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Synthesis, characterization, biological and catalytic activity of carboxymethyl chitosan schiff base metal complexes

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The Schiff base of carboxymethyl chitosan/p-dimethylaminobenzaldehyde (CMC-SB), as well as its complexes with cobalt (CMC-SB-Co), nickel (CMC-SB-Ni), and zinc (CMC-SB-Zn), were synthesised and studied using FTIR, XRD, TGA, DSC, and SEM. Schiff base metal complex production has been verified by the FTIR and XRD data. According to the thermal studies, the CMC-SB Zinc combination has better thermal stability than other complexes. The material is porous and rough, and the SEM results show that it has several potential uses in the biological field. The zinc complexes have increased activity when it comes to catalysis. It has been determined by the MTT test and ALP activity that the synthesised sample is non-toxic, compatible, and has good antioxidant activity.

[**Keywords**: Carboxymethyl chitosan, Schiff base, Catalytic activity]

Introduction

The second most prevalent biopolymer after cellulose is chitin[1,2]. A cationic polysaccharide generated from chitin is chitosan, has special physical, chemical, and biological characteristics. The most straightforward method of modifying chitosan chemically is carboxymethylation, which also has the advantages of being biodegradable, non-toxic, and biocompatible[3,4]. High water solubility and distinctive biological, chemical, and physical features characterise carboxymethyl chitosan[5,6]. As a result, it has many uses, mostly in biomedical sectors, such as a bactericide, blood anticoagulant, wound dressing, moisture-retention agent, and drug administration[7-9]. Carboxymethyl chitosan can be utilised in the manufacturing of drugs and cosmetics because of its ability to absorb and retain moisture. In the agricultural and food industries, carboxymethyl chitosan is used to protect fruits and vegetables against bacteria and fungi during storage[10].

The Schiff bases are created when the free amino groups of chitosan combine with an active carbonyl molecule, such as aldehyde or ketone[11–13]. The hydrophilicity, flexible structure, and presence of amino and hydroxyl groups in CMC schiff base make it a good candidate for metal complexation. In the past few years, many Schiff base compounds have been created and are now being used to introduce novel catalysts and treatments. The coordination of the Schiff bases with the transition metals plays a crucial role in the use of catalytic oxidation[14].

As a result, the present effort involved the fabrication of carboxymethyl chitosan Schiff base metal complexes, nickel, zinc, and cobalt are the metals used. FTIR, XRD, DSC, TGA, and SEM analyses were used to characterise the Schiff base metal complexes. For the synthesized metal complexes, biological activity including antioxidant activity, alkaline phosphatase activity, and cytotoxicity were studied. Additionally, the samples' catalytic oxidation was investigated.

Materials and Methods

India Sea Foods in Cochin, Kerala, India, was the manufacturer and supplier of carboxymethyl chitosan. Sigma Aldrich in India is where the p-dimethylmino Benzaldehyde aldehyde was purchased. Cobaltous chloride, nickel chloride, and zinc chloride were only a few of the compounds used, and they were all of analytical quality.

Synthesis of CMC-SB

The p-dimethyl amino benzaldehyde and carboxymethyl chitosan have already been synthesised and published in the literature[15].

Synthesis of CMC-SB metal(II) complex

A flask containing 0.5 m mol of the purified CMC-SB was magnetically swirled in ethanol for 5 h. This previously treated methanolic suspension was agitated once more for 15 h with a 0.5 mol ethanolic solution of $CoSO_4$. After the solution had been filtered, the end product (CMC-SB-Co) was thoroughly washed with ether and dried under vacuum at 50 °C. Carboxymethyl chitosan Nickel (II) (CMC-SB-Ni) and Carboxymethyl chitosan Zinc (II) (CMC-SB-Zn) complexes were made using a similar procedure. Figure 1 depicts the structure of the prepared Schiff base metal complexes.

Characterization of the samples

AVATAR 330 FTIR spectra from Thermo Nicolet using the KBr pellet technique. X-ray powder diffractometer with a Ni filter and a Cu K α radiation source (XRD). The relative intensity was measured in the scattering range 2θ between 10° and 90°. The

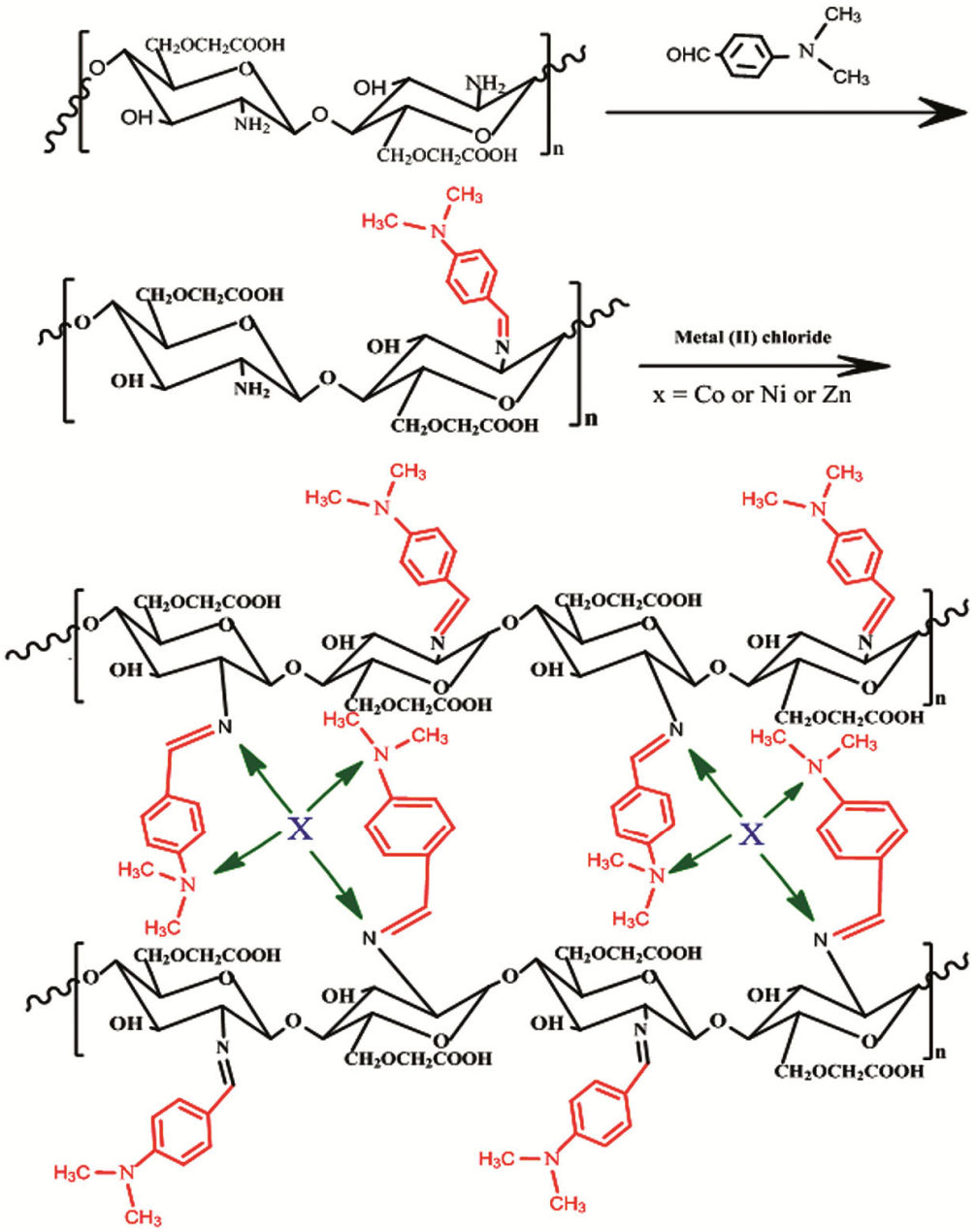

Fig. 1 — Structure of carboxymethyl chitosan Schiff base and its Co(II), Ni(II) and Zn(II) complexes

thermogravimetric investigation, which measured the subjects' weight loss at various temperatures in the heating range of 20° - 850 °C at a heating rate of 20 °C per min, was conducted using SOT Q600 V8.0 Build 95 equipment. The thermal behaviour of NET 2 SCH DSC thermal analyzer was examined. The results were recorded and looked through. SEM (Leica, Cambridge, UK).

In-vitro cytotoxicity and cell proliferation assay

HeLa cell lines were used in this analysis. The human cervical cancer cell line was donated by the National Centre for Cell Science in Pune, India (HeLa). All cell lines were grown in DMEM (Dulbecco's Modified Eagle Medium), which was enhanced with 10 % Foetal Bovine Serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin, 0.14 percent sodium bicarbonate, and 0.1 mM sodium pyruvate. The cell lines were maintained in a 37 °C, 95 percent humidity, and 5 percent CO_2 environment in a CO_2 incubator (N-Biotech). MTT (3-(4-(5,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) test was used to measure the cell viability on the Schiff base metal complex films.

The amount of bright yellow MTT changed into by cutting the tetrazolium rings into dark blue formazan is directly proportional to the number of metabolically active cells. This decline can only take place when the mitochondrial reductase enzymes are activated. On the designated days, the culture media from the scaffolds was removed and incubated with 400 L of MTT for 4 hrs at 37 °C in the dark (5 mg ml^{-1} medium). After removing the unreacted dye, 400 L of DMSO was used to dissolve the intracellular insoluble purple formazan product into a coloured solution. The absorbance of this solution was determined with a spectrophotometer set to 540 nm and an aGENios® micro plate reader.

Alkaline phosphatase assay

Cells were grown on the films in the cell toxicity experiment to determine the activity of alkaline phosphatase (ALP). The cells were homogenised in 25 mM carbonate buffer (pH = 10.3) containing 0.1 percent Triton X-100 after being rinsed with PBS solution and the necessary incubation on the specified days. The ALP activity was then assessed by incubating the pretreatment cells for 30 min at 37 °C in 250 mM carbonate buffer containing 1.5 mM $MgCl_2$ and 15 mM para-nitro phenyl phosphate (p-NPP). When ALP is present, p-nitrophenol and inorganic phosphate are created. Using a spectrophotometer's absorbance at 405 nm, the scaffolds' ALP activity was determined.

Antioxidant activity

The modified approach was used to determine the antioxidant activity of the samples by measuring their ability to scavenge DPPH (diphenyl-picryl-hydrazyl). Test samples in various amounts were incubated in 0.1 mL of an ethanol solution containing 0.1 m mol/L of DPPH (0.1 mL). After 20 min of sitting at 30 °C with vigorous stirring, the reaction mixture's absorbance at 517 nm was measured in comparison to a control. The radical scavenging activity was determined as a reduction in DPPH absorbance using the equation below:

Scavenging effect (%) = $[1 - A_{sample} / A_{control}] \times 100$ %

Catalytic activity

Cyclohexane oxidation took place in an aerobic environment. The Schiff base complex (0.05 g) was dissolved in 10 ml of acetonitrile, 10 mmol of a 30 percent hydrogen peroxide solution, and 25 ml of a flask with a magnetic stirrer and a water condenser. 5 m mol of cyclohexane was added to this reaction mixture and stirred magnetically under 70 °C and air pressure for 12 h. For the product analyses, aliquots were taken separately at 8 and 12 h. To demonstrate the significance of the catalyst and H_2O_2 in the reaction, two separate blank experiments were also conducted, one without a metal complex and the other without H_2O_2. The product samples were collected, and an HP 6890 gas chromatograph with a FID detector was used to examine them. The following parameters were used in the product analysis: HP-5 capillary column, nitrogen carrier gas, and 0.5 cm^3 min^{-1} flow rate. The cyclohexane conversion % was computed as follows:

Conversion % of Cyclohexane = 100 × [Initial %-Final%] /Initial%

Results and Discussion

FTIR studies

Figure 2(a) displays the FTIR spectrum of the carboxymethyl chitosan/p-dimethylamino benzaldehyde Schiff base. The peak at 1642 cm^{-1} confirms the Schiff base development. Figure 2(b – d) show the carboxymethyl chitosan Schiff base cobalt, nickel, and zinc complexes. The peak at 3418, 3432, and 3444 cm^{-1} is due to the stretching vibrations of the -OH and -NH atoms in the cobalt, nickel, and zinc complexes. The peak changes from 3393 cm^{-1} to

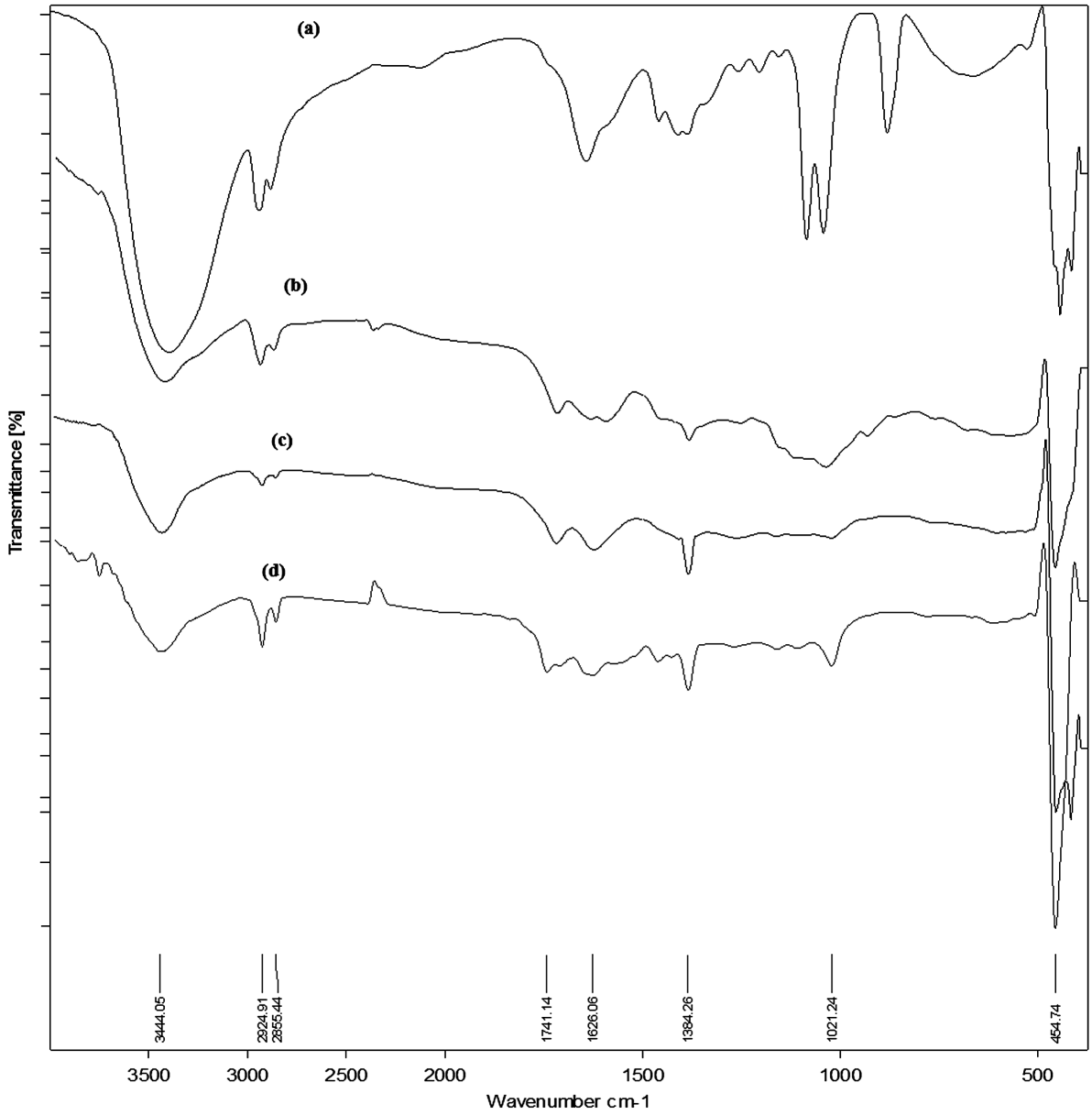

Fig. 2 — FTIR spectrum of (a) Carboxymethyl chitosan Schiff base; (b) Carboxymethyl chitosan Schiff base cobalt complex; (c) Carboxymethyl chitosan Schiff base nickel complex; and (d) Carboxymethyl chitosan Schiff base zinc complex

anywhere between 3400 and 3450 cm^{-1} as a result of complexation. The band at 400-600 cm^{-1} shows that the metal and the Schiff base complex have coordinated stretching of the M-O and M-N atoms.

XRD studies

The XRD examination is essential for determining the sample's structure, crystalline nature, and degree of polymer complexation[16]. The carboxymethyl chitosan Schiff base exhibits a weak and broad peak at $2\theta = 40°$, as seen in Figure 3(a). However, the peak shift and their intensity increased as a result of the metal's complexation with the Schiff base, as shown in Figure 3(b – d). When compared to Schiff base, the intensity of the cobalt and zinc combination peaked at $2\theta = 40°$. But for nickel complex, the summit has been moved. All of them obviously show that the metal and Schiff base have been successfully combined[17].

DSC analysis

The broad endothermic peak around 100 °C that can be seen in the Figure 4(a) of the carboxymethyl

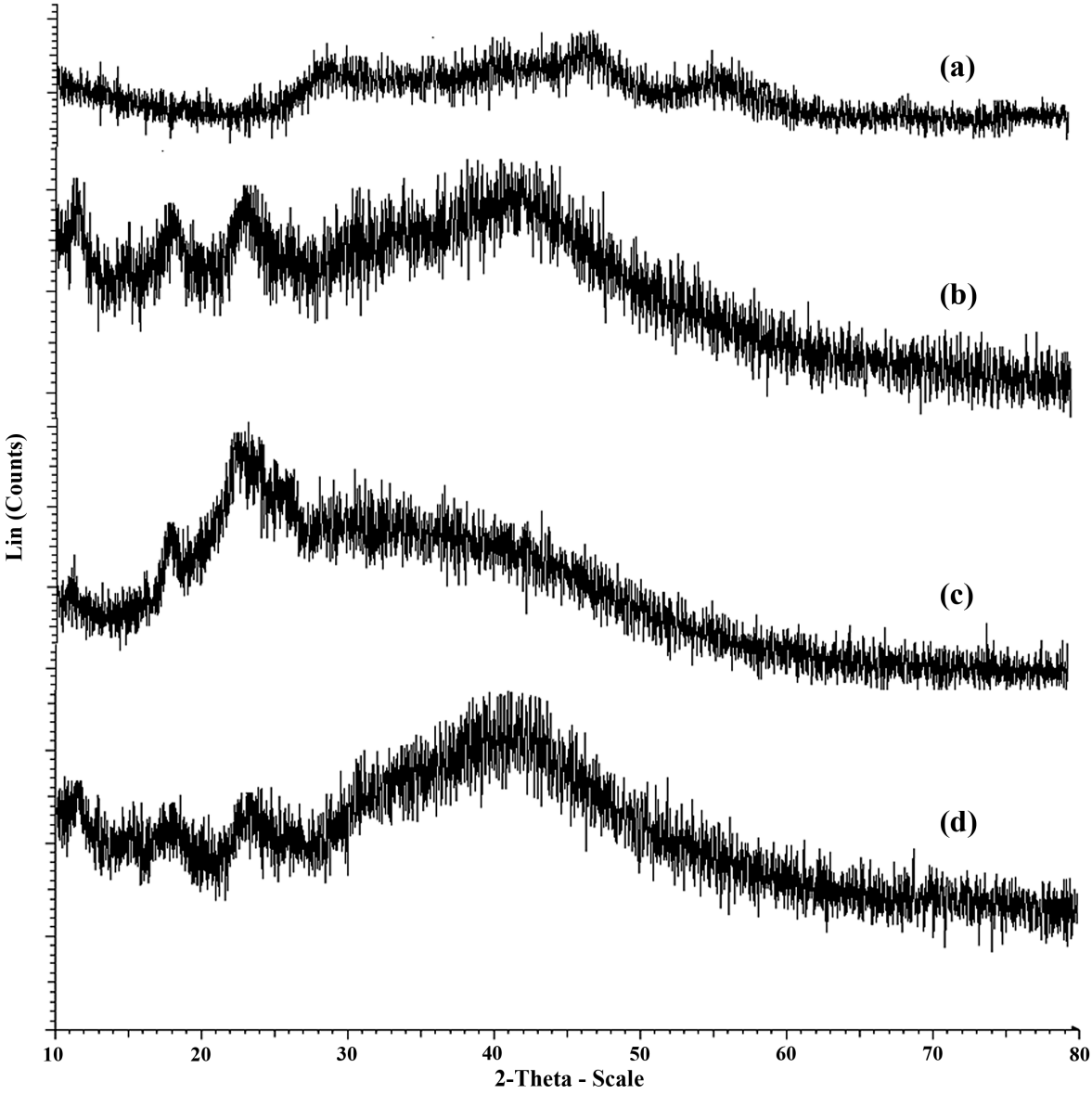

Fig. 3 — XRD pattern of (a) Carboxymethyl chitosan Schiff base; (b) Carboxymethyl chitosan Schiff base cobalt complex; (c) Carboxymethyl chitosan Schiff base nickel complex; and (d) Carboxymethyl chitosan Schiff base zinc complex

chitosan Schiff base DSC curves is caused by the evaporation of water. At 251 °C and 303 °C, respectively, there are significant exothermic and endothermic peaks. The breakdown of polymers is shown by this exothermic peak. The Schiff base sample is amorphous, as evidenced by the lack of a glass transition temperature. The DSC curves of the carboxymethyl chitosan Schiff base complexes with cobalt, nickel, and zinc were shown in Figure 4(b – d). For the Schiff base cobalt and nickel complexes, a broad endothermic peak develops at 110 °C, and a broad exothermic peak about 290 °C.

TGA analysis

The TGA thermogram for carboxymethyl Schiff base is shown in Figure 5(a); the narrow curve at 100 °C is due to adsorbed humidity. The curve at 300 °C results from the breakdown of the carboxylic group and the glucosamine group. The thermogram of the carboxymethyl chitosan schiff base cobalt, nickel, and zinc complexes is shown in Figure 5(b – d). The breakdown of glucosamine and the carboxylic group causes the three metal complexes to exhibit the same curve at about 300 °C. The breakdown of metal may be the cause of the curve at 580 °C. In contrast to the other

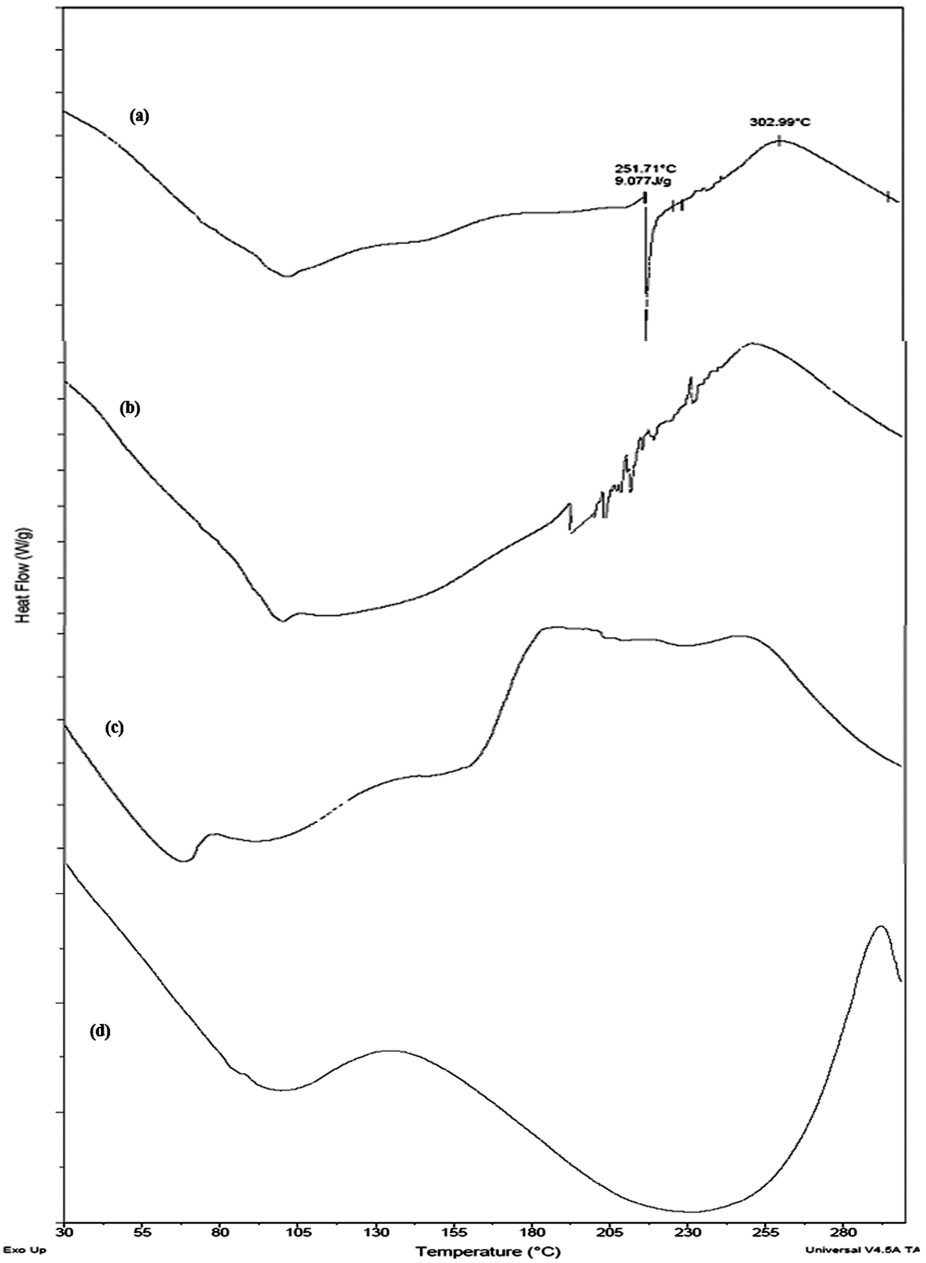

Fig. 4 — DSC thermogram of (a) Carboxymethyl chitosan Schiff base; (b) Carboxymethyl chitosan Schiff base cobalt complex; (c) Carboxymethyl chitosan Schiff base nickel complex; and (d) Carboxymethyl chitosan Schiff base zinc complex

metal complexes, which were more widely apparent, the zinc complex of carboxymethyl chitosan has a strong peak.

SEM analysis

The morphology of the materials can be predicted using the SEM analysis. Figure 6(a) displays a SEM view of the rough surface of carboxymethyl chitosan Schiff base. The rough and porous surface grew with continuous matrix on complexation with metals like cobalt, nickel, and zinc, as shown in Figure 6(b – d). Zinc complexed with carboxymethyl chitosan Schiff base exhibits needle-shaped crystals among the Schiff base metal complexes[18].

Invitro cytotoxicity

The findings of the MTT experiment used to test the cytotoxic effect of carboxymethyl chitosan Schiff

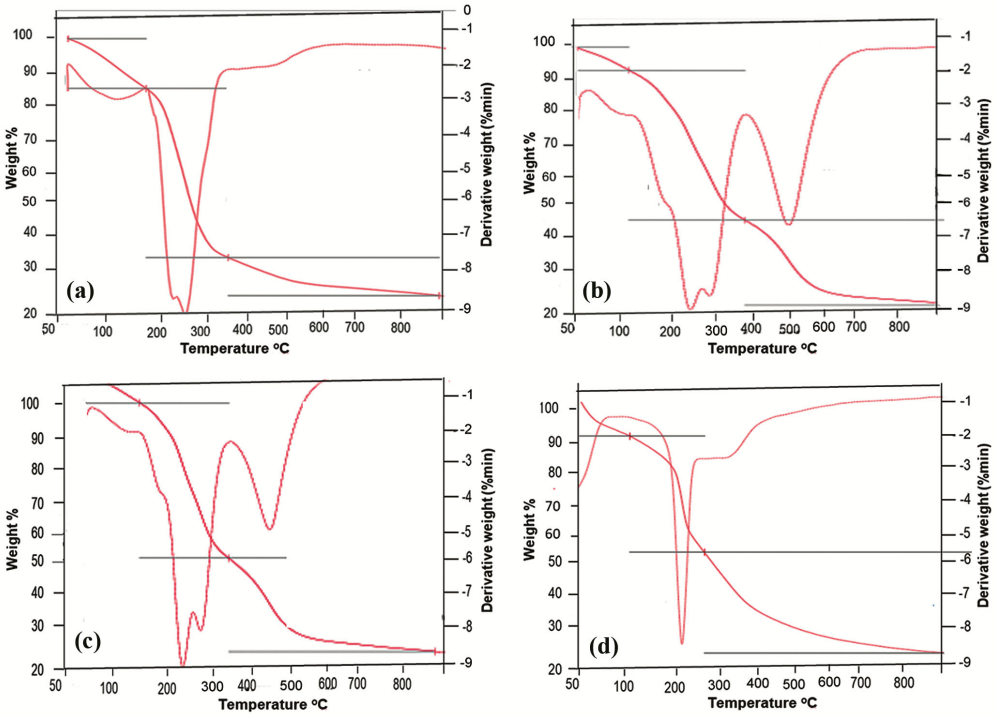

Fig. 5 — TGA thermogram of (a) Carboxymethyl chitosan Schiff base; (b) Carboxymethyl chitosan Schiff base cobalt complex; (c) Carboxymethyl chitosan Schiff base nickel complex; and (d) Carboxymethyl chitosan Schiff base zinc complex

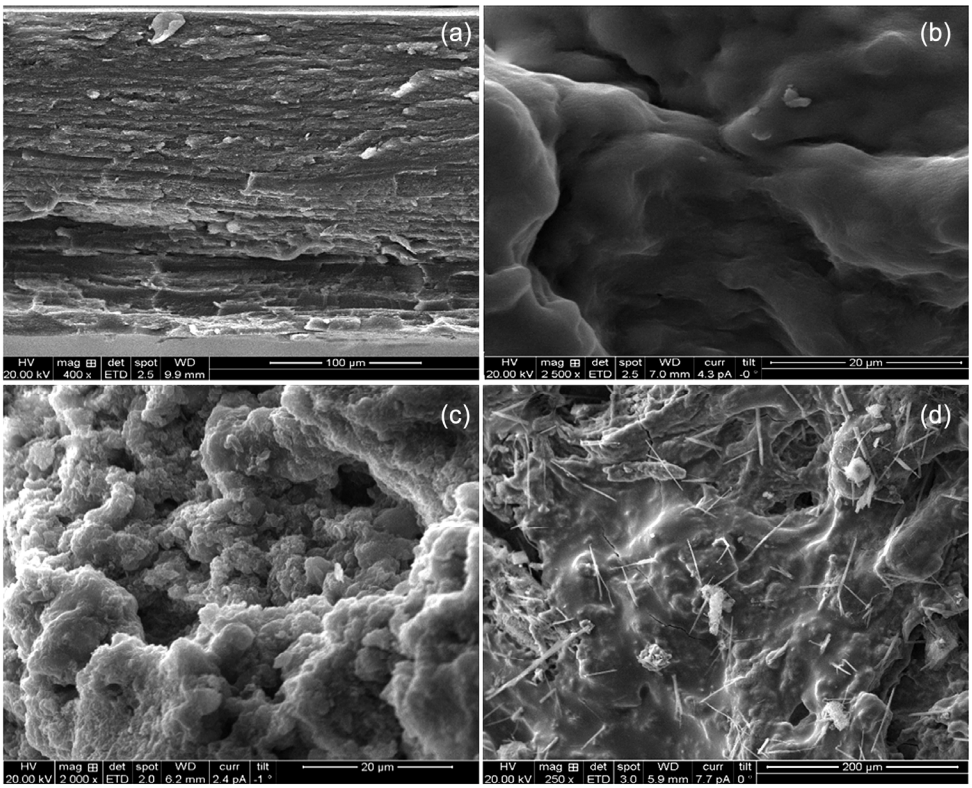

Fig. 6 — SEM image of (a) Carboxymethyl chitosan Schiff base; (b) Carboxymethyl chitosan Schiff base cobalt complex; (c) Carboxymethyl chitosan Schiff base nickel complex; and (d) Carboxymethyl chitosan Schiff base zinc complex

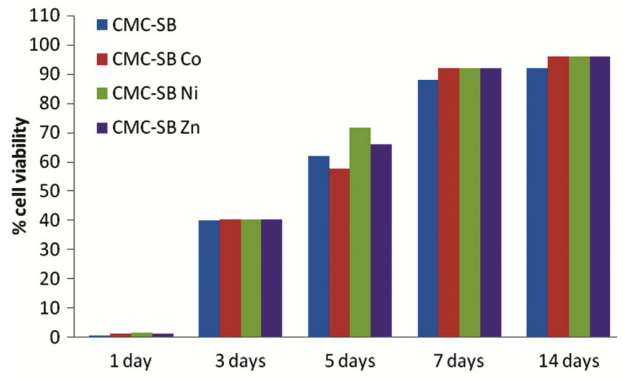

Fig. 7 — Cell proliferation assay

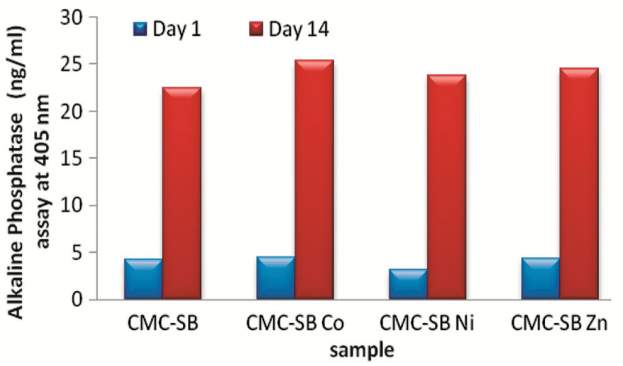

Fig. 8 — ALP activity

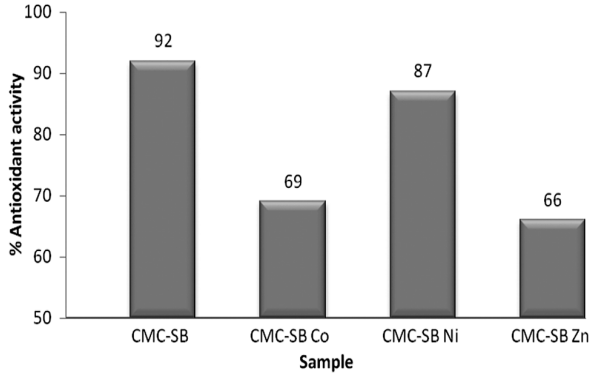

Fig. 9 — Antioxidant activities

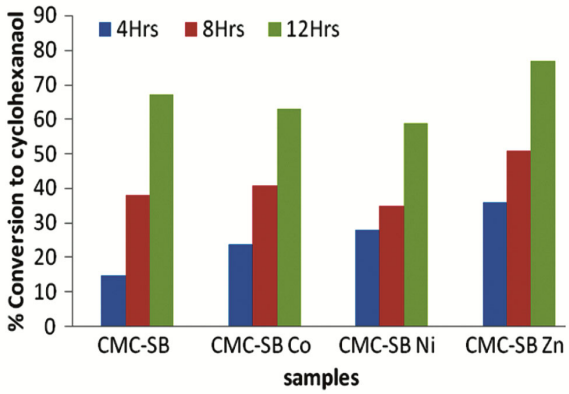

Fig. 10 — Catalytic oxidation

bases and their cobalt, nickel, and zinc complexes against human breast cancer (MCF-7) cell lines from day 1 to day 14 are shown in Figure 7. The percentage of viability also keeps growing as the days go by. The carboxymethyl chitosan Schiff base cobalt, nickel, and zinc complexes show higher survivability when compared to the carboxymethyl chitosan Schiff base[19].

Alkaline phosphatase assay

ALP activity measurements were used to investigate and display the impact of phosphorylated carboxymethyl chitosan Schiff base cobalt, nickel and zinc complexes on the MCF-7 cell line (Fig. 8). Up till day 14, it is discovered that the MCF-7 Cell Line's ALP activity increased. When compared to the other metal complexes, the carboxymethyl chitosan Schiff base cobalt complex exhibits higher activity[20].

Antioxidant activity

Antioxidants are essential for the health-protective factor since they are primarily responsible for the coexisting lipids' oxidation rate and the peroxide value of oxidation prodents. Figure 9 displays the scavenging activity of the produced Schiff bases and their cobalt, nickel, and zinc complexes. According to the findings, the Schiff base has higher antioxidant properties than metal complexes. Nickel Schiff base exhibits the highest level of activity among the metal Schiff bases. The generated samples may be employed as a suitable wound healing material in the future because they have good anti-oxidant properties[21].

Catalytic activity

In today's industries, catalytic oxidation - the conversion of cyclohexane to cyclohexanol is crucial[22]. Figure 10 displays the produced samples' catalytic activity. As the number of hours increases up to 12 h, the catalytic oxidation increases. The carboxy-methylchitosan zinc complex has better catalytic activity than the others when compared to the Schiff base. The Schiff base zinc complex has many industrial uses[23].

Conclusion

Chitosan/p-dimethyl amino carboxymethyl FTIR, SEM, DSC, TGA, and XRD were used to produce and analyse benzaldehyde metal complexes. The effectiveness of the Schiff base metal coordination's

formation is supported by all of these findings. Future industrial applications for the increased activity catalytic oxidation of zinc complex are numerous. The MTT assay and ALP activity demonstrate that the produced samples may be used in the future for medication administration. The nickel complex and Schiff base's antioxidant properties make them potential materials for wound healing. As a result, the produced material is mostly employed for biomedical applications and is highly non-toxic and compatible.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Statement

This work was not published in any mean and no endangered species are being used in this study.

Author Contributions

RS: Experimental data analysis and manuscript writing; MD: Data analysis and data interpretation; PNS: Conceptualization and editing the manuscript; TG: Manuscript formatting and result interpretation; SP: Plagiarism reduction; PM: Reference formatting.

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