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Synthesis, Spectroscopic Studies and Biological Activities of Iron (II), Cobalt (II) and Zinc (II) Schiff Base Complexes

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ABSTRACT

Schiff base complexes have attracted wide attention due to their important role in analytical chemistry, organic synthesis, metallurgy, refining of metals, electroplating and photography. It has been proved that azomethine linkage (C=N) of Schiff base provides the opportunity for the stupendous biological activities such as antitumor, antibacterial, antifungal and herbicidal activities. In the present study Schiff bases of Carboxymethyl Chitosan and 4-hydroxy benzaldehyde Schiff bases were prepared and characterized by FTIR, XRD, TGA and DSC studies. The results show the formation of Schiff bases with the amine of carboxymethyl chitosan and 4-hydroxy benzaldehyde. The Iron (II), Cobalt(II) and Zinc (II) metal complexes were prepared by sol gel method. The complexes were again characterized by FTIR, XRD, TGA and DSC studies. The antioxidant activity of the complexes was studied by DPPH studies. The cytotoxicity of the complexes towards HeLa cell lines was also studied. From the results it can be concluded that the complexes are less toxic and have good antioxidant activity. The results are discussed.

Keywords: Antioxidant, Carboxymethyl Chitosan, Cytotoxic studies, 4-Hydroxy benzaldehyde, Iron, Cobalt, Zinc schiff bases.

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Website: http://heb-nic.in/cass-studies	
Received on 03/03/2020	
Accepted on 11/03/2020 © HEB All rights reserved	

INTRODUCTION

Wide ranges of naturally occurring polymers that are derived from renewable resources are available for various applications (Charles et al., 1983; Fuller et al., 1996; Kaplan, 1998; Scholz and Gross, 2000; Gross and Scholz, 2001). The polymers such as polysaccharides, proteins and nucleic acids etc. control various life processes in plants and animals. In that, an amino polysaccharide, chitosan acts a crucial role in various applications.

Schiff base ligands are considered as “privileged ligands” containing azomethine group (-HC=N-). They are formed by condensation of a primary amine and carbonyl compound. Chitosan anchored Schiff base complexes have been amongst the most widely studied coordination compounds in the past few years, since they are becoming increasingly important as biochemical, antimicrobial and catalytic reagents. There are some reports available with transition metal complexes obtained from Schiff base modified chitosan (Liu et al., 2007; Wang et al., 2009). The affinity of chitosan and its derivatives to metal ions, such as Cu, Cd, Pb, Ni, Co and Ca has extending their applications in various fields.

As reported in many investigations, carboxymethyl chitosan (CMC) has several desirable characteristics. For instance, CMC has good ability to form films, fibers, and hydrogels (Wongpanit et al., 2005). Moreover, CMC exhibit low toxicity, biocompatibility, biodegradable, antibacterial property and apoptosis inhibitory activity (Tokura et al., 1996; Hjerde et al., 1997; Seyfarth et al., 2008). On considering the properties of CMC, in the present work the Schiff bases and its Cu and Ni complexes were prepared using CMC and 4-hydroxy benzaldehyde. The Schiff base metal complexes have promising applications in the biomedical field.

The antimicrobial properties of the complexes have been recognized for centuries and have initiated the most fundamental breakthrough in medicinal history. Schiff bases characterized by the -N=CH- (imine) groups are active against a wide range of organisms, including bacteria, fungi, and even algae (Rehman *et al.*, 2004; Gu *et al.*, 2007; Slavica *et al.*, 2010; Varghese *et al.*, 2010). Complexes of transition metal ions with various ligands have been proved to exhibit antimicrobial activity against a spectrum of microbes and also they have been shown to possess toxicity against a number of cell lines of human and rodents in cell culture (Lakshmi et al., 2009).

This study includes an efficient method to synthesize the Schiff base and Schiff base metal complexes by the reaction of Carboxymethyl Chitosan and 4-hydroxy benzaldehyde under sol gel method. Schiff bases and its complexes were by FTIR, XRD, TGA and DSC studies. The antioxidant activity and cytotoxicity of the complexes was investigated.

Materials and Methods

Materials

Chitosan and carboxymethyl chitosan were purchased from India Sea Foods, Cochin, Kerala, India. The 4-hydroxy benzaldehyde was purchased from Sigma Aldrich, India. All the chemicals used were of analytical grade.

Preparation of carboxymethyl chitosan Schiff base with 4-hydroxy benzaldehyde

Synthesis of Carboxymethyl Chitosan Schiff Bases (CMC-SB1)

Carboxymethyl chitosan was dissolved in a mixed solution of ethanol with a small amount of water and stirred at room temperature for 30 min. Then, 4-hydroxy benzaldehyde was added to the mixture. The mixture was stirred

and heated at 60°C for 12 h under water bath heating. After cooling, the crude product was washed with ethanol to the point of colorless filtrate. The product was dried at 60°C in vacuum for 24 h whose yield was computed as the following equation (Guinesi and Cavalheiro, 2006).

Synthesis of Carboxymethyl Chitosan Schiff Base of Iron(II) (CMC-SB1-Fe), Cobalt(II) (CMC-SB1-Co) and Zinc(II) (CMC-SB1-Zn) Complexes

0.5 mmol of the purified CMC-SB was taken in a flask and magnetically stirred for 5 h in ethanol. This pre-treated methanolic suspension was again stirred with 0.5 mol ethanolic solution of ferrous sulphate for 15 h. The resulting product after the filtration of the solution was washed well with ether and dried at 50°C in vacuum. Similar procedure was adopted for the syntheses of (CMC-SB-Co), and Zinc (II) (CMC-SB-Zn) Complexes The yield of the schiff bases and Fe, Co and Zn Schiff base complexes were calculated. The prepared samples were analysed by the following methods.

Fourier transform infrared studies

Fourier transform infrared spectra of chitosan Schiff base derivatives using KBR pellet method were recorded in the frequency range of 400 – 4000 cm^{-1} using Thermo Nicolet AVATAR 330 spectrophotometer.

X – Ray diffraction studies

X – ray diffractograms of samples were obtained using an X – ray powder diffractometer (XRD – SHIMADZU XD – D1) with Ni – filter and Cu $K\alpha$ radiation source. The relative intensity was recorded in the scattering range 2θ , varying from 10° to 90°.

Thermogravimetric analysis

Thermogravimetric analysis of the chitosan Schiff base derivatives was conducted using the instrument SOT Q600 V8.0 Build 95, to measure their weight loss at different temperatures in the heating range 20° - 850° C at a heating rate of 20°C per minute.

Differential scanning calorimetric analysis

The thermal behavior of the chitosan Schiff base derivatives was studied using NET 2 SCH DSC thermal analyzer. The samples were inserted into the Al pan and DSC scan was made from 30°–300°C in nitrogen atmosphere at a heating rate of 20°C per minute. The results were recorded and analyzed.

Evaluation of Biological activity

Antimicrobial assay

Antibacterial analysis was followed using standard agar well diffusion method to study the antimicrobial activity of compounds (Bagamboula et al., 2004). Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10⁵ colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 μL (5 μg compound in 500 μL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 h at 37°C for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used

as solvent control. Ciprofloxacin was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent. The tests were carried out in triplicates.

In vitro cytotoxicity and cell proliferation assay

Cell viability on the polymer films was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Sigma Aldrich, Bangalore). The assay is based on the reduction of mitochondria reductase of living cells. Cleaving the tetrazolium rings turns the pale yellow MTT into dark blue formazan, the concentration of which is directly proportional to the number of metabolically active cells. This reduction takes place only when mitochondrial reductase enzymes are active. The media on the scaffolds was removed on the respective days and incubated with fresh culture medium containing 400 μ L of MTT (5 mg ml⁻¹ medium) at 37°C for 4 hrs in darkness. Then the unreacted dye was removed and 400 μ L of DMSO was added to dissolve the intracellular insoluble purple formazan product into a colored solution. The absorbance of this solution was quantified by spectrophotometer at 540 nm with aGENios® microplate reader (Tecan Austria GmbH, Austria). Cell viability and proliferation of cells was quantified as a percentage compared to that of control.

Results and discussion

FTIR studies

Figure 1: FTIR spectrum of carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base (CMC-SB1)

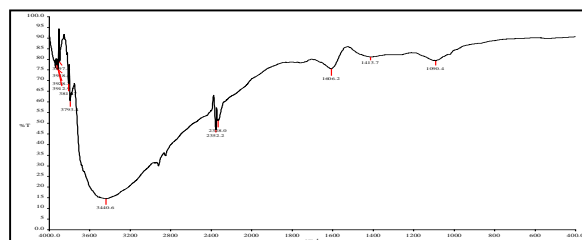


Table 1: FTIR Spectral details of carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base (CMC-SB1)

Wave number cm ⁻¹	Responsible group
3440.6	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2900 & 2870	C–H asymmetric and symmetric stretching vibration
1606.2	C=N Stretching of imine
1413.7	Aromatic C–C stretching, Asymmetric deformation of COO ⁻
1090.4	Glycosidic bonds C–O–C, C–O stretching

Table – 1 and Figure – 1, represent the FT–IR spectral information of Carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base (CMC-SB1). From the above spectral information of the prepared binary blend it is evident that the prominent peak obtained at 3477.66 cm^{-1} corresponds to intermolecular hydrogen bonded O–H stretching and amine N–H symmetrical stretching vibrations (Pushpika Katugampola et al., 2014). The peaks obtained at 2900 cm^{-1} and 2870 cm^{-1} corresponds to asymmetric and symmetric C–H Stretching in $-\text{CH}_2$ (Hua-Cai Ge et al., 2005).

A peak originated due to C=N stretching of imine was observed at 1606.2 cm^{-1} confirms the formation of carboxymethyl chitosan Schiff base. A peak observed at 1413 cm^{-1} is due to aromatic C–C stretching and asymmetric deformation of COO^- (Chen et al., 2007). The peaks originated at 1090.4 cm^{-1} corresponds to C–O stretching and glycosidic bonds of C–O–C skeletal vibration modes (Mourya et al., 2010).

The IR spectroscopy confirms the imine formation represented by the absorption band at 1606.2 cm^{-1} which is not present in the original carboxymethyl chitosan.

Figure 2: FTIR spectrum of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

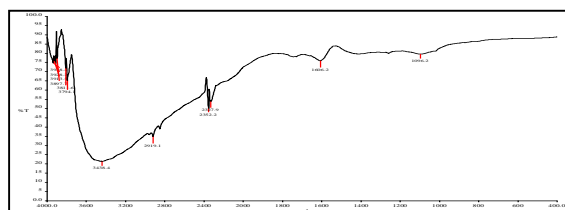


Table 2: FTIR spectral details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

Wave number cm^{-1}	Responsible group
3438.4	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2919.1	Asymmetric and symmetric C–H stretching
1606.2	C=N of imine
1096.2	Glycosidic bonds C–O–C, C–O stretching

Table – 2 and Figure – 2 shows the FT–IR spectral details of the carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe). The prominent peaks are at 3452.58 cm^{-1} , 2919.1 cm^{-1} , 1606.2 cm^{-1} and 1090.4 cm^{-1} . The broad peak at 3452.58 cm^{-1} corresponds to intermolecular hydrogen bond O–H, N–H stretching of polymeric association (Depuy and Rinehart et al., 1975).

The peak obtained at 2919.1 cm^{-1} indicates the aromatic and aliphatic C–H stretching vibration (Chen et al., 2003). A characteristic band observed for C=N stretching is observed at 1620.21 cm^{-1} confirms the formation of Schiff base (Sarawat et al., 1977).

The IR spectrum of the Schiff base showed a peak at 1606.2 cm^{-1} assigned to (C=N) of the azomethine stretching vibration and a peak at 1090.4 cm^{-1} due to C–O–C stretching frequency (Mourya et al., 2010). These two bands are shifted to lower or higher wave numbers in the complexes indicating the participation of azomethine nitrogen in

coordination (Masoud et al., 1991). The peak is shifted for C-O-C stretching vibration at 1096 cm^{-1} confirms the formation of iron Schiff base complex.

Figure 3: FTIR spectrum of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

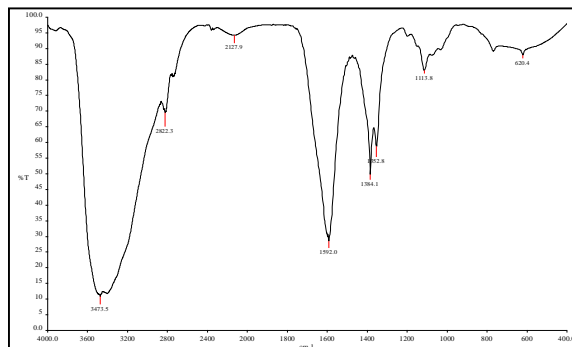


Table 3: FTIR spectral details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

Wave number cm^{-1}	Responsible group
3473.5	Intermolecular hydrogen bonded O-H stretching, N-H stretching in secondary amides
2822.3	Asymmetric and symmetric C-H stretching
1592	C=N of imine
1384.1, 1352.8	O-H stretching
1113.8	C-O stretching
800, 620.4	M-O and M-N stretching

Table – 3 and Figure – 3 shows the FT-IR spectrum of carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co). From the spectrum the prominent peaks are obtained at 3473.5 cm^{-1} , 2822.3 cm^{-1} , 1592 cm^{-1} , 1384.1 cm^{-1} , 1352.8 cm^{-1} , 1113.8 cm^{-1} and 620.4 cm^{-1} corresponds to intermolecular hydrogen bonded O-H stretching, N-H symmetrical stretching vibrations (Taylor et al., 1973), asymmetric and symmetric C-H Stretching in $-\text{CH}_2$ (Pushpika Katugampola et al., 2014), C-H bending in aromatic, C=N stretching of imine, O-H deformation, C-O stretching, M-O and M-N stretching vibrations respectively. The C=N imine stretching band at 1606 cm^{-1} in the free Schiff-base is shifted to lower frequency and is observed at around 1592 cm^{-1} for the complexes (Riyadh, Ahmed et al., 2013). New vibrations at 800 cm^{-1} , 690 cm^{-1} which are not present in the free Schiff base attribute to the existence of M-O and M-N stretching vibrations (Hamil et al., 2009). The C-H stretching frequency appears at 2893.66 cm^{-1} , the C-O stretching frequency appears at 1113.8 cm^{-1} . Also these shift in peaks was observed in the Schiff base metal complexes when compared with the FTIR spectrum of MA1 – MA4 confirming the coordination of the metal cobalt with carboxymethyl chitosan Schiff bases.

Figure 4: FTIR spectrum of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

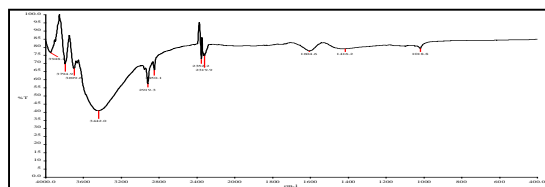


Table 4: FTIR spectral details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

Wave number cm^{-1}	Responsible group
3948.2, 3794.9, 3699, 3442	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2919.3, 2850.1, 2352.2, 2319.9	Aromatic and aliphatic C–H stretching
1604.6	C=N of imine
1416.2	Aromatic C=C stretching, O–H stretching
1018.8	CH_2 –OH in primary alcohol, C–O–C stretching

Table – 4 and Figure – 4 shows the FT–IR spectrum of carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn). From the spectrum the prominent peaks are obtained at 3442 cm^{-1} corresponds to intermolecular hydrogen bonded O–H stretching, N–H symmetrical stretching vibrations (Raman et al., 2015), 1604.6 cm^{-1} corresponds to C=N stretching. The band at 1416.2 cm^{-1} corresponds to aromatic C=C stretching (Ti Feng Jiao et al., 2011).

The C=N imine stretching band at 1606.2 cm^{-1} in the free Schiff-base is shifted to lower frequency and is observed at around 1602.7 cm^{-1} for the complexes (Varun et al., 2014) confirms the formation of zinc Schiff base complex. Thus the FTIR result clearly shows the interaction which was formed between carboxymethyl chitosan Schiff base with the metals. The shift in peaks was observed in the Schiff base metal complexes when compared with the FTIR spectrum of CMC-SB1 and CMC-SB2, confirming the coordination of the metal with carboxymethyl chitosan Schiff bases.

TGA analysis

Figure 5: TGA thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

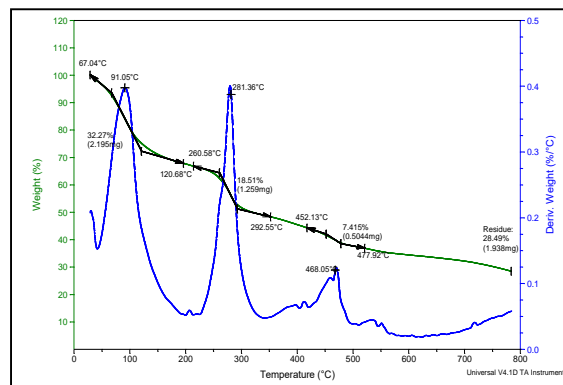


Table 5: TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

Percentage decomposition	Decomposition Temperature °C
10	70
20	100
30	170
40	270
50	340
60	470

Figure 5(a): TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

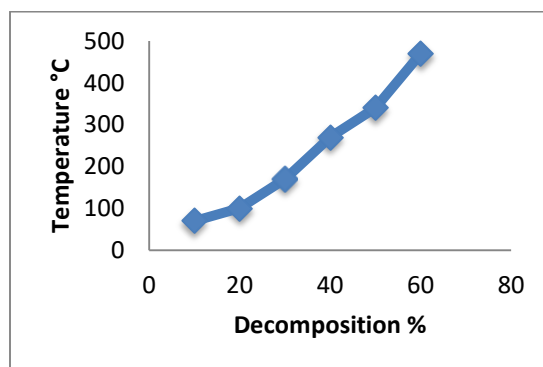


Table – 5 and Figure – 5, 5(a) represents the TGA thermogram details of Carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe). Initial decomposition temperature was found to be 70 °C. At 470 °C about 60% of the sample disintegrated leaving behind 28.49% of the sample as residue. Around 71.51% of the sample is disintegrated at the end of the experiment. In the first stage around 10% weight loss at 70° C had taken place due to the loss of water. In the second stage maximum weight loss occurred at the temperature range 200 °C to 500 °C, due to the decomposition of polymer linkage.

Figure 6: TGA thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

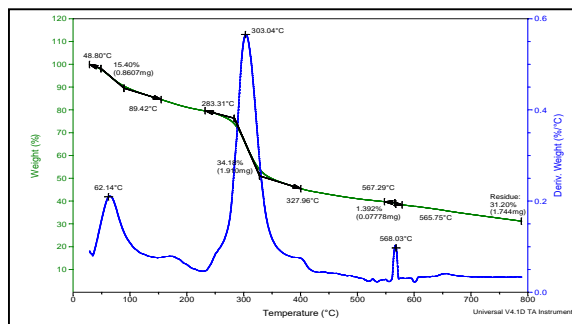


Table 6: TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

Percentage decomposition	Decomposition Temperature °C
10	90
20	220
30	280
40	310
50	330
60	460

Figure 6(a): TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

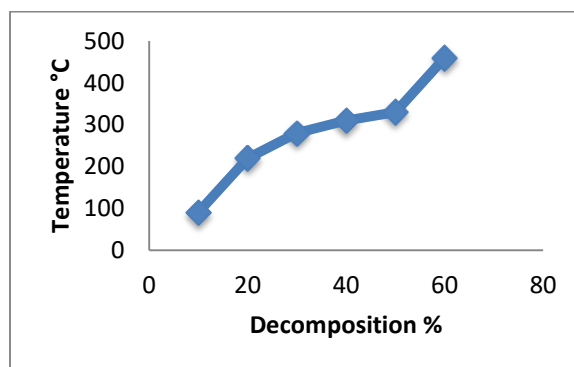


Table – 6 and Figure –6, 6(a) represent TGA thermogram details of carboxymethyl chitosan/4- hydroxy benzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co). From the figure it is evident that about 60% decomposition took place at 460 °C. Around 31.20% of sample remained as residue and 68.80% of the sample disintegrated at the end of the experiment. In the first stage around 10% weight loss at 90° C had taken place due to the loss of water. In the second stage maximum weight loss occurred at the temperature range 200 °C to 500 °C, due to the decomposition of polymer linkage.

Figure 7: TGA thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

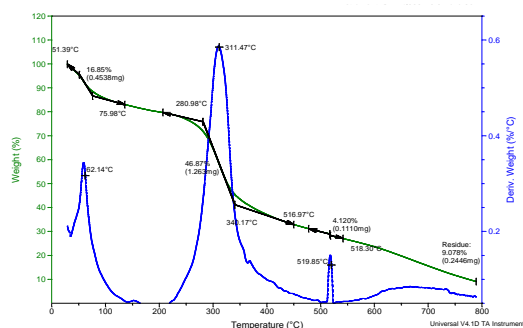


Table 7: TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

Percentage decomposition	Decomposition Temperature °C
10	70
20	220
30	280
40	310
50	330
60	400
70	530
80	650

Figure 7(a): TGA thermogram details of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

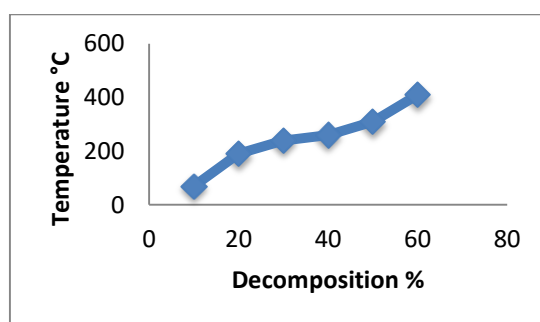


Table – 7 and Figure – 7, 7(a), represent the TGA thermogram details of carboxymethyl chitosan/4- hydroxy benzaldehyde Schiff base zinc(II) complex (CMC-SB1-Zn). Initial decomposition temperature was found to be 70 °C. At 650 °C about 80% of the sample disintegrated leaving behind 9.078% of the sample as residue. Around 90.922% of the sample is disintegrated at the end of the experiment.

On comparing the complexes which was prepared by using Carboxymethyl chitosan Schiff base the nickel complex was found to be highly thermally stable. The initial decomposition temperature is higher for nickel complex and its value is found to be 130 °C. It confirms that the nickel Schiff base complex was thermally more stable.

DSC studies

DSC is an analytical tool which helps to understand the thermal behavior of polymers and copolymers. It helps in finding the glass transition temperature of polymers, and copolymers. The glass transition temperature (T_g) is the temperature at which the material undergoes a structural transition from an amorphous solid state (glassy state) to a more viscous (rubbery) state. The direct information obtainable from a DSC thermogram is the enthalpy associated with the process.

Figure 8: DSC thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

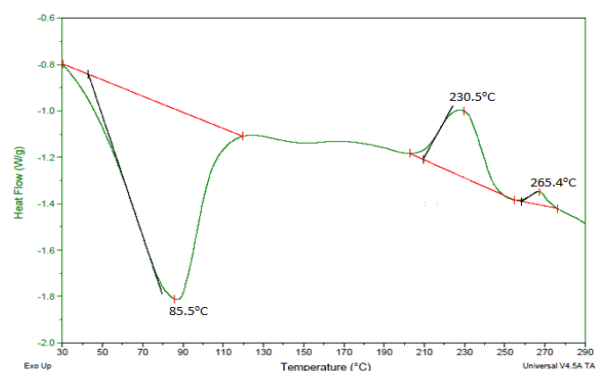


Figure – 8 represents the DSC thermogram of carboxymethyl chitosan/4-hydroxy benzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe). From the DSC measurements, glass transition temperature (T_g) was taken as the mid point of the transition region (Chellaian Justin Dhanaraj et al., 2014) and it is found to be 162 °C confirming the compatibility of the prepared material (Dos Santos et al., 2005).

The DSC curve showed one broad endothermic peak at 85.5 °C showing the crystallization of the blend at a lower temperature and two exothermic peaks at 230.5 °C and 265.4 °C showing the melting temperature of the complex.

Figure 9: DSC thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

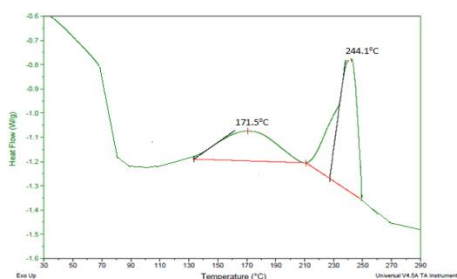


Figure – 9 represents the DSC thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co). Heat of melting gives idea about percent crystallinity of polymer, the melt of amorphous polymer has been range of melting points result from different crystalline regions in polymer structure (Bunian Shareef et al., 2013).

The DSC curve of the sample shows two broad exothermic peaks at 171.5 °C and 244.1 °C. The exothermic peak tells that there is more than one melting temperature for the complex. The glass transition temperature was observed to be 133 °C.

Figure 10: DSC thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

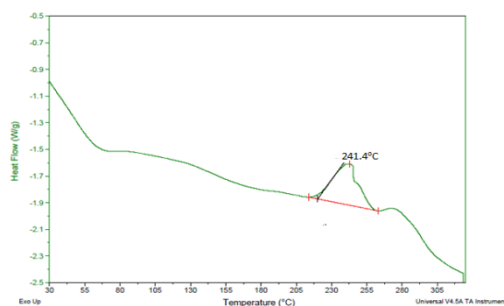


Figure – 10 represents the DSC thermogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn). The DSC curve shows one exothermic peak at 241.4 °C showing the melting temperature of the complex. The glass transition temperature of the complex was observed to be 144 °C.

On comparing the glass transition temperatures of carboxymethyl chitosan/ 4-hydroxybenzaldehyde Schiff base metal complexes of iron (CMC-SB1-Fe), cobalt (CMC-SB1-Co), nickel (CMC-SB1-Ni), copper (CMC-SB1-Cu) and zinc (CMC-SB1-Zn) the Tg value of Nickel Schiff base complex is higher and its value was found to be 178 °C which confirms it has high thermal stability and compatibility than the other complexes (Mastan vali shaik and Dr.M.S Dastageer, 2016). The results of DSC are more correlate with the TGA.

DSC is an analytical tool which helps to understand the thermal behaviors of the polymers. Figure 4a, 4b and 4c shows the DSC thermogram of CMC/4-hydroxy benzaldehyde Schiff base metal complexes(Fe,Co,Zn). The glass transition temperature of the Schiff base metal complexes(Fe,Co,Zn) showed a single Tg at 162°, 133°, and 178°C respectively, confirming the attractive physico-chemical interaction and high degree of compatibility. The change in the crystallization and melting temperatures showed that change in thermal behavior during coordination with different metals.

XRD studies

X-Ray diffraction analysis (XRD) investigates crystalline material structure, including atomic arrangement, crystalline size and imperfections. X-ray diffraction patterns of various samples were obtained to investigate the change of crystalline nature of modified carboxymethyl chitosan Schiff base complexes.

Figure 11: XRD pattern of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base Iron (II) complex (CMC-SB1-Fe)

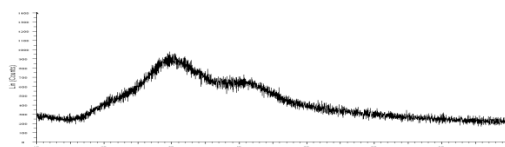


Figure – 11 shows the XRD diffractogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex. From the figure, the theta values obtained were 30° and 40°. Only 12.67% of crystallinity was observed when the iron was added which shows the significance of forming the complex. Thus the ordered structure of the polymers used was disrupted to a greater extent making the complex amorphous. The low crystallinity indicates that the complexes are more amorphous than the free carboxymethyl chitosan Schiff base.

Figure 12: XRD pattern of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

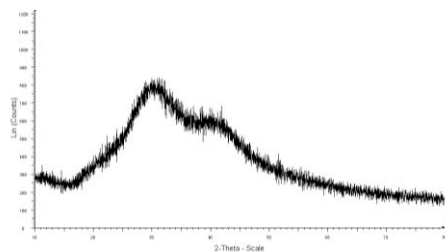


Figure – 12 shows the XRD diffractogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base Cobalt (II) complex. From the figure, it was evident that the intensity of crystalline peaks was increased and thus the percentage of crystallinity was found to be 14.29%. The theta values obtained were 30° and 40°. The increase in the number of peaks and the increment in the percentage of crystallinity value confirm the amorphous nature of the formed cobalt Schiff base complex.

Figure 13: XRD pattern of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base Zinc (II) complex (CMC-SB1-Zn)

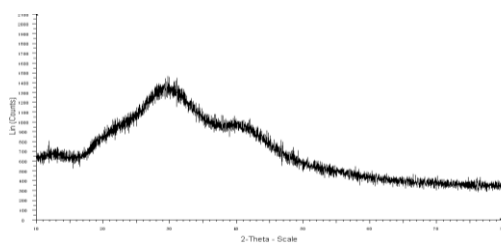


Figure – 13 shows the XRD diffractogram of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base Zinc (II) complex. From the figure, the theta values obtained were 30° and 40°. Only 10% of crystallinity was observed when the Zinc was added which shows the significance of forming the complex. Thus the ordered structure of the polymers used was disrupted to a greater extent making the complex amorphous. The low crystallinity indicates that the complexes are more amorphous than the free carboxymethyl chitosan Schiff base. XRD results suggested that the destruction of crystalline regularity of carboxymethyl chitosan Schiff base during complex formation was due to the intermolecular interaction between the two compounds and also indicated that there was good miscibility.

The X-ray diffraction analysis is used to determine the structure, complexation and crystallization of the polymer matrix (Pradhan et al., 2005). The X-ray pattern of the Schiff base and its copper and nickel complexes are shown in Figure 5. As compared with CMC alone, the CMC/4-hydroxy benzaldehyde shows the weaker and broader peak at $2\theta = 40^\circ$. For Iron, Cobalt and Zinc Schiff base complexes the 2θ values are at 30° and 40° are same. This

observed change in the peak positions are due to the coordination of metal with CMC/4-hydroxy benzaldehyde Schiff base.

SEM studies

The carboxymethyl chitosan Schiff base metal complexes were observed using a VG – Microtech super scan scanning electron microscope, UK. The samples were gold coated by sputtering technique and observed under different magnifications ranging from 20X to approximately 30,000X, spatial resolution of 50 – 100 nm. Complex fracture structures were analyzed after immersing the films in the liquid nitrogen for 10 min. The surface morphology of carboxymethyl chitosan Schiff base metal complexes characterized by SEM indicates homogeneous and continuous matrix without any pores (or) semi-pores (or) cracks on the surface with good structural integrity.

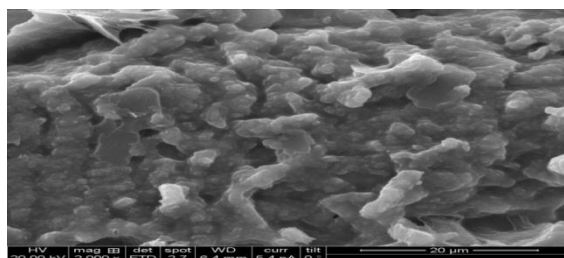


Figure 14: SEM image of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base iron (II) complex (CMC-SB1-Fe)

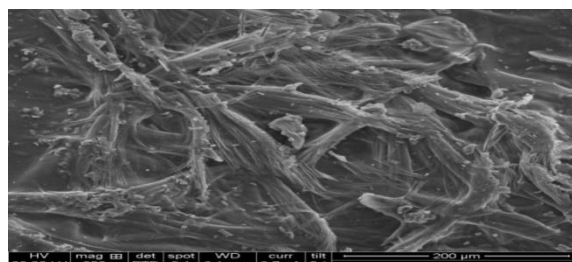


Figure 15: SEM image of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base cobalt (II) complex (CMC-SB1-Co)

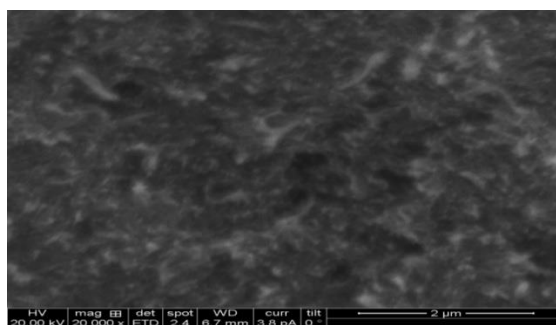


Figure 16: SEM image of carboxymethyl chitosan/4-hydroxybenzaldehyde Schiff base zinc (II) complex (CMC-SB1-Zn)

Carboxymethyl chitosan Schiff base metal complexes have rough and dense surfaces as shown in **Figures – (14 – 16)**. The reason for irregular surface may be due to higher viscosity of the chitosan Schiff base solution. From the morphology of the products we can observe uneven structure and rough surfaces. From SEM micrographs, it is

proven that the surface characteristic of the complexes plays important role to allow water uptake and cell proliferation.

In the present paper, the formation and characterisation of Carboxymethyl chitosan Schiff base metal complexes has been dealt with. The results prove the formation of the products with improved properties.

Antimicrobial activity

Both antibacterial and antifungal activities of carboxymethyl chitosan Schiff's base metal complexes were studied for its suitability in biomedical applications. Chitosan anchored Schiff base complexes have been amongst the most widely studied coordination compounds in the past few years, since they are becoming increasingly important as biochemical, antimicrobial and catalytic reagents. The data indicated that Carboxymethyl Chitosan + 4-hydroxy benzaldehyde Schiff's base and its transition metal complexes have a good inhibiting effect on both bacterial and fungi. (Mastan vali shaik et al., 2016)

Table 8: Antibacterial activity of Carboxymethyl chitosan Schiff base metal complexes (Diameter in mm)

Microorganisms	<i>Ciprofloxacin</i>	CMC-SB1-Fe	CMC-SB1-Co	CMC-SB1-Ni	CMC-SB1-Cu	CMC-SB1-Zn
<i>Streptococcus faecalis</i> Gram +VE	24	18	19	21	28	22
<i>Staphylococcus aureus</i> Gram +VE	23	18	20	20	27	23
<i>Escherichia coli</i> 2Gram -VE	26	19	21	22	28	22
<i>Klebsiella pneumonia</i> Gram -VE	22	16	19	21	25	19
<i>Bacillus cereus</i> Gram +VE	26	19	18	21	26	24
<i>Pseudomonas areuginosa</i> Gram -VE	25	18	17	22	28	25

Figure 17: Antibacterial activity of Carboxymethyl chitosan Schiff base metal complexes

Carboxymethyl Chitosan + 4-hydroxy benzaldehyde Schiff's base (CMC-SB1) ligand was complexed with various transition metals like Iron, Cobalt, Nickel, Copper and Zinc. The antibacterial activities of these complexes were studied comparatively by keeping *Ciprofloxacin* drug as the control. The results are shown in Table and Figure. The result reveals that CMC-SB1-Cu showed high antibacterial activity for all the six selected bacterial species. Ancient civilizations exploited the antimicrobial properties of copper long before the concept of microbes became understood in the nineteenth century (Dollwet and Sorenson, 1985). These effect is well pronounced here when

copper is complexed with the CMC-SB1 ligand. The antibacterial property for both the ligand and the metal were during blending and complexation. The order of activity of CMC-SB1-Cu was *Streptococcus faecalis* = *Escherichia coli* = *Pseudomonas areuginosa* > *Staphylococcus aureus* > *Bacillus cereus* > *Klebsiella pneumonia*.

On comparing the antibacterial activity of CMC-SB1 metal complexes with bare CMC-SB1 ligands, in case of metal complexes increases the antibacterial activity. This increase in antibacterial activity is due to the participation of metal ions with electrostatic interaction with the functional groups present in the ligands during blending. Also the results reveals the following order of antibacterial activity in the carboxymethyl chitosan Schiff base metal complexes CMC-SB1-Cu > CMC-SB1-Zn > CMC-SB1-Ni > CMC-SB1-Co > CMC-SB1-Fe.

Table 9: Antifungal activity of Carboxymethyl chitosan Schiff base metal complexes (Diameter in mm)

<i>Organisms (Fungi)</i>	<i>Amphotericin B</i>	CMC-SB1-Fe	CMC-SB1-Co	CMC-SB1-Ni	CMC-SB1-Cu	CMC-SB1-Zn
<i>Candida albicans</i>	14	18	19	21	28	22
<i>Mucor Sps</i>	18	18	20	20	27	23
<i>Rhizopus sps</i>	14	19	21	22	28	22

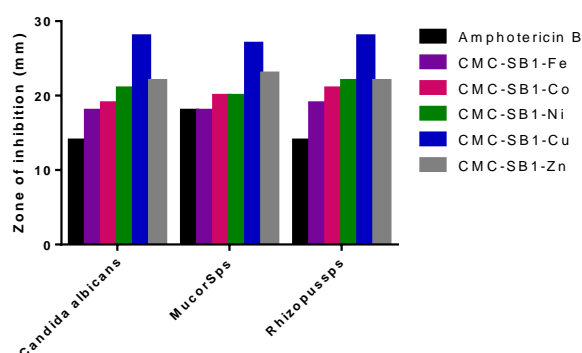


Figure 18: Antifungal activity of Carboxymethyl chitosan Schiff base metal complexes

Also, the effect of carboxymethyl chitosan Schiff's base metal complexes in inhibiting the growth of the *Candida albicans*, *Mucor Sps* and *Rhizopus sps* are listed in **Table 9** and **Figure – 18**. The results shows the significant inhibiting effect at all observed for the prepared complexes against the *Candida albicans*, *Mucor Sps* and *Rhizopus sps*. It was also shown that the antifungal activity of the Schiff's base against *Rhizopus sps* was stronger than that of other *sps*.

Moreover, chitosan Schiff base have higher inhibition efficiency than the non-modified chitosan. From the results, it can be indicated that the inhibition activity of both carboxymethyl chitosan Schiff base and its metal complexes were higher against all gram positive bacteria tested comparing with that on gram negative bacteria. The results compared with standard drugs (chloramphenicol and *Amphotericin B* for bacteria and fungi), have been indicated that the synthesized material was more active and showed good activity to the standard drug, especially, it exhibited highest inhibition activity against microorganisms more than the standard drug. Antimicrobial activity is a property of both organic and inorganic substances and the exploitation of such activity is of considerable importance in the development of antiseptics, sanitizers, germicides, bactericides and disinfectants (Maurer and Shringapurey, 1977).

Antioxidant activity

DPPH has been used extensively as a free radical to evaluate reducing substances (Cotelle et al., 1996). The scavenging activity for CMC-SB1-Fe, CMC-SB1-Co, CMC-SB1-Ni, CMC-SB1-Cu and CMC-SB1-Zn are 88%, 67%, 79%, 66% and 71% respectively. Among the five complexes prepared, Fe carboxymethyl chitosan Schiff base complexes shows higher activity than others. The antioxidant capacity of materials depends on the steric and electronic effect, besides the presence of the hydroxyl group and amine group, which is capable of donating hydrogen atoms.

Cytotoxicity studies

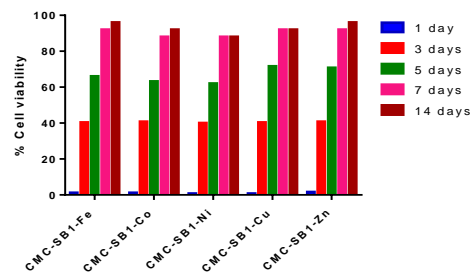


Figure 19: Cytotoxicity studies of Carboxymethyl chitosan Schiff base metal complexes

For cell survival and function, on the material surface the cell adhesion, growth, and proliferation are the sequential response. The mechanism of necrosis or apoptosis determines the cell viability. The destruction of the plasma membrane by external damage is the result of necrosis. Apoptosis, which is much slower than necrosis, leads to highly controlled cell death by biochemical and morphological events. The cellular response to a toxicant is determined by viability assays which are the vital steps in toxicity studies. Viability assays give information on cell death and metabolic activities. The MTT assay used in this work is a quick and effective method for testing mitochondrial activity, which correlates quite well with cell proliferation. It is seen that the absorbance index of the tested group increased with the increase of culture time. Significant differences were observed in the cell activity of Cu and Zn derivative, in the first five days, which implied that the derivative was beneficial to cell development. After that there are no significant differences in the prepared material.

Carboxymethyl chitosan Schiff base metal complexes are successfully prepared and characterized using FTIR, TGA, DSC and XRD to study its physical and chemical interactions formed. The FTIR results reveal that is a new peak for imine linkage, which confirms the Schiff base formation its coordination with metal complexes. The thermal analysis results suggested that there was a change in thermal behavior while Schiff base coordinate with metal and XRD results showed that there was a change in crystalline nature of the prepared material. The biological evaluation was done for the prepared sample to study its compatibility for biomedical applications. The results revealed that the prepared material have good antimicrobial, antioxidant and non-toxicity properties.

Cytotoxicity studies

The level of cell growth and proliferation on the carboxymethyl chitosan Schiff base films were assessed using MTT assay in vitro (Figure-). MTT cell viability test was performed on HeLa cell lines. Cells were cultivated in RPMI with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in humidified environment containing of 5% CO₂. The cytotoxicity of the polymers used to study the biocompatibility of the material using the MTT assay. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) is a monotetrazolium salt that is widely used to detect cell proliferation and cytotoxicity of materials (Mosmann, 1983). The MTT assay measures the activity of living cells mainly via mitochondrial dehydrogenases, which is supported by NADH-linked mitochondrial substrates, such as malate, glutamate or pyruvate (Liu et al., 1997).

It can be seen that the absorbance index of the tested groups increased with the increase of culture time. In the first five days, significant differences were observed in the cell activity between CMC-SB and its metal complexes, which implied that the derivatives were beneficial to cell development. It was found that there was no big difference in the cell activity of all the test groups after 5-day culture. The percentage viability was remarkably higher for copper complex than nickel complex.

Conclusion

Carboxymethyl Chitosan/4-hydroxy benzaldehyde Schiff bases (CMC-SB1) and its Iron (CMC-SB1-Fe), Cobalt (CMC-SB1-Co) and Zinc (CMC-SB1-Zn) complexes were prepared and characterized by FTIR, XRD, TGA and DSC studies. The results show the formation of Schiff bases and its metal complexes were formed effectively with modified properties. The antioxidant activity of the complexes Iron, Cobalt and Zinc complexes were studied. The results reveal that both the metal complexes have antioxidant activity. The cytotoxicity of the complexes towards HeLa cell lines were showed that the prepared compounds are beneficial to cell development.

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