Synthesis and characterization of nano hydroxyapatite with dextran nano composite for biomedical applications

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Abstract

Hydroxyapatite/Dextran (HD) Nano composites are important biocompatible materials. The title compound was successfully synthesized by a wet chemical method at room temperature with different mass ratio. The composite structure and morphologies of the synthesized hydroxyapatite were analyzed by X-ray diffraction(XRD), Fourier transformation infrared spectroscopy(FTIR), Transmission electron microscope(TEM), Thermo gravimetric analysis(TGA) and antibacterial activity of the synthesized Hydroxyapatite/Dextran nano composite related with gram negative and gram positive were examined. The obtained results indicate good thermal stability. The physical and chemical properties agreed well with previous report. Lattice parameter and volume density are matched with JCPDS NO:09-0432. Antibacterial test exhibits antimicrobial activity. It can potentially be applied in biomedical and bone tissue engineering materials.

Key words: HD, XRD, FTIR, TGA,TEM, Antibacterial activity.

Introduction

Calcium phosphate are primarily used as bone substitutes in biomedical industry due to their bio compatibility, low density, chemical stability and their compositional similarity to the mineral phase of bone[1]. Hydroxyapatite is a kind of calcium phosphate bio ceramic material and having a composition of $Ca_{10}(PO_4)_6(OH)_2$[2]. Hydroxyapatite is a main component in bone. The number of medical applications of HAp are limited, primarily due to its relatively poor mechanical properties, small amounts of biopolymer and/or silicon are introduced during the HAp synthesis to improve the mechanical properties[3]. To Increase the biocompatibility[4] of the composite, particle size should be taken into consideration [5]. This biomaterial is widely used to repair, fill, extend and reconstruct damaged bone tissue. It can also be used in the preparation of soft tissue[6]. Hap being the main inorganic composition of the hard tissue in natural bones, it has been extensively studied for medical application due to its excellent bioactivity and bio compatibility[7]. It is an ideal material for its excellent properties like biocompatibility, non-toxic, low cost. HAp with hytrophilic polymeric matrix expected to have enhanced properties of inorganic and organic components[8]. Various methods of the preparation of synthetic HAp have used Hydrothermal method[9], chemical precipitation technique[10], biomimetric preparation[11], sol-gel method, etc.,. The most widely used

technique for synthesis of HAp is chemical precipitation technique. This technique used raw materials at a reasonable cost. It is possible to improve the properties of HAp ceramic by controlling the process parameters such as particle size and shape, particle distribution and agglomeration.

Dextran is a natural linear polymer of glucose linked by a 1-6 linked glucoyranoside and some branching of 1,3 linked side chains. Dextran is synthesized from sucrose by cerain lactic acid bacteria, the best known being leuconostons mesenteroides and streptococcus mutans[12]. Dextran polymers have a number medical applications. Dextran has been used as model of drug delivery due its characteristics such as water solubility, biocompatibility and bio degradability. It is a potential polysaccharide polymer that can sustain the delivery of proteins, vaccines and drug.

The objective of the present work is to synthesis nano HAp, Hydroxyapatite/Dextran composite material with mass ratio prepared by wet chemical method at room temperature and carried out its characterization.

Experimental design

Materials:

The raw materials required to start the processing of the composite were: analytical grade calcium hydroxide and ammonium di hydrogen phosphate obtained from Merck and dextran purchased from Loba. Doubly distilled water was used as the solvent.

Methods:

Synthesis of nano HAp

Nano HAp was synthesized by following a modified 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) and was stirred. A solution of 6.7 g ($NH_4H_2PO_4$) was dissolved in volume of water and then added to the $Ca(OH)_2$ solution over a period 24h.

Synthesis of HAp/ Dextran nano composite

The HAp/Dextran nano composite were coded as nano HAp/Dex-20(HD8:2), HAp/Dex-30(HD7:3), HAp/Dex-40(HD6:4). Water was used as the solvent to prepare the polymer solution. Dextran was dissolved by using magnetic stirrer. Then suitable amount of HAp was dispersed in deionized water. HAp in water was mixed with polymer solution. The homogenously mixed solution is immediately taken into heat process.

Result and discussion

FTIR

The FTIR spectra of HAp/ Dextran composite are shown in figure 1. The O-P-O bending bands have been observed at 566 cm^{-1},565 cm^{-1} and 601 cm^{-1},603 cm^{-1} due to presence of nano HAp. The bands at 3386-3401 cm^{-1} and 1634 cm^{-1} in HAp are owing to the presence of lattice water in the solid. The presence of carbonate ions in the synthesized HAp practical have been assigned at 1338-1340 cm^{-1} which may comes from the atmosphere carbon dioxide during the synthesis. The bands observed between 2924 cm^{-1} corresponds to C-H stretching band of dextran. A new peak of C-H stretching bands observed at 2944 cm^{-1} indicate that the chemical bond interactions between HAp/Dextran have been carried out in the solution.

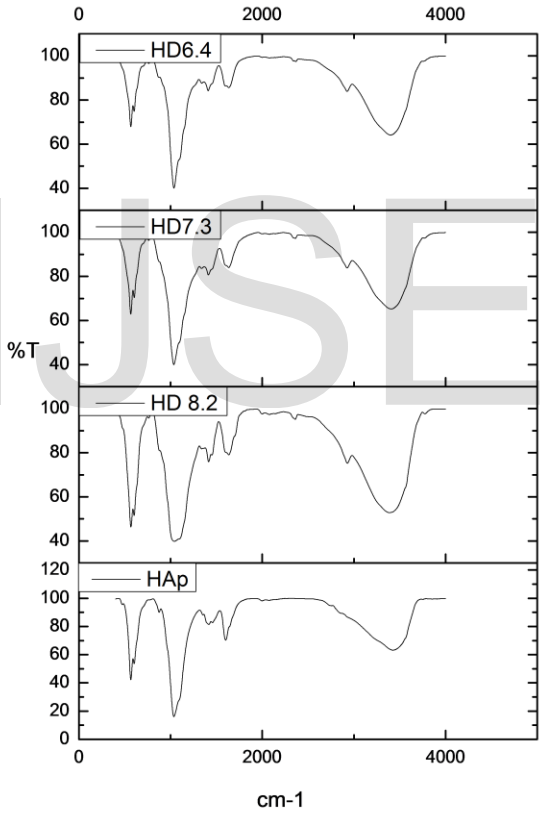

Fig:1 FTIR graph for HAp and HAp\Dextran composite.

X ray powder diffraction

Diffraction data were obtained on a Rigaku Diffratometer at 40kv and 15mA. The measurement were made over the scattering angle 2θ range from 10 to 90˚. Phase were identified using the International centre for diffraction data powder diffraction file No 09-0432. All XRD patterns show diffraction lines characteristics of hydroxyapatite present. They agree well the

values observed in literature. The major phase, as expected is HAp , which is confirmed by comparing data obtained with the ICDD-PDF 2 Card 09-0432. The substitutions in HAp structure appeared in the XRD charts show there is a small change in the peak position and intensity. The broadening of peak in XRD revealed there is a very slight shift of the peak position in all HAp/Dextran samples compared the JCPDS. The XRD patterns show diffraction peaks with line broadening and high intensities, which confirms the nano size with crystalline nature. The diffraction peaks particularly, in the planes 002,211,112, 300 are high and narrow implying that the HAp crystallizes well. The intensity diffraction peak 211 were chosen to estimate the following parameters. The crystallite size of the nano composite was calculated using Scherrer's formula. The fraction crystallinity, specific surface area, micro strain, lattice parameters and unit cell volume are tabulated in table 1. Nanosized composite increased specific surface area and micro strain values. Lattice parameters value and density of volume values are matched with JCPDS Card No.09-0432.

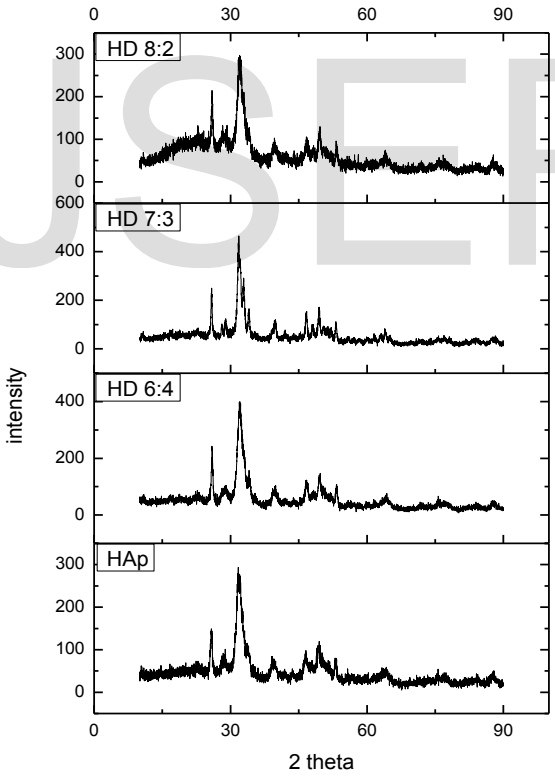

Fig : 2 XRD pattern for HAp and HAp/Dextran composite.

Properties Sample	Crystalline size (nm)	Degree of crystallinity	Micro strain	Specific surface area (m^2/g)	Lattice parameters		Density of volume(A^3)
					a	C	
HAp	4.48	0.00422	0.4450	423.31	9.4865	6.8511	533.95
HD 8:2	4.63	0.0024	0.4103	409.21	9.4529	6.8444	529.65
HD7:3	18.48	0.1517	0.1082	102.75	9.4277	6.9003	531.14
HD6:4	4.604	0.0023	0.4351	412.76	9.4626	6.8368	530.15
ICDD 09-0432	-	-	-	-	9.4180	6.8840	528.80

Table 1: diffraction peak (211) parameter values.

Thermal analysis

Thermo gravimetric analysis (TGA) which provides an indication of the amount of weight loss during the decomposition process. The weight loss profiles indicate that multistage decomposition process are occurring . TGA analysis was performed in the range 0 to 800˚C. The initial weight loss from 80˚C to 220˚C is about 7%, which may be due to the evaporation of water and hydroxylation of HAp. The second weight loss is observed about 220˚C to 550˚C. This is due to decomposing of organic phase in the composite. The third stage higher than 600˚C, there was no weight loss for the composite material. The result indicates that the composite material exhibit good thermal stability.

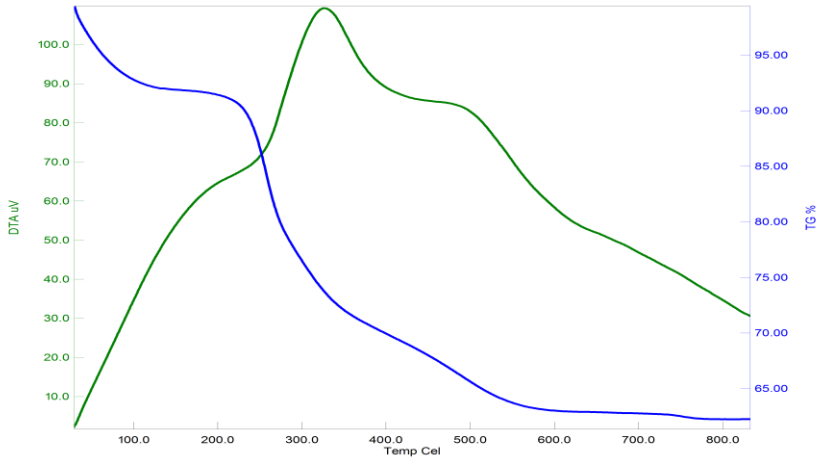

Fig 3: TGA graph for HD 7: 3 composite.

TEM:

TEM image of pure nano HAp and different weight percentage of Dextran compositions are as shown in Figure 3 and 4. Transmission electron microscope (TEM) experiments were performed on a Tecnai T20 electron microscope with an acceleration voltage of 200kV. TEM image shows that particle exhibit nano rod morphology. The particle size of HAp is nm. Where added to Dextran, the rod like morphology starts disappear. Increase in the Dextran composition change from rod like to an irregular morphology. SAED pattern is identification of inorganic phase. Dextran was increase semi crystalline nature into crystalline nature.

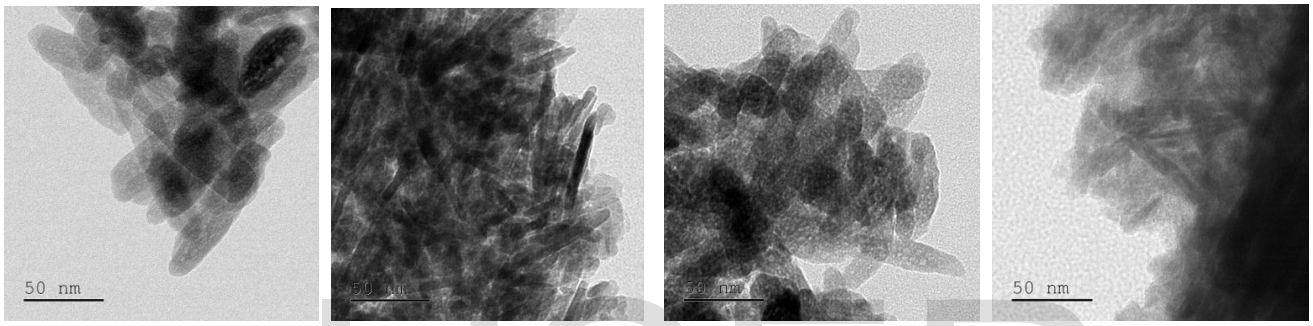

Fig 3 a: HAp Fig 3b: HD8:2 Fig 3c: HD7:3 Fig 3d:HD6:4

Fig 3: 50 nm image for HAp and HAp/Dextran nano composites.

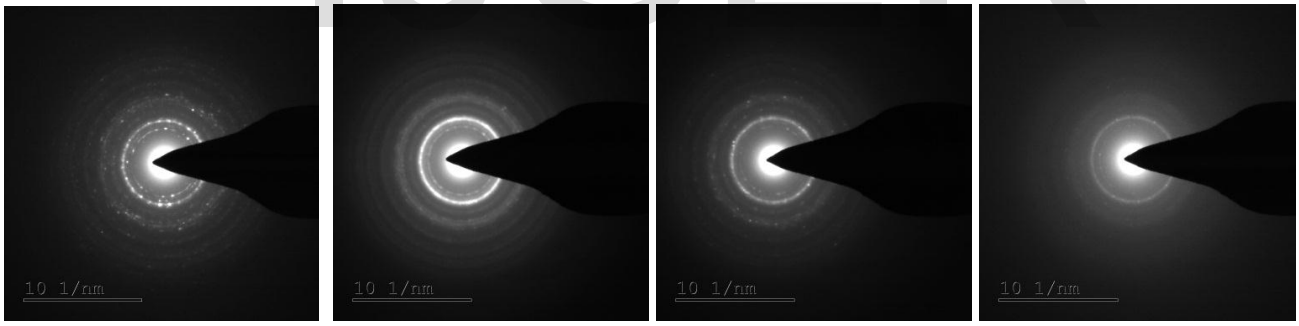

Fig 4a : HAp Fig 4b: HD8:2 Fig 4c: HD 7:3 Fig 4d: HD6:4

Fig 4: SAED pattern for HAp and HAp/Dextran nano composites.

Anti microbial activity

HAp and HAp/Dextran were subjected to antimicrobial studies with gram positive and gram negative bacteria. The antibacterial activity was performed by disc diffusion method. The plate were incubated at 37˚C for 24 h for the bacteria and at room temperature for 24-48 hr.All the prepared samples exhibited antimicrobial activity. The resultant data is shown in table 2. The composite of HD 7:3 showed higher antibacterial activity against E.Coli and S.coccus. The

antibacterial activity is dependent on the molecular structure of the compound and show medium the bacterial stain.

Microorganisms	Name of the compounds				Standard (Chloromphenical for bacteria)
	HD8:2	HD 7:3	HD6:4	HAP	
Escherichia coli (mm)	8 ± 0.56	9 ± 0.63	6 ± 0.42	5 ± 0.35	15 ± 1.05
Streptococcus pyogenes (mm)	4 ± 0.28	8 ± 0.56	7 ± 0.49	6 ± 0.42	12 ± 0.84

Table 2: Antibacterial activity of the HAp, HAp/Dextran composite.

Escherichia coli *Streptococcus pyogenes*

Fig 5. Antibacterial activity of the HAp ,HAp/Dextran composites

Conclusion

The nHAp/Dextran composite material with different mass ratio, were prepared using wet chemical method at room temperature. The composite exhibit excellent structural and thermal stability. The particle size of composite is observed a 20 nm with a rod like shape. FTIR spectrum confirm the functional group and formation of the HAP. XRD results exhibit crystalline nature. Lattice parameter and volume density were matched with JCPDS No: 09-0432. The highest antimicrobial activity was observed in HD7:3 ratio composites against E.Coli and S.coccus. The physical and chemical performance of the synthesized composite material satisfy the requirements of biomedical applications and bone tissue engineering materials.

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