

A BIOCOMPATIBLE AND LOAD BEARING COMPOSITE OF MULTIWALLED CARBON NANOTUBES CHITOSAN AND NATURAL HYDROXYAPATITE DERIVED FROM THE CHICKEN BONES WASTED IN THE SLAUGHTER HOUSES

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Received: 08 Jun 2012, Revised and Accepted: 18 July 2012

ABSTRACT

A large amount of chicken bone is produced as waste from the protein industry all over the world and discarding it has become one of the important environmental concerns in recent time. The value addition to the waste bones including the isolation of hydroxyapatite (HAP) will reduce the dumping of these wastes on to the environment. HAP has been isolated from the waste chicken bones and the composite of functionalized multiwalled carbon nanotube (f-MWCNT)-chitosan-HAP has been prepared to improve the mechanical and thermal properties of HAP by the incorporation of chitosan and multiwalled carbon nanotube (MWCNT). MWCNT add mechanical and tensile strength whereas chitosan add binding and biocompatibility to the composite.

Keywords: Hydroxyapatite, Multiwalled carbon nanotube, Chitosan, Composite, Chicken bone.

INTRODUCTION

HAP is widely used in surgery to mimic and replace the broken bone because of its biocompatibility, bioactivity, osteoconductivity and similar structure to the inorganic component present in the bone.^{1,2} HAP can be synthesized in the laboratory by chemical method or isolated from natural source including bovine bone,³ bovine teeth,⁴ pig bone⁵ and fish bone.^{1,6} Natural HAP with the carbonate group has more biocompatibility, osteoconduction and bioresorption when compared with synthetic HAP without the carbonate group.⁷ The poor mechanical strength and brittleness of the HAP make it unfit for major load bearing applications. Recently, several HAP-polymer composites with increased mechanical strength have been developed and reported for orthopedic applications. Even though synthetic composites of HAP including poly (methyl meth acrylate), poly-L-lactide, high density polyethylene are there, the natural biopolymer based composite material gained special attention due to its biocompatibility and biodegradability.⁸

Chitosan is a natural linear polysaccharide obtained by deacetylation of chitin present in the exoskeletons of shrimp, crab and cell wall of fungi.^{9,10} Chitosan has many applications including medicine, biosensors, tissue engineering, paper, food, agriculture and electrochemistry because of its biocompatibility, biodegradability, multiple functional group and low price.^{11, 12} The mechanical properties of HAP-chitosan polymer composite can be further improved by controlling interfacial bond between the matrix and adding small amount of carbon nanotube (CNT) as reinforcement. CNTs can act as an excellent reinforcement material for polymer matrix due to its higher Young's modulus value. In the in-vitro studies the CNTs promote the cell adhesion and proliferation of osteoblast but the only problem that has been observed is the aggregation of CNTs in polymer matrix which could be overcome by the organic functionalization of the CNTs.¹³⁻¹⁵ Hence we prepare a composite of the isolated HAP from chicken bone along with chitosan and MWCNT as a good candidate for the load bearing applications with the added advantage of biocompatibility.

MATERIALS AND METHODS

Materials

Chitosan powder (degree of deacetylation 90% with molecular weight 310 kDa) was purchased from Wako Pure Chemical Industries Ltd., Japan. HAP was isolated from chicken bone (obtained from local slaughterhouse waste) by alkaline hydrolysis method. MWCNT (outer diameter <8 nm, length 10–30µm) was purchased from cheap tubes.com, USA. Glacial acetic acid, NaOH and acetone AR grade was purchased from the local suppliers.

Isolation of HAP from chicken bone

The chicken bones were washed with concentrated NaOH solution followed by rinsing with water to remove the traces of meat, skin and other impurities present on the surface of the bones. Then the bones were dried in hot air oven at 100 °C and grained in to small pieces. 16.954 g of grained chicken bone was stirred with 200 ml of 2N NaOH solution at room temperature for 44 h. Then it was kept in an ice water bath and con.HCl was added drop wise with stirring for neutralization. The obtained white precipitate was filtered, washed many times with distilled water followed by acetone and dried in air oven at 100 °C till a constant weight is obtained.

Oxidation of MWCNT

MWCNTs were oxidized by a modified procedure described by Lee *et al.*¹⁶ The MWCNTs (100 mg) were sonicated in a 3:1 mixture of con.HNO₃ and con.H₂SO₄ for two and half hour. The f-MWCNTs were centrifuged washed with distilled water and dried in oven at 100 °C for overnight.

Preparation of f-MWCNT-chitosan-HAP composite

The composite has been prepared by the procedure reported by Venkatesan *et al.*⁷ 1.74 g of chitosan was stirred in 220 ml of 2% acetic acid solution for 6 h and then it is sonicated for 1 h. In the mean while 10 mg of f-MWCNT was dispersed in distilled water by sonication for 30 min. To the chitosan solution dispersed f-MWCNTs were added drop wise and the mixture was kept under stirring for 6 h. The HAP (4.07 g) obtained from hydrolysis of chicken bone was dispersed in distilled water and added slowly to the f-MWCNT-chitosan solution then stirred for 48 h. It was neutralized with 10% NaOH and finally washed with water and filtered to obtain grayish white precipitate.

Characterizations

The thermal stability of the composite and chicken bones was studied using thermo gravimetric (TG) and differential thermal (DTA) (SDTQ 600 TA Instrument, USA) analysis with scan range of 50 °C to 800 °C at constant heating rate of 10 °C/min in the presence of nitrogen atmosphere. The vibrational frequency of the samples were studied by Fourier transformed infrared spectroscopy (FTIR) (Jasco FTIR4100, Japan) and the spectrum was recorded within the range of 4000 cm⁻¹ to 600 cm⁻¹. The phase and crystallinity of the samples were examined by X-Ray diffraction (XRD) (Bruker, D8 Advance X-ray Diffraction spectrophotometer, German). It was carried out at room temperature using CuKα as the radiation source in the wavelength 1.504Å, over the angle range 10° to 80°, step size 0.02° and scan speed 0.5°/min. The resultant XRD value for isolated HAP was

compared with the standard Joint Committee on Powder Diffraction Standards (JCPDS) cards available in the system software. The morphology of the composite and isolated HAP was studied by field emission electron microscopy (FE-SEM) (JSM-6700F, JEOL, Japan) equipped with an in-situ energy dispersive X-Ray (EDX) spectrophotometer.

RESULTS AND DISCUSSION

TG/DTA analysis of composite

The TG and DTA analysis served as an important tool for identifying thermal stability of the composite material. The

TG/DTA analysis for the composite material is shown in Fig. 1. The weight loss for the composite material observed in the temperature range of 280 °C -342 °C is attributed to the decomposition of the chitosan molecule. This is supported by the endothermic peak observed at 314 °C in DTA curve.^{7, 17} Further, there was a weight loss indicated by the two endothermic peaks at 540 °C and 687 °C respectively. The peak at 540 °C was predominant over the other peak at 687 °C. These transitions may be due to the polymorphic change in HAP. The absence of any significant weight loss when calcined above 700 °C indicated the stability of f-MWCNT-chitosan-HAP composite.

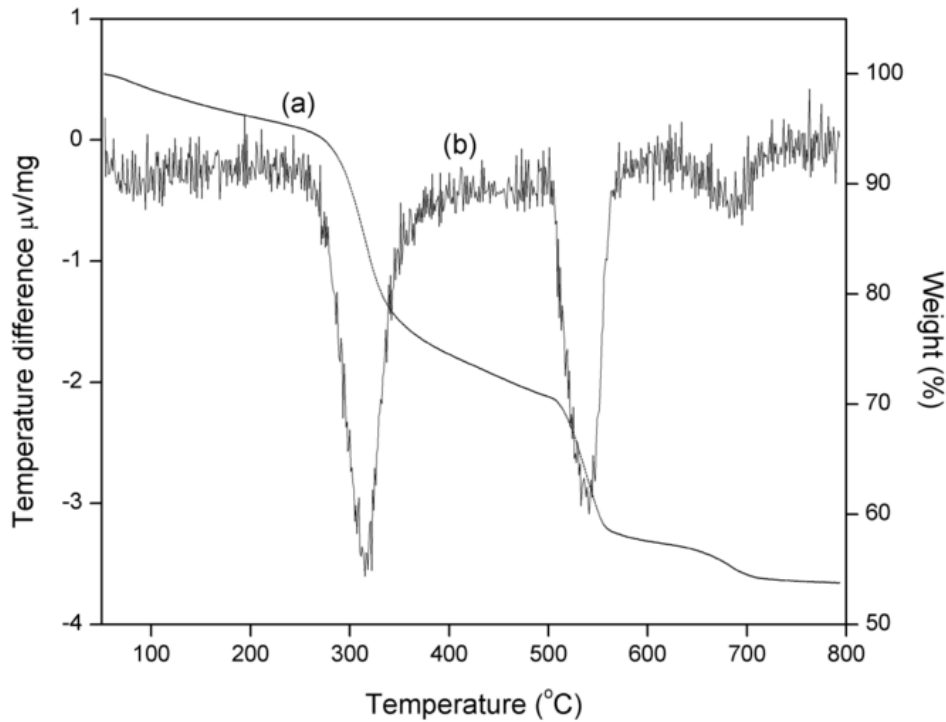


Fig. 1: a) TGA and b) DTA curve for f-MWCNT-chitosan-HAP composite from 50 °C to 800 °C.

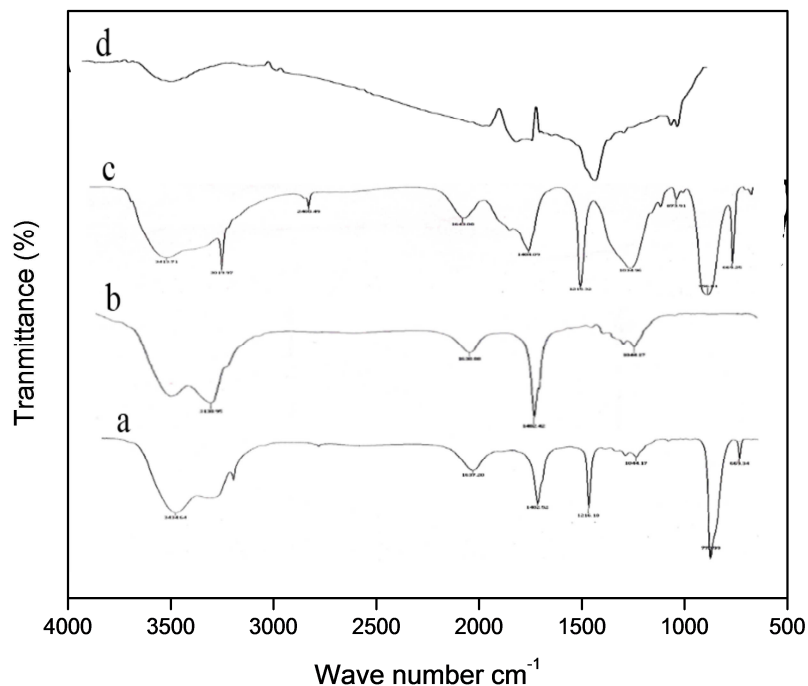


Fig. 2: FTIR spectra of a) chitosan, b) f-MWCNT, c) HAP, and d) f-MWCNT-chitosan-HAP composite.

FTIR analysis

The FTIR is an important tool for identifying the functional groups. The FTIR spectra of chitosan, f-MWCNT, HAP and f-MWCNT-chitosan-HAP composite are shown in Fig. 2. The three characteristic bands 1034 cm^{-1} due to triply degenerate asymmetric (P-O) stretching ($\nu_3\text{ PO}_4^{3-}$), the 962 cm^{-1} symmetrical non-degenerate P-O stretching ($\nu_1\text{ PO}_4^{3-}$) and 602 cm^{-1} antisymmetric triply degenerate (O-P-O) bending ($\nu_4\text{ PO}_4^{3-}$) for the PO_4^{3-} were observed in isolated HAP.¹⁸⁻²² The peak at 1251 cm^{-1} is characteristic peak of the non-degenerate deformation of hydrogen groups (H- PO_3 , O- PO_3 , HPO_4^{2-}), which may reflect the interaction with water molecule in the structure. The peak at 2400 cm^{-1} is assigned to the hydrogen-phosphate group.^{19, 21-22} The association of water molecule can be confirmed from the broad peak at $3500\text{-}3300\text{ cm}^{-1}$ for hydrogen bonded hydroxyl group and at 3019 cm^{-1} for free OH^- , the bending mode of OH^- was observed at 669 cm^{-1} . The peak at 1643 cm^{-1} was also assigned to the bending mode of associated water molecule.^{19, 23} The nonstoichiometric HAP contain water molecule in its unit cell which has been confirmed from the EDX result of isolated HAP.²⁴ The three characteristic band of stretching mode ($\nu_3\text{ CO}_3^{2-}$) at 1470 cm^{-1} and 1404 cm^{-1} and bending mode ($\nu_1\text{ CO}_3^{2-}$) at 873 cm^{-1} has confirming the B-type carbonate present in the isolated HAP.^{3, 19}

For chitosan the FTIR spectrum showed the following characteristic bands as reported earlier; hydroxyl OH^- at 3434 cm^{-1} , carbonyl (C=O) group at 1637 cm^{-1} , C-H stretching of rocking at 1402 cm^{-1} , C-O group at 1216 cm^{-1} , amine stretching frequency at 1140 cm^{-1} and 1070 cm^{-1} and pyranose C-O-C ring stretching at 1044 cm^{-1} .^{7, 25}

Furthermore the characteristic stretching frequency for f-MWCNTs was observed at 3500 cm^{-1} for OH^- stretching vibration, carbon skeleton at 3138 cm^{-1} , carbonyl (COOH) at 1638 cm^{-1} , C-C stretching vibration at 1402 cm^{-1} and C-O stretching vibration at 1044 cm^{-1} .²⁶ The FTIR spectrum of the composite f-MWCNT-chitosan-HAP contains the characteristic peaks of all the raw materials like chitosan, HAP and f-MWCNT. Moreover a new strong absorbance band at 1672 cm^{-1} (amide group) confirms the amide formation between the f-MWCNT and Chitosan in the f-MWCNT-chitosan-HAP composite.⁷

XRD analysis

The XRD pattern of chitosan, f-MWCNTs, HAP and f-MWCNT-chitosan-HAP are shown in Fig. 3. The XRD values of the isolated HAP from chicken bone were found to be in good conformity with the standard HAP (JCPDS-09-0432/1996). The HAP shows major peaks at 25.09° , 32.04° , 32.2° , 33.9° and 47.08° . Two major peaks at 10.7° and 20.0° for chitosan and the characteristic peak at 26.05° for f-MWCNTs were also observed. The shift in the peaks of HAP from 25.09° to 25.88° , 32.04° to 32.03° , 32.2° to 32.19° , 33.9° to 33.06° and 47.08° to 46.81° and also the reduction in intensity of the peak for the chitosan confirm the formation of f-MWCNT-chitosan-HAP composite.^{7, 27}

FE-SEM and EDX analysis

The morphology of the isolated HAP and the f-MWCNT-chitosan-HAP composite material was studied using FE-SEM analysis and the chemical composition of the isolated HAP was obtained from EDX analysis.

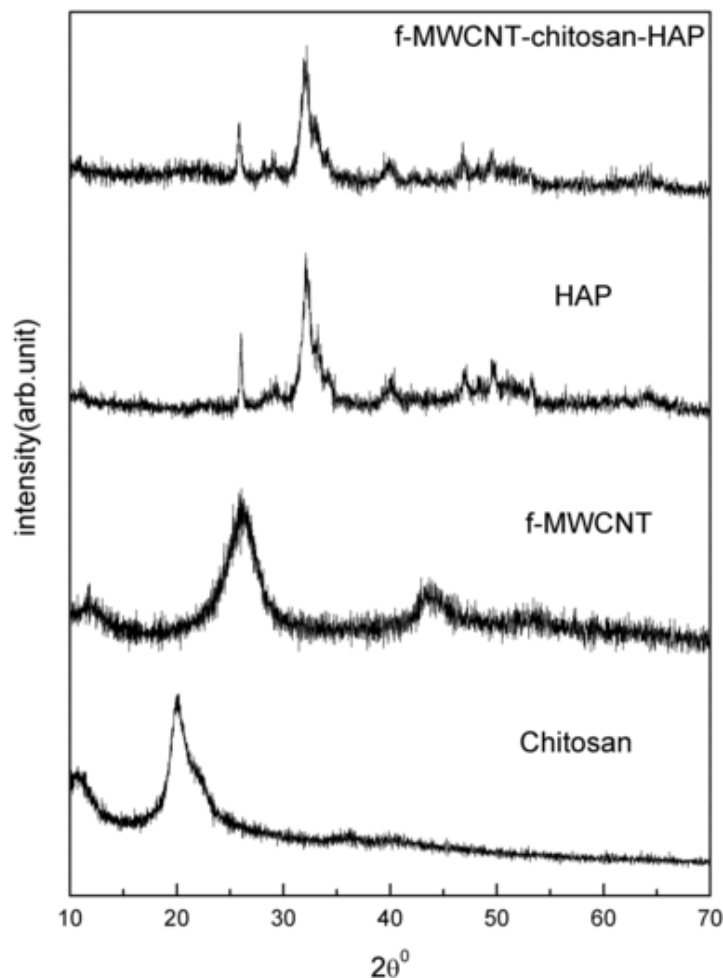


Fig. 3: XRD spectrum of chitosan, f-MWCNT, HAP and f-MWCNT-chitosan-HAP.

The FE-SEM and EDX analysis of isolated HAP is shown in Fig. 4. The SEM images confirm the isolated HAP has flake like structure and the size of the HAP was found to be 50-100 nm. The EDX result of the isolated HAP proves the isolated HAP having Ca and P with Ca/P ratio of 1.88 which is higher than that

of stoichiometric HAP value of 1.67.¹⁷ Fig. 5 shows the FE-SEM image of composite with the magnification of 500 and 2,500. The morphology of the FE-SEM image of f-MWCNT-chitosan-HAP confirms the uniform dispersion of HAP particle on the chitosan polymer matrix.⁷

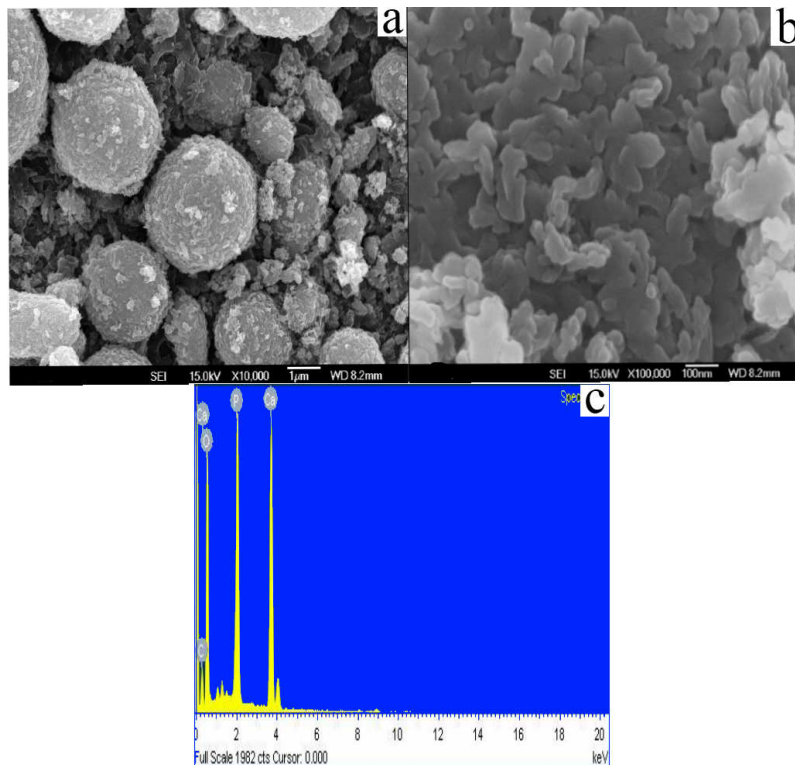


Fig. 4: Magnification at a) 10,000 and at b) 100,000 of FE-SEM and c) EDX images of HAP.

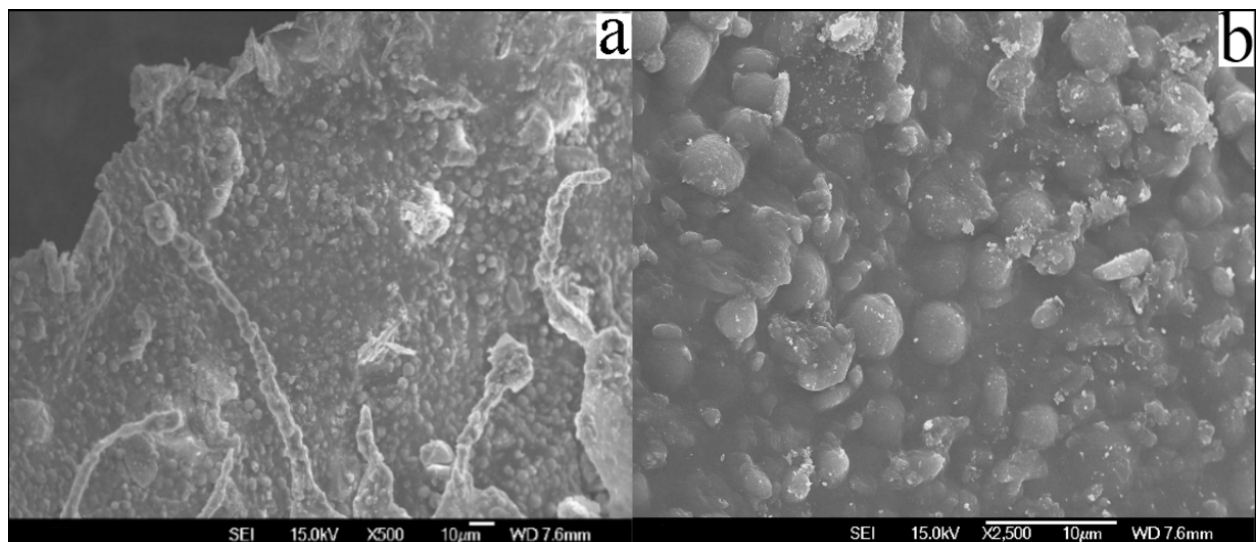


Fig. 5: Magnification at a) 500 and at b) 2,500 of FE-SEM image of f-MWCNT-chitosan-HAP composite.

CONCLUSIONS

The value addition to the chicken bone will decrease the dumping of waste bones on to the environment and thereby will reduce the environmental problem. A composite with the isolated HAP with increased mechanical strength has been prepared for load bearing applications including bone engineering and characterized using TG/DTA, FTIR, powder XRD, FE-SEM and EDX.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the management of the VIT University, Vellore, India for the facilities offered to carry out this piece of work. The authors also acknowledge the powder XRD facility at SAS, VIT jointly funded by VIT and FIIST, DST India and the instrumentation facility for recording the spectra.

REFERENCES

- Ivankovic H, Orlic S, Kranzelic D, Tkalcec E. Highly porous hydroxyapatite ceramics for engineering applications. *Adv Sci Technol* 2010;63 :408-413.
- White AA, Best SM, Kinloch IA. Hydroxyapatite-carbon nanotube composite for biomedical applications: a review. *Int J App Ceram Technol* 2007;4 :1-13.
- Ooi CY, Hamdi M, Ramesh S. Properties of hydroxyapatite produced annealing of bovine bone. *Ceram Int* 2007;33 :1171-1177.
- Elkayra A, Elshazly Y, Assaad M. Properties of hydroxyapatite from bovine teeth. *Bone Tissue Regene Insights* 2009;2 :31-36.
- Lombardi M, Palmero P, Haberko K, Pyda W. Gelcast components having controlled porosity features obtained from a natural hydroxyapatite powder. *Ceram Materials* 2010;62 :342-348.
- Venkatesan J, Kim SK. Effect of temperature on isolation and characterization of hydroxyapatite from Tuna (Thunnus obesus) bone. *Materials* 2010;3 :4761-4772.
- Venkatesan J, Qian ZJ, Ryu BM, Kumar NA, Kim SM. Preparation and characterization of carbon nanotube-grafted-chitosan-natural hydroxyapatite composite for bone tissue engineering. *Carbohydr polym* 2011;83 :569-577.
- Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. *Biomaterials* 2001;22 :165-173.
- Kulkarni PV, Keshavayya J, Baek SH, Kim B, Suh KD. Chitosan-sodium alginate biodegradable interpenetrating polymer network (IPN) beads for delivery of ofloxacin hydrochloride. *Int J Pharm Pharm Sci* 2010;2 :77-82.
- Jaafari K, Ruiz T, Elmaleh S, Coma J, Benkhrouja K. Simulation of a fixed bed adsorber packed with protonated cross-linked chitosan gel beads to remove nitrate from contaminated water. *Chem Eng J* 2004;99 :153-160.
- Liu YL, Chen WH, Chang YH. Preparation and properties of chitosan/carbon nanotube nanocomposites using poly(styrene sulfonic acid)-modified CNTs. *Carbohydr Polym* 2009;76 :232-238.
- Tang C, Chen N, Zhang Q, Wang K, Fu Q, Zhang X. Preparation and properties of chitosan nanocomposites with nanofillers of different dimensions. *Polym Degrad Stab* 2009;94 :124-131.
- Sambarkar PP, Patwekar SL, Dudhgaonkar BM. Polymer nanocomposites: An overview. *Int J Pharm Pharm Sci* 2012;4 :60-65.
- Yang J, Yao Z, Tang C, Darvell BW, Zhang H, Pan L et al. Growth of apatite on chitosan-multiwall carbon nanotube composite membranes. *App Surf Sci* 2009;255 :8551-8555.
- Shieh YT, Yang YF. Significant improvements in mechanical property and water stability of chitosan by carbon nanotubes. *Eur polym J* 2006;42 :3162-3170.
- Lee J, Kim M, Hong Ck, Shim SE. Measurement of the dispersion stability of pristine and surface-modified multiwalled carbon nanotubes in various nonpolar and polar solvents. *Meas Sci Technol* 2007;18 :3707-3712.
- Liu J, Shi F, Yu L, Niu L, Gao S. Synthesis of chitosan-hydroxyapatite composites and its effect on the properties of bioglass bone cement. *J Mater Sci Technol* 2009;25 :551-555.
- Joschek S, Nies B, Krotz R, Gopferich A. Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. *Biomaterials* 2000;21 :1645-1658.
- Koutsopoulos S. Synthesis and characterization of hydroxyapatite crystals: A review study on the analytical methods. *J Biomed Mater Res* 2002;62 :600-612.
- Lemos AF, Rocha JHG, Quaresma SSF, Kannan S, Oktar FN, Agathopoulos S et al. Hydroxyapatite nano-powders produced hydrothermally from nacreous material. *J Eur Ceram Soc* 2006;26 :3639-3646.
- Rajesh R, Hariharasubramanian A, Ravichandran YD, Chicken bone as a bioresource for the bioceramic (Hydroxyapatite). *Phosphorus, Sulfur Silicon Relat Elem* 2012;187 :914-925.
- Wawro D, Pighinelli L. Chitosan fibers modified with HAp/ β -TCP nanoparticles. *Int J Mol Sci* 2011;12 :7286-7300.
- Balamurugan A, Michel M, Faure J, Benhayoune H, Wortham L, Sockalingum G et al. Synthesis and structural analysis of sol gel derived stoichiometric monophasic hydroxyapatite. *Ceram-Silikaty* 2006;50 :27-31.
- Bouyer E, Gitzhofer F, Boulous MI. Morphological study of hydroxyapatite nanocrystal suspension. *J Mater Sci - Mater Med* 2000;11 :523-531.
- Pawlak A, Mucha M. Thermogravimetric and FTIR studies of chitosan blends. *Thermochimica Acta* 2003;396 :153-166.
- Tahermansouri H, Chobfroch KD, Meskinfam. Functionalization of carboxylated Multi-walled carbon nanotubes with 1,2-phenylenediamine. *Int J Nano Dimen* 2010;1 :153-158.
- Mugadza T, Nyokong T. Covalent linking of ethylene amine functionalized single-walled carbon nanotubes to cobalt (II) tetracarboxyl-phthalocyanines for use in electro catalysis. *Synth Met* 2010;160 :2089-2098.