

# Synthesis and Characterization of Nano Hydroxyapatite with poly ethylene oxide nanocomposite for biomedical applications

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**Abstract:** The aim of this study is to prepare and characterization of hydroxyapatite with polymer nano composite. In this present study, we have developed wet chemical precipitation method for preparing nHAp with Polymer powder. It is well known that wet chemical precipitation method have several advantages for producing ceramic particularly high crystallinity, high purity, nano size and high reactivity. HAp with polymer is an important inorganic biomaterial which has attracted the attention of researchers related to biomaterial field in recent years. In this work, poly ethylene oxide with hydroxyapatite nanocomposite were analyzed and confirmed by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Transmission Electron Microscopy (TEM), thermal analysis of TG/DTA and the consequence of this investigation suggest that the nHAp with poly ethylene oxide can be used to antibacterial agent for developing new pharmaceutical to control studied human pathogenic bacteria responsible for severe illness.

**Keywords:** FTIR, TEM, TG/DTA, XRD, Antibacterial Activity.

## 1. Introduction

Hydroxyapatite (HAp) is the major component of bone and has long been used as orthopedic and dental materials [1, 2]. HAp is well known for its excellent biocompatibility, osteoconductive, slow degradation, non-cytotoxicity, non-inflammantry as well as non-immunogenic properties [3, 4]. HAp is synthetically obtained in the laboratory by several methods, such as hydrothermal process, sol-gel, wet chemical precipitation, among others and depending on the applications. The most common method to obtain HAp is the wet chemical precipitation method [5, 6]. Since large amounts of materials could be collected; it is inexpensively and easily reproducible. Hydroxyapatite with polymer nanocomposies has been used as a biocompatible and osteoconductive substitute for bone repair and implantation [7, 8]. As the inorganic component of hard tissue (HAp) has long been used in orthopedic surgery. However, HAp is difficult to shape because of it brittleness and lack of

flexibility. HAp powder can migrate from implanted sites, thus making them in appropriate for use. Moreover, these powders do not disperse well and agglomerate easily [9]. Therefore, the incorporation of HAp with polymeric nanocomposites to overcome processing and dispersion challenge of great interest to the biomedical community.

In the present study, we have attempted to prepare HAp with poly ethylene oxide (PEO) nanocomposite through a wet chemical precipitation method, which has the potential to provide much better dispersion of HAp nano particles onto the polymer, yielding a composite with a uniform microstructure. Poly ethylene oxide (PEO) has been chosen as the polymer, because the phosphate group of PEO can be act as coupling/anchoring agent, which has higher affinity toward the HAp nanoparticle [10]. Some synthetic polymer such as polyurethane, (PU) poly vinyl acetate (PVAc) and poly ethylene propylene (PVP) are employed for combination with HAp to apply in bone tissue engineering.

Therefore, many studies have been reported on combination of nHAp with biodegradable synthetic materials, such as poly oxymethylene, poly orthoester, and poly dioxanone and poly anhydrides. Among the available biodegradable synthetic polymer – based composites have been focused with more attention than other synthetic polymer composite for bone tissue engineering applications [11].

## 2. Materials and Methods

### 2.1 Materials

The entire chemical was synthesized through a high frequency microwave accelerated with wet chemical precipitation method. The raw materials required to start the processing of the composite were: analytical grade calcium hydroxide, ammonium dihydrogen phosphate were purchased from Sigma Aldrich and poly ethylene oxide was procured from Alfa Aesar. Ethanol and doubly distilled water were used as the solvent.

### 2.2 Methods

#### 2.2.1. Synthesis of HAp with PEO nano composites

The First calcium hydroxide was slowly added to a solution of ammonium dihydrogen phosphate and after proper mixing, the HAp was precipitated by adding ammonia and pH of the solution was maintained from 9 to 11. The solution was constantly stirred for 24h by magnetic stirrer, allowing the reaction to complete. The resultant precipitate was separated and ammonia was removed by repeated washing. The precipitate was allowed to dry in an oven at 90 °C subsequently; aggregates formed were crushed into fine powder. In a separate study as - synthesized HAp powder (80) was mixed with polymer

solution (20) where number denotes the wt% and the resultant HAp – PEO nano composite powder was thoroughly mixed using a mortar and pestle for 30 min.

## 3. Results and Discussion

### 3.1. FTIR

The functional groups present in the prepared nHAp with PEO powder. The FTIR spectra of pure nHAp/PEO nanocomposites are shown in fig.1. The FTIR spectrum investigation was carried out using Perkin Elmer spectrometer in the range of 400 – 4000  $\text{cm}^{-1}$ . The bond observed at 3427.97  $\text{cm}^{-1}$  (kannan et al., 2006) the –OH group. The band at 2915.62  $\text{cm}^{-1}$  and 2374.21  $\text{cm}^{-1}$  are assigned to the hydroxyl group present in poly ethylene oxide (PEO). The characteristic bands of PEO absorption are observed at 1599.10, 1454.05 and 876.41  $\text{cm}^{-1}$ . The band located at 1034.82  $\text{cm}^{-1}$  is attributed to the  $\text{PO}_4^{3-}$  group. The strong peaks at 1407.34  $\text{cm}^{-1}$  corresponds to the stretching vibration of  $\text{CO}_2^{3-}$ . The observed bands a 602.44  $\text{cm}^{-1}$  are due to phosphate stretching mode is appeared at 565.54  $\text{cm}^{-1}$  corresponds to  $\text{PO}_4^{3-}$  groups.

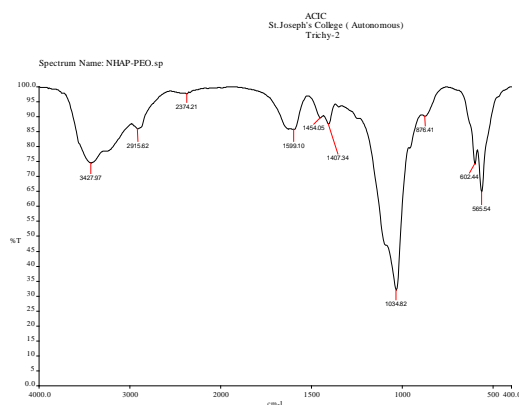


Fig.1. FTIR spectrum of nHAp with poly ethylene oxide.

### 3.2. XRD

Fig.2. shows that the collected XRD data was perfectly matched with standard JCPDS no: 09-432. Crystallite size and crystalline of HAp/PEO were investigated with powder XRD spectrum and broaden diffraction peaks are  $2\theta = (26.5^\circ), (29.0^\circ), (33.5^\circ)$  and  $(40.0^\circ)$  these peaks are assigned to the miller's indices reflection planes are (002), (210), (112), and (212) with the XRD pattern of complete polymer (PEO) showed four characteristic reflection planes. These plane (101), (111), (210) and (004) indicates that the amorphous phase HAp / PEO and crystallite size is very small from 50 nm. This XRD spectrum indicates most of the high intensity peaks positioned between  $2\theta = 25 - 34^\circ$  and we observed that peak broadening was indicated synthesized HAp/PEO crystallite size present in nano scale level.

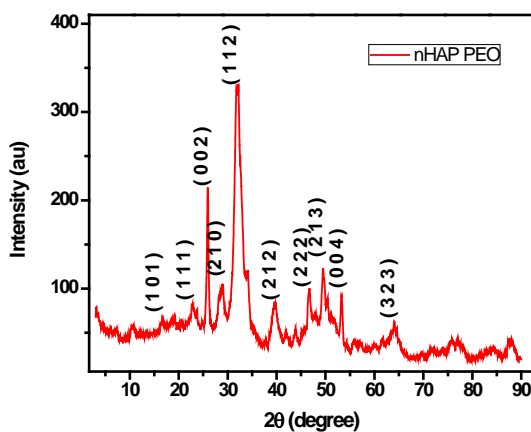
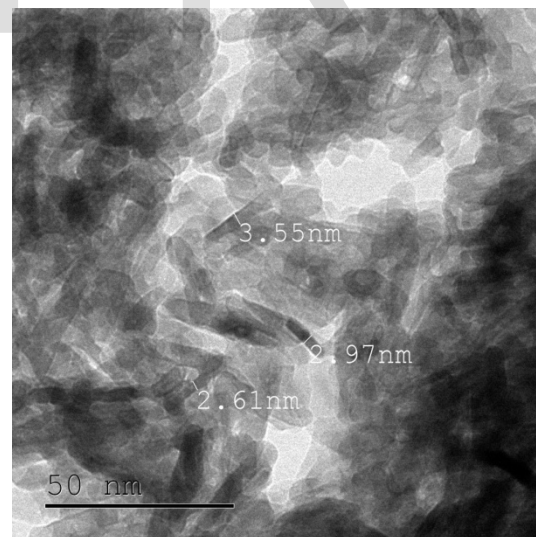


Fig.2. XRD spectrum of nHAp with poly ethylene oxide.

### 3.3. TEM

The structure and morphology of the samples were further confirmed by the TEM images (fig.3) of the prepared nano hydroxyapatite with poly ethylene oxide. In order to confirm the XRD results concerning the presence of HAp crystallites in the composite samples, TEM measurements were performed. The TEM micrographs, revealed the structural features of the composites. The nHAp with poly ethylene oxide of the sample (Fig.3) reveal the existence of nano crystallites ranging 50 nm length and 2.61 nm to 3.55 nm width, while TEM image associated these nano crystallites with the presence of HAp, through Miller indices (hkl) confirming the XRD results. The particles size is also found to be in agreement with previous report Ferraz et.al. (2004). In addition the selected area electron diffraction (SAED) of the precipitates shows diffraction ring of patterns, which implies that the precipitates are crystalline nature. This is agreed with XRD results.



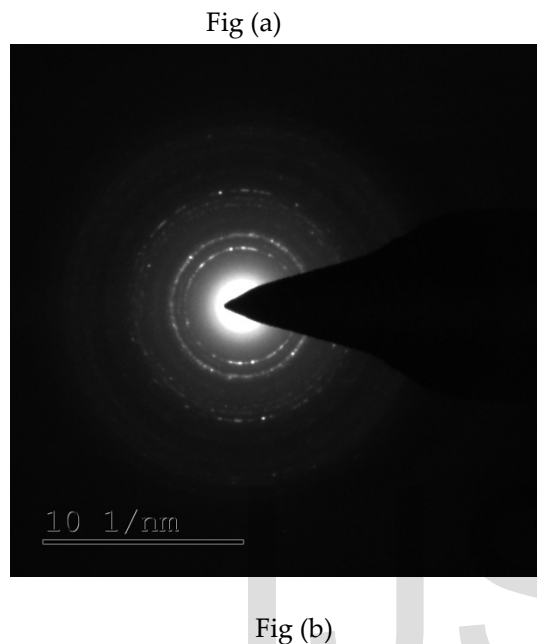


Fig.3. (a) is TEM image and (b) is Selected Area Electron Diffraction (SAED) image of nHAp/Poly ethylene oxide.

### 3.4 TGA/DTA

TGA/DTA studies have been carried out on freeze dried HAp/PEO nanocomposite sample. Experiments have been performed using simultaneous TGA/DTA analysis by heating the sample at 25° C/Min in the temperature range 30° C to 800 ° C in air atmosphere. First order derivative of TGA curved reveals the temperature at which the maximum decrease of mass occur. The temperature at the maximum loss rate is 70°C for the 7.66% HAp/PEO composite, 250°C for the 6.15% HAp/PEO composites and 340°C for the 9.90% HAp/PEO composites. Almost stable

curve was noticed with in this temperature range, which indicates thermal stability of HAp powder. This supported by the endothermic peaks observed at 317°C in DTA curve. Further, there was a weight loss indicated by the two endothermic peaks at 287°C and 348°C respectively. The transitions may be due to the polymer to HAp interaction.

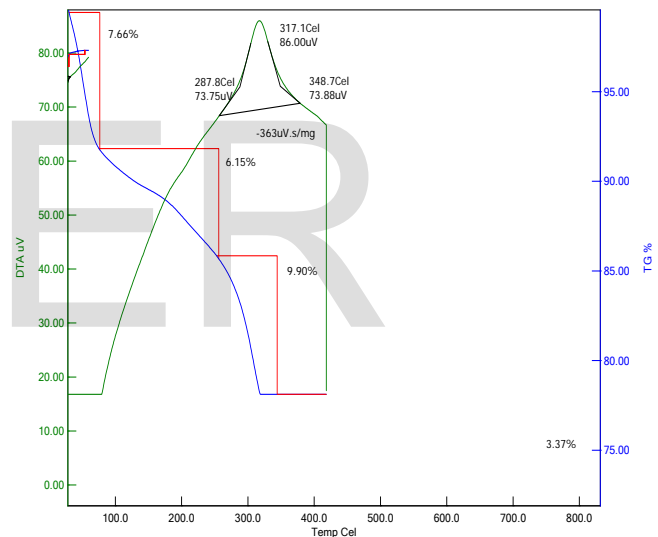


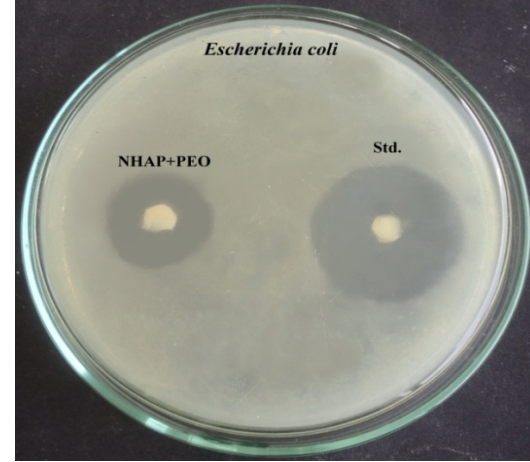
Fig.4. TG-DTA analysis of synthesized nHAp/Poly ethylene oxide.

### 3.5 Antibacterial Activity

The antibacterial activity test were performed against the bacterial Escherichia coli

(E. Coli) and streptococcus pyogenes (Str. pyogenes). Fig. 5 show the photographs of the antibacterial activity of Hap with polymer nanocomposites. The inhibition zones with diameter of a few millimeters around each well with Hap with polymer composite is used to study the activity. The highest antibacterial activity was observed against Str. Pyogenes and E.Coli. Str.pyogenes has a thick cell wall and E.Coli has a relatively thin cell wall. The cell wall attributed differ between gram positive (Str. pyogenes) and gram negative (E. Coli). Additionally cell – wall properties, like cell permeability and capability of solubilizing Hap with polymer entire bacteria might also be considered. Finally, the antibacterial activity of the Hap with polymer nanoparticles compared to different bacterial species, is listed in table. It can be seen in table that gram positive (Str. pyogenes) and gram negative (E. Coli) due to differences in their membrane structure. The gram positive bacteria have more peptidoglycan than gram negative bacteria because of their thicker cell wall and because peptidoglycan are negatively charged and HAp with polymer nanoparticles are positively charged. Hence more Ca ions may get trapped by peptidoglycan in gram positive bacteria than in gram negative bacteria. The results in table also show that the gram negative bacteria is slightly more resistant than gram positive bacteria to the action of HAp with polymer nanoparticles.

*Escherichia coli*



*streptococcus pyogenes*

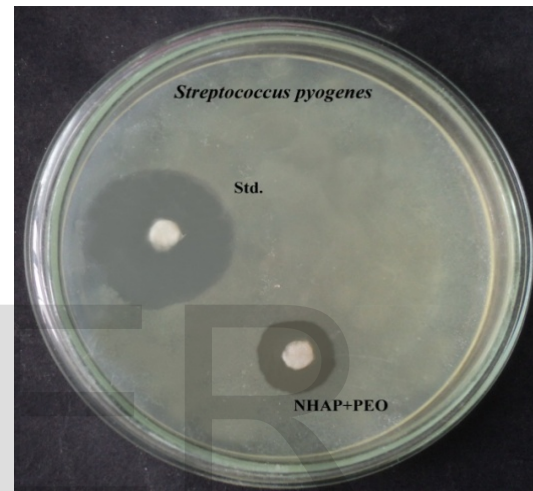


Fig. 5. Antibacterial activity of nHAp with Poly ethylene oxide.

Microorganisms	NHAp + PEO (mm)	Standard (Chloromphenical For bacteria)
Escherichia Coli	7±0.49	14 ± 0.98

<b>Streptococcus Pyogenes</b>	<b>6±0.36</b>	<b>13 ± 0.91</b>

**Table for Antibacterial activity**

#### 4. Conclusion

The above presented work used the wet chemical precipitation method due to high reproducibility and simplicity. One of the main advantages of the method is that the water is its only byproduct. This process show that high purity of nano HAp with polymer powder could be obtained at room temperature. The size and morphology of the sample were characterized using transmission electron microscopy with the particle size 50 nm. The formation of HAp with polymer nanocomposites was confirmed by X-ray diffraction and Fourier transform infrared spectroscopy (FT-IR).The thermo gravimetric analysis and Differential thermal analysis (TGA/DTA) measurements were carried out for thermal properties of prepared samples. Antibacterial activity results show that HAp with poly ethylene oxide is active against most common gram positive and gram negative bacteria making it useful for medical application and environmental field. Therefore it can be concluded that HAp based polymer materials are certainly among the most promising challenges in bioactive ceramic for the near future and consequently the research effort put in their development will continue to increase.

#### Acknowledgements

Authors thanks to the management of Urumu Dhanalakshmi College, Tiruchirappalli for providing research facilities in the campus.

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