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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 cinnamaldehyde 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 cinnamaldehyde. 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.

Key words: Antioxidant, Carboxymethyl Chitosan, Cytotoxic studies, cinnamaldehyde, Iron, Cobalt, Zinc schiff bases.

Introduction

Wide ranges of naturally occuring 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 cinnamaldehyde. 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 algaes (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 cinnamaldehyde 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 cinnamaldehyde was purchased from Sigma Aldrich, India. All the chemicals used were of analytical grade.

Preparation of carboxymethyl chitosan Schiff base with cinnamaldehyde

Synthesis of Carboxymethyl Chitosan Schiff Bases (CMC-SB2)

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, cinnamaldehyde 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-SB2-Fe), Cobalt(II) (CMC-SB2-Co) and Zinc(II) (CMC-SB2-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^{o}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 difractometer (XRD – SHIMADZU XD – D1) with Ni – filter and Cu Kα radiation source. The relative intensity was recorded in the scattering range 2θ, varying from 10^o to 90^o.

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^o - 850^0 C at a heating rate of 20^0C 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^o–300^oC in nitrogen atmosphere at a heating rate of 20^oC 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 (5µg 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 400µL of MTT (5 mg ml^{-1} medium) at 37°C for 4 hrs in darkness. Then the unreacted dye was removed and 400µL 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/cinnamaldehyde Schiff base (CMC-SB2)

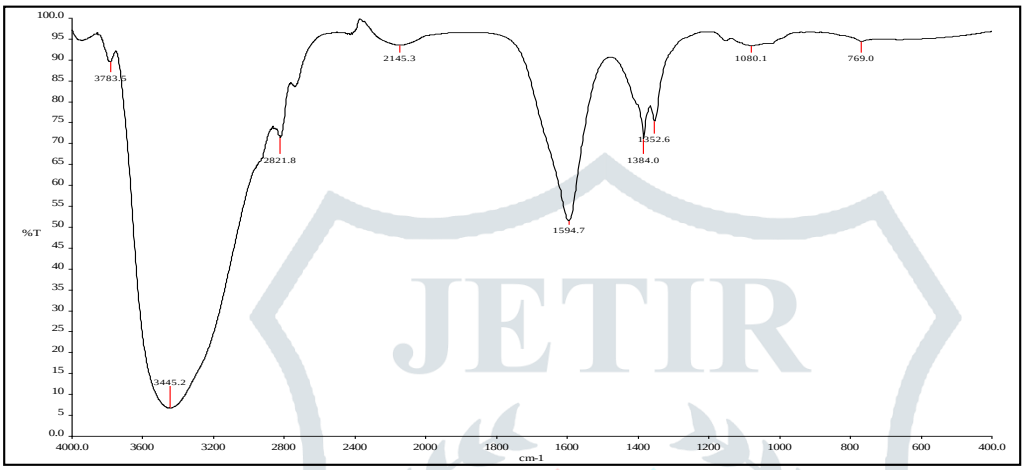


Table 1: FTIR Spectral details of carboxymethyl chitosan/cinnamaldehyde Schiff base (CMC-SB2)

Wave number cm^{-1}	Responsible group
3442	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2821.8	Aromatic and aliphatic C–H stretching
1594.7	C=N of imine
1384, 1352.6	C-H and O-H bending
1080.1	Glycosidic bonds C–O–C, C–O stretching
769	C–H out of plane bending for mono substituted benzene

Table – 1 and Figure – 1, represent the FT–IR spectral details of carboxymethyl chitosan/ cinnamaldehyde Schiff base (CMC-SB2). It shows a sharp peak at 3442 cm^{-1} corresponds to intermolecular hydrogen bond O–H, N–H stretching of polymeric association (Mourya et al., 2010).

The peak obtained at 2821.8 cm^{-1} is due to aromatic and aliphatic C–H stretching vibration. A characteristic peak observed at 1594.7 cm^{-1} corresponds to C=N imine which confirms the formation of Schiff base (Thatte et al., 2014). A peak obtained at 1384 cm^{-1}, 1352.6 cm^{-1} represents C-H and O-H bending modes of vibration. Carboxymethyl chitosan primary amino groups react with the active carbonyl groups of cinnamaldehyde to produce corresponding Schiff base (Mohy Eldin et al., 2015).

The peak originated at 1080.1 cm^{-1} corresponds to Glycosidic bonds C–O–C and C–O stretching. An additional strong peak which was observed at 769 cm^{-1} corresponds to C–H out of plane bending for mono substituted benzene and confirms phenyl groups were observed (Mohy Eldin et al., 2015).

On comparing the FTIR spectrum of carboxymethyl chitosan/cinnamaldehyde Schiff base (CMC-SB2) with carboxymethyl chitosan, the appearance of new peak for the imine (C=N) group and aromatic C=C and C–H groups confirms the effective blending for the formation of Schiff base. The FTIR spectrum of carboxymethyl chitosan Schiff base with 4- hydroxy benzaldehyde and cinnamaldehyde showed the characteristic peaks. It can be seen that the characteristic absorption bands of Schiff base formation were in the range of 1598 – 1650 cm^{-1}, confirming the presence of C=N (imine) bond which was formed between the –NH group of the CMC matrix and with the C=O group of corresponding aldehyde.

There was decrease in the intensity of the peak for NH_2 group content which indicates the participation of the amino group of the polymer matrix with the aromatic aldehyde to form Schiff bases (Riham and Ferky, 2011). Colthup et al., (1990) reported the strong absorption band at 1631 cm^{-1} for biopolymer Schiff bases. Similar observations were seen in CMC-SB2 and CMC-SB2 FTIR spectrum.

Figure 2: FTIR spectrum of carboxymethyl chitosan/cinnamaldehyde Schiff base iron (II) complex (CMC-SB2-Fe)

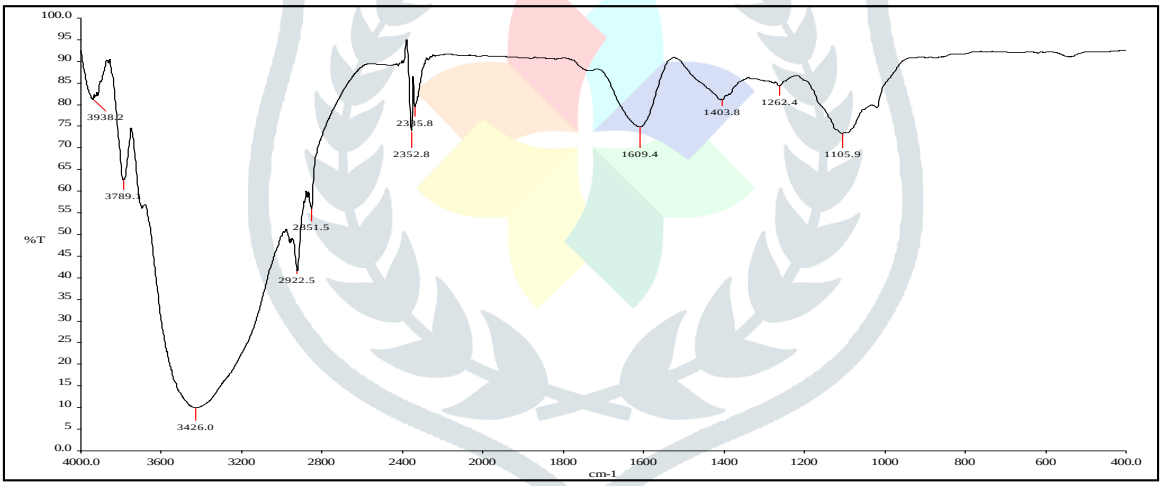


Table 2: FTIR spectral details of carboxymethyl chitosan/ cinnamaldehyde Schiff base iron (II) complex (CMC-SB2-Fe)

Wave number cm^{-1}	Responsible group
3426	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2922.5 & 2851.5	Aromatic and aliphatic C–H stretching
1609.4	C=N of imine and Aromatic C=C stretching
1403.8, 1262.4	C-H and O-H bending
1105.9	Glycosidic bonds C–O–C, C–O stretching
500	M–O and M–N stretching

Table – 2 and Figure – 2 shows the FT–IR spectral details of the carboxymethyl chitosan/cinnamaldehyde iron complex (CMC-SB2-Fe). It shows a sharp peak at 3426 cm^{-1} corresponding to intermolecular hydrogen bond O–H, N–H stretching of polymeric association. The peak obtained at 2922.5 cm^{-1} and 2851.5 cm^{-1} corresponds to aromatic and aliphatic C–H stretching in CH_2. A characteristic peak observed at 1609.4 cm^{-1} is due to C=N imine which confirms the formation of Schiff base and aromatic C=C stretching. The bands observed at 1403.8 cm^{-1}, 1262.4 cm^{-1} corresponds to C-H and O-H bending. The peak originated at 1105.9 cm^{-1} corresponds to Glycosidic bonds C–O–C, C–O stretching.

New vibration at 500 cm^{-1} which was not present in the free Schiff base attribute to the existence of metal–ligand bonding. The appearance of these vibrations confirmed the involvement of nitrogen and oxygen atoms in chelation (Najila Taher et al., 2008).

Figure 3:FTIR spectrum of carboxymethyl chitosan/cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co)

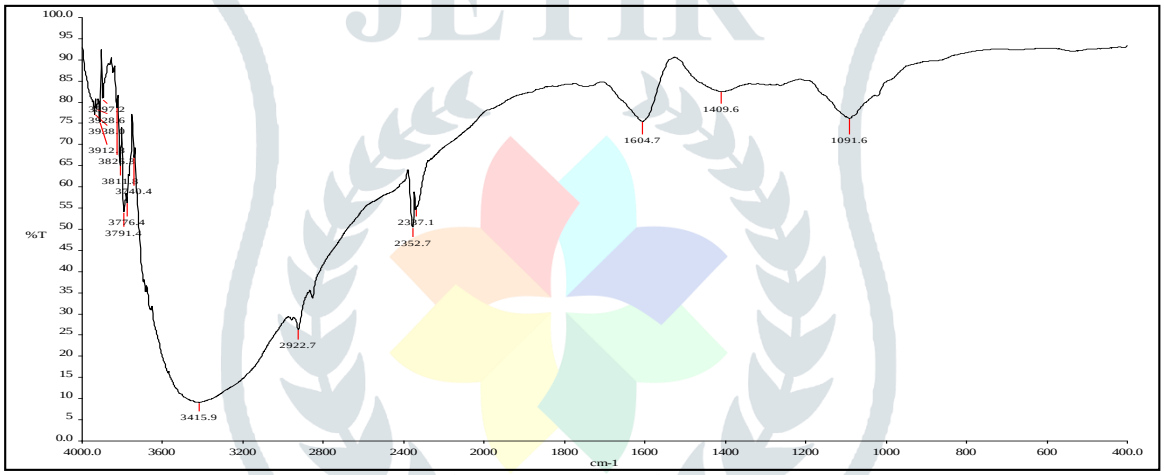


Table 3: FTIR spectral details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base cobalt (II) complex (CMC-SB2-Co)**

Wave number cm^{-1}	**Responsible group**
3415	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2922.7 & 2820	Asymmetric and symmetric C–H stretching
1604.7	C=N of imine
1409.6	C-H and OH bending
1091.6	CH–OH in cyclic alcohol, C–O stretching
480	M–N stretching

Table – 3 and Figure – 3 represents the FT–IR spectrum of carboxymethylchitosan/cinnamaldehyde Schiff base cobalt complex (CMC-SB2-Co). From the spectrum the prominent peaks are obtained at 3415 cm^{-1}, 2922.7 cm^{-1}, 2820 cm^{-1} ,1604.7 cm^{-1}, 1409.6 cm^{-1}, 1091.6 cm^{-1} and 480 cm^{-1} corresponds to intermolecular hydrogen bonded O–H

stretching, N–H symmetrical stretching vibrations (Talat Barana et al., 2015), asymmetric and symmetric C–H Stretching in $–CH_2$, C=N stretching of imine, C–OH stretching, O–H deformation and M–N stretching vibrations respectively. The bonding of the ligand to the metal ions is confirmed by the appearance of band at 480 cm^{-1} due to M–N vibration (Ferraro et al., 1971).

Figure 4: FTIR spectrum of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

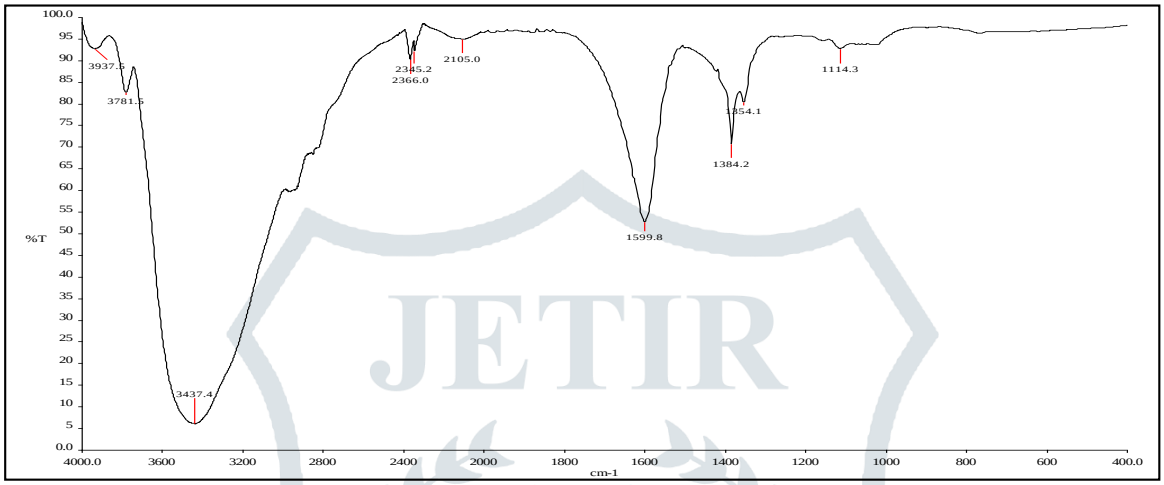


Table 4: FTIR spectral details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

Wave number cm^{-1}	Responsible group
3415	Intermolecular hydrogen bonded O–H stretching, N–H stretching in secondary amides
2922.7	Asymmetric and symmetric C–H stretching
1599.8	C=N of imine and Aromatic C=C stretching
1384.2, 1354.1	C-H and O-H bending
1114.3	Glycosidic bonds C–O–C, C–O stretching

Table – 4 and Figure – 4 shows the FT–IR spectrum of carboxymethyl chitosan/cinnamaldehyde Schiff base zinc (II) complex (CMC-SB2-Zn). From the spectrum the prominent peaks are obtained at 3415 cm^{-1}, 1384.2 cm^{-1}, 1354.1 cm^{-1} and 1114.3 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 $–CH_2$ (Pushpika Katugampola et al., 2014), Glycosidic bonds C–O–C stretching.

The peak at 1599.8 cm^{-1} corresponds to imine formation. The peak is shifted for C-O-C stretching vibration at 1114.3 cm^{-1} confirms the formation of zinc Schiff base complex. Thus the FTIR result clearly shows the interaction 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-SB2 confirming the coordination of the metal with carboxymethly chitosan Schiff bases.

TGA analysis

Figure 5: TGA thermogram of carboxymethyl chitosan/ cinnamaldehyde **Schiff base iron (II) complex (CMC-SB2-Fe)**

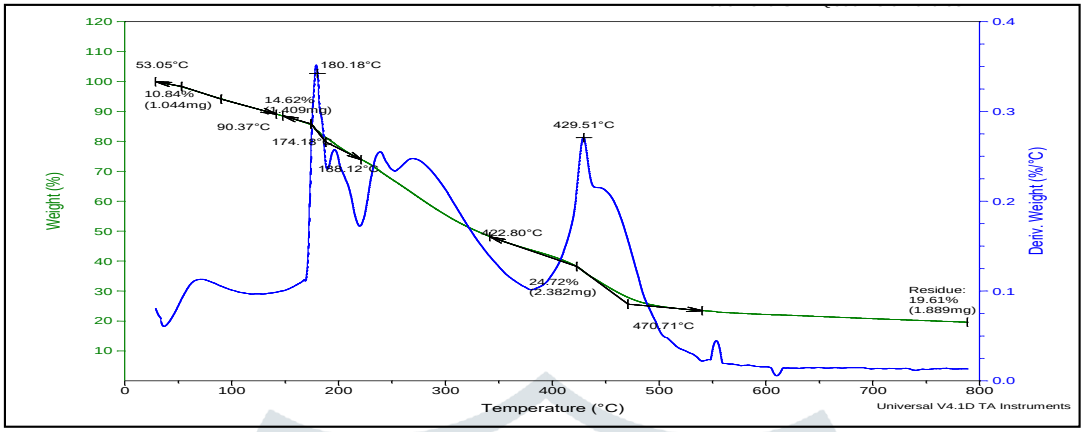


Table 5: TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base iron (II) complex (CMC-SB2-Fe)**

Percentage decomposition	Decomposition Temperature °C
10	130
20	190
30	240
40	270
50	340
60	410
70	460

Figure 5(a): TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base iron (II) complex (CMC-SB2-Fe)**

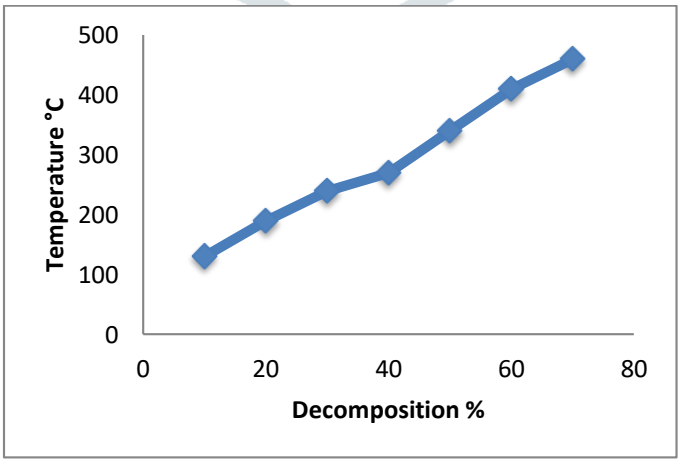


Table – 5 and Figure – 5, 5(a) represents the TGA thermogram details of Carboxymethyl chitosan/cinnamaldehyde Schiff base iron (II) complex (CMC-SB2-Fe). Initial decomposition temperature was found to be 130 °C. At 460 °C about 70% of the sample disintegrated leaving behind 19.61% of the sample as residue. Around 80.39% 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 400 °C, due to the decomposition of polymer linkage.

Figure 6: TGA thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co)

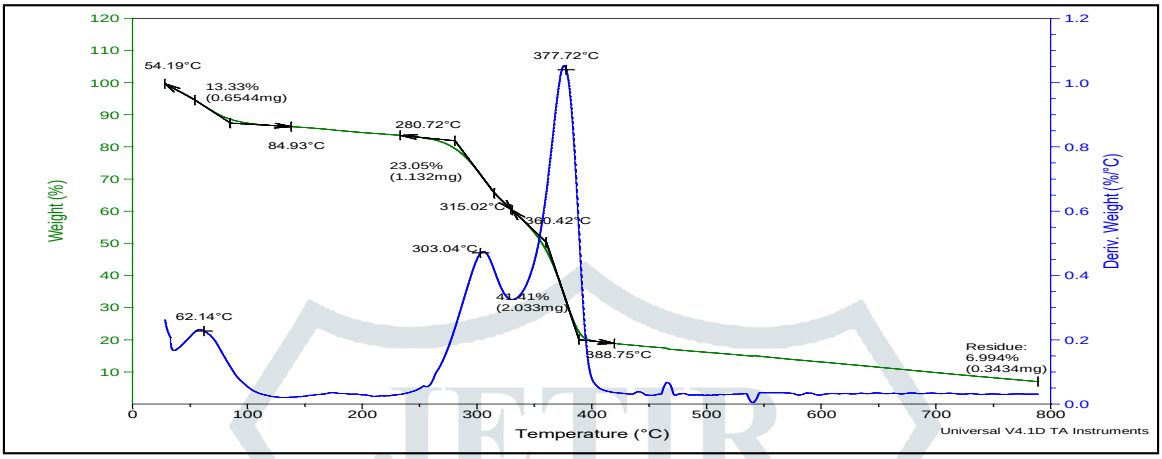


Table 6: TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co)

Percentage decomposition	Decomposition Temperature °C
10	70
20	270
30	300
40	330
50	350
60	360
70	370
80	400

Figure 6(a): TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co)

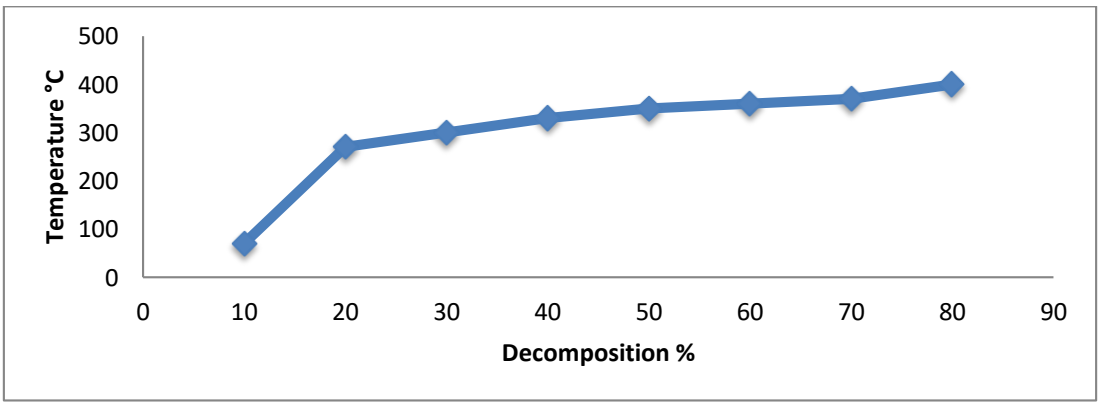


Table – 6 and Figure –6, 6(a represents the TGA thermogram details of Carboxymethyl chitoson/cinnamaldehyde Schiff base Cobalt (II) complex (CMC-SB2-Co). Initial decomposition temperature was found to be 70 °C. A steep increase in temperature is observed initially from 70 °C to 270 °C at 10% to 20% decomposition. Around 93.006% of the sample is disintegrated at the end of the experiment leaving a residue of 6.994%.

On comparing the initial decomposition temperature of iron and cobalt complexes, the initial decomposition temperature is higher for iron complexes which indicates that cobalt Schiff base complex is less thermally stable than iron Schiff base complex.

Figure 7: TGA thermogram of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

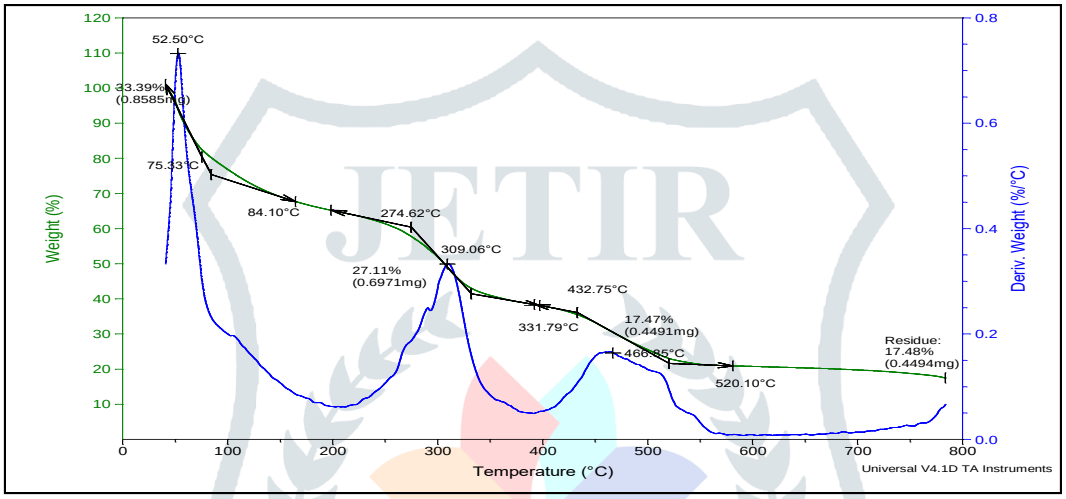


Table 7: TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

Percentage decomposition	Decomposition Temperature °C
10	50
20	70
30	150
40	280
50	300
60	350
70	470

Figure 7(a): TGA thermogram details of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

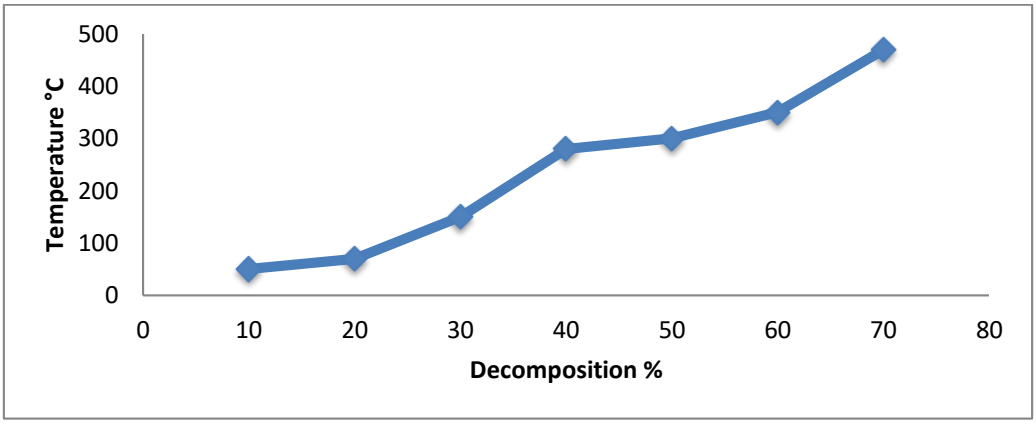


Table – 7 and Figure – 7, 7(a), represents the TGA thermogram details of carboxymethyl chitosan/cinnamaldehyde zinc (II) complex (CMC-SB2-Zn). Initial decomposition temperature was found to be 50 °C. At 470 °C about 70% of the sample disintegrated leaving behind 17.48% of the sample as residue. Around 82.52% of the sample is disintegrated at the end of the experiment.

When comparing the complexes of carboxymethyl chitosan Schiff base with metal ions. The initial decomposition temperature of nickel Schiff base complex was found to be higher and thus confirms nickel complex possess higher thermal stability.

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 (Tg) 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.

DSC thermal analysis also helps us to find out the miscibility of the two polymers which are involved in blending. A baseline step in a DSC curve has appeared due to the different heat capacity below and above the glass transition temperature of a polymer (Wunderlich, 1976). From the DSC measurements, glass transition temperature (Tg) was taken as the mid point of the transition region (Chellaian Justin Dhanaraj et al., 2014).

Figure 8: DSC thermogram of carboxymethyl chitosan/ cinnamaldehyde **Schiff base iron (II) complex (CMC-SB2-Fe)**

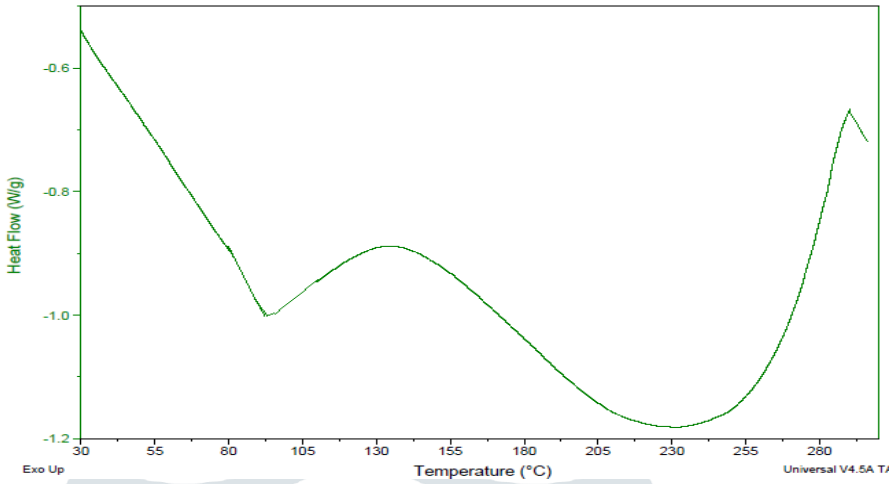


Figure – 8 represent the DSC thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base iron (II) complex (CMC-SB2-Fe). The DSC curve shows two broad endothermic peaks at 97 °C and 230 °C. The endothermic peak tells that there is more than one crystallization form. The glass transition temperature was observed to be 295 °C. There is no exothermic peak below 300 °C.

Figure 9: DSC thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co)

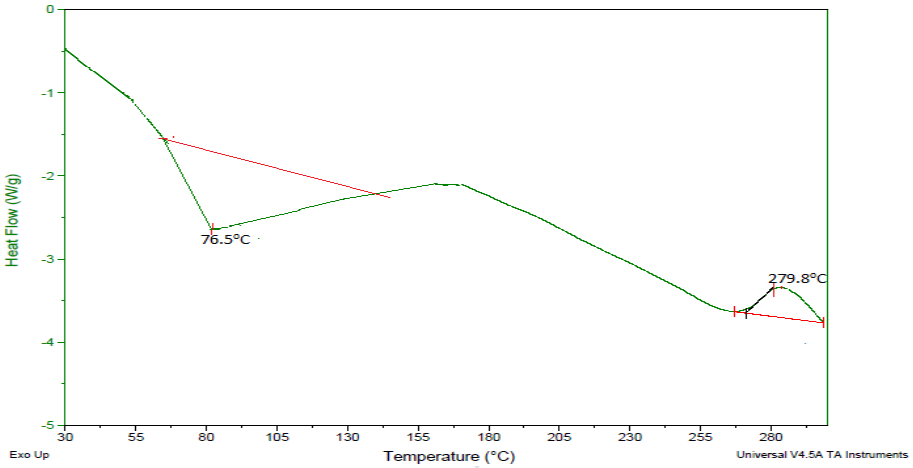


Figure – 9 represent the DSC thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base cobalt (II) complex (CMC-SB2-Co). The DSC curve showed one broad endothermic peak at 76.5 °C showing the crystallization of the blend at a lower temperature and one exothermic peak at 279.8 °C showing the melting temperature of the complex. The glass transition temperature of the sample was observed to be 213 °C.

Figure 10: DSC thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base zinc (II) complex (CMC-SB2-Zn)

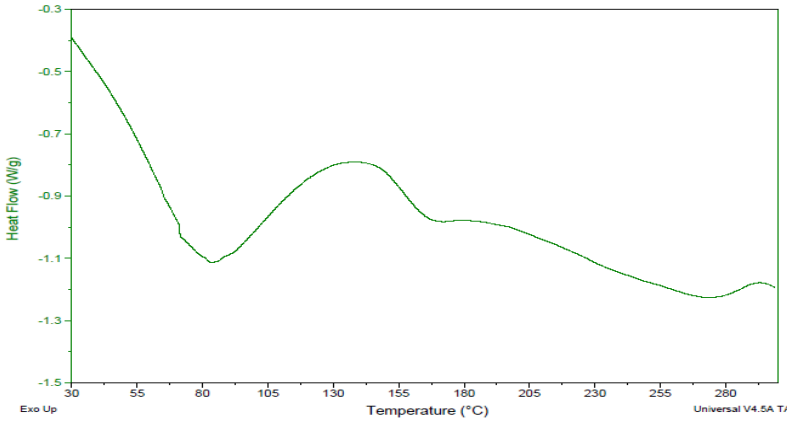


Figure – 10 represent the DSC thermogram of carboxymethyl chitosan/cinnamaldehyde Schiff base zinc (II) complex (CMC-SB2-Zn). The DSC curve shows two endothermic peaks at 83°C and 172°C. The endothermic peak tells that there is more than one crystallization form. The glass transition temperature was observed to be 172 °C. There is no exothermic peak below 300 °C.

On comparing the glass transition temperatures of carboxymethyl chitosan/ cinnamaldehyde Schiff base metal complexes of iron (CMC-SB2-Fe), cobalt (CMC-SB2-Co), nickel (CMC-SB2-Ni), copper (CMC-SB2-Cu) and zinc (CMC-SB2-Zn) the Tg value of Iron Schiff base complex is higher and its value was found to be 295 °C which confirms it has high thermal stability and compatibility than the other complexes (Mastan vali shaik and Dr.M.S Dastageer, 2016).

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/cinnamaldehyde 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 295°, 213°, and 172°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 of carboxymethyl chitosan/cinnamaldehyde Schiff base Iron (II) complex (CMC-SB2-Fe)

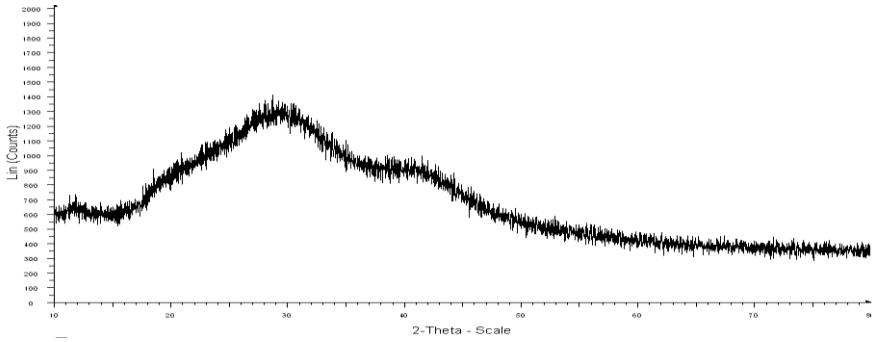


Figure – 11 shows the XRD diffractogram of carboxymethyl chitosan/ cinnamaldehyde Schiff base iron (II) complex. From the figure, the theta values obtained were 29° and 10°. Only 14.67% of crystallinity was observed when the iron was added which shows the significance of forming the complex. Thus the 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/ cinnamaldehyde **Schiff base cobalt (II) complex (CMC-SB2-Co)**

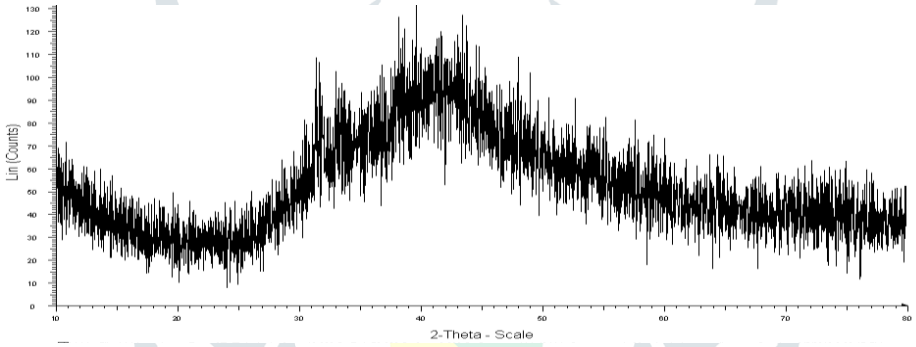


Figure – 12 shows the XRD diffractogram of carboxymethyl chitosan/ cinnamaldehyde Schiff base Cobalt (II) complex. The 2 theta values obtained was 38°. From the figure, it was evident that the intensity of crystalline peaks was increased and thus the percentage of crystallinity was found to be 11.02 %.

Figure 13: XRD pattern of carboxymethyl chitosan/ cinnamaldehyde **Schiff base Zinc (II) complex (CMC-SB2-Zn)**

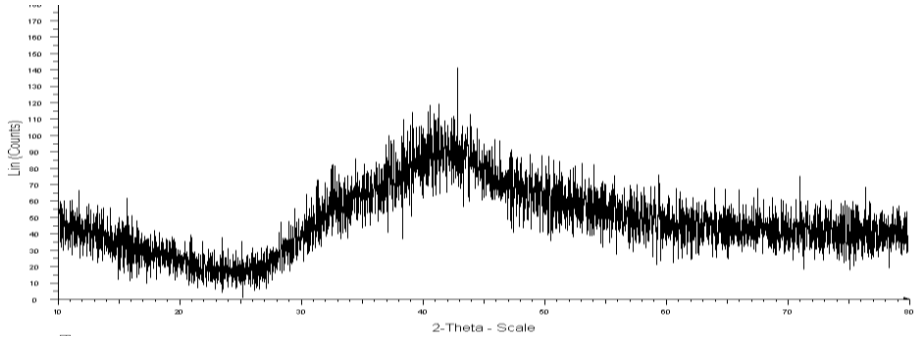


Figure – shows the XRD diffractogram of carboxymethyl chitosan/ cinnamaldehyde Schiff base Zinc (II) complex. Only 9 % of crystallinity was observed when the Zinc was added which shows the significance of forming the complex. Thus the ordered structure of the polymers used was disrupted to a greater extent making the complex amorphous. The low crystallinity indicates that the complexes are more amorphous than the free carboxymethyl chitosan Schiff base. From the figure, the theta value obtained was 40°.

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 iron,cobalt and zinc complexes are shown in Figures 11,12,13. As compared with CMC alone, the CMC/cinnamaldehyde shows the weaker and broader peak at 2θ = 20° & 42°. For Iron, Cobalt and Zinc Schiff base complexes the 2θ values are at 29° & 10°,38° and 40º are almost same. This observed change in the peak positions are due to the coordination of metal with CMC/cinnamaldehyde 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.

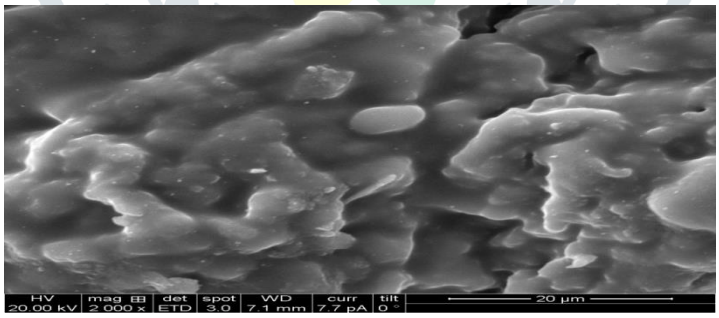


Figure 14: SEM image of carboxymethyl chitosan/ cinnamaldehyde **Schiff base iron (II) complex (CMC-SB2Fe)**

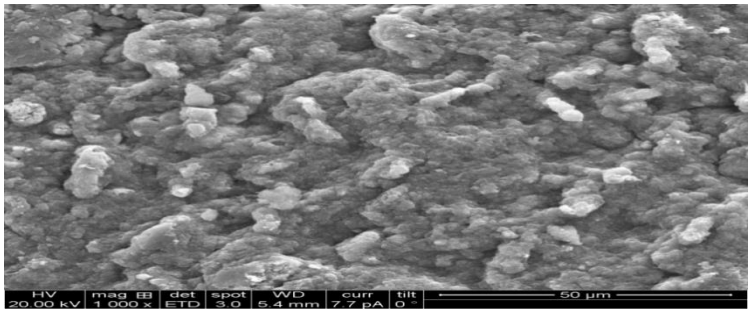


Figure 15: SEM image of carboxymethyl chitosan/ cinnamaldehyde **Schiff base cobalt (II) complex (CMC-SB2-Co)**

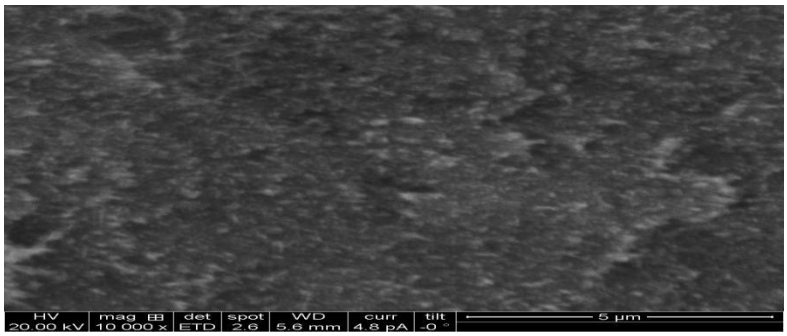


Figure 16: SEM image of carboxymethyl chitosan/ cinnamaldehyde **Schiff base zinc (II) complex (CMC-SB2-Zn)**

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.

It is possible to conclude that reaction coordination occurs between CMC-SB2 and metal which results in a complete change in the surface morphology of the prepared materials. The rough surface morphology with more number of voids proves that the prepared material can be more suitable for the better adsorption and cell culture 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

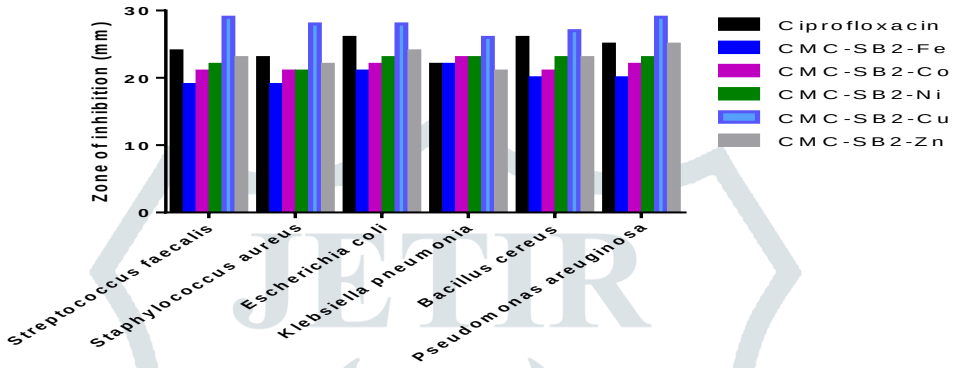
Both antibacterial and antifungal activities of carboxymethyl chitosan Schiff's base metal complexes were studies for its suitability in biomedical applications. Chitosan anchored Schiff base complexes have been amongst the most widely studied coordination compounds in the past few years, since they are becoming increasingly important as biochemical, antimicrobial and catalytic reagents. The data indicated that Carboxymethyl Chitosan + cinnamaldehyde 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	***Ciprofloxacin***	**CMC-SB2-Fe**	**CMC-SB2-Co**	**CMC-SB2-Ni**	**CMC-SB2-Cu**	**CMC-SB2-Zn**
Streptococcus faecalis *Gram +VE*	24	19	21	22	29	23
Staphylococcus aureus *Gram +VE*	23	19	21	21	28	22
Escherichia coli *Gram -VE*	26	21	22	23	28	24
Klebsiella pneumonia	22	22	23	23	26	21

Gram –VE						
Bacillus cereus *Gram +VE*	26	20	21	23	27	23
Pseudomonas areuginosa *Gram –VE*	25	20	22	23	29	25

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



Antibacterial activity of carboxymethyl chitosan Schiff base metal complexes

Carboxymethyl Chitosan + cinnamaldehyde Schiff's base (CMC-SB2) 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-SB2-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-SB2 ligand. The antibacterial property for both the ligand and the metal were during blending and complexation. The order of activity of CMC-SB2-Cu was *Streptococcus faecalis = Pseudomonas areuginosa > Escherichia coli = Staphylococcus aureus > Bacillus cereus > Klebsiella pneumonia.*

On comparing the antibacterial activity of CMC-SB2 metal complexes with bare CMC-SB2 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-SB2-Cu > CMC-SB2-Zn > CMC-SB2-Ni > CMC-SB2-Co > CMC-SB2-Fe.**

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

Organisms (Fungi)	*Amphotericin* B	CMC-SB2-Fe	CMC-SB2-Co	CMC-SB2-Ni	CMC-SB2-Cu	CMC-SB2-Zn
Candida albicans	14	11	17	18	19	18
MucorSps	18	16	16	15	18	17
Rhizopussps	14	13	12	14	16	15

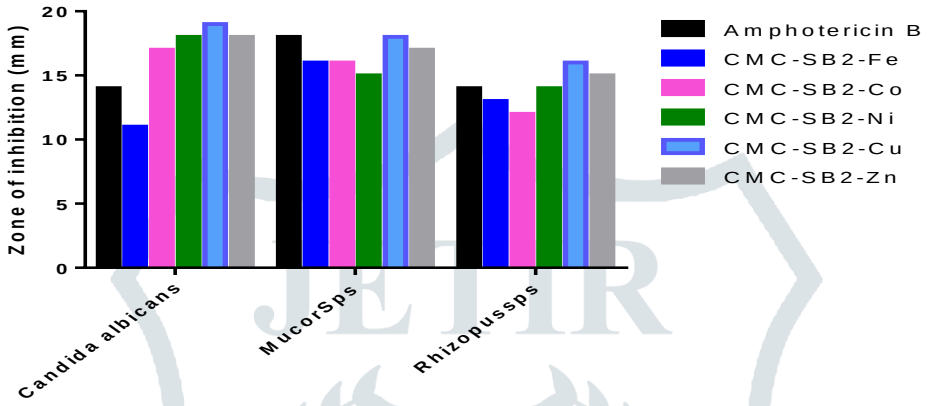


Figure 18: Antifungal activity of Carboxymethyl chitosan Schiff base metal complexes

Also, the effect of carboxymethyl chitosan Schiff's base metal complexes 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 *Candida albicans* was stronger than that of other *sps*.

It is seen that in the case of activity against fungi the inhibition was stronger in the order, Candida albicans > Mucorsps > Rhizopussps as shown by carboxymethyl chitosan Schiff's base metal complexes. Here for fungi also the electronic effect plays a role in decreasing antifungal activity.

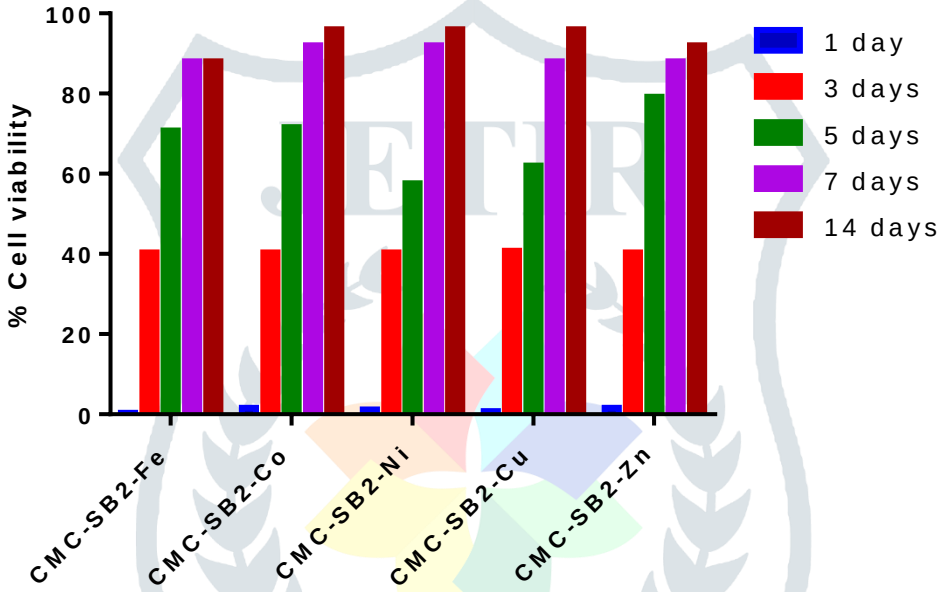
Moreover, chitosan Schiff base have higher inhibition efficiency than the non-modified chitosan. From the results, it can be indicated that the inhibition activity of both carboxymethyl chitosan Schiff base and its metal complexes were higher against all gram positive bacteria tested comparing with that 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

The anti-oxidant activity of chitosan and its derivatives has indicated that the active hydroxyl and amino groups in the polymer chains may take part in free radical scavenging and contributed to the anti-oxidant activity. The contents of active hydroxyl, amino, amido groups in their polymer chains as well as molecular weight affect the anti-oxidant activity of chitosan and derivatives (Feng et al., 2008; Guo et al., 2005). The process of wound healing includes

inflammation, cell proliferation and contraction of collagen lattice formation. When a wound is incurred, it is accompanied, within a short time by pain, reddening and edema, which are the typical symptoms of inflammation caused by the release of eicosanoids, prostaglandins, leukotrienes and ROS. These oxygen derived species consist of oxygen radicals and certain non-radicals that are oxidizing agents and/or are easily converted into radicals. Antioxidants have been shown to play a role in delaying or preventing oxidative stress caused by free radicals (Hermans et al., 2007). The scavenging activity for CMC-SB2-Fe, CMC-SB2-Co, CMC-SB2-Ni, CMC-SB2-Cu and CMC-SB2-Zn are 56%, 59%, 44%, 74% and 86% rspectively. Among the five complexes prepared, Zn carboxymethyl chitosan Schiff base complexes shows higher activity than others.

Cytotoxicity studies



The ultimate goal of our research is the application of the antioxidant and antibacterial chitosan derivatives in mammalian systems, the issue of cytotoxicity has to be addressed. Pure carboxymethyl chitosan has been reported to be non-toxic. Our results also show that the cytotoxicity value of carboxymethyl chitosan Schiff base and its complexes with higher percentage cell viability. Figure shows the cytotoxicity results for carboxymethyl chitosan Schiff's base metal complexes on HeLa cell lines using direct contact test. Results demonstrated that, carboxymethyl chitosan Schiff base and its complexes derivatives exhibit high percentage of cell viability in terms of their biocompatibility.

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 Co, Ni and Cu followed by Zn and Fe 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.

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 37ºC 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/cinnamaldehyde Schiff bases (CMC-SB2) and its Iron (CMC-SB2-Fe), Cobalt(CMC-SB2-Co) and Zinc (CMC-SB2-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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