

Synthesis and Characterization of Nano Hydroxyapatite with Poly Vinyl Pyrrolidone Nano Composite for Bone Tissue Regeneration

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Abstract

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), the main mineral component of bone and teeth, is native to the human body. Hydroxyapatite (HAp) is a desirable implant material due to its biocompatibility and osteoconductivity properties. In this study, nano hydroxyapatite (nHAp) with poly vinyl pyrrolidone (PVP) was synthesized at room temperature condition. The synthetic nano hydroxyapatite (nHAp) prepared by wet chemical precipitation method was investigated. Hydroxyapatite is biocompatible with the human organism and is capable of integrating biologically into bone tissue. The synthesized sample were characterized by Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), Transmission electron microscope (TEM), Energy dispersive analysis of x-rays techniques (EDAX) and Micro hardness test.

Keywords: XRD, FT-IR, TEM, EDAX, MICRO HARDNESS

I. Introduction

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) has received considerable attention as a bone substitute due to its similar biocompatibility, bioactivity, and osteoconductivity to bone [1]. Hydroxyapatite is a mineral that has significant research applications, especially in the biomedical field [2]. Various methods have been employed for the preparation of hydroxyapatite either from natural sources coral or bone or through chemical synthesis [3-5]. Hydroxyapatite is manufactured in many forms and can be prepared as a dense ceramic [6], powder [7], ceramic [8], or porous ceramic [9] as required for the particular applications. Hydroxyapatite has been widely used in biomedicine as filler [10], for bone repair and bone tissue regeneration [11], and as drug delivery carrier owing to its good osteoinductive properties [12]. Hydroxyapatite also has many important industrial and biomedical applications in catalysis, ion exchange, sensors, and bioceramics [13]. Hydroxyapatite can be manufactured synthetically by using number of different method. The process for the preparation of hydroxyapatite and other calcium phosphate powder may be classified in synthesis from manual bone (or) coral. It can be also be synthesized by reactions in solid state [14], co-precipitation [15, 16], hydrothermal method [17], sol-gel process [18], microwave processing [19].

Polymeric materials have been used in medical and surgical applications. This paper has shown to improve the performance of poly vinyl pyrrolidone along with HAp for medical applications. In the present study, we have attempted to prepare a HAp/poly vinyl pyrrolidone (PVP) nano composite

through a chemical method, which has potential to provide much better dispersion of nHAp particles on the polymer matrix, yielding a composite with a uniform microstructure. Several authors have investigated equivalent materials and most recent attempts have used polymer additives such as poly acrylic acid (PAC), poly lactic acid (PLA), collagen, and gelatin due to efficiency of their contained calcium binding properties [20-23]. It has also been shown that the poly vinyl pyrrolidone (PVP) have very interesting applications in the biomedical field (24-27) Poly vinyl pyrrolidone (PVP) has been phosphate group of PVP can act as coupling/anchoring agent which has a higher affinity towards the nHAp particles moreover, it is easy to prepare from cheaper ingredient, we have also studied morphology, nano-particle polymer matrix internal bonding, mechanical properties of the synthesized HAp/ PVP nano composite.

II. Materials and Methods

2.1 Materials

The raw materials required to start the processing of the composite were: analytical grade Calcium hydroxide ($\text{Ca}(\text{OH})_2$) was purchased from MERCK (Mumbai, India) and ammonium dihydrogen phosphate ($(\text{NH}_4)_2\text{H}_2\text{PO}_4$) procured from MERCY (Mumbai, India). Poly vinyl pyrrolidone (mol.wt 40000) were purchased from Sigma Aldrich. Doubly distilled water with ethanol was used as the solvent.

2.2 Methods

Synthesis of nHAp/PVP

The synthesis of nano-hydroxyapatite (n-HAp) was carried out in the presence of polymer: poly vinyl pyrrolidone (PVP). The first step in the synthesis of the composite is the preparation of 1.39M Calcium hydroxide solution and a 0.84M solution of ammonium dihydrogen phosphate. The calcium hydroxide solution was slowly added to the polymer solution under continuous stirring for an hour. Then, ammonium dihydrogen phosphate solution was added gradually to the above mixture. A milky white coloration was observed almost instantaneously. The rotating time was then raised with constant stirring for 5 hours. The pH of the slurry was measured digitally during the precipitation reaction, reaching a final value of pH11. The suspension was left at room temperature for 24 hours. The solution was dried in a micro wave oven at 60⁰ C.

III. Results and Discussion XRD Analysis

The XRD patterns of nano HAp and PVP nano composites were taken. The structure of the samples was analyzed by X-ray diffraction (XRD) using a RIGAKU X-ray diffractometer. The patterns indicate the presence of amorphous HAp. The broad peaks reveal that the nano particles sized are very small in the range of 10-50 nm. The reflection planes corresponding to the characteristic XRD spectral peaks of pure nano HAp and PVP nano composites are shown in fig.1. The observed diffraction peaks are identified by standard JCPDS file no.(09-0432) and are assigned as crystalline HAp. The sharp diffraction characteristic peaks that appeared at around 26⁰, 33⁰ and 40⁰ are the HAp/PVP nano composites corresponding to the peaks of HAp powder. The XRD patterns show diffraction peaks with high intensities which conform the nano size with crystalline nature.

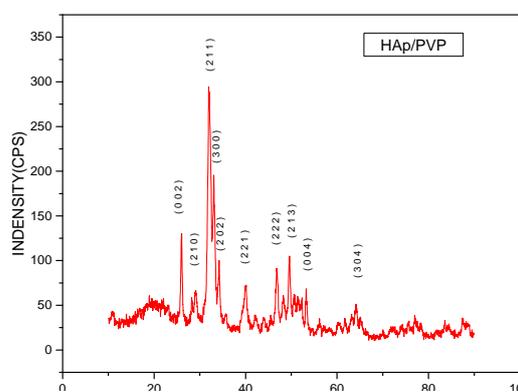


Fig.1. XRD of HAp/PVP Nano composite

FTIR

The FTIR spectra of pure nano HAp and PVP nano composites are shown in fig.2. The FTIR

spectrum was investigations and carried out using PERKIN ELEMER spectrometer in the range of 400 cm⁻¹ to 4000 cm⁻¹. The functional groups were identified using the peak assignments. A strong peak at 3416.20 cm⁻¹ was assigned to the O-H stretching in alcohol group. The strong band at 2958.29 cm⁻¹ was assigned to carboxylic acid group. The observed band -C≡C- usually occurred at 2143.62 cm⁻¹. The strong peak at 1663.20 cm⁻¹ showed stretching vibration of C=O aldehydes group. The medium peak C-C stretching of 1574.23 cm⁻¹ and 1407.60 cm⁻¹ appeared at aromatic groups region. The strong peaks 1289.34 cm⁻¹ was presented to C-N stretching of aromatic amines. The medium peak at 1085.67 cm⁻¹ showed C-N stretching of aliphatic amines. The observed band at 858.03 cm⁻¹ corresponds to C-H bend alkenes group. The strong peak 757.53 cm⁻¹ was observed in C-H at aromatic group. The medium peaks of 602.99 cm⁻¹ and 566.72 cm⁻¹ were appeared in C-Br stretching of alkynes group which indicate peaks interaction between HAp and PVP.

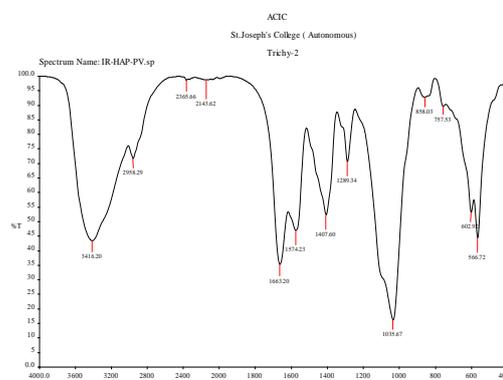
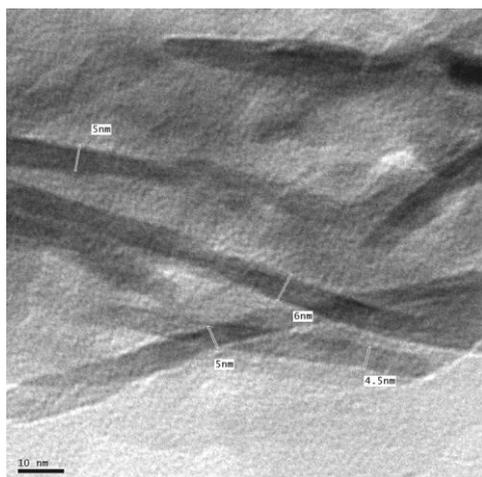


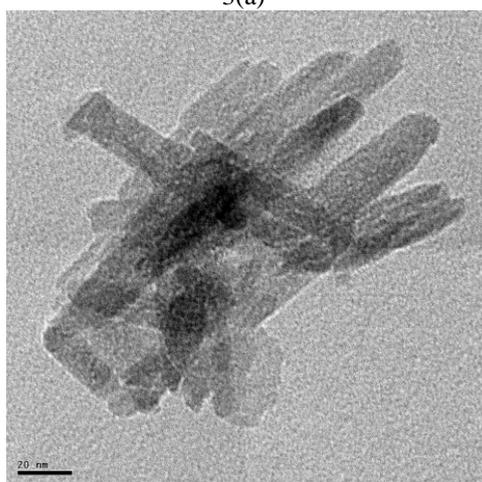
Fig.2. FTIR of HAp/PVP nano composite

TEM

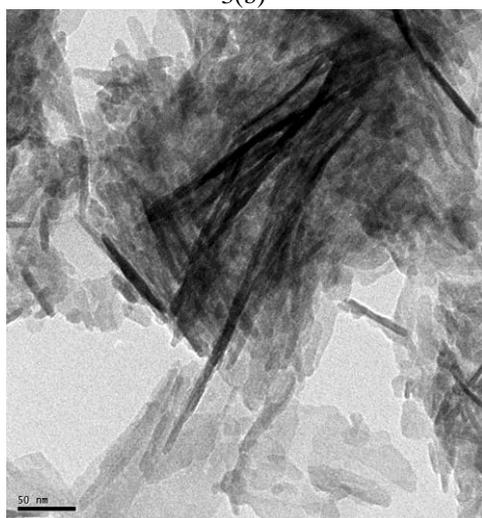
The TEM photograph of nano HAp/PVP nano composite powder are shown in the figure 3(a), (b) &(c). Nano HAp/PVP composite particles were a bit thicker (10-50 nm) and longer with clear contour. In addition the particles showed less agglomeration. The particles of nano HAp have the typical rod-like morphology and their length is 10 nm. In addition the selected area electron diffraction (SAED) in fig. 3(d) of the precipitates shows the diffraction dots or rings reflect, which implies that the precipitates are crystalline in nature. The SAED results of nano HAp and PVP are in good agreement with the lattice structure of hydroxyapatite and exhibit excellent crystallinity. This is agreed with XRD results.



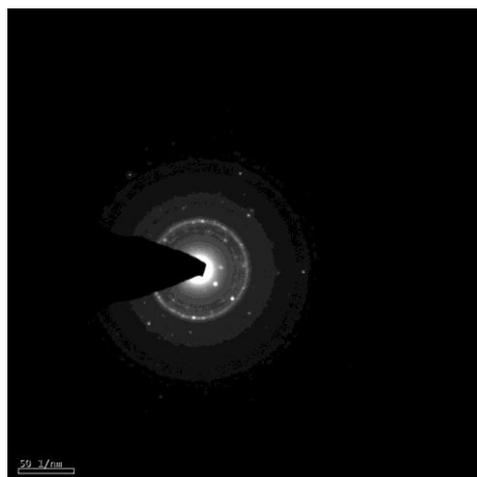
3(a)



3(b)



3 (c)



3(d)

Fig.3(a), (b) and (c) TEM image of HAp/PVP different nanometer range and (d) is selected area electron diffraction SAED of HAp/PVP nano composite.

EDAX

The Energy Dispersive Analysis of X-rays (EDAX) of nano HAp/PVP is shown in fig.4. The mineral composition of calcium phosphate Ca, O, P and organic content C are present in both nano composite materials. The presented spectrum shows that the Ca/P value of synthesized HAp-with PVP composite to be 1.72 which is closer to the Ca/P ratio of human bone (Trommer et.al., 2007).

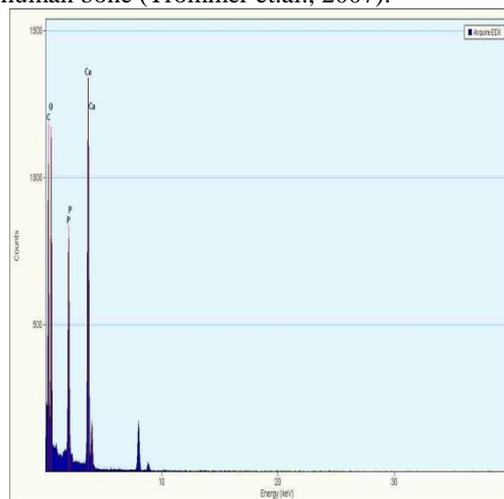


Fig.4. EDAX of HAp/PVP nano composite

Micro hardness Test

The micro hardness tests are used to determine the resistance of a material to deformation. This test can be performed on a macroscopic (or) microscopic scale. The pellet indentation hardness correlates linearly with tensile strength. With the controlled test force, the specimen is pressed by using the indenter with a dwell time of 10 to 15 seconds. Micro hardness of pure HAp and PVP based composites (HAp/PVP-10g to 100g) is as shown in fig.5. A

maximum increasing the peak value (33.7HV at 50g) is observed for the composition. The same blocks containing the residual part of the implanted HAp were used to measure the hardness of bone by means of an indentation test (Micro hardness MVHT; Wilson wolpert- Germany). Brief measurements of micro hardness were made tangential to the interface with a vickers indenter applied to the bone at a load of 50g. For the load of 10g, 25g, 50g, and 100g the hardness number was in the range 18.1, 32.4, 33.7 and 29.9. The hardness of the specimens used in this study increases with the increasing test load and it comparable is with others have reported. The tendency of hardness of materials decrease with the increasing test load above 50gm.

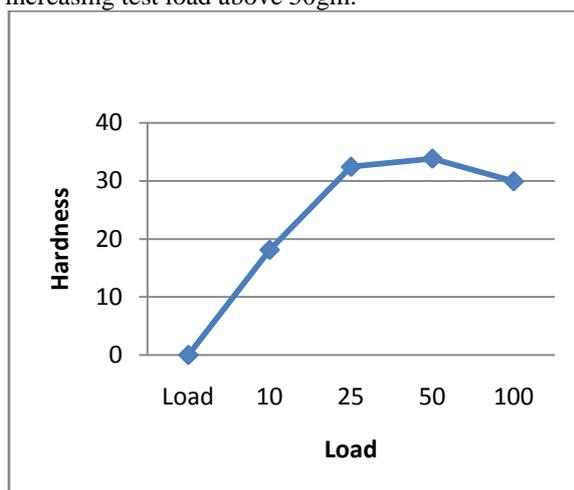


Fig.5. Micro hardness tester

IV. Conclusion

In this study, nano hydroxyapatite (n-HAp) with poly vinyl pyrrolidone (PVP) has been synthesized with homogeneous composition using wet chemical precipitation method. This Composite material can be used for bone surgery because of their biocompatibility and osteoconductivity properties. The formation of hydroxyapatite nano particle was confirmed by X-ray diffraction (XRD) and functional groups of the compound are identified using Fourier transforms infrared spectroscopy (FT-IR). The elemental compositions were examined using the EDAX analysis. The size and morphology of the samples were characterized using transmission electron microscopy (TEM). The transmission electron microscopy analysis confirms the presence of HAp/PVP nanoparticles with the particle size of around 10-50 nm. The prepared HAp/PVP nanocomposite might be more effective material for the treatment of bone defects and bone replacement.

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Reference

- [1] R.V. Silva, J.B. Bertran, N.H. Moreira, International journal of oral and maxillofacial surgery 34(2005) 178-184.
- [2] A. Stoch, W. Jastrzebski, E. Diugon, W. Lejda, B. Trybalska, G.J.Stoch, A. Adamczyk, Journal of molecular structure 744-747 (2005) 633-640.
- [3] T.M. Fillion, Lix, Mason-Savas A, Kreider JM, Goldstein SA, Ayers DC, et al. tissue Eng pt A 2011: 17:503.
- [4] W. Chen, Y. Liu, H. Countney, M. Bettenga, C, Agrawal, J. Bungardner and J. Ong, Biomaterials. 2006, 27, 5512-5517.
- [5] Joschek, S., Nies, B., Krotz, R. & Gopfrerich, A. (2000). *Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. Biomaterials*, 21, 1645.
- [6] Kong, L. B., Ma, J. & Boey, F. (2002). Nanosized hydroxyapatite powders derived from coprecipitation process. *J. Mater. Sci.*, 37, 1131.
- [7] Kweh, S. W. K., Khor, K. A. & Cheang, P. (1999). *The effect of processing parameters on the characteristics of plasma sprayed hydroxyapatite (HAp) coatings. J. Mater. Process. Technol.*, 89, 373.
- [8] Ben-Nissan, B., Milev, A. & Vago, R. (2004). *Morphology of sol-gel derived nano-coated coralline hydroxyapatite. Biomaterials*, 25, 4971.
- [9] Ozgur, N. E. & Cuneyt Tas, A. (1999). *Manufacture of MacroPorous Calcium Hydroxyapatite and Tri-Calcium Phosphate Bioceramics. J. Euro. Ceram. Soc.*, 19, 2569
- [10] Nunes CR, Simske SJ, Sachdeva R, Wolford LM. *Long-term growth and apposition of porous hydroxyapatite implants. J Biomed Mater Res* 1997;36:5603.
- [11] Heise U, Osborn JF, Duwe F. *Hydroxyapatite ceramic as a bone substitute. Int Ortho* 1990; 14:329-38.
- [12] Bagambisa, F.B., Joos, U. and Schilli, W. (1993). *Mechanisms and Structure of the Bond between Bone and Hydroxyapatite Ceramics, J. Biomed. Mater. Res.*, 27(8): 1047-1055.
- [13] F. Favier, E. C.Walter, M. P. Zach, T. Benter, and R. M. Penner, "Hydrogen sensors and switches from electrodeposited palladium mesowire arrays," *Science*, vol. 293, no. 5538, pp. 2227-2231, 2001.
- [14] I. H. Arita, V.M.Castano, and D.S. Wilkison, *synthesis and processing of hydroxyapatite ceramic tapes with controlled porosity, Journal of Materials*

- science: *Materials in medicine*, Vol.6, No. 1, 1995, pp. 19-23
- [15] M.Akao, H. Aoki, K. Kato, *Mechanical properties of sintered hydroxyapatite for prosthetic applications*, *journal of material science*, Vol.16, No. 3, 1981, PP.809-812
- [16] L.B. Kong, J. Ma, F. Boey, *Nanosized hydroxyapatite powders derived from coprecipitation process*, *Journal of Materials Science*, Vol. 37, No.6, 2002, pp. 1131-1134.
- [17] X. Zhang, And K.S. Vecchio, *Hydrothermal synthesis of hydroxyapatite rods*, *J. cryst. Growth*, Vol. 308, No.1, 2007, pp. 133-140.
- [18] J. Wang and L. L. Shaw, *Synthesis of high purity hydroxyapatite nanopowder via sol-gel combustion process*, *Journal of Medicine*, vol. 20, 6, pp.1223-1227.
- [19] Y. Yang, J.L. Ong, J. Tian, *Rapid sintering of hydroxyapatite by microvave processing*, *Journal of Materials Science Letters*, Vo. 21, 2002, pp.67-69.
- [20] M. C. Chang, C. C. Ko, W. H. Douglas, *Biomater.* 2003; 24(17):2853–2862.
- [21] C. Schiller, E. Matthias. *Biomater.* 2003;24(12): 2037–2043.
- [22] S. Z. Chian Liou, S. Y. Chen, D. M. Liu. *Biomater.* 2003;24(22):3981–3988.
- [23] N. Spanos, V. Deimede, P. G. Koutsoukos. *Biomater.*, 2002;23(3):947–953.
- [24] S. Mollazadeh, J. Javadpour, A. Khavandi, *Ceram. Intern*, 2007;33:1579–1583
- [25] Yanjie Zhang and Jinjun Lu. *Crys Grow and Desg* 2008; 8 (7): 2101-2107.
- [26] H. Kobayashi. *Advances in Sci and Tech* 2006; 53:9-16.
- [27] A. Mostafa, H. Oudadesse, M. B. Mohamed, E. S. Foad, Y. Le Gal, G.Cathelineau.; *Chem. Eng. J.* 2009; 153:187-192.