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Research Article

SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIBACTERIAL APPLICATIONS OF CALCIUM NANOPARTICLES

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ABSTRACT

Inorganic nanoparticles for biomedical applications have undergone extensive investigations in recent years. Among different inorganic drug carriers, calcium carbonate ($CaCO_3$) nanoparticles show unique advantages due to their ideal biocompatibility and the potential as delivery system for loading different categories of drugs. Calcium derivatives have great potential to be applied in bone and teeth related disorders due to their ideal biocompability with the natural bone and teeth structures and the biodegradability as well. Calcium carbonate and calcium hydrogen phosphate nanoparticles are synthesized by wet chemical precipitation method. X-ray diffraction studies are made to determine the structural properties. The optical behavior of both calcium nanoparticles are studied by UV characterization. The band gaps of both the samples are also found. The antibacterial activity was tested on Gram negative and Gram positive bacterial strains.

Keywords: Calcium carbonate; Calcium Hydrogen phosphate; Antibacterial activity

1. INTRODUCTION

Calcium derivatives are the most important natural constituents of bone and teeth. In fact, the primary tissues of bone, osseous tissue is mostly made up of a composite material consist of the inorganic calcium derivatives [1]. Furthermore, tooth enamel is one of the four tissues that compose the tooth which contains the highest percentage of Calcium compound [2]. Therefore, different calcium derivatives have shown a great potentials to be applied in bone and teeth related disorders due to their ideal biocompability with the natural bone and teeth structures and the biodegradability as well [3,4]. Filling dental caries, treatment of early dental caries lesions and generating neo-formed bone tissue using by different types of calcium derivatives has also shown a potential applications.[5,6] One of the most common calcium derivatives with long history of applications in various fields is $CaCO_3$. It has been used in plastics, paint, paper, inks, food as well as pharmaceutical industries applications [4, 7, 8]. Medical applications of $CaCO_3$ in modern health care systems have mostly attracted the attention of researches due to its great potentials and capabilities. This material is low cost, safe, accessible and biocompatible [9-12].

This paper presents the preparation, structural, optical characterization and antibacterial applications of calcium carbonate and calcium hydrogen phosphate nanoparticles.

2. SYNTHESIS AND CHARACTERIZATION

2.1. Synthesis of Calcium carbonate nanoparticles

Calcium carbonate is synthesized from egg shell. Bulk amount of hen egg shells are collected. Then the egg shell is dried at room temperature. The egg shells are slightly crushed and taken in a crucible and kept in the muffle furnace at 900°C for 2 hours. Then it is grained into fine powder. Thus Calcium carbonate nanoparticles are synthesized.

2.2. Synthesis of Calcium Hydrogen phosphate nanoparticles

Calcium Hydrogen phosphate nanoparticles are prepared by wet chemical precipitation method. The Calcium carbonate powder which is prepared already is used for the synthesis of $Ca(HPO_4)$. 1.5g of Calcium carbonate powder is mixed with 50ml of distilled water and is stirred for 30 minutes using magnetic stirrer. 1.097ml of orthophosphoric acid is added to 50ml of distilled water. The orthophosphoric acid solution is added drop by drop to the calcium carbonate solution and stirred steadily for two hours. Then it is washed 3 to 4 times using distilled

water. Then the sample is dried by applying heat to remove moisture. Thus Calcium Hydrogen Phosphate nanoparticles are obtained.

2.3. Characterisation

The structural characterization of Calcium Carbonate and Calcium Hydrogen Phosphate nanoparticles were carried out by powder X-ray diffractometer system with XPERT-PRO software. The intensity of the diffracted beam against 2θ is recorded in the range $10\text{-}80^0$ with Cuk_α radiation (λ=1.54056Å) and step size 0.0500.

The grain sizes for the calcium carbonate nanoparticles were calculated using Debye Scherer's equation

$$D=0.9\lambda / \beta \cos\theta \quad (1)$$

Where, λ is the X-ray wavelength used (1.54060Å), β is the full width half maximum(FWHM). The dislocation density is found out using the formula

$$\delta = 1/D^2 \ lines/m^2 \quad (2)$$

The UV visible spectrum of the synthesized Calcium nanoparticles have been investigated by the UV-Vis spectrometer EVOLUTION 220 in DRS mode with a wavelength range 190-1100nm. Energy band gap of the nanoparticle is calculated by the formula

$$E_g=h\nu = hc/\lambda \quad (3)$$

The antibacterial activity was tested on Gram negative (Escherichia coli and Pseudomonas aeruginosa) and Gram positive (Enterococcus feacalis and Bacillus subtilis) bacterial strains. The qualitative assessment of the antibacterial effect was done using agar disc diffusion (KirbyBauer) method. The contact biocidal property can be determined by measuring the diameter of the zone of inhibition (ZOI) around the well. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres.

3. RESULT & DISCUSSION

The powder sample of $CaCO_3$ nanoparticles was characterized by powder XRD analysis is shown in Fig. 1. The diffraction data are in good agreement with the JCPDS file No:85-1108. From the JCPDS file it is confirmed that the prepared nanoparticles are Calcium Carbonate belongs to Rhombohedral structure with a = 4.980Å, b =4.980Å, c = 17.018Å, $\alpha = \beta \neq \gamma = 90^0$ and Volume =365.57Å^3. The grain size and dislocation density for calcium hydrogen phosphate nanoparticles is 53.9934 nm and 0.34301×10^{15} lines/m^2, respectively.

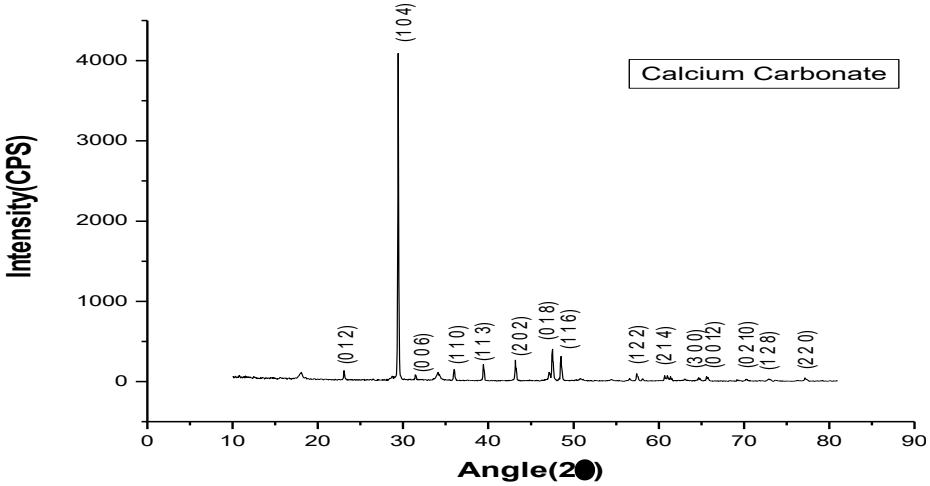

Fig. 1: XRD pattern of Calcium Carbonate

The X-ray powder diffraction patterns of $Ca(HPO_4)$ is show in Fig 2. The diffraction datas are in good agreement with the JCPDS file No: 89-5969. From the JCPDS file it is confirmed that Calcium Hydrogen Phosphate nanoparticles are formed, belongs to Triclinic structure with a = 6.9Å, b =7.0Å, c = 6.650Å, $\alpha \neq \beta \neq \gamma = 90^0$ and Volume =309.84Å^3. The grain size and dislocation density for calcium hydrogen phosphate nanoparticles is 53.9837 nm and 0.34314×10^{15} lines/m^2, respectively.

The transmittance and absorbance spectra of Calcium Carbonate nanoparticle is shown in Fig. 3 and Fig. 4 respectively. It was observed that the Calcium Carbonate nanoparticles have good transmittance and the lower cut off wavelength is found to be 320 nm and the maximum absorbance is 0.7 %.

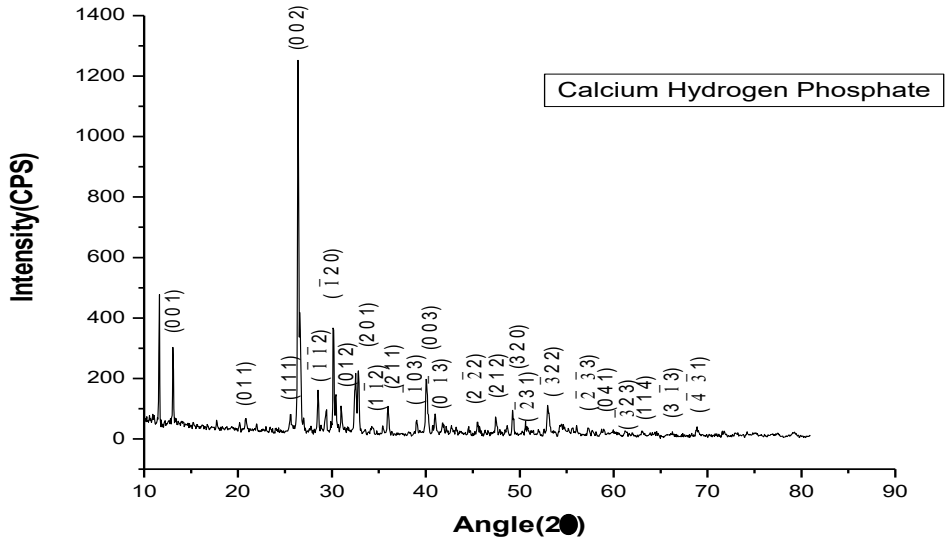

Fig. 2: XRD pattern of Calcium Hydrogen Phosphate.

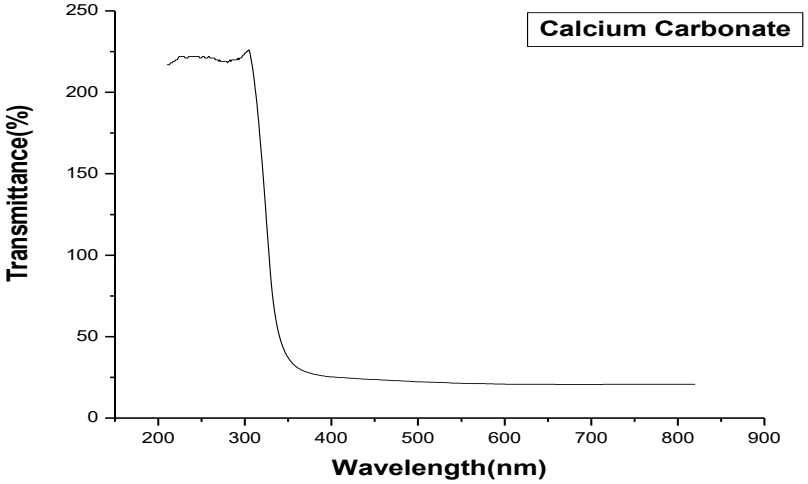

Fig. 3: Optical transmittance spectra for Calcium Carbonate nanoparticles

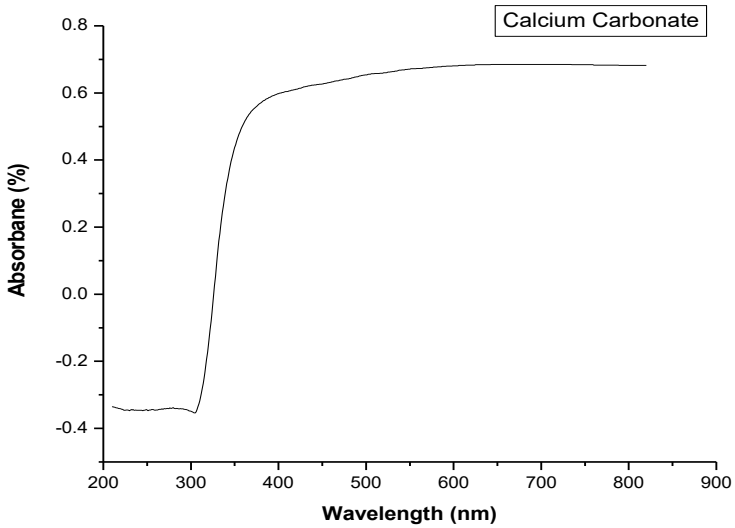

Fig. 4: Optical absorbance spectra for Calcium Carbonate nanoparticles

The transmittance and absorbance spectra of Calcium Hydrogen Phosphate nanoparticle is shown in Fig.5 and Fig.6 respectively. It was observed that the Calcium hydrogen phosphate have good transmittance and the lower cut off wavelength is found to be 325 nm and the maximum absorbance is 0.35%.

The optical energy band gap of $CaCO_3$ and Ca (HPO_4) is found to be 3.87 eV and 3.82 eV respectively. The diameters of the ZOI are determined and these are tabulated in Table 1. The absence of growth around the nanoparticles is an indirect measure of the ability of the material to inhibit the growth.

Table1: Diameters of the ZOI produced by the samples against the microbes

Synthesized Nanoparticles	Zone of inhibition(mm)			
	Enterococcus feacalis	*Bacillus subtilis*	*Escherichia Coli*	*Pseudomonas aeruginosa*
$CaCO_3$	21	20	19	21
Ca(HPO_4)	12	11	17	11
Positive Control	17	25	20	23

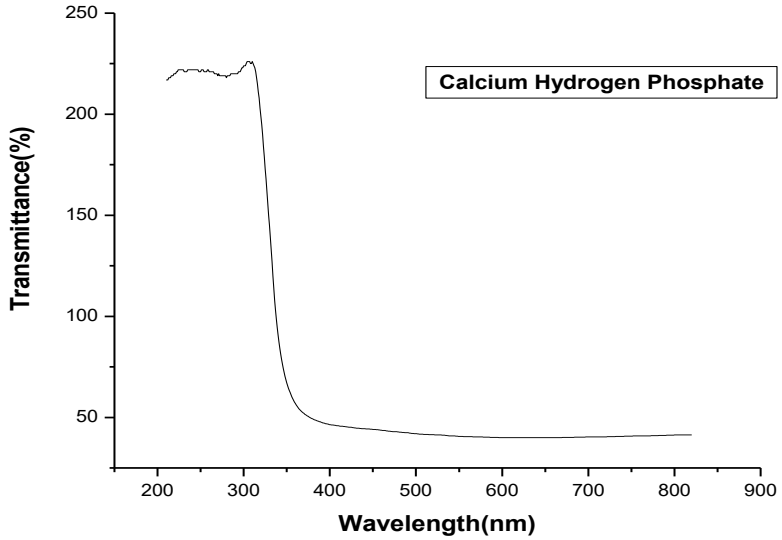

Fig. 5: Optical transmittance spectra for Ca (HPO_4) nanoparticle

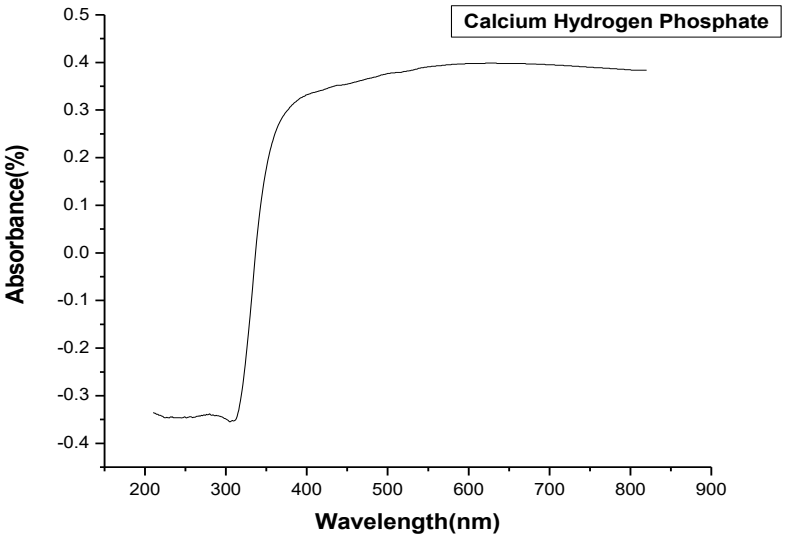

Fig. 6: Optical absorbance spectra for Ca (HPO_4) nanoparticle

4. CONCLUSION

In the present investigation Calcium nanoparticles are synthesized from egg shell by wet chemical precipitation method. $CaCO_3$ is prepared initially from eggshell and then $Ca(HPO_3)$ nanoparticle is prepared by adding orthophosphoric acid by wet chemical precipitation method. From XRD studies, it reveals that Calcium carbonate has rhombohedral structure and Calcium Hydrogen Phosphate has triclinic structure. The grain size and dislocation density of both the nanoparticles are also investigated from the powder XRD characterization. The optical properties are studied using UV studies. The antibacterial action of Calcium nanoparticles was studied and the inhibition zones were examined around the disc is measured with transparent ruler in millimeters. It has been found that the calcium nanoparticles are highly antibacterial on gram positive bacteria's.

5. REFERENCES

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