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New anti-bacterial polychelates: synthesis, characterization, and anti-bacterial activities of thiosemicarbazide–formaldehyde resin and its polymer–metal complexes

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New polymeric ligand (resin) was prepared by the condensation of thiosemicarbazides with formaldehyde in the presence of acidic medium. Thisemicarbazide–formaldehyde polymer–metal complexes were prepared with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) in 1 : 2 metal : ligand molar ratio. The polymeric ligand and its polymer–metal complexes were characterized by elemental analysis, thermogravimetric analysis (TGA), FTIR, ^{13}C NMR and ^{1}H NMR. The geometry of central metal ions was conformed by electronic (UV–vis) and EPR spectra. The antibacterial activities of all the synthesized polymers were investigated against *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* and *Salmonella typhi* (Gram-negative). These compounds showed excellent activities against these bacteria using the shaking flask method. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: thiosemicarbazides; polymer–metal complexes; elemental analysis; shaking flask method.

Introduction

The synthesis of new polymers with reactive functional groups has evoked considerable interest in recent years. These polymers are synthesized, tested and used not only for their macromolecular properties but also for their properties of functional groups.[1] The functional groups containing oxygen, nitrogen, phosphorus and sulfur present in the resin matrix are capable of coordinating with different metal ions and form polymers metal complexes.[2,3] The polymer–metal complexes have found widespread applications in nuclear chemistry, pre-concentration and recovery of trace metal ions, pollution control, hydrometallurgy, polymer drug grafts and waste water treatments.[4–7] In addition they are also used as mechano-chemical systems and models for bio-inorganic systems.[8,9] Thiosemicarbazide are of considerable interest because of their chemistry to form stable chelates with essential metal ions and potentially beneficial biological activities, such as antitumor, antibacterial, antiviral and anti-malarial.[10–18] The potential biological activity of compounds containing sulfur and nitrogen may be responsible for this increased interest. Formaldehyde is an extremely reactive chemical[19] and has been proposed as a mutagenic agent[20] and as an alkylating agent by reaction with carboxyl, sulfydryl and hydroxyl groups.[21] Formaldehyde also interacts with protein,[22,23] DNA and RNA[24] *in vitro*. It has long been considered to be sporicidal by virtue of its ability to penetrate into the interior of bacterial spores.[25] The interaction with protein results from a combination with the primary amide as well as with the amino groups, although phenol groups bind little formaldehyde. Several formaldehyde-releasing agents have been used in the treatment of peritonitis.[26,27] They include noxythiolin (oxymethylenethiourea), tauroline (a condensate of two molecules of the aminosulponic acid taurine with three molecules of formaldehyde), hexamine (hexamethylenetetramine, methenamine), the resins melamine and urea formaldehydes, and imidazolone derivatives such as dantoin. All of these agents are claimed to be microbicidal on account of the release of formaldehyde. However, because the antibacterial activity of these formaldehyde-based compounds is greater than that of free formaldehyde, so the synthesis of new formaldehyde-based compounds is one way in which it may be hoped to gain a greater anti-microbial activity. The targeted synthesis of polymer–metal complexes requires an appreciation not only of the variety of possible anti-microbial activities that can be prepared but also of other potential uses. We now report the synthesis, characterization and anti-bacterial activities of thiosemicarbazide formaldehyde resin and its polymer–metal complexes As will be seen, the lower minimum inhibition (MIC) values of Cu(II)-chelated polymer and the higher thermal stability of this polymer are due to the higher stability constant.

Experimental Section

Materials and bacterial strains

Thiosemicarbazide, formaldehyde and acetic acid were purchased from s.d. Chemical Co. All solvents used in synthesis were

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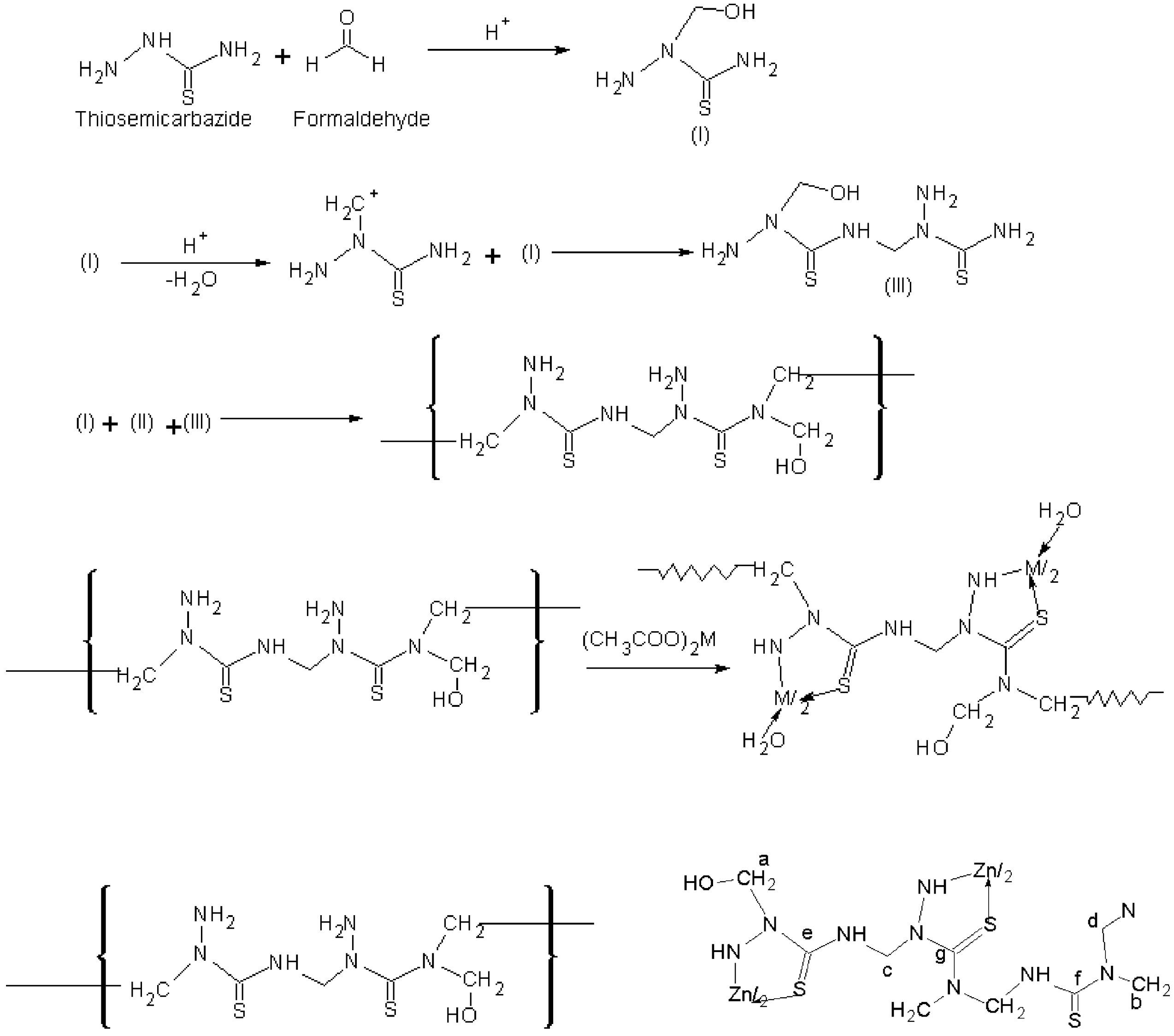
purchased from s.d. Fine Chemicals and recrystallized from methanol before use. All other chemicals were used as received. Tryptic soy agar (TSA) was purchased from Difco Laboratories. It contained 15.0 g pancreatic digest of casein, 5.0 g enzymatic digest of soybean meal, 5.0 g sodium chloride and 15.0 g agar. Tryptic soy broth (TSB) was also purchased from Difco Laboratories. It contained 17.0 g pancreatic digest of casein, 3.0 g enzymatic digest of soybean meal, 2.5 g dextrose, 5.0 g sodium chloride and 2.5 g dipotassium phosphate. Bacterial strains used for antimicrobial activity tests included *S. aureus* (IFO 2340), *B. subtilis* (IFO 24 370), *S. thypi* (IFO 3807) and *E. coli* (IFO 3628) strain. The strains were kept at −80 °C in a freezer.

Measurements

The elemental analyses of polymeric ligand and its polymer–metal complexes were carried out on a Perkin Elmer Model-2400 elemental analyzer (CDRI, Lucknow). The metal contents were determined by complexometric titration against EDTA after decomposing with concentrated nitric acid (HNO_3). The FT-IR spectra were recorded over the 4000–500 cm^{-1} range on a Perkin Elmer infrared spectrophotometer model 621 using KBr pallets. The UV–vis spectra were determined on a Perkin Elmer Lambda EZ-201 spectrophotometer using DMSO as a solvent and the magnetic susceptibility measurements of these resins were carried out on a Gouy balance using $Hg[Co(SCN)_4]$ as a celebrant. Proton and carbon-13 nuclear magnetic resonance spectra (^{1}H NMR and ^{13}CNMR) were recorded on a Jeol–GSX 300 MHz FX -1000 FT-NMR spectrometer using DMSO as a solvent and tetramethylsilane (TMS) as an internal standard. Thermal behavior of the polyester (thermogravimetric analysis, TGA) was determined using an analyzer 2000 in nitrogen atmosphere at a heating rate of 20 °C/min.



Where M= Mn(II), Co(II), and Ni(II)
M'= Cu(II) and Zn(II).

Scheme 1. Synthetic route of polymer–metal complexes.

Synthesis

Synthesis of polymeric ligand

Thiosemicarbazide (9.14 g, 0.1 mol) and 37% aqueous solution of formaldehyde (22.5 ml, 0.3 mol) were mixed in 60 ml *N,N*-dimethylformamide (DMF) in a 100 ml round-bottom flask. The flask was closed with a rubber septum, and the mixture was stirred at 40 °C for 24 h. The reaction mixture was evaporated using a rotary evaporator, and the final mixture was cooled and precipitated into deionized water. A solid light yellow product was obtained, which was dried in a vacuum oven to remove trapped solvents to give polymeric ligand (TSCFR) (14.16 g) in 73% yield. The polymeric ligand was insoluble in water, methanol, ethanol and nonpolar solvent but soluble in THF, DMF and dimethyl sulfoxide (DMSO) at room temperature.

Synthesis of polymer–metal complexes:

Polymer metal complexes were synthesized by mixing a hot solution of polymeric ligand (0.02 mol) with metal acetate (0.01 mol) in a 100 ml round-bottom flask at 40 °C for 24 h. The reaction mixture was cooled and precipitated into a 75 : 50 (v/v) water–acetone mixture. The solid colored product was filtered, and then reprecipitated from DMF into ethanol. The solid product was filtered and washed with water and ethanol, respectively. Finally, the product was dried in a vacuum oven to remove trapped solvents and gave a colored powder of polymer–metal complexes in 70–75% yield.

Anti-bacterial assessment

The antibacterial activity tests were performed using the shaking flask method[28] and the number of viable cells was counted using the spread plate method.[29] *S. aureus, B subtilis, S. typhi* and *E. coli* were streaked out on tryptic soy agar plates and incubated at 37 °C for 24 h. A representative colony was lifted off with a wire loop and placed in 5 ml of tryptic soy broth, which was then incubated with shaking at 37 °C for 24 h. At this stage, the cultures of *S. aureus, B subtilis, S. typhi* and *E. coli* each contained approximately 10^9 colony-forming units (CFU) per ml. Cultures of *S. aureus* and *E. coli* containing 10^5 CFU/ml were prepared by dilution with TSB, and these were used for antimicrobial tests. The antibacterial activities of the new polymeric ligand and its polymer–metal complexes were determined by testing 30 mg/ml concentration of the compounds against these two types of bacteria using the aforementioned methods. Only one concentration of these polymers was tested since these polymers were not soluble in TSB. The polymeric ligand and its polymer–metal complexes were in a powder form and were not soluble in water; they formed suspensions upon mixing with TSB. Each suspension containing antimicrobial agent was mixed with 10^5 CFU of the test organism in a 10 ml culture tube (Falcon). The tubes were incubated at 37 °C for 24 h. The test was repeated at least three times for each antimicrobial agent. Samples were taken from each tube and diluted with TSB. The diluted solutions were spread on agar plates and the plates were incubated at 37 °C for 24 h. The number of bacterial cells was calculated by multiplying the number of colonies by the dilution factors.

Results and Discussion

Characterization of the polymer and its polymer metal complex

The polymeric ligand was prepared according to the synthetic route shown in Scheme 1. In this reaction the protonation takes place on the oxygen atom of formaldehyde as (I) in protonation of formaldehyde the Cannizzaro reaction is favored and the polymeric ligand occurs in excellent yield. Scheme 1 shows the synthetic route for the polymer–metal complexes. The elemental analysis results of the synthesized compound are also in very good agreement with the calculated values given in Table 1. FTIR spectroscopy was used for the analysis of the polymeric ligand and its polymer–metal complexes, illustrated in Table 2. The IR spectrum of the polymeric ligand showed a very broad band in the 3520–3200 cm^{-1} region. In this region two strong bands due to asymmetric and symmetric ν N–H are generally observed near 3340 cm^{-1} for Thiosemicarbazide-formaldehyde resin TSCFR. This peak, at around 3400 cm^{-1} in the polymer–metal complexes spectrum, is broader and more intense compared with

Table 1. Elemental analysis data of the polymeric ligand and its polymer–metal complexes

Compound abbreviation	Empirical formula	Elemental analysis				
		Carbon	Hydrogen	Nitrogen	Sulfur	Metal
Metal–TSCFR	$(C_4H_{10}N_6S_2)_x$	23.30	4.85	40.77	31.06	–
		(23.28)	(4.89)	(40.76)	(31.04)	–
TSCFR–Mn(II)	$(C_4H_8N_6S_2)_x{}^{-}x\mathrm{Mn(II)}^{\bullet}2H_2O$	16.27	4.06	28.48	21.70	18.62
		(16.25)	(4.05)	(28.49)	(21.71)	(18.63)
TSCFR–Co(II)	$(C_4H_8N_6S_2)_x - x\mathrm{Co(II)}^{\bullet}2H_2O$	16.05	3.34	28.10	21.41	19.70
		(16.04)	(3.34)	(28.08)	(21.40)	(19.71)
TSCFR–Ni(II)	$(C_4H_8N_6S_2)_x - x\mathrm{Ni(II)}^{\bullet}2H_2O$	16.06	3.34	28.12	21.43	19.69
		(16.05)	(3.35)	(28.09)	(21.41)	(19.70)
TSCFR–Cu(II)	$(C_4H_8N_6S_2Cu)$	17.94	2.99	31.91	23.92	23.74
		(17.95)	(2.97)	(31.92)	(23.90)	(23.75)
TSCFR–Zn(II)	$(C_4H_8N_6S_2Zn)$	17.82	2.97	31.11	23.76	24.24
		(17.83)	(2.95)	(31.10)	(23.78)	(24.25)

x = Number of repeating units of polymeric chain. Calculated (observed) value, metal to ligand stoichiometry.

Table 2. FT-IR spectral bands with their assignments

Assignment	TSCFR	TSCFR–Mn(II)	TSCFR–Co(II)	TSCFR–Ni(II)	TSCFR–Cu(II)	TSCFR–Zn(II)
NH (*asym* and *sym*)	3460–3200(s)	3440–3320(s,b)	3435–3325(s,b)	3440–3200(s,b)	3430–3250(s,b)	3430–3200(s,b)
CH_2 (Asym and Sym)	2970–2855(s)	2940–2840(s)	2960–2850(s)	2950–2845(s)	2940–2850(s)	2940–2850(s)
C=S (thiomide)	1675(s)	1650(b)	1645(b)	1640(b)	1645(b)	1650(b)
δ HOH (water)	–	1660(s)	1655(s)	1660(w)	–	–
δ (CH) bending	1480(s)	1470(m)	1465(s)	1465(w)	1475(s)	1470(s)
δ (C–N) (oxamide)	1430(s)	1430(m)	1430(s)	1430(s)	1420(m)	1430(s)
ν M–S	–	630(s)	625(s)	625(s)	630(s)	625(b)
ν M–N	–	510(w)	500(s)	490(s)	510(m)	520(s)

s, strong; vs, very strong; m, medium; b, broad; w, weak.

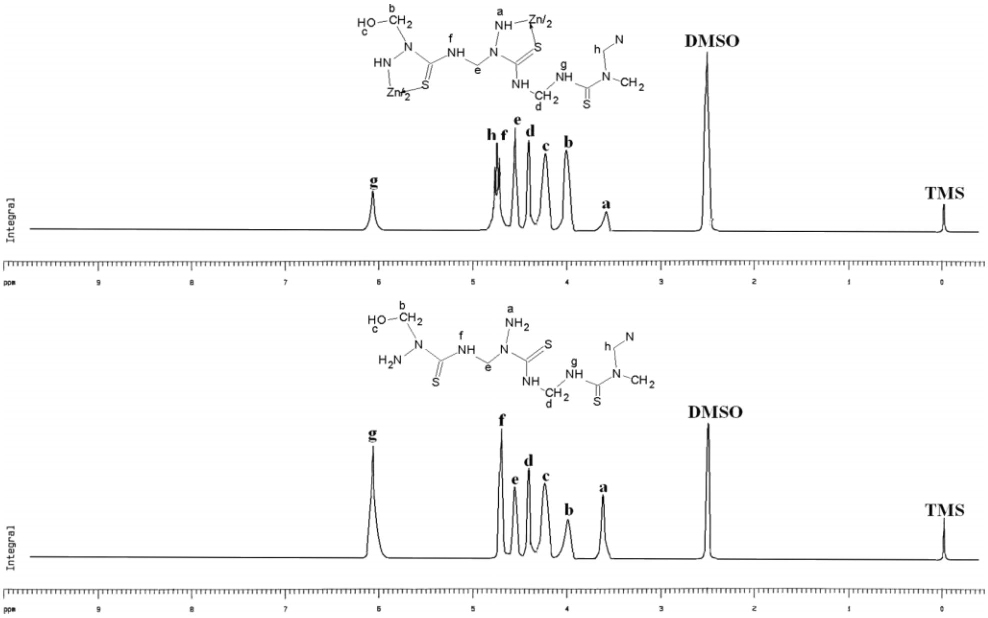


Figure 1. ^{1}HNMR spectra of TSCFR and TSCFR–Zn(II).

the peak in the polymeric ligand spectrum and indicate that the amount of NH (amine) group is lower than that of the parental ligands. This region, with a maximum broadness around 3420 and 3430 cm^{-1}, is attributed to OH stretching vibration of the terminal hydroxyl group or coordinated water molecules in TSCFR–metal complexes. Furthermore, the lowering and broadening in this region suggest the intermolecular hydrogen bonding,[30] which is possible between the sulfur of thionyl group and hydrogen of amide and amine groups in case of TSCFR. Two strong and sharp bands appear in all the synthesized polymers at 2970–2940 and 2850–2840 cm^{-1} due to the *asym* and *sym* stretching vibrations of the –CH_2 groups. In TSCFR, the ν C=S stretching frequency appears at 1675 cm^{-1} but it is shifted (25–30 cm^{-1}) to lower frequency after chelation. All the polymers give a weak absorption band around 1480–1465 cm^{-1} regions; this absorption is most likely due to the CH bending mode of CH_2-N → groups. The presence of coordinated water molecules in the Mn(II), Co(II) and Ni(II) metal complexes was further confirmed by appearance of δ HOH deformation bands in the regions 1640–1620 and 670–660 cm^{-1} for the rocking modes of coordinated water; this band was not found in the absorption spectra of the Cu(II) and Zn(II) metal complexes, which is also supported by analytical and thermal analysis data. In all the polymer–metal complexes, coordination of metal ion to the polymeric ligand was further supported by the appearance of ν M–S and ν M–N stretching vibrations in 630–620 and 510–490 cm^{-1} regions, respectively.[31]

The synthesized polymers were followed by ^{13}C NMR and ^{1}H NMR. Figure 1 shows ^{13}C NMR spectra of polymeric ligand and its Zn(II) polychelates. The chemical shifts for the formaldehyde –CH_2 groups of NH–CH_2–NH, NH–CH_2–N → and <N–CH_2–N → appear at 65.3, 75.6 and 81.2 ppm, respectively. The thionyl peak of the polymeric ligand also shifts from 180.17 to 186.09 ppm. In the polymer–metal complexes the thionyl peaks are shifted at 178, 182 and 183 ppm due to NH–CS–NH, NH–CS–N→ and <N–CS–N→ respectively. All of the other peaks of polymer–metal complexes are seen at the same chemical shifts in the polymeric ligand spectrum. ^{1}H NMR was also utilized to follow the synthesis. Figure 2 shows the ^{1}H NMR of TFRs and polymer–metal complexes. In the spectrum of the polymeric ligand, the amine and amide NH resonance shifts appear at 3.78 and 5.79 ppm, respectively. The NH bond peaks at 3.78 ppm become less intense as the reaction proceeds to complexation with metal ions. The methylene group of the synthesized complexes shows resonance signals at

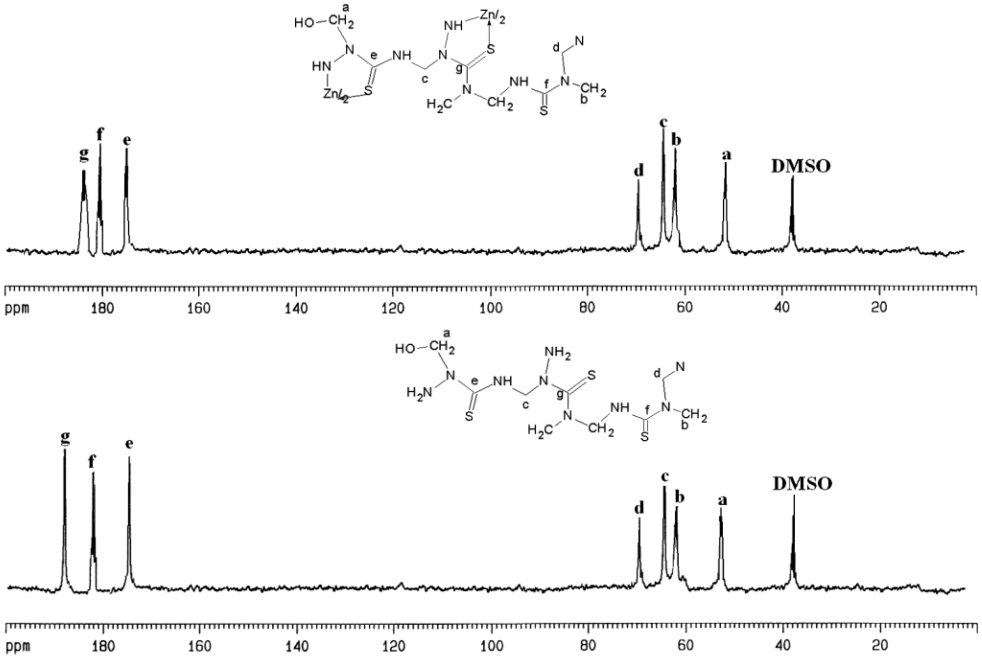


Figure 2. ^{13}CNMR spectra of TSCFR and TSCFR–Zn(II).

Table 3. Magnetic susceptibility and electronic and ESR spectral parameters of polymer–metal complexes

Abbreviation	Magnetic[a] moment (μ_{eff})	ESR g^{H}	ESR $g^{\perp}$	Electronic spectral data: Electronic transition (cm^{-1})	Electronic spectral data: Assignment	10 Dq	B'	β	β%
TSCFR–Mn(II)	5.38	2.61	2.15	24 580	$^4A_{1g}(G) \leftarrow {}^6A_{1g}(F)$	7680	642	.82	18%
				22 790	$^4T_{2g}(G) \leftarrow {}^6A_{1g}(F)$				
				18 598	$^4T_{1g}(G) \leftarrow {}^6A_{1g}(F)$				
TSCFR–Co(II)	4.89	2.32	2.25	19 590	$^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$	9620	816	.83	16%
				16 260	$^4A_{2g}(F) \leftarrow {}^4T_{1g}(F)$				
				8560	$^4T_{2g}(F) \leftarrow {}^4T_{1g}(F)$				
TSCFR–Ni(II)	2.92	2.26	2.13	24 325	$^3T_{1g}(P) \leftarrow {}^3A_{2g}(F)$	8530	868	.84	16%
				13 940	$^3T_{1g}(F) \leftarrow {}^3A_{2g}(F)$				
				9 000	$^3T_{2g}(F) \leftarrow {}^3A_{2g}(F)$				
TSCFR–Cu(II)	1.81	2.30	2.07	25 065	Charge-transfer				
				15 370	$^2A_{1g} \leftarrow {}^2B_{1g}$				

[a] Bohr magneton.

4.62, 4.71 and 4.82 ppm for NH–CH$_2$–NH, NH–CH$_2$–N → and <N–CH$_2$–N →, respectively.

The electronic spectra of all the synthesized polymers were recorded in DMSO solution. The various crystal field parameters Dq, B, β and β^0 were calculated using known equations and the values are given in Table 3. The magnetic moment of TSCFR–Mn(III) is 5.83 BM, which suggests the presence of five unpaired electrons. The electronic spectrum of this complex exhibits three absorption bands at 18 598, 22 792 and 24 580 cm^{-1}, which may be assigned to $^4T_{1g}(G) \leftarrow {}^6A_{1g}(F)(\nu_1)$, $^4T_{2g}(G) \leftarrow {}^6A_{1g}(F)(\nu_2)$ and $^4A_{1g}(G) \leftarrow {}^6A_{1g}(F)(\nu_3)$ transitions, respectively, suggesting octahedral geometry.[32] The polymer complex of Co (II) has a magnetic moment of 4.89 BM due to four unpaired electrons and shows three bands at 8570, 16 260 and 19 590 cm^{-1} due to $^4T_{2g}(F) \leftarrow {}^4T_{1g}(F)(\nu_1)$, $^4A_{2g}(F) \leftarrow {}^4T_{1g}(F)(\nu_2)$ and $^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)(\nu_3)$ transitions, respectively.[29] The TSCFR–Ni(II) complex show three bands at 9000, 13 940 and 24 325 cm^{-1} assigned to the spin-allowed transitions $^3T_{2g}(F) \leftarrow {}^3A_{2g}(F)(\nu_1)$, $^3T_{1g}(F) \leftarrow {}^3A_{2g}(F)(\nu_2)$ and $^3T_{1g}(P) \leftarrow {}^3A_{2g}(F)(\nu_3)$, respectively, which showed that nickel (II) complex has an octahedral structure.[33] The electronic spectrum of the PEODA–Cu (II) complex exhibits bands at 15 370 and 25 065 cm^{-1}, assigned to $^2A_{1g} \leftarrow {}^2B_{1g}$, and a charge transfer band that indicates a square planar geometry.[34] Thus the electronic spectra study further supports the structure proposed for the polymeric complexes.

The ESR spectrum of paramagnetic metals chelated polymers recorded in DMSO is shown in Fig. 3(a, b). The ESR spectrum provides information on the importance of studying the Cu(II) metal ion environment. The ESR spectrum of TSCFR–Cu(II) is anisotropic with resolved hyperfine structure. This anisotropic

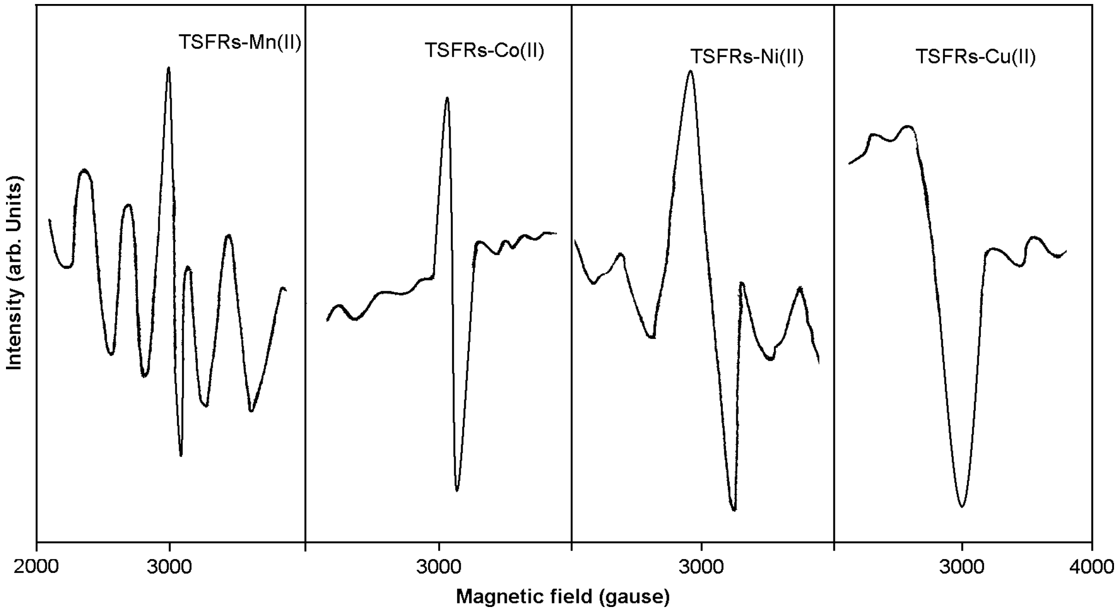


Figure 3. ESR spectra of polymer–metal complexes.

spectrum shows a $g_{\parallel} > g_{\perp}$ with the following values: $g_{\parallel} = 2.306$, $g_{\perp} = 2.0720$, in which $g_{\parallel} > g_{\perp}$. These values indicate that the ground state of Cu(II) is predominately $dx^2 - y^2$, which supports a square planar structure.[35] From the above results, it is found that the bonds between the polymer ligand and metal ion have an ionic character more than a covalent character. The covalent character of a bond becomes more pronounced when the parameters $g_{\parallel}$ and $g_{\perp}$ are decreased. The most sensitive parameter is $g_{\parallel}$; the variation in the $g_{\parallel}$ value is the best indication of the covalent character. According to Kivelson and Neimen,[35] for an ionic environment the $g_{\parallel}$ value is normally >2.3 and for the covalent character the value is less than 2.3. The g-values can be used to calculate the G-value, with this factor indicating that the ligand is a weak field or strong field ligand. The equation used is:

$$G = (g_{\parallel} - 2.002)/g_{\perp} - 2.002$$

Where G is less than 4.0, the ligand forming Cu^{2+} complex is regarded as a strong field ligand.[36] In these resins, the G-value is 5.114, indicating that the resin forms a weak field ligand.[37]

Figure 4 shows the TGA thermograms of all the synthesized polymers and the data are tabulated in Table 4. The initial 2–3% weight loss for the polymeric ligand is 125–150 °C. This decomposition is mainly due to absorbed water and other solvents. Above 200 °C a rapid weight loss was observed in the polymeric ligand and 60% of weight was lost up to 310–400 °C; the ligand decomposed completely into volatile products up to 500 °C, where the complexes were not completely decomposed up to 800 °C. For Mn(II) and Co(II) complexes, initial 4–6% weight loss up to 150–160 °C corresponded to the theoretical value for two coordinated water molecules.[38] Therefore, it is suggested that the coordinated water molecules are lost up to this temperature range. In the case of TSCFR–Ni(II), the weight corresponding to two water molecules was gradually lost up to 225 °C. It is suggested that Ni(II) complexes did not suddenly lose all the coordinated water molecules present in the polymer–metal complexes. The Cu(II) and Zn(II) complexes did not show any further weight loss up to 380 °C, suggesting the absence of any coordinated water molecules. After the loss of coordinated water molecules, the polymer–metal complexes showed two-step degradation, where the first step was faster than the second. This may be due to the fact that the non-coordinated part of the complexes decomposes first, while the actually coordinated part of all the polymer–metal complexes decomposes later.[39] The results of thermogravimetric analysis revealed that the polymer complex

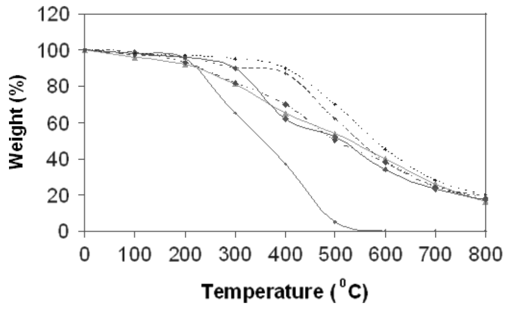


Figure 4. TGA spectra of polymer–metal complexes.

Table 4. Thermal behaviors of chelated polyester their metal complexes

	Temperature (°C) corresponding to weight loss of					
Materials	100	200	300	400	600	800
TSCFR	98.4	95.3	65	37	0	0
TSCFR–Mn(II)	96	92	81.3	65.8	40	11.4
TSCFR–Co(II)	98.5	93	82	70	38	18
TSCFR–Ni(II)	98	96.2	90	62.4	34.6	17
TSCFR–Cu (II)	98	97	95	90	45	20
TSCFR–Zn(II)	97	96	90.4	87	38	19.6

of Cu(II) is comparatively more thermally stable than the other complexes. The thermal stability of TSCFR–Cu(II) is higher owing to the higher stability constant of Cu(II) ions. The order of stability on the basis of thermal residual weight at 800 °C appears to be TSCFR–Cu(II) > TSCFR–Zn(II) > TSCFR–Ni(II) > TSCFR–Co(II) > TSCFR-Mn(II), this order matching Irving and Williams's order of stability for the complexes of divalent metal ions.[40]

Antimicrobial assessment

All the polymers were tested for anti-bacterial activity against *S. aureus* and *B. subtilis* (Gram-positive) and *S. typhi iand E. coli* (Gram-negative) bacteria, and the results MICs are given in Table 5 and Figure 5. The shaking flask method was utilized here in order to determine the antimicrobial activities of the polymeric ligand and its polymer–metal complexes. According to this method, each antimicrobial agent (30 mg/ml) was mixed with a certain number of bacteria (1×10^5 CFU/ml) in a flask (culture tube) and the flask was incubated at 37 °C for 24 h. Then, a 100 μL sample was taken from each tube and spread onto agar plates. The agar plates were incubated at 37 °C for 24 h, and the number of viable bacteria on the plates was counted at the end of the incubation period. The number of viable bacteria and percentage reduction of the number of bacteria are shown in Fig. 4. Table 5 shows the MICs evaluated by the conventional spread plat method. The two figures for each strain indicate the range of MICs: growth of the bacterium cold be seen as visible colonies below the lower concentration limit of MIC, whereas no colonies were observed above the higher limit. Consequently, the exact MIC is supposed to lie between these two values. A general trend can be seen from the table that the Cu(II)-chelated polymer complexes were more active then other metal chelated polymers. It is also evident that all the polymer–metal complexes were more active than the polymeric ligand. The lethal action of formaldehyde biocides is an outcome of their ability to inhibit the microbes. Formaldehyde is an extremely reactive chemical that interacts with protein, DNA and RNA, resulting from a combination with the primary amide as well as with the amino groups, although phenol groups bind little formaldehyde. Formaldehyde also reacts extensively with nucleic acid[41] (e.g. the DNA of bacteriophage T2[42]). As pointed out above, it forms protein–DNA cross-links in SV40, thereby inhibiting DNA synthesis.[43] Low concentrations of formaldehyde are sporostatic and inhibit germination.[44] Formaldehyde alters the HBsAg and HBcAg of HBV.[45]

Table 5. Antibacterial activity of polymeric ligand and its metal complexes

	MIC[a] (μg/ml)			
Abbreviation	E. coli	B. subtilis	S. aureus	S. typhi
TSCFR	250–350	250–350	>500	>600
TSCFR–Mn(II)	225–350	225–350	260–350	230–415
TSCFR–Co(II)	225–350	225–350	>500	230–415
TSCFR–Ni(II)	220–325	200–325	250–325	230–415
TSCFR–Cu(II)	200–300	200–250	140–226	140–225
TSCFR–Zn(II)	215–325	200–250	150–226	230–350

[a] Minimum inhibitory concentration. Determine by spread plat method.

It is difficult to pinpoint accurately the mechanism(s) responsible for formaldehyde-induced microbial inactivation. Clearly, its interactive, and cross-linking properties must play a considerable role in this activity. Most of the other aldehydes (glutaraldehyde, glyoxyl, succinaldehyde and *o*-phthalaldehyde) that have sporicidal activity are dialdehydes (and of these, glyoxyl and succinaldehyde are weakly active). The distance between the two aldehyde groups in glutaraldehyde (and possibly in *o*-phthalaldehyde) may be optimal for interaction of these CHO groups in nucleic acids and especially in proteins and enzymes.[46]

There are a few possibilities for the cleavage of formaldehyde or active groups from the polymers. One is that the Canizaro reaction can be reversed to release formaldehyde. Polymer–metal

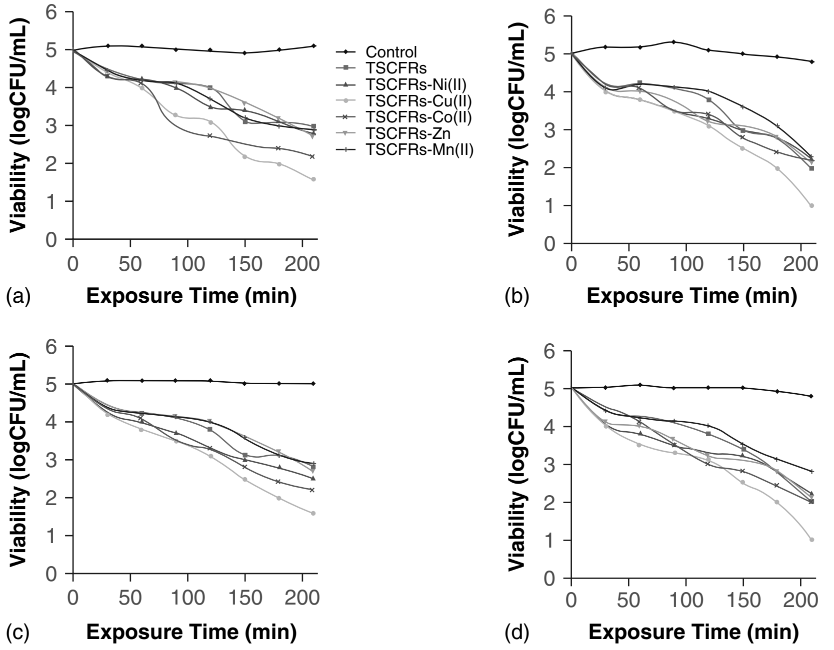


Figure 5. Antimicrobial activity against (a) *E. coli*, (b) *B. subtilis*, (c) *S. aureus* and (d) *S. typhi*.

complexes are not dangerous to eukaryotic cells at low concentrations. The new polymers containing metal complexes are not expected to have toxicities to humans; however, a toxicity study for these polymers should be carried out before their use *in vivo*. Although allergies may develop to formaldehyde, in general, this should not be a concern for formaldehyde-based polymers. Formaldehyde-based polymers cause fewer allergic responses compared with the formaldehyde monomers; however, this issue should also be clarified before any internal use of these polymers.

Conclusions

The new polymeric ligand (TSCFR) has been synthesized by condensation polymerization. The polymeric ligand also coordinated with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) to give polymer–metal complexes. All the synthesized polymers showed excellent antimicrobial activities against *S. aureus, B. subtilis, S. typhi* and *E. coli*. These results indicate that the polymer–metal complexes show more antibacterial activity than the polymeric ligand. The Cu(II) chelated polymers show more antibacterial activity and more thermal stability than other polymers due to its higher stability constant. Since these agents are relatively stable to high temperatures, they can be used for medical and biomaterial applications requiring thermal sterilization.

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