SYNTHESIS AND CHARACTERIZATION OF NANO HYDROXYAPATITE WITH POLY (VINYL ALCOHOL) AND CARBOXYL METHYLCELLULOSE COMPOSITE FOR PHARMACEUTICAL APPLICATIONS

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Abstract

Hydroxyapatite with poly (vinyl alcohol) and Carboxyl methylcellulose composites was synthesized by wet chemical method. Di ammonium hydrogen phosphate, calcium hydroxide and poly (vinyl alcohol) and Carboxyl methylcellulose were used as starting materials. The powder sample was characterized by the Fourier Transform infra-red (FTIR), Transmission electron microscope (TEM), Thermal gravimetric analysis, X-ray diffraction, antibacterial activity and anti-inflammatory test. With respect to the result achieved from the above analysis, it was found that NanoHAp/PVA/CMC composite can successfully be produced through the wet chemical precipitation method. TEM technique demonstrated that the powder were nano sized and had a rod-like morphology. FTIR reveals the functional groups. XRD pattern illustrate the crystallite size and lattice parameter values. Antibacterial activity exhibits the activity in E. coli and S.aureus bacteria.

Key words: Hydroxyapatite, poly (vinyl alcohol) (PVA), Carboxyl methylcellulose (CMC), XRD, TEM.

1. INTRODUCTION

Tissue engineering is an interdisciplinary and multidisciplinary field that aims at the development of biological substitutes that restore, maintain or improve tissue function...
Bone tissue is composed of minerals and proteins. The minerals are mostly apatite such as hydroxyapatite, fluoroapatite and carbonate apatite [2]. Hydroxyapatite is widely accepted as a bioactive material for guided regeneration [3]. Hydroxyapatite which has molecular stoichiometric formula $\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2$, has been extensively investigated due to its excellent bio compatibility, bioactivity and osteoconductivity as well as its similarities to the main mineral component of bone. However, the poor compressive strength and fatigue failure limits its applicability to the low or non-load bearing sites in human body [4,5]. Additionally, it has been reported that Hydroxyapatite in the form of powders, used for the treatment of bone defects, has problem associated with migration to places other than implanted areas. It varies in its applications, such as shells and pearls are all organic/inorganic composites with good mechanical properties, which may provide a route to resolve the problems. Extensive research has been carried out in this regard and composite materials based on hydroxyapatite and a variety of polymers have been worked out [6]. Polysaccharides are widely applied in many fields, particularly in the food. Pharmaceutical and medicine. Polysaccharide applicable in tissue engineering are usually combined with other natural or synthetic polymers, or are reinforced with inorganic particles. [7]. Carboxyl methyl cellulose (CMC) is used as a thickener, binder, stabilizer, suspending agent or flow control agent. It is also used in coating pharmaceutical tablet and ceramic industry [8]. The poly (vinyl alcohol)(PVA) hydrogel is an excellent artificial articular cartilage repair material due to its biocompatibility and biological properties [9,10,11]. It possesses high porous structure and high content of free water, similar to that of nature articular cartilage, PVA hydrogel has increasingly attracted interest in application as an articular repairing materials [12].

This work describes the preparation and physical, chemical morphology characterization of hydroxyapatite with CMC and PVA composites for pharmaceuticals applications.

2. Experimental Details

2.1 Materials

The raw materials required to start the processing of the composite were: calcium hydroxide $\text{Ca(OH)}_2$ and Ammonium dihydrogen phosphate $(\text{NH}_4)_2 \text{PO}_4$ were obtained from Merk(India). Carboxyl methyl cellulose and poly (vinyl alcohol) was purchased from Loba(India). Ethanol and double distilled water were used as the solvent.
2.2 Synthesis of nano HAp

Nano HAp was synthesized by following a modified wet chemical method. At room temperature, 5.56g of calcium hydroxide was dissolved in a ethanol-water mixed solution and stirred for 4h. A solution of 6.7g Ammonium dihydrogen phosphate was dissolved in a 100 ml volume of water and then added to the Ca (OH)$_2$ solution over a period of 24 hours. The liquid products were then subjected to Microwave exposure for various timings under trial and error method. The products were grained by mortar vessel to get fine products nano composite.

2.3 Synthesis of nHAp with PVA and CMC.

Carboxyl methyl cellulose and poly (vinyl alcohol) were dissolved in double distilled water and purified by filtering the sample several times. Then added suitable amount of nHAp periodically by keeping it a mechanical stirrer. The products were dried using by Microwave oven and Final products were obtained.

3. RESULT AND DISCUSSIONS

3.1 FTIR analysis

Functional groups were investigated by FTIR spectrometer in the range from 4000-400 cm$^{-1}$. Spectrum was obtained in the transmission mode. The figure shows the FTIR spectrum of prepared HAp/PVA/CMC composite. 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 characteristics peaks at 602.38cm$^{-1}$ and 1038.07cm$^{-1}$ correspond to the stretching vibration of PO$_4^{3-}$. OH$^{-}$ ions are identified by observation of the broadband from 3700 to 2500cm$^{-1}$. The peak of this band at 3412.91 cm$^{-1}$ is a typical assignment of the stretching mode of OH$^{-}$ ions. The stretching vibrations, ascribed to CO$_3^{2-}$ at around 1417.14 cm$^{-1}$ which an indication of the presence of carbonate apatite. This might have originated through the absorption of carbon dioxide from the atmosphere. The band at 1608 cm$^{-1}$ corresponds to adsorbed H$_2$O.
3.2 XRD analysis

Synthesized sample was analysed by X-ray diffractometry (XRD) using CuKα Ni-filtered radiation. The registered pattern range for 2theta value was from 10° to 80°. The obtained XRD pattern indicated the amorphous nature of HPC sample.
3.3 Thermal analysis

To investigate the thermal properties of the sample, HAp/PVA/CMC were subjected to TGA/DTA studies. Thermogravimetric Analysis (TGA) provides the quantitative measurement of any weight change associated with a transition of the sample. It can directly record the loss in weight with the time or temperature due to dehydration and decomposition. Differential Thermal Analysis (DTA) is a thermoanalytical technique to record the difference in temperature between substance and a reference when they are subjected to identical heating at the controlled rate. The record obtained is known as DTA cure, this series of peaks whose positions are determined by the composition and crystal structure of the sample. These experiments were performed in the nitrogen atmosphere in the temperature range of 80 to 800°C with the heating rate of 20°C/minute. The exothermic peak is observed at the DTA curve at 450°C could be due to a crystallization process of the powder.

![TGA/DTA curves of nHAp/PVA/CMC nanocomposites](image)

**Fig 3.3 TGA/DTA curves of nHAp/PVA/CMC nanocomposites**

3.4 TEM

The structure and morphology of the sample were further confirmed by the TEM and TEM images of the prepared nano-hydroxyapatite, as shown in a figure. The transmission electron microscopic analysis confirms the presence of the rod-like morphology of the prepared HPC nanoparticles. In addition, the selected area electron diffraction (SAED) of the
precipitates shows diffraction ring of patterns, this implies that the precipitates are amorphous nature.

![TEM/SAED image of nHAp/PVA/CMC nanocomposites](image)

**Fig 3.4 TEM/SAED image of nHAp/PVA/CMC nanocomposites**

### 3.5 In-vitro antibacterial activity

Antimicrobial activities of the sample for HAp and PVA with CMC with various concentrations were investigated against *S.aureus* bacteria, gram positive bacteria and *E.Coli* bacteria, gram-negative bacteria. The investigation was done by agar diffusion method in deionized water solution. The inoculums of all microorganisms were prepared from fresh overnight bath culture that was incubated at 37°C. The diffusion technique was carried out by pouring Agar solution about 3 to 4 mm thickness in a Petri dish and adding dense inoculum of the tested micro organism in order to maintain semi-confluent of growth. The Petri plates were left for 10 minutes to dry in air and the prepared powders in the form of a solution mixed with deionized water were arranged on the agar surface. It was incubated for 24 hours at 37°C and the reading was carried out by measuring the width of the zone of inhibition (mm). The results exhibit that the concentration of the sample has been directly proportional to the inhibition activity. *Specifically, E.Coli shows better activity when compared with S.aures bacteria.*
Table 3.1 In vitro antibacterial activity of nHAp/PVA/CMC nanocomposites

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>50 µl</th>
<th>100 µl</th>
<th>150 µl</th>
<th>Standard Chloromphenical for bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli (mm)</strong></td>
<td>1.50± 0.10</td>
<td>3.50± 0.24</td>
<td>5.70± 0.39</td>
<td>10.20± 0.71</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus (mm)</strong></td>
<td>1.20±0.08</td>
<td>3.10±0.21</td>
<td>5.30±0.37</td>
<td>10.00± 0.70</td>
</tr>
</tbody>
</table>

Fig 3.5 In vitro antibacterial activity of nHAp/PVA/CMC nanocomposites

3.6 In-vitro Anti-Inflammatory activity test

The reaction mixture (5mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2mL of varying concentrations of HAp/PVA/CMC which reveal final concentration of 100,200,300,400,500 µg/mL in double–distilled water. Then the mixture was incubated at 37°C in a BOD incubator for 10 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660nm by using double distilled water. The percentage inhibition of protein denaturation was calculated by using the following formula

\[
\% \ of \ inhibition = 100 \times \left( \frac{A_t}{A_C} - 1 \right)
\]

The results indicate in-vitro anti-inflammatory of HPC was equated against denaturation of egg albumin. The results summarized in table 1. The present findings exhibited a concentration-dependent inhibition of protein denaturation by HPC throughout
the concentration range of 100 to 500µg/mL. This was further confirmed by comparing their EC50 values HPC with is standard value of Diclofenac sodium.

Table 3.2 In vitro anti-inflammatory of nHAp/PVA/CMC nanocomposite

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>% of inhibition (HPC)</th>
<th>% of inhibition Standard (Diclofenac sodium)</th>
</tr>
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<tbody>
<tr>
<td>100µg/ml</td>
<td>11.84±0.82</td>
<td>21.03 ± 1.47</td>
</tr>
<tr>
<td>200µg/ml</td>
<td>21.05±1.47</td>
<td>32.54 ± 2.27</td>
</tr>
<tr>
<td>300µg/ml</td>
<td>42.10±2.94</td>
<td>51.65 ± 3.61</td>
</tr>
<tr>
<td>400µg/ml</td>
<td>63.15±4.42</td>
<td>62.35 ± 4.36</td>
</tr>
<tr>
<td>500µg/ml</td>
<td>77.63±5.43</td>
<td>76.25 ± 5.33</td>
</tr>
<tr>
<td>EC50</td>
<td>340.75</td>
<td>309.36</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Hydroxyapatite/poly (vinyl alcohol) and Carboxyl methyl cellulose were synthesized by the wet chemical method. The hydroxyapatite powder was characterized at macroscopic level by XRD, FTIR, TGA/DTA and in-vitro tests. FTIR investigations also showed all typical absorption characteristics of nHAp/PVA/CMC. The XRD analysis showed that the prepared nHAp/GG sample was amorphous nature. The TEM images confirm that the composites show rod-like morphology. The thermal behavior of hydroxyapatite was studied by TGA/DTA. Antibacterial activity results show that nHAp/PVA/CMC is active against gram-negative bacteria. Anti-inflammatory nature of the compound is observed at the highly concentration of the sample. The physical and chemical performance of synthesized composite materials meet at some extent the requirements of bone tissue engineering material. *In vivo* study will confirm the its compatibility with the bone tissue engineering materials. The nano sized HAp/PVA/CMC composite can be useful as pharmaceutical applications.
REFERENCES


