International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 3 Issue 9, September- 2014

Synthesis and Characterization of Hydroxyapatite and Gelatin Doped with Magnesium Chloride for Bone Tissue Engineering

K. Senthilarasan[1], A. Ragu[2], P. Sakthivel*
[1,2,*]Department of Physics ,
UrumuDhanalakshmi College,
Tiruchirappalli,Tamilnadu,
India.

***Abstract* - Hydroxyapatite(HAp) is effectively used as bone substitutes and tissue Engineering because it closely resembles bone apatite and exhibits good biocompatibility and bioactivity. Hydroxyapatite and gelatin doppedwithmagnesium chloride powder was synthesized through wet chemical method. The obtained powder were physically characterized using Powder X-ray analysis, Transmission Electron Microscope(TEM) and Fourier Transform Infrared Spectroscopy (FT-IR) to reveals its phase content, morphology and types of bond present within it. Thermal analysis (TG-DTA) was carried out to investigate the thermal stability of the powder. The crystallite size, lattice parameters, specific surface area , volume density, microstrain and dislocation density also measured by using XRD data.**

Keywords: HAp, XRD,FT-IR,TEM,TG-DTA.

INTRODUCTION

Tissue Engineering presents an alternative approach for the healing of diseased, damaged and traumatised bone tissue.[1]. The biomedical composite have been investigated or are currently under investigation as replacement materials for diseased or damaged tissues in the human body. Polymer matrix composites, metal matrix composites and ceramic matrix composites have all been made for possible human tissue replacement. However, polymer matrix composites are the most widely studied composite materials for tissue substitution due to a number of reasons: resemblance in composition and structure to the natural tissue, good bio compatibility, relative ease of manufacture, etc.,. There are a number of natural polymers that can be used in the medical field.[2]. Bone tissue is composed of minerals and proteins. The minerals are mostly apatites such as Hydroxyapatite, fluorapatite, and carbonateapatite.[3] It's stoichmetry is represented by the formula $Ca_{10}(Po_4)_6OH_2$. It is comprised of calcium and phosphorus present in the ratio Ca/p of 1.67. Hydroxyapatite has attracted much interest as biomaterials for use in prosthetic application due to its similarity in crystallography and chemical composition to that of human hard tissue.[4]HAp has excellent biocompatibility with hard tissues and high osteo conductivity and bioactivity despite its low degradation rate, mechanical strength and osteo inductive potential. Collagen is biocompatible, biodegradable and osteo inductive, acting as an excellent delivery system for bone morphogenetic proteins.[5]

Gelatin is a natural biopolymer obtained as a hydrolysis product of collagen, which is a fibrous protein found abundantly in the animal kingdom in the form of hides, skins, bones and connective tissues. Gelation is one of the natural polymer used as support material for gene delivery, cell culture and more recently tissue engineering. Gelatin –based systems have the ability to control release of bioactive agents such as drug, protein and dual growth factors.The natural water soluble biopolymer has to its credit a large number of applications in pharmaceuticals, medicine, food, and other allied filed.[6]nHAp / Gel composite have been developed into a good candidate material for hard tissue repairs because of their similar composition to the hard tissue good biocompatibility and high osteoconductive activity. Very recently they have been also used as a drug delivery system for the treatment of bone infection and defects[7]. Magnesium is a fundamental element and prevents possible risk factors for osteoporosis in humans.[8] Magnesium chloride is a natural mineral. The various hydrates of Magnesium chloride such as $MgCl_2(H_2O)_x$are typical ionic halides and are highly soluble in water.

EXPERIMENTAL DESIGN

Materials

The raw materials required to start the processing of the composite were: Calcium hydroxide $Ca(OH)_2$ and Ammonium dihydrogen phosphate $(NH_4)_2PO_4$were obtained from Merk (India). GelatinPowder was purchased from Spectrum(India). Magnesium Chloride purchased from Loba (India) Ethanol and double distilled water was used as the solvent.

Synthesis of nanoHAp

Nano HAp was synthesized by following a modified wet chemical method. At room temperature, 5.56

g of calcium hydroxide was first dissolved in a 100 ml volume of an ethanol-water mixture (50:50%,v/v) and stirred for 3h. A solution of 6.7 g $(NH_4)H_2PO_4$ was dissolved in 100 volume of water and then added to the $CaOH_2$ solution over a period of 24 hours. The amount of reagents in the solution was calculated to obtain a Ca/P molar ratio value equals 1.67, Corresponding to a Stoichiometric HAp. The pH of the slurry was measured digitally during the precipitation reaction, reaching a final value of pH 11.

Synthesis of nHAp / Gelatin / Magnesium chloride composite

Gelatin was dissolved in double distilled water and then nHAp powder continuously rotated using Maganetic stirrer. Now was added a little amount of Magnesium Chloride .Finally, The sample was dried using by microwave ovenafter the sample was powderd using morter vessel.

FTIR

The FTIR spectra of nHAp/Gel/$MgCl_2$ composite are shown in Fig(1). The FTIR Spectrum was investigated and carried out using PERKIN ELEMER Spectrometer in the range of 400 Cm^{-1} to 4000 Cm^{-1}. The function group associated with hydroxyapatite were identified by FTIR (Fourier Transform Infra red)spectroscopy. The composite spectrum are similar to the spectra of real bone[8]. The sepcrtrum clearly indicate the peaks at 602.91 Cm^{-1},1032.74Cm^{-1} corresponding to Po_4^{-3} ion. The peak observed at 3269.69 Cm^{-1}, corresponds to the stretching mode of (-OH) group, which characterizes the presence of hydrated calcium phosphate such as (HAp). The peak 1452.64Cm^{-1} corresponds to the stretching vibration of Co_3^{2-} ions. C-O stretching was observed at 1660.14Cm^{-1} . N-H Stretching is observed at 1547.18Cm^{-1}.

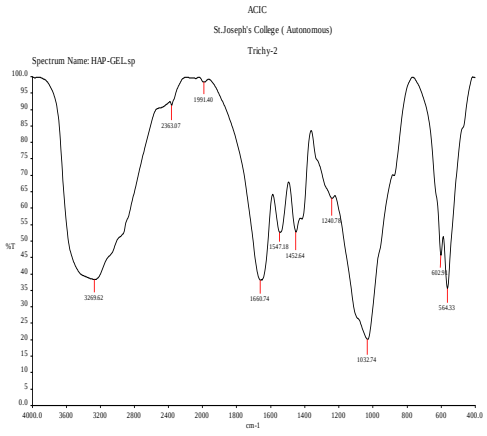

Fig1: FTIR spectrum for nHAp/Gel/$Mgcl_2$.

XRD

X-Ray diffraction studies of the powdered sample were carried out for phase identification using X-ray diffractometerRigaku with monochromatic CuKα radiation (λ=1.5405A°) and scan range of 2θ=10° to 90°.XRD data was matched with IICD no 09-0432. The diffraction peaks are 2θ = 25.74, 31.748, 32.815 these peaks are assigned to the Miller's indices reflection planes are (0 0 2),(2 1 1),(3 0 0) indicates that the amorphous phase hydroxyapatite. This XRD spectrum (Fig:2) indicates most of the high intensity peaks positioned between 2θ = 25°-34°. We observed from the peak broadening, that the synthesized HAp crystallite size present in Nano scale level. The d-space values of 2.812 , 2.72 and the other d-spacing value match with the hexagonal system with primitive lattice.

Line width(FWHM), 2θ , Miller indices value and Crystallite size are shown in the table 1. Fraction Crystallinity, Specific surface area , Micro Strain and dislocation density are shown in the table 2.

The Crystallite size were determined using Scherrer's equation.

$$D = \frac{K\,\lambda}{\beta\, Cos\theta}$$

Where, D is the crystallite size, K is the Shape constant (0.9), λ is the X-ray wavelength,θ in the diffraction angle in degrees and β (in radians) is the half width full maximum.

The crystal lattice parameters a and c, were calculated using the formula.

$$\frac{1}{d^2} = \frac{4}{3}\frac{(h^2 + k^2 + hk)}{a^2} + \frac{I^2}{C^2}$$

Where, d is obtained from the formula d= nλ/ 2 Sinθ.[9] The lattice parameter values are $a = 9.4241\ and\ c = 6.900$.

The degree of crystallinity (X_c) corresponding to peaks determined using the relation.

$$X_c = (K/\beta)^3$$

Where, K is a constant with a value of 0.24 for HAp.

Specific surface area of the HAp determined by the formula [10]

$$S = 6\times10^3/\ d^*\rho$$

Where ρ is the crystallite size and d is the theoretical density of HAp = (3.16 g/cm^3)

The volume (v) of the hexagonal unitcell of each HAp formulation was calculated using the following relation. [9]

$V = 2.589\ a^2c$

$V = 375.68$

The micro strain (ε) is calculated using the relation

$$(\varepsilon) = \frac{\beta\ Cos\theta}{4}$$

The volume of dislocation density (δ) is calculated using the relation .[11] . . $(\delta) = 1 / D^2$

2 θ	FWHM	(h k l)	Crystal size (nm)
25.794	0.274	0 0 2	5.191
31.748	0.825	2 1 1	1.7467
32.815	0.384	3 0 0	3.7629

Table 1: FWHM, 2 θ , Miller induces value and Crystallize Size for nHAp/Gel/$Mgcl_2$.

Specific surface area	Micro strain	Dislocation density	Fraction of crystallinity
365.91	0.0667	0.0371	0.6719
1087.04	0.1983	0.3277	0.0246
504.59	0.0920	0.0706	0.2441

Table 2: Fraction Crystallinity, Specific surface area , Micro Strain and dislocation density for nHAp/Gel/$Mgcl_2$.

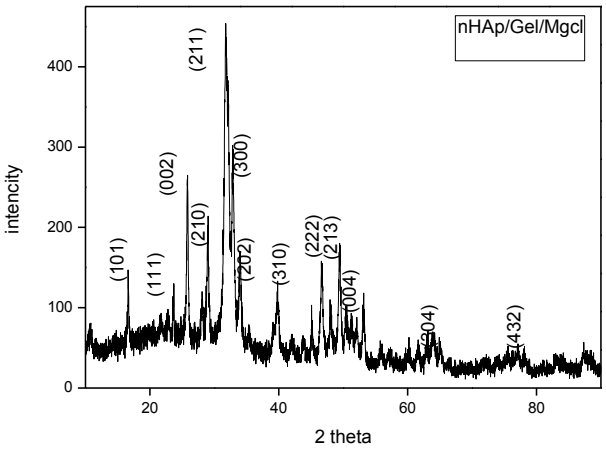

Fig 2: XRD spectru for nHAP / Gel / Mgcl.

TEM

Transmission Electron Microscope (TEM) is a technique which is used to find the morphology and the particle size of the sample. Surface morphology is very important to investigate nanostructure or nano particles. The morphology of the sample was investigated by TEM. Fig. **(3) (4) (5)** represented a typical TEM image of the HAp/Gel/$Mgcl_2$powder at different magnification. From the TEM image it could be seen the agglomeration of rod like type nano composite. The morphological structures of the samples observed in this study were very similar to those of the mineral phase present in bone and teeth. It may create a bioactive bone between the material and tooth structure, such as enamel and dentine, and provides better mechanical properties due to its high-surface area to volume ratio, superior chemical homogeneity and micro structural uniformity morphology . The TEM image also confirm that the particle sizes varies from 20-100nm. The inorganic phase was further identified as HAp/Gel/$Mgcl_2$ from the SAED [selected area electron diffraction] pattern of the sample fig.6. The diffraction rings reflect the crystallinity of sample SAED results of good agreement with the lattie structure and exhibits excellent crystallinity, however there is obvious diffraction pattern for HAp which indicaes that its main composition is aamorphous phase.polycrystalline rings for the (002), (211) plane of hydroxyapatite are in good agreement with XRD analysis

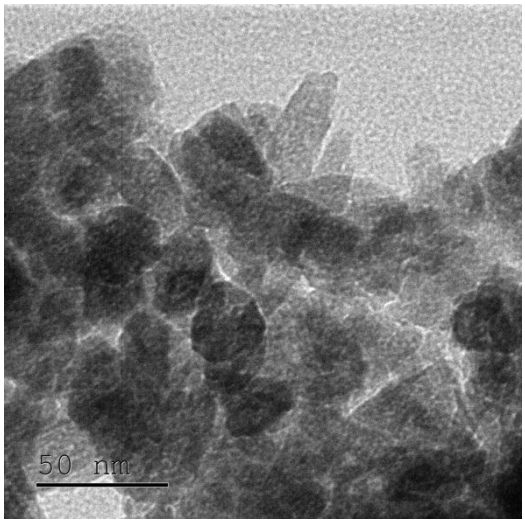

Fig (3).TEM image for 50nm.

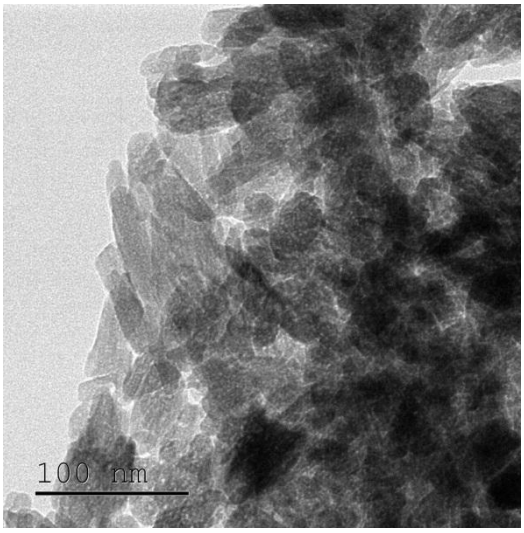

Fig .(4) TEM image for 100nm.

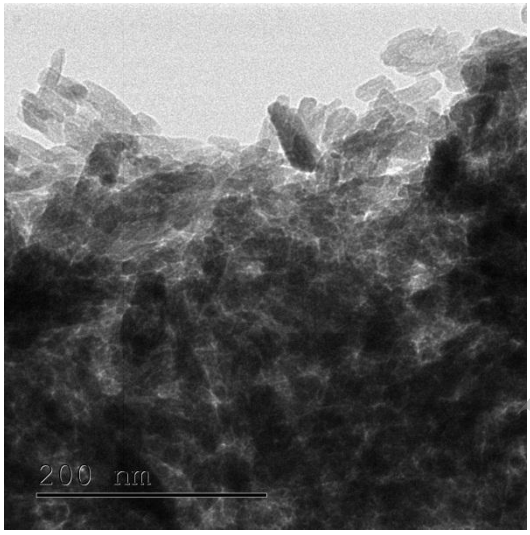

Fig.(5) TEM image for 200nm.

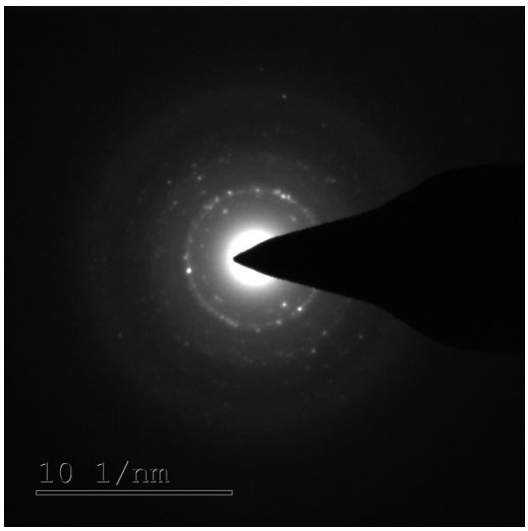

Fig: (6) SAED pattern

Thermal analysis

Thermal characterization is important to know material reactivity under specific temperature. The thermo gravimetric analysis (TGA) and Differential thermal analysis (DTA) (Fig:7) were carried out for a sample of weight6.379mg. The sample were heated starting from room temperature (29°C) and going upto 900°c, at heating rate 30°C/min, on an aluminum support under a dynamic nitrogen atmosphere (N2). There was an initial loss of mass of the order of 5.4% between room temperature and 180°C. second loss occurred between 180°C to 280°C,equivalent to 3.9 %. The third loss between 280°c to 420°c, equivalent to 14.6 %. The maximum loss between 420°c to 700°c, equivalent to 15.8%.Beyond 700°c to 900°c no significant weightloss was observed. Almost stable cure was noticed within this temperature range, which indicates thermal stability of HAp/Gel/$Mgcl_2$ powder. The DTA curves shows first endothermic peak at 380°c the melting point of the sample. The secondendothermic peak at 475°c in the DTA curve indicates the decomposition of the sample.

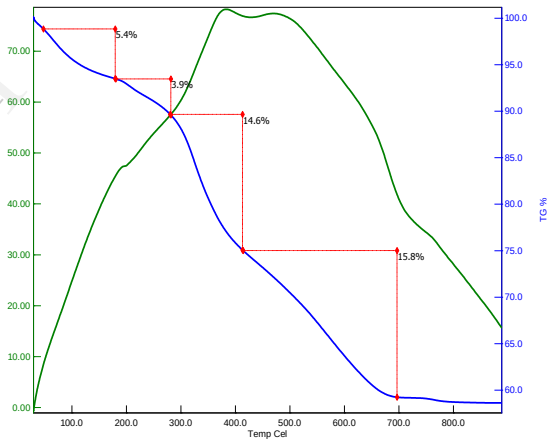

Fig 7: TG/DTA for nHAP / Gel / $Mgcl_2$.

CONCLUSION

nHAp/Gel/$Mgcl_2$ composite have been successfully synthesized using the modified wet chemical method. FTIR result conforms functional groups. The formation nanoparticles was confirmed by XRD analysis and XRD indicated the phase purity and crystallinity of powder sample. The size and morphology of the powder were characterized using TEM. SAED pattern are in good agreement with XRD. The crystallite size, lattice parameters, specific surface area , volume density, microstrain and dislocation density also measured by using XRD data. The nanosizednHAp/Gel/$Mgcl_2$ powder produced can be highly useful as a bone replacement material.The future application of nHAp/Gel/$Mgcl_2$ in the human biomedical was expected. Further studies is needed to carried out in order to proof its safety and efficacy in the human body.

ACKNOWLEDGEMENTS

The Management of UrumuDhanalakshmi College, Tiruchirappalli for providing research facilities in the campus.

REFERENCE

[1]. S.Mobini, J.Javadpour, M.Hosseinalipour, M.Ghazi-Khansari, A.Khavandi and H.R.Rezaie. "Synthesis and Characterisationofgelatinnanohydroxyapatite composite scaffolds for bine tissue Engineering", Advances in Appliled Ceramics.2005 Vol.101.

[2] Y.Liu, M.Wang "Fabrication and characteristics of Hydroxyapatite Reinforced polypropylene as a Bone Analogue Biomaterial", Journal of applied polymer Science, Vol.106,2780-2790 (2007).

[3]. Mehdi KazemzadehNarbat, MehranSolatiHashtin, Mohammad Pazouki, "Fabrication of porous hydroxyapatite-gelatin scaffolds crosslinked by glutaraldehyde for bone tissue engineering".

[4] Gineste L, Gineste M Ranz X, Ellefterion A, Rouquet N, Frayssinet P (1999). "Degradation of hydroxyapatite, fluoroapatite and flurohydroxyapatite coating of dental implants in dogs". J Biomed Mater Res.48:224-34.

[5] Mehdi KazemzadehNarbat, FaribaOrang ,MehranSolatiHashtjin and AzadehGoudarzi."Fabrication of porous Hydroxyapatite-gelatincomposite Scaffolds for bone Tissue Engineering".Iranian Biomedical Journal 10(4): 215-223 (October 2006).

[6] H.Bundela, A.K. Bajpai. "Designing of hydroxyapatite-gelatin based porous matrix as bone Substitute: Correlation with biocompatibility aspects". eXPRESS polymer Letters Vol.2, No.3 (2008) 201-213.

[7] ShadabBagheri-Khoulenjani , Mohammad Etrati-Khosroshahi and Hamid Mirzadeh. "Particle Size and Distribution Modelling of Nanohydroxyapatite-in-gelatinNanocompositeMicrospheres By Surface Response Method". Iranian polymer Journal 19(10),2010,743-755.

[8]. Md. JakirHossan , M.A Gafur , M.R Kadir and Mohammad MainulKarim , "Preparation and Characterization of gelatin–Hydroxyapatite composite for bone Tissue Engineering". International Journal of Engineering& Technology IJET-IJENS Vol:14 No:01.

[9] AzadehRezakhani and M.M KashaniMotlagh., "Synthesis and Characterization of hydroxyapatite Nanocrystal and gelatin doped with Zn^{2+} and cross linked by glutaraldehyde". International Journal of physical science vol.7(20),pp.2768-2774,23 may,2012.

[10]. Palanivel.R, A. Rubankumar. "Synthesis and Spectroscopic Characterization of Hydroxyapatite By Sol-Gel Method". International journal of chem tech Research CODEN (USA): IJCRGG ISSN : 0974-4290, Vol.5, No.6, pp 2965-2969, Oct-Dec 2013

[11]. M.Jothibas,C.Manohaaran,S.Dhanapandian,S.Johnsonjeyakumar, "Influence of precursor concentration on sprayed In_2O_3 Thin Films", Asian journal of Chemistry; Vol.25,Supplementary Issue(2013),S59-S64.