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Synthesis and Characterization of Nano Hydroxyapatite with Agar-Agar Bio-Polymer

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Abstract
Hydroxyapatite used for bone replacement is one of the most active areas of ceramic biomaterials research currently. It is a desirable implant material due to its biocompatibility and osteoconductive properties. Agar agar is a biological polymer frequently used in tissue engineering and pharmaceutical for potential use in bone replacement. Nano hydroxyapatite was successfully synthesized by wet chemical precipitation method . In this work nHAp/agar composite were synthesized and characterization of the compound were done by using characterization Fourier transform infrared(FTIR), X-ray diffract ration (XRD), Transmission electron microscope(TEM) and Energy dispersive analysis of X-ray spectrum(EDAX).
Key words: HAp, Agar, FTIR, XRD, TEM, EDAX.

I. 1.Introduction

Hydroxyapatite also known as hydroxylapatite is the major component of tooth enamel and bone mineral. Bone consists of approximately 8 wt% water, 22 wt% protein and 70 wt% minerals[1]. The mineral component of bone is a form of calcium phosphate known as hydroxyapatite with chemical formula $Ca_{10}(PO_4)_6OH_2$ and the hexagonal crystalline structure[2]. The hydroxyl ion $(OH)^-$ can be replaced by Cl^-.F^-,etc in the collagen fiber matrix[3]. HAp with bone bonding properties is widely used in hard tissue replacement due to their biocompatibility and osteoconductive properties [4,5]. Hydroxyapatite is non-inflammatory and it causes no immunological and irritating response [6]. This bio ceramic has been widely used in dental materials, constituent implants and bone substituent materials due to its excellent biocompatibility, bioactivity, osteoconductive, non-toxicity and non-inflammatory nature[7,8]. To prepare fine Hydroxyapatite powders many chemical processing routes have been employed including hydrothermal reaction[9,10],Sol-gel synthesis[11,12], pyrolysis of aerosols[13,14], micro emulsion[15,16], biomimetic process[17,18], Chemical precipitation method[19,20], Solid state[21], Co precipitation method[22,23], microwave process[24]. A widely variety of polymers are used in medical as biomaterials. Their application range from facial prostheses to tracheal tubes from dentures to hip and knee joint. Natural polymer is developing the most promising therapeutic systems, namely drug delivery system which provides an effective therapy to the patients for prolonged periods. A natural polymers have lots of advantage over synthetic polymers as safe and non-toxic in nature, highly biocompatible and bio degradable very much environment and eco-friendly[25]. Natural polymers are environmentally benign substance and also possess great potential to developed for industrial and medical applications by themselves or incombination with other supplementary organic or inorganic compound. The use of natural polymer has been extended to the nanotechnology and biomedical fields, due to its biocompatibility for in vivo applications as well as its stabilization of nanostructures [26].

Agar is biological polymer. It is actually the resulting mixture of two components the linear polysaccharide agarose and a heterogeneous mixture of smaller molecules called agaropection. Agar is gelatinous, non-toxic and biodegradable substance derived from marine algae. It has been used in a floating controlled-release tablet. Agar can also be used as a base for non-melting and non distintegrating suppositions. It is a hydrophilic natural polysaccharide and can be easily fabricated by thermo crosslinking to hydrogel that can also be used as a drug delivery system.

This work describes the synthesis and characterization of nano hydroxyapatite with agar agar biopolymer composite which can be tried for bone regeneration and tissue repair engineering.

II. Materials and methods

2.1 Material

The raw materials required to start the processing of the composite were: calcium hydroxide $Ca(OH)_2$ and Ammonium dihydrogen phosphatete $(NH_4)_2PO_4$ obtained from Merk (India), Agar bacterrliogial grade purchased from Nice (India). Ethanol and double distilled water were used as the solvent.

2.2 Methods

Synthesis of nano HAp

Nano HAp was synthesized by following a modified wet chemical method. At room temperature, 7.48g of Calcium hydroxide was first dissolved in a 100 ml volume of an ethanol-water mixture (50:50%, v/v) and was stirred for 3h. A solution of 6.7g $(NH_4)H_2PO_4$ was dissolved in 100ml volume of water and then added to the $Ca(OH)_2$ solution over a period of 24 hours. The pH of the slurry was measured digitally during the precipitation reaction, reaching a final value of pH 11.

Synthesis of Agar/HAp nano composite:

Agar was dissolved in double distilled water. Then nHAp powder was added in small amount periodically by keeping it an a mechanical stirrer. Finally, The sample was dried using by microwave oven and after that the sample was powdered using mortar vessel.

III. Result and discussion

FTIR

The FTIR spectrum investigations were carried out using PERKIN ELEMER Spectrometer in the range of 400 cm^{-1} to 4000 cm^{-1}. The functional groups associated with hydroxyapatite were identified by FTIR spectroscopy. The FTIR spectra of the prepared sample are given in fig1. The strong peak at 3405.01cm^{-1} confirms the presence of a –OH stretching (hydroxyl) groups. The peak at 1479.33cm^{-1} corresponds to the stretching mode frequency of the CO_3^{2-} group. The peak at 1041cm^{-1} and 603.96cm^{-1} are assigned to vibration of the phosphate group, PO_4^{3-}. The peak 1257.93cm^{-1} assigned to ester group. The above peaks indicate that the chemical bond interactions between HAp and agar bio polymer.

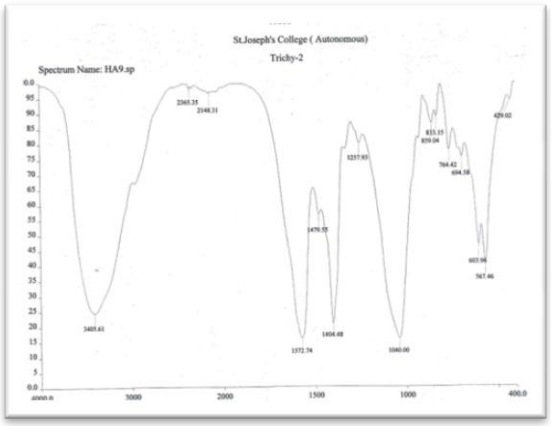

Fig 1: FTIR Spectra of Agar/nHAp

XRD

The XRD Patterns of nano Agar/nHAp composites were taken. The structure of the sample was analyzed by X-ray diffraction (XRD) using RIGAKU X-ray Diffractometer. The structural analysis of sample was done by the powder diffraction with monochromatic CuKα radiation of λ=1.5405 A^o and scan range of 2θ=10^o to 90^o . The patterns indicate the presence of amorphous HAp. The XRD patterns of the synthesized agar /nHAp are shown in fig.2. The d-spacing values at 2.82Å, 2.79Å, 2.72Å and the values assigned to the Miller's indices of reflection plane are (002),(211) and (300). This indicate the amorphous phase of Hydroxyapatite. The XRD patterns show diffraction peaks with high intensities and the mage obtained from SAED conforms the nano crystalline nature of the compound.

The particle size is determined by the Scherrer equation.

$$D = K\lambda / B_{1/2} \cos \theta$$

In the above equation, D is the particle size, K is the shape constant 0.9, λ is the wave length of X-rays, which is 1.5405 Å, θ is Bragg angle of peak from diffraction and $B_{1/2}$ is in terms of radians.

The fraction of crystallinity Xc of the HAp nanoparticle is calculated from the equation.

$$Xc = (0.24/\beta)^3 .$$

where β is the full width half maximum (FWHM)value.

Specific surface area of the HAp is determined by the formula

$$S = 6 \times 10^3 / d^*\rho.$$

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

2 θ	FWHM	Crystal size (nm)	Fraction of crystallinity	Specific surface ares (m^2/g)
31.813	0.657	13.13	0.0487	144.61
32.893	0.429	20.17	0.1750	94.13
33.99	0.37	23.45	0.2729	80.96

Table.1: Line width and crystalline size, fraction of crystallinity and specific surface area.

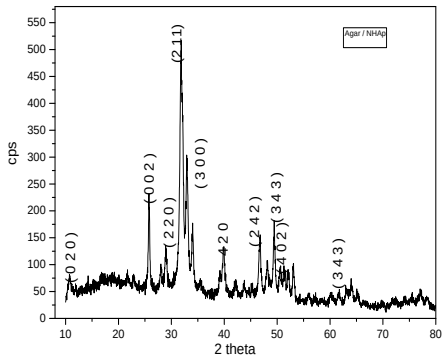

Fig 2: XRD Spectrum of Agar/nHAp.

TEM

The morphology of the sample was investigated by transmission electron microscope. Figs. 3a, 3b and 3c represented a typical TEM image of the Agar/nHAp composite at different magnification. From the TEM image it could be seen the agglomeration of rodlike-type nano composites of agar and nHAp. 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. Moreover, these nHAp / Agar samples had good dispersive properties and displayed a relatively uniform morphology. The TEM image also confirm that the particle sizes varies from 20-100nm. The inorganic phase was further identified as agar/nHAp from the SAED [selected area electron diffraction] pattern of the sample fig.4. The polycrystalline rings for the (002), (211) and (300) plane of hydroxyapatite are in good agreement with XRD analysis.

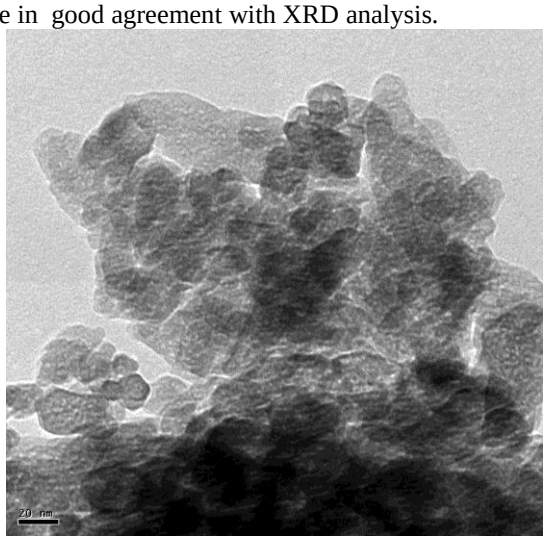

Fig 3a: TEM image for 20nm.

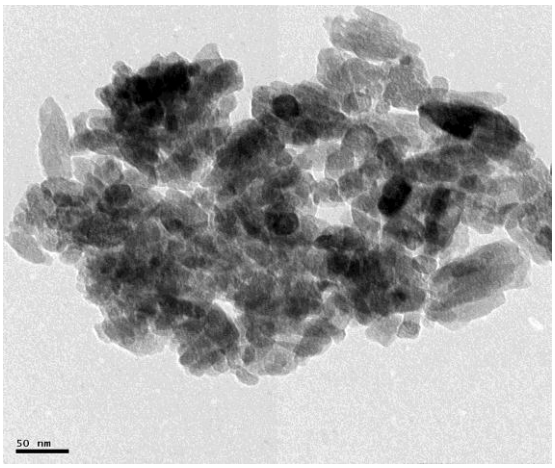

Fig 3b:TEM image for 50nm

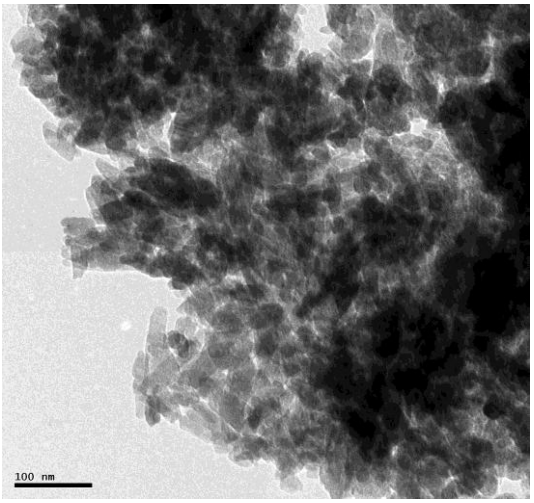

Fig 3c: TEM image for 100nm.

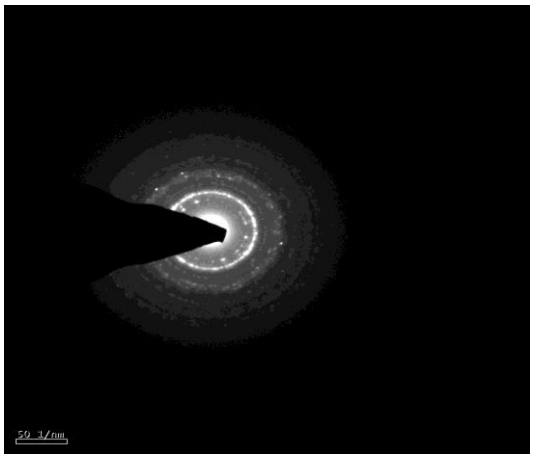

Fig 4: SAED pattern of Agar/nHAp. EDAX

The energy dispersive analysis of X-ray of nano HAp/ agar is shown in fig.5. Energy dispersive X-ray analysis identifies the elemental composition of materials. EDAX systems are attached to TEM instrument. The mineral composition of Ca, O, P, C are present in Agar/nHAp composite. The weight percentage of Ca, O, P and C focused to be 21.42%, 27.40%, 9.75%, 41.40% respectively.

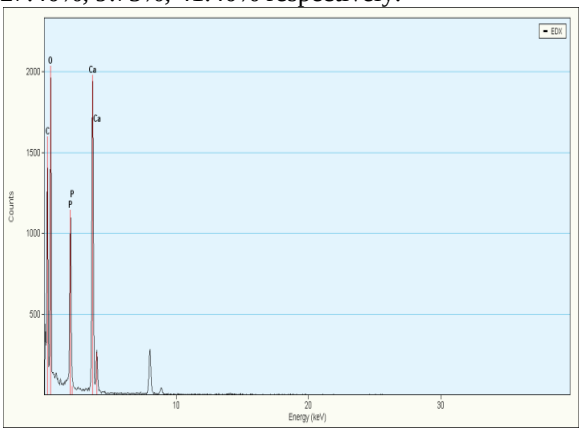

Fig 5: SAED spectrum of Agar/nHAp composite.

IV. Conclusion

Agar / nHAp composite have been successfully synthesized using the modified wet chemical method. FTIR result conforms functional groups like (-OH), ester group and presence of a phosphate group. The XRD patterns reveal that hydroxyapatite is a major phase presented in Agar/nHAp composite. The particle size is measured by Scherrer equation and fraction of crystallinity and specific surface area are also measured. The TEM image show the shape and size of the prepared nano composite which varies from 20-100 nm. The Elemental compositions were examined using the EDAX analysis. The developed Agar/nHAp composite may have applications in making a biocomposite, drug delivery and bone tissue reconstruction engineering.

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