J. Indian Chem. Soc.,
Vol. 96, January 2019, pp. 52-55

# Physico-chemical characterization and antimicrobial efficiency of beta-cyclodextrin/ hydroxyapatite composite

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*Manuscript received online 27 August 2018, accepted 09 October 2018*

β-CDs are biocompatible, biodegradable and non-toxic materials and the central empty cavity of CDs (host) is capable of loading hydrophobic molecules (guest) through Van der Waals force and hydrogen bonds. Because of this unique structure, the physicochemical properties of the guest molecule, such as poor solubility, instability and undesired side effects can be masked. β-Cyclodextrins (CDs) are cone-shaped α-1,4-linked macrocyclic oligosaccharides with a hydrophilic exterior and a hydrophobic inner cavity that allow the formation of inclusion complexes with hydrophobic compounds. For enhancing the biological or mechanical properties of cyclodextrins incorporating it with other polymers like chitosan, gelatin and pectin has been widely investigated. To boost mechanical properties and bioactivity activities, a number of bioactive inorganic minerals and hydrophilic biopolymers have been developed for various applications. Hydroxyapatite (HAp) is one of the most extensively employed calcium phosphates owing to its similarity to the main mineral constituent of bone tissue. The bio-adaptability and versatility of β-CD and HAp makes them capable of alleviating the undesirable properties of various areas like adsorption, drug delivery and bone tissue engineering through the formation of inclusion complexes. The prepared composite has been characterised by using FT-IR, XRD, TGA and DSC analytical techniques and also the effect of β-CD/HAp composite towards bacterial species like *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* and fungal species like *Aspergillus niger, Aspergillus flavus* and *Mucor mucedo*. Using characterisation techniques it was concluded that intermolecular interactions between the two components and chemical compositions of the prepared material. The enhanced antimicrobial activity of the composite may thus provide an opportunities for potential use as an alternative biomaterial for bone tissue engineering applications.

Keywords: Cyclodextrins, bioactive mineral, hydroxyapatite, characterisation, bacterial species fungal species.

## Introduction

Polymer composites are excellent materials for electronic and biomedical applications. Because of high stiffness-to-weight and strength-to-weight ratios, polymer based composite materials are being used in different fields of science and technology[1,2].

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and antimicrobial susceptible tests. Nowadays, discovery of new antibiotics is an exclusively important objective because of the exciting antibiotics are in danger of losing their efficacy due to the increase in microbial resistance[3,4].

Among the biomacromolecules, cyclodextrins (β-CDs) are valuable multifunctional tools that have been extensively used in pharmaceutical industry. Cyclodextrins are oligosaccharides composed of 6,7,8 or 9-glucopyranose units (α-, β-, γ- or δ-CD, respectively), with a relative hydrophilic surface and

a hydrophobic central cavity[5,6]. The cyclodextrin inclusion complex formation has been successfully applied to enhance the chemical stability, solubility and bioavailability of poorly soluble compounds[7,8]. Like compounds possess low solubility, hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is the main mineral component of biological hard tissues (such as bone and teeth) and constitutes about 70% by weight of the human bone[9]. Bacterial infection is one of the most serious complications of implant surgery, and it always leads to severe physiological damage and additional costly surgical procedures[10–12].

*Materials and methods:*

*Materials:*

β-Cyclodextrin (β-CD) and hydroxyapatite (HAp) were purchased from MPM Scientific Chemicals India Private Ltd. All chemicals were of reagent grade and were used as received, and distilled water was used in all solutions and reagents throughout the experiment.

*Methods:*

*Preparation of β-cyclodextrin and hydroxyapatite (β-CD-HAp) composite:*

About 0.5 g of β-cyclodextrin was dissolved in hot water and kept aside. Then, 0.5 g of hydroxyapatite was weighed and dispersed in minimum amount of distilled water. This dispersed HAp was slowly added to β-cyclodextrin in hot water with constant stirring. The mixture was agitated at room temperature using ultrasonicator for 45 min. The homogenous solution was poured into petridish and kept for air drying.

*Characterization:*

The FT-IR spectra of β-CD/HAp composite was recorded by using the Shimadzu FT-IR Spectrophotometer in the wavelength range of 400–4000 $cm^{-1}$. The X-ray diffraction pattern of the prepared sample was tested by an X-ray scattering D8 ADVANCE Diffractometer using Ni filter Cu Kα radiation source (λ = 0.154 nm), set at scan rate = 10°C/min, using a voltage of 30 kV and a current of 40 mA. The TGA study of the prepared samples was carried out using SDT Q600 V8.0 Build 95 instrument at a heating rate of 10°C per minute in nitrogen atmosphere to analyze the weight losses at different stages. The differential scanning calorimeter (DSC) was used to examine the thermal property of the composite. The measurements were performed with NETZSCH DSC 200 PC in a pan Al, pierced lid in the $N_2$ atmosphere at a heating rate of 10°C/min.

*Antimicrobial activity (in vitro):*

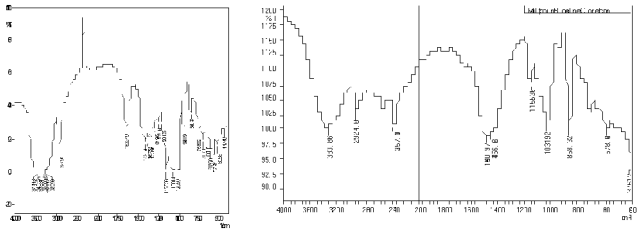
The antimicrobial activities of β-CD/HAp composite were screened against bacterial strains such as *Staphylococcus aureus*, *Klebsiella pneumonia* and *E. coli* and three fungal strains such as *Aspergillus flavus, Aspergillus niger* and *Mucor.* The studies were carried out by Muller Hilton agar for bacterial strains and Sabouraud dextrose agar for the fungal strains. Stock solution of the composite were prepared by dissolving 1 mg/mL of the composite in DMSO and tested against microbial species using ampicillin as positive control and polymyxin B sulphate as antifungal drug. Finally the bacterial strains were incubated at 37°C for 24 h, whereas the fungal strains were incubated at room temperature for 48 h.

## Results and discussion

*FT-IR spectrometry:*

The FT-IR spectral details of pure β-CD and β-CD/HAp composite was represented in Fig. 1a and Fig. 1b. The FT-IR spectra shows all the characteristic bands of functional groups present in the pure β-CD and β-CD/HAp composite. The absorption peaks observed at 3444.87 $cm^{-1}$ and 2927.24 $cm^{-1}$ in case of pure β-CD (Fig. 1a) corresponds to the intermolecular hydrogen bonded -OH stretching frequency of hydroxyl group[13] and aliphatic methylenic-CH stretching. The sharp peak observed at wavenumber such as 1639.49 $cm^{-1}$ corresponds to the presence of C=O stretching, the absorption bands at 1155.36 $cm^{-1}$ and 1029.99 $cm^{-1}$ revealed the presence of C-O-C stretching and O-H bending vibrations respectively.

The FT-IR spectrum of the β-CD/HAp composite has shown in the Fig. 1b. The figure shows that the broad peak appeared in the range of 3363 $cm^{-1}$ may be due to the overlapping of OH stretching band of HAp with the OH stretching band of β-CD. The shifting of bands to lower values indicates the strong interaction between β-CD and HA during ultrasonication and also with composite formation. The appearance of new bands in the range of 2357 $cm^{-1}$ and 2924 $cm^{-1}$ in case of β-CD/HAp composite may be attributed to the coordinative interactions between -OH of β-CD and $Ca^{2+}$ of HAp that becomes the basis of nucleation and growth point of apatite crystals[14]. Furthermore, the less intense peaks observed at about 1490 $cm^{-1}$ and 1456 $cm^{-1}$ should be attributed to the absorption bands of $CO_3^{2-}$, indicating the pres-



(a) (b)

**Fig. 1.** FT-IR spectrum of (a) pure β-cyclodextrin and (b) β-CD/HAp composite.

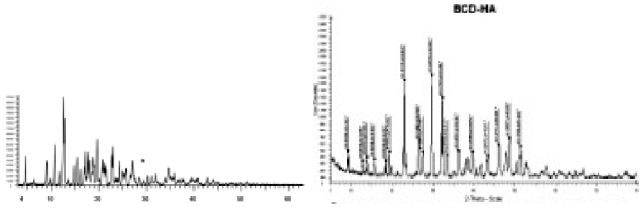
ence of carbonate ions. The intense peaks located at 1031 $cm^{-1}$ and 578 $cm^{-1}$ in case of β-CD/HAp composite can be attributed to the $PO_4^{3-}$. Then, intensive absorption band in 858 $cm^{-1}$ corresponds to a characteristic band to $HPO_4^{2-}$. The appearance of these new peaks in case of β-CD/HAp composite confirms that the strong interaction had taken place effectively between β-CD and HAp.

*XRD studies:*

The XRD diffractogram of pure β-CD and β-CD/HA composite has shown in Fig. 2a and Fig. 2b. The spectrum of pure β-cyclodextrin indicates high crystallinity in cyclodextrin due to the characteristic sharp peaks at (041), (141), (180), (162), (223) and (044) corresponds to the 2θ values at 13.1°, 15.8°, 17.9°, 20.9°, 22.8° and 35.9° respectively[15,16]. The characteristic peaks at 2θ = 26.12° and 32.132° were those expected well from HAp structure which indicate the high crystallinity of the HAp and there is no impurity phase. In Fig. 2b, XRD diffractogram of the β-CD/HA composite exhibits appearance of new and intense peaks at 26.5°, 27.4° and 29.6°, which confirms that the interaction between HA and β-CD is of coordinative nature involving the -OH of β-CD and the $Ca^{2+}$ of the HA[17], the overall number of crystalline structures is reduced and slightly increases amorphous nature of the composite.

*Thermal studies:*

The prepared β-CD/HA composite was subjected to TGA and DSC analysis. TGA thermogram of prepared β-CD/HA




(a) (b)

**Fig. 2.** XRD spectrum of (a) pure β-CD and (b) β-CD/HAp composite.

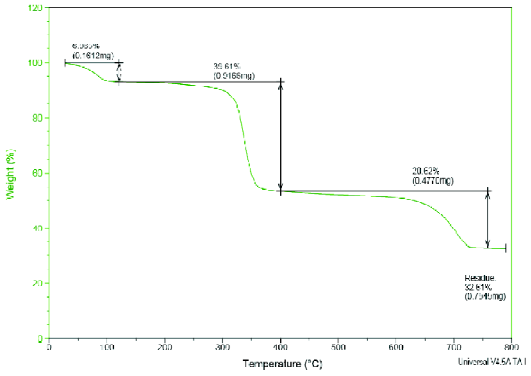



**Fig. 3.** TGA thermogram of β-CD/HAp composite.

composite Fig. 3 showed the various stages of decomposition. The weight loss occurred below 100°C can be assigned for desorption of adsorbed water molecules and weight loss occurred at this temperature is very less (6.9%), in that stage decomposition of the composite has taken place. The TGA graph of β-CD/HAp composite involves two step weight loss in the range of 300–400°C and 600-700°C which can be due to the dehydration reaction of -OH groups in HAp molecule with total weight loss of 68% at the end of the experiment, which indicated that HAp raised the thermal stability of β-CD reflecting the interaction between β-CD and HAp[18]. In the DSC curve of β-CD/HAp composite a single endothermic (Tc) and exothermic (Tm) peak was observed in both the curves. The presence of endothermic peak around 100.7°C due to the presence of trace amounts of water in the sample and also the melting temperature (Tm) of HAp was observed at 332°C. Pure β-CD has lower glass transition temperature and the glass transition temperature of the composite is 213°C which attributes mainly due to the composite formation[19].

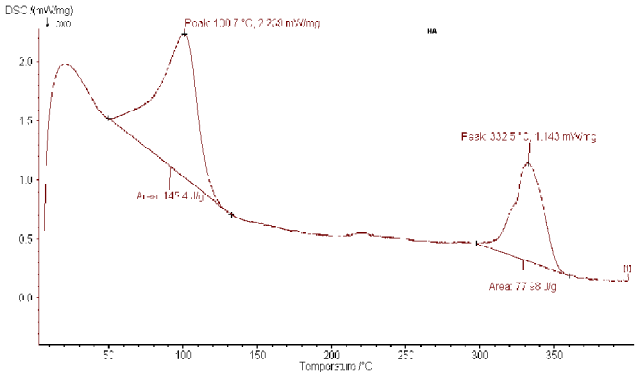



**Fig. 4.** DSC curve of β-CD/HAp composite.

*Antimicrobial studies:*

The antibacterial activity of the prepared β-CD/HAp composite were tested against Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Klebsiella pneumonia* and *E. coli* and also antifungal activity against *Aspergillus flavus, Aspergillus niger* and *Mucor.* The zone of inhibition values of the prepared β-CD/HAp composite against the growth of the selected microbial species are measured in mm. The results of screening of antimicrobial activities of the composite is represented in Fig. 5 and Fig. 6. The prepared β-CD/HAp composite shows good antibacterial activity[20] against *E. coli, Staphylococcus aureus* and *Klebsiella pneumonia* with the zone of inhibition in diameter 49 mm, 47 mm and 44 mm and also antifungal activity[21] against *Aspergillus flavus, Aspergillus niger* and *Mucor* with the zone of inhibition 55 mm, 34 mm and 47 mm.

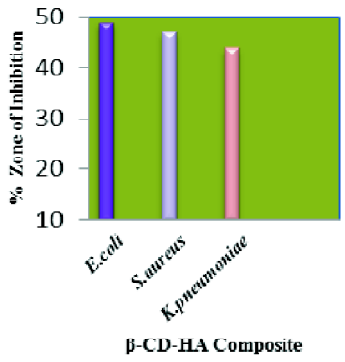



**Fig. 5.** Growth inhibition (%) of selected bacterial strains.

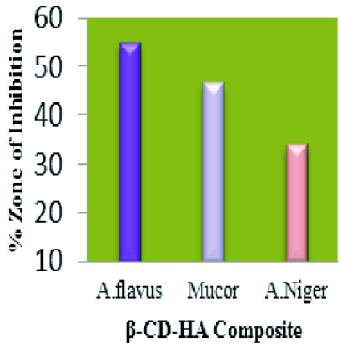



**Fig. 6.** Growth inhibition (%) of selected fungal strains.

## Conclusions

The preparation of β-CD/HAp composite was carried out successfully by using Ultrasonication method. The FT-IR and XRD results evidenced that intermolecular interaction and high crystallinity of the composite. TGA and DSC studies confirmed that the prepared composite was thermally stable with very high decomposition temperature. The biological *in vitro* assays revealed that the prepared composite would be highly suitable for biomedical and tissue engineering applications.

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