Int J Pharm Bio Sci 2015 April; 6(2): (P) 526 - 531

Research Article **Polymer Science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SYNTHESIS AND CHARACTERIZATION OF NANO HYDROXYAPATITE WITH PECTIN CITRUS (BIO-POLYMER) FOR BIOMEDICAL APPLICATION

K. SENTHILARASAN AND P. SAKTHIVEL*

Department of physics, Urumu Dhanalakshmi College, Tiruchirappalli-620019.

ABSTRACT

Hydroxyapatite (HAp) is a biocompatible ceramic that is widely used in a number of biomedical applications and devices. Due to the close similarity between nanometer scale forms of HAp and the mineral phase found in the natural bone matrix, it has gained increased importance in recent years. Pectin is a naturally occurring polysaccharide. The benefits of pectin are appreciated by scientists due to its biodegradability. HAp/PC nano composite could be more useful for treatment of oral bone defects in comparison with conventional of nHAp/Pc and could be more effective as a bone replacement material to promote bone formation. FTIR, XRD, TEM, TG/DTA was used to identify the functional groups, phase structure, morphology and thermal stability of the synthesized composite. The result of these studies indicated that the powders were biocompatible and would not cause toxic reactions. This powder sample could be applied for biomedical application.

KEYWORDS: **Hydroxyapatite,Pectin Citrus, FTIR, XRD, TEM, TG/DTA.**

*Corresponding author

P. SAKTHIVEL
Department of physics, Urumu Dhanalakshmi College, Tiruchirappalli-620019.

INTRODUCTION

Bone is a dynamic, highly vascularised tissue with a unique capacity to heal and remodel without leaving a scar[1]. Bone tissue in the adult skeleton is arranged in two architectural forms: trabecular also called cancellous or spongy bone and cortical or compact bone.[2] Bone is involved in a series of processes which are found to be essential for the human body. Most of the outstanding properties of bone are related to its matrix constitution. Bone matrix has two components: a mineral part constituted by hydroxyapatite which contributes with 65-70% to the matrix and an organic part, composed of glycoproteins, proteoglycans, and sialoproteins, bone "gla" proteins, that comprises the remaining 25-30% of the total matrix.[1] Hydroxyapatite is the major mineral component of human bones. It is calcium orthophosphate which has in its structure hydroxyl group. Biological properties of hydroxyapatite are very favorable. It is not only biocompatible (which means that after implantation it causes no side effect) but also bio active (this means that implants made with this material bond with the surrounding bone tissue through formation of a chemical bond).[3] These properties made hydroxyapatite an excellent material for production of ceramic materials intended for use as implants in dentistry and treatment of orthopedic injuries. Biological hydroxyapatite always contain in their structure carbonate groups and various other cationic and ionic substitutions in the crystallographic network and hence exhibit deviations from the stoichiometry.[4] Natural polymers possess highly organized structures, some of them containing functionalities capable of binding cell receptors, thus inducing cell adhesion. Furthermore, anionic polysaccharides such as alginate, gellan, hyaluronic acid and pectin are good mucoadhesive materials and therefore, as carriers may prolong the residence and the exposure time of drug, allowing their improved absorbance.[5] Pectin is obtained from inner portion of the ring of citrus fruits. Pectin is a very promising biomaterial. Pectin is a biocompatible anionic polysaccharide that constitutes 30% of the plant cell. It is almost entirely composed of three polysaccharidic domains: homonogalacturonan(HGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-I (RG-II). HGA is the major component of pectic polysaccharides and contains α-(1→4) -D-linked galacturonic acids (1,4-α-D-Gal A) that are partially methyl-esterified and sometimes partially acetyl-esterified. It is rich in carboxyl and hydroxyl groups which can promote the binding of $Ca^{+}$from the solution to carboxylate ions and this intitates the apatite nucleation process.[5-6] Pectin is an interesting constituent for pharamaceutical use, e.g as a carrier of a variety of drugs for controlled release applications. Pectin recently exploited for various biomedical applications, including gene delivery, wound healing and tissue engineering. It has also used as an emulsion stabilizer. Experimentally, pectin has been used in gel formulations for the oral sustained delivery of ambroxol.[7-8] Modified citurs pectin (MCP) is promoted with claims it can help treat prostate cancer and melanoma.[9] The objective of this work is the synthesis of Pectin Citrus/nHAp powders by wet chemical method. The heat treated powders were characterized by X-ray diffraction in order to identify the phase composition. Functional groups are identified using FTIR, the morphology of synthesized powders has been studied by TEM and Thermal stability was measured by TG/DTA studies.

MATERIALS AND METHODS

MATERIAL

Water soluble pectin citrus was obtained from Alfa Aesar. All chemicals needed for synthesis of nano hydroxyapatite with Ammonium dihydrogen phosphate, calcium hydroxide and ethanol were purchased from Merck. Double distilled water was used as the solvent.

SYNTHESIS NANO HYDROXYAPATITE

Nano HAp was synthesized by following wet chemical method. At room temperature. 5.6 g of $Ca(OH)_2$ was first dissolved in a 100ml volume of an ethanol-water mixture(50:50%, v/v). A solution of 6.7 g $(NH_4)\ H_2PO_4$ was dissolved in 100 ml volume of water and then added to the $Ca(OH)_2$ solution over a period of 26h. The pH of the slurry was measured digitally during the precipitation reaction, reaching a final value of pH 11.

SYNTHESIS OF HAp/PC NANO COMPOSITES

Water was used as solvent to prepare polymer solution. Pectin Citrus was dissolved by using

mechanical stirrer for 4 hours. Then suitable amount of HAp mixed with polymer solution. The homogeneously mixed solution is immediately taken to high energy microwave heat process.

RESULTS AND DISCUSSION

FTIR

The Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Spectrometer, in the range of 400 cm^{-1} to 4000 cm^{-1}. The phosphate ions, PO_4^{3-} are the principal molecular components of HAp giving to the IR absorbance in the 550-1200cm^{-1} region. The characteristic peaks at 1035 cm^{-1} correspond to the stretching vibration of PO_4^{3-} .The O-P-O bending bands have been observed at 565.30cm^{-1} and 602.69 cm^{-1} due to presence of Nano Hap. The bands at 3527.59 cm^{-1} is due to stretching vibration of the HAp hydroxyl group. The presence of carbonate ions in the sample have been assigned at 1419.65cm^{-1}, which may be due to presence of the atmospheric carbon dioxide during the synthesis. (Figure: 1).

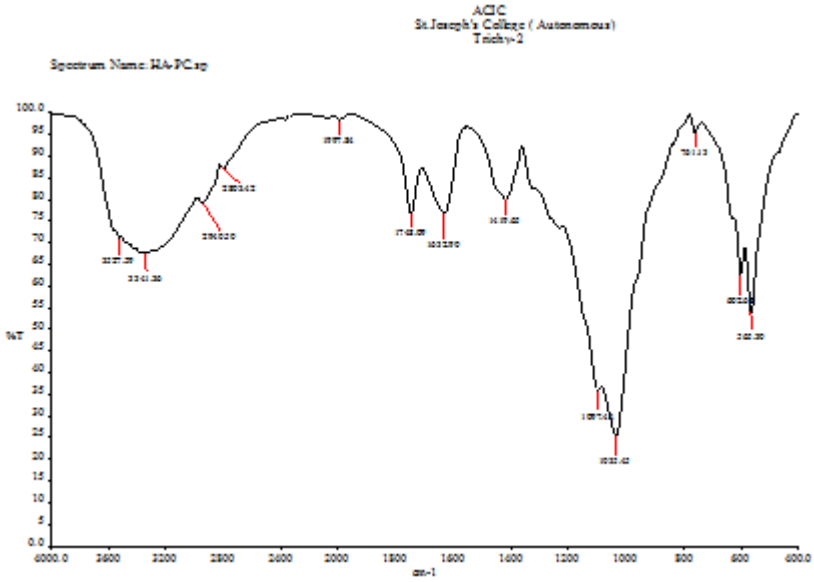

Figure 1
FTIR Spectrum for nHAp/PC

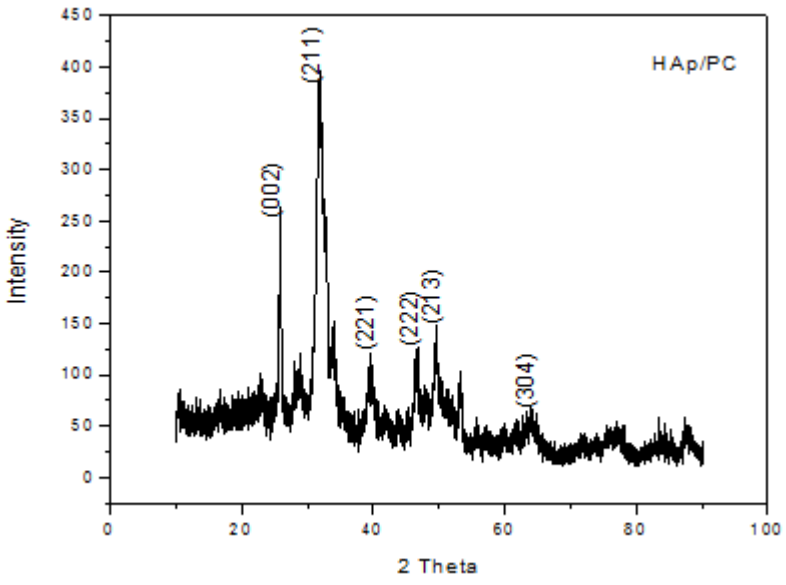

Figure 2
XRD Pattern for nHAp/PC.

XRD

X-Ray diffraction studies of the powdered sample carried out for phase identification using X-ray diffractometer Rigaku with monochromatic Cu-Kα radiation (λ=1.5405Å). The powder sample were scanned in the Bragg angle,2θ range from 10˚-90˚. The crystalline phase of the synthesized HAp/PC Nano composites was investigated by XRD and is depicted in Figure 2. The XRD patterns showed the HAp crystalline phase, which reflected the characteristics of the (002), (211), (221), (222), (213) and (304) planes. The results are in good agreement with the ICDD card No. 09-0432. The crystallite size of HAp is determined using the Scherrer formula.

$$D = \frac{0.9\lambda}{\beta cos\theta} \qquad \text{--- (1)}$$

Where, D is the crystallite size calculated for the (h k l) reflection, λ the wave length of Cu-Kα radiation, β the full width of the peak at half of the maximum intensity and θ the diffraction angle of the corresponding reflection. The fraction of crystallinity (X_c) of the HAp nanoparticles calculated from the equation.

$$X_C = \left(\frac{0.24}{\beta}\right)^3 \qquad \text{--- (2)}$$

Crystallite size, Fraction of crystallinity, specific surface area, micro strain, Dislocation density are calculated in Table1. The lattice parameters, unit cell volume were calculated and compared with ICDD Data, where Micro Strain, Dislocation density and Specific surface area are increased, the crystallite size and Fraction of crystallinity are decreased. This property of reciprocity is various miller indices, is a unique phenomenon in nano composite material.

Table 1
Crystallite Size, Fraction of crystallinity, Specific surface area, Micro Strain, Dislocation density

h k l	FWHM(deg)	Crystallite Size	Fraction of crystallinity	Specific surface area	Micro strain	Dislocation-density
0 0 2	0.335	4.245	0.6719	447.27	0.081	0.0554
3 1 0	1.04	1.415	0.2441	1341.2	0.244	0.4989
2 1 1	1.67	0.862	0.0246	2201.3	0.401	1.3441

TEM

Transmission electron microscope (TEM) experiments were performed on a Tecnai T20 electron microscope with an acceleration voltage of 200kV. The TEM pattern and electronic diffraction of nano-HAp/PC are shown in figure 3a,3b. It could be seen that the needle-like type morphology with a mean length and width of about 70 and 4 nm. These nano-HAp/PC composite had good dispersive properties and displayed a relatively uniform morphology. The inorganic phase was further identified from the selected area electron diffraction (SAED) pattern of the powder, where in the polycrystalline rings could be detected (Figure 4). This is agreed with XRD result and confirmed the Nano size components of HAp/PC nanocomposite.

TG-DTA

TG-DTA of nanoHAp/PC powder was carried out from30 ˚C to 800 ˚C in air atmosphere using Perkin Elmer analyzer at heating rate 25 ˚C per minute. The decomposition behavior of nHAp/PC composite is as shown in Figure 5. In the TGA curves, three steps were observed. The first step is a small decrease in weight is associated with adsorbed water removing when heated above 90 ˚C. The second step from 220 ˚C to 500 ˚C, the curve which rapidly decrease may be due to the dehydration reaction of organic residues in Pectin citrus chains. In 500 ˚C to 800˚C, no significant weight loss was observed. Almost stable curve was noticed within this temperature range, which indicates thermal stability of nHAp/PC powder. In the DTA curve there is a sharp curve occur which is followed by a broad curve between approx.(315˚C to 400 ˚C). This is occurring because of evaporation of water. The endothermic peak in the curve is related to the removal or addition of other groups during the synthesis of nHAp/ PC.

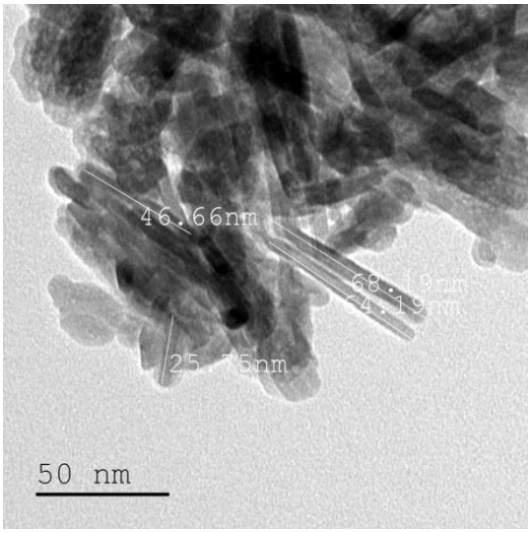

Figure 3 a
TEM image for 50nm.

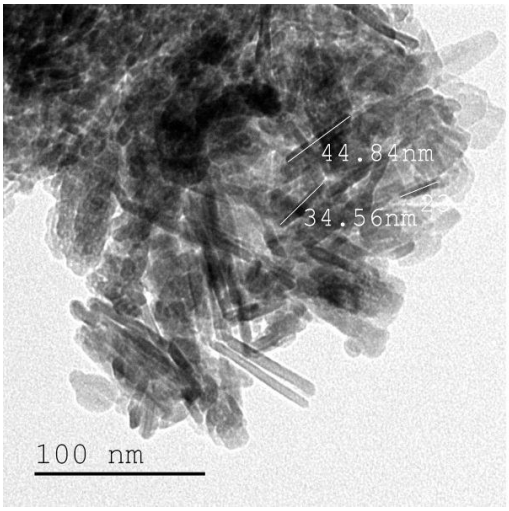

Figure 3 b
TEM image for 100 nm

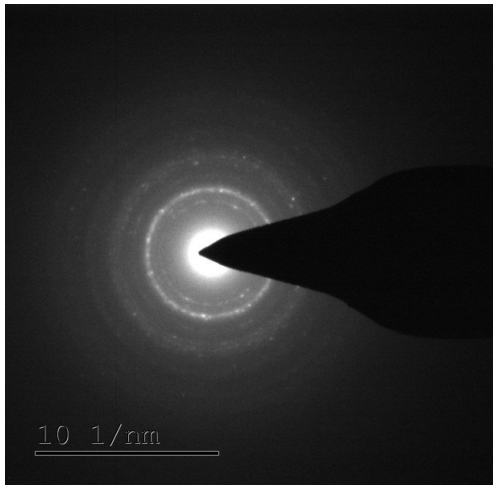

Figure 4
SAED pattern for nHAp/PC composite.

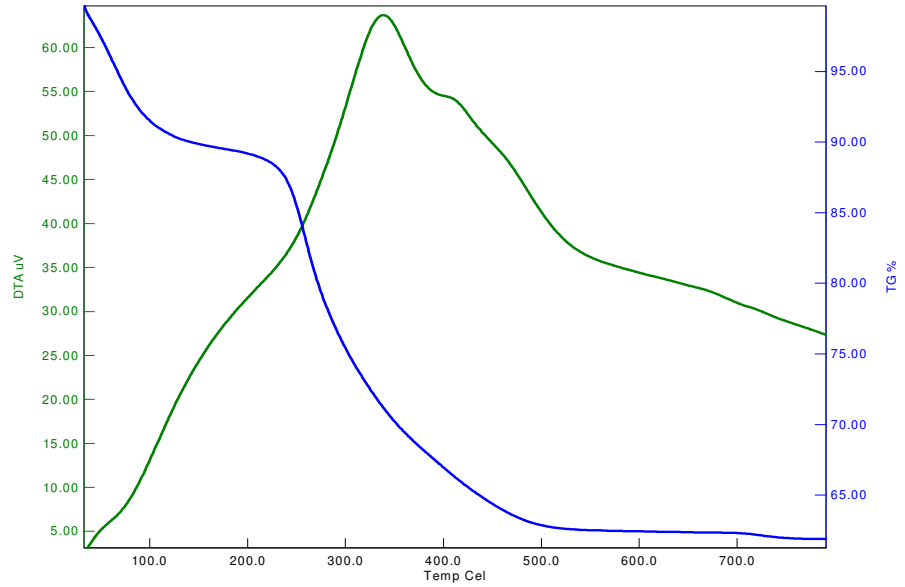

Figure 5
TG/DTA graph for nHAp/PC composite.

CONCLUSION

HAp/ PC nano composites were synthesized by modified wet chemical method. At room temperature,the XRD peaks, lattice parameter and volume density are matched with ICDD card no(09-0432). The FTIR analysis confirms functional groups. TG/DTA indicates a thermal stability. TEM image confirms the needle-like morphology with a mean length and width of about 70 and 4 nm. SAED pattern confirms the crystalline nature of composite. HAp/PC with optimum properties can be used in various biomedical applications. Efforts are being done to study *in vivo* and *invitro*behavior of the compound to confirm the biomedical interaction of the compound.

ACKNOWLEDGEMENT

The management of Urumu Dhanalakshmi College, Tiruchirappalli for providing research facilities in the campus.

REFERENCES

1. Sommerfelt, D.W, Rubin. C.T, Eur. Spine J.,10, S86, 2001.
2. Sikavavitsas. V.I, Temenoff. J.S, Mikos.A.G, Biomaterials, 22, 2581, 2001.
3. Hench L.L., Wilson J., An Introduction to bio ceramics, world Scientific, Singapore, New Jersy, London, Hong Kong 139-199, (1993).
4. Janus A.M., Faryna M., Haberko K., Rakowaka A., Panz T., Chemical and microstructural characterization of natural hydroxyapatite derived from pig bones, Microchimica Acta, 161 349-353, (2008).
5. Helena R. Moreira, FabiolaMunarin, Roberta Gentilini, LiviaVisai, Pedro L. Granja, Maria Cristina Tanzi, Paola Petrini., Injectable pectin hydrogels produced by internal gelation: pH dependence of gelling and rheological properties., Carbohydrate polymers 103,339-347, ,(2014).
6. Mazz Marry, Maureen C Mccann, Frank Kolpak, Alan R White, Nicola J Stacey and Keith Roberts., Extraction of pectic polysaccharides from Sugar-beet cell walls, J Sci Food Agric 80:17-28 (2000).
7. PranatiSrivstava , RishabhaMalviya., Source of pectin, extraction and its applications in pharmaceutical industry - an overview., Indian Journal of Natural products and Resources Vol.2(1)., pp. 10-18, March 2011.
8. Akanksha Gupta Natasha Sharma, M.P. Khinchi, DilipAgrawal., A Review on natural polymer., Asian Joranal of Pharmaceutical Research and Development Vol.1(5):134-145, sept-oct.2013.
9. What is modified citrus pectin? Cancer Research UK. Retrieved September 2014.