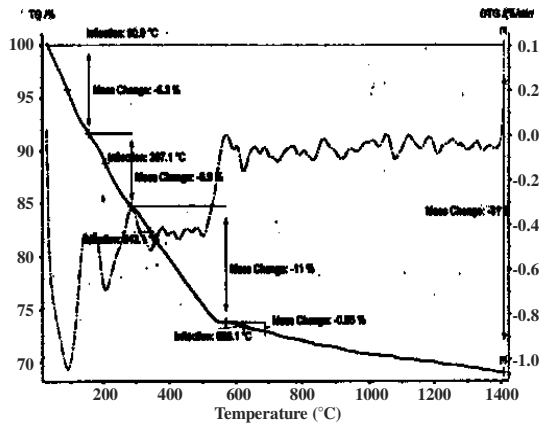


Fig. 4. TGA Curve of nano hydroxyapatite/poly(vinyl alcohol) 40 Composite

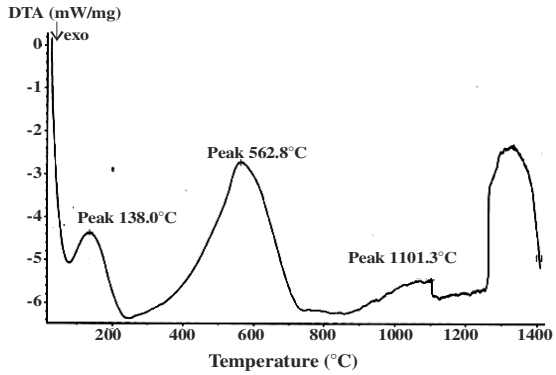


Fig. 4a. DTA Curve of nano hydroxyapatite/poly(vinyl alcohol) 40 Composite

^{31}P MAS-NMR analysis: The ^{31}P MAS-NMR spectra for the nano hydroxyapatite appears at 2.568 ppm After the development of poly(vinyl alcohol)/hydroxyapatite nano composites, the ^{31}P characteristic peak moves to 2.645 ppm, indicating that after the formation nano composites, the chemical environment of the phosphorus atom in nano hydroxyapatite crystal has been changed. This shift is due to the interaction of hydroxyapatite with poly(vinyl alcohol) in poly(vinyl alcohol)/ hydroxyapatite nano composite. The chemical interaction may be due the hydrogen bonding interaction between the PO_4^{3-} ions of hydroxyapatite and the -OH functional groups of poly(vinyl alcohol)[13].

Antiinflammatory potential analysis: The compound poly(vinyl alcohol)20/hydroxyapatite and poly(vinyl alcohol) 60/hydroxyapatite showed significant protection towards HRBC membrane rupture which is induced by hypotonic saline. The effect may be due to the resistance caused by polymers in the destruction of erythrocyte membrane. From the results it was proved that nano poly(vinyl alcohol)20/ hydroxyapatite composition was more effective than nano poly(vinyl alcohol)60/hydroxyapatite composition and also nano hydroxyapatite (Table-1).

TABLE-1
ANTI-INFLAMMATORY ACTIVITY BY HRBC MEMBRANE STABILIZATION METHOD

Concentration (µg/mL)	Inhibition of nano HAp (%)	Inhibition of nano PVA20/ HAp composite (%)	Inhibition of nano PVA60/ HAp composite (%)
1000	93.00	98.12	97.14
800	93.03	99.21	98.12
400	98.67	99.85	98.81
200	98.57	99.90	99.23
100	98.43	99.44	99.10
50	98.72	98.76	98.74
10	99.25	99.52	98.94

Conclusion

In the present work, a novel poly(vinyl alcohol)/hydroxyapatite nanocomposite is prepared by simple chemical route. The reduction in particle size with increase in concentration of poly(vinyl alcohol) is due to the size control effect of poly(vinyl alcohol) molecular structure. The rod-like morphology becomes as an irregular morphology with increase in poly(vinyl alcohol) additives. It is inferred that the composition of poly(vinyl alcohol) shows significant influence on particle size, thermal stability and antiinflammatory activities which facilitate to optimize the composition of composite for particular applications. Nanomaterials are greatly promising in the development of more valuable orthopedic and dental implants.

ACKNOWLEDGEMENTS

The authors are grateful for the financial supports from the University Grants Commission and Council of Scientific and Industrial Research, New Delhi, India.

REFERENCES

1. M. Li, X. Xiao, R. Liu, C. Chen and L. Huang, *J. Mater. Sci: Mater. Med.*, **19**, 797 (2008).
2. S. Bose and S.K. Saha, *Chem. Mater.*, **15**, 4464 (2003).
3. Y. Ding, J. Liu, H. Wang, G. Shen and R. Yu, *Biomaterials*, **28**, 2147 (2007).
4. H. Wang, Y. Li, Y. Zuo, J. Li, S. Ma and L. Cheng, *Biomaterials*, **28**, 3338 (2007).
5. V.S. KomLev, S.M. Barinov and F. Rustichelli, *J. Mater. Sci. Lett*., **22**, 1215 (2003).
6. N.M. Sundaram, E.K. Girija, M. Ashok, T.K. Anee, R. Vani and R. Suganthi, *Mater. Lett.*, **60**, 761 (2006).
7. V. Rajendran, A.N. Begum, M.A. Azooz and F.H. EI Bata, *Biomaterials,* **23**, 4263 (2002).
8. S. Kannan, A.F. Lemos and J.M.F. Ferreira, *Chem. Mater*., **18**, 2181 (2006).
9. A. Lak, M. Mazloumi, M. Mohajerani, A. Kajbafvala, S. Zanganeh, H. Arami and S.K. Sadrnezhaad, *J. Am. Ceram. Soc*., **91**, 3292 (2008).
10. L. Bertinetti, A. Tampieri, E. Landi, C. Ducati, P.A. Midgley, S. Coluccia and G. Martra, *J. Phys. Chem. C,* **111**, 4027 (2007).
11. R. Murugan and S. Ramakrishna, *Biomaterials,* **25**, 3829 (2004).
12. N. Pramanik, S. Mohapatra, S. Alam and P. Pramanik, *Polym. Compos*., **29**, 429 (2008).
13. J. Zhan, Y.H. Tseng, J.C.C. Chan and C.Y. Mou, *Adv. Funct. Mater*., **15**, 2005 (2005).