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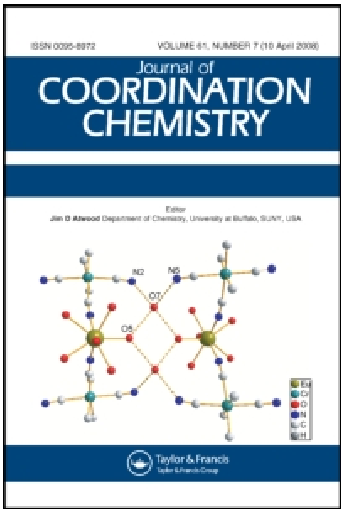

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Antimicrobial polyesters containing Schiff-base metal complexes

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Antimicrobial polyesters containing Schiff-base metal complexes (PSB) were prepared by polycondensation of adipoyl chloride with chelated Schiff-base diol {*bis*-(2-hydroxy-5-methylol-benzaldehyde)ethylenediamine}. All the metal chelated polyesters were characterized by elemental analysis, UV–Visible, FTIR, ^{13}C and ^{1}H NMR spectra and thermogravimetric analysis. The analytical data of the polyesters agreed with 1 : 1 molar ratio (metal chelated diols to adipoyl chloride). The geometry of the chelated polyesters was confirmed by magnetic susceptibility measurements and UV–Visible spectroscopy. The thermal behaviors of these chelated polyesters were studied by TGA (Thermogravimetric analyzer) in a nitrogen atmosphere up to 800°C. The TGA results revealed that the Cu(II) chelated polyester has better heat resistant properties than the other polyesters. The antimicrobial properties of these polyesters were investigated with agar diffusion methods against selected microorganisms *Bacillus subtelillis*, *Bacillus megaterium*, *Streptococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella boydii* and for antifungal activity against *Candida albicans*, *Trichophyton longifusus*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Microsporum canis*, *Puccinia graminis*. The antimicrobial activity of these polyesters was higher than standard drugs *Kanamycin* and *Miconazol*.

Keywords: Polyester; Schiff base; Ethylenediamine; Thermogravimetric analysis

1. Introduction

Interest in coordination polymers has arisen for enhanced electronic, magnetic, optic, sensor and catalytic properties [1–3]. Coordination polymers containing metal ions play an important role in inhibiting growth of microbes. Antimicrobial coordination polymers have been reported by complexation of polymeric ligand with metal ions as well as by polymerization of monomeric metal complexes [4–6]. Metal ions inhibit the growth of bacterial cells by affecting protein synthesis or by binding the ribosomal subunits of the pathogens [7]. An alternative route was adopted for synthesis of coordination polymers by using metal complexes containing bifunctional chain extender groups such as Schiff-base complexes [8], crown ether complexes [9] and ferrocene complexes [10]. Several Schiff-base containing metal has been synthesized due to their biological, pharmacological, clinical and analytical importance [11].

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Schiff-base polymers can be easily processed to suitable materials for biomedical applications as antibacterial and antifungal agents [12, 13]. Antimicrobial polymers enhance the efficiency of existing antimicrobial agents and minimize the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity. We have modified monomeric metal complexes into polymers with superior properties over conventional organic polymers.

In the present study we have synthesized Schiff-base diol {*bis*-(2-hydroxy-5-methylol-benzaldehyde)ethylenediamine} by reaction of methylolsalicylaldehyde and ethylenediamine to prepare new metal chelated polyesters of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) acetates. The polyesters containing Schiff-base metal complexes (PSB) were prepared by polycondensation of adipoyl chloride with chelated Schiff-base diol. The spectral, magnetic and thermal properties and geometry around the metal are discussed. The synthesized polyesters were also screened for antimicrobial activity against selected microorganisms *B. subtelillis*, *B. megaterium*, *S. aureus*, *E. coli*, *S. typhi*, *P. aeruginosa*, *S. boydii* and for antifungal activity against *C. albicans*, *T. species*, *A. flavus*, *A. niger*, *F. species*, *M. species*, *P. species*.

2. Experimental

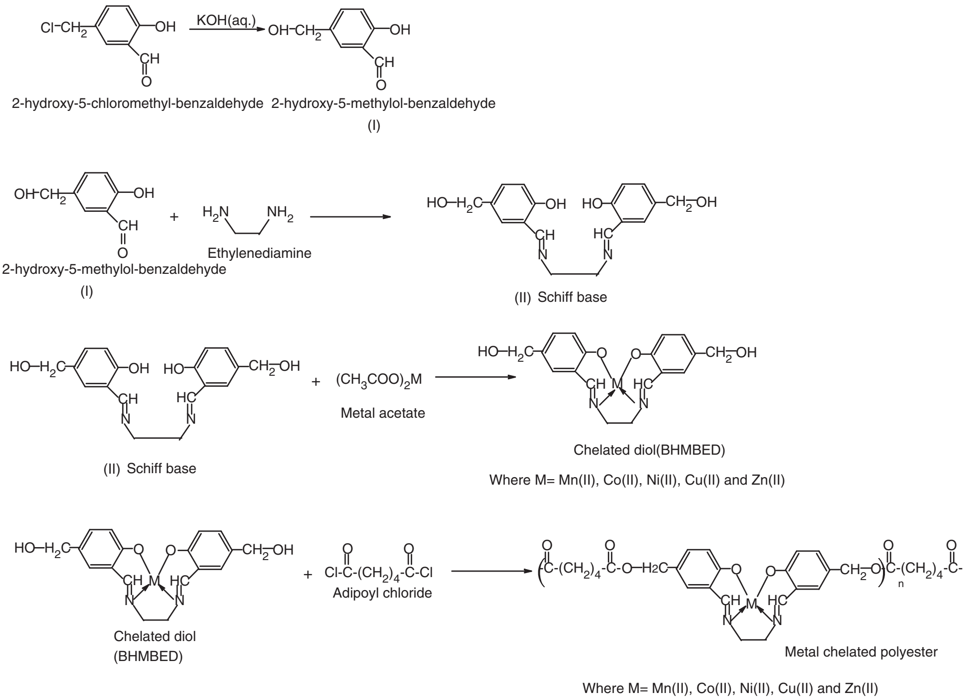
2.1. *Materials*

Salicylaldehyde, ethylenediamine; adipoyl chloride (Merck, India); potassium hydroxide, hydrogen chloride; formaldehyde (37% aqueous solution); manganese(II) acetate tetrahydrate; $Mn(CH_3COO)_2 \cdot 4H_2O$; copper(II) acetate monohydrate, $Cu(CH_3COO)_2 \cdot H_2O$; nickel(II) acetate tetrahydrate, $Ni(CH_3COO)_2 \cdot 4H_2O$; cobalt(II) acetate tetrahydrate, $Co(CH_3COO)_2 \cdot 4H_2O$; and zinc(II) acetate dihydrate, $Zn(CH_3COO)_2 \cdot 2H_2O$ (s.d. Fine Chem. India. Ltd.) were of analytical grade and used as received. DMF, DMSO, ethyl alcohol, methanol, acetone (Merck, India) etc. were purified by standard procedures before use. The strains were kept at −80°C in a deep freezer. 2-Hydroxy-5-chloromethyl benzaldehyde was prepared and purified according to a procedure described [14]. 2-Hydroxy-5-methylol benzaldehyde was prepared by hydrolysis of 2-hydroxy-5-chloromethyl benzaldehyde in alkaline solution according to scheme 1.

2.2. *Synthesis*

2.2.1. Synthesis of Schiff-base diol {*bis*-(2-hydroxy-5-methylol-benzaldehyde) ethylenediamine}. The Schiff-base diol was prepared by mixing 5-methylolsalicylaldehyde (6.08 g, 0.04 mol) and ethylenediamine (0.68 mL, 0.02 mol) in 50 mL of methanol. The mixture was refluxed at 80°C for 3 h. After that, excess solvent was removed and the mixture was poured into ice-cooled water to precipitate the Schiff base. Obtained precipitate was filtered, washed with water and petroleum ether, and dried in vacuum at 70°C for 2 h. The product was a yellow powder in 80% yield (2.62 gm).

^{1}H NMR-(300 MHz, DMSO, δ) 13.10 (s, 2H, OH), 7.52–6.87 (m, 14H, Ar–H), 4.82 (s, 2H, OH); 8.26 (s, 2H, CH=N); 4.01–3.60 (m, 8H, Ar–CH_2–, =N–CH_2); FT-IR



Scheme 1. Reaction scheme of metal chelated polyester.

(KBr pellets $\nu_{(\max)}$ cm^{-1}) 3324–3235, 3055, 2962–2850, 1630, 1502, 1265. Anal. Calcd [$C_{18}H_{20}N_2O_4$]%: C, 65.83; H, 6.13; N, 8.53. Found: C, 65.80; H, 6.14; N, 8.51.

2.2.2. Preparation of Schiff-base metal complexes. Metal chelated diols of M(II) [M(II)=Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] were synthesized by reaction of Schiff-base diol (BHMBED) with metal acetate in 1 : 1 molar ratio (for example 3.28 g 0.01 mol of Schiff-base diol and 1.99 g. 0.01 mol of copper acetate monohydrate using methanol as a solvent). This mixture was refluxed at 60°C for 6 h with continuous stirring. The resulting brown precipitate was filtered, washed with water and ethanol, and dried at 60°C in vacuum for 2 h. A similar procedure was adopted for preparation of all chelated diols.

^{1}H NMR-(300 MHz, DMSO, δ) 5.38 (s, 2H, OH), 7.30–6.74 (m, 14H, Ar–H); 8.31 (s, 2H, CH=N); 4.51–4.20 (8H, Ar–CH_2–, =N–CH_2); FT-IR (KBr pellets $\nu_{(\max)}$ cm^{-1}) 3314–3245, 3050, 2960–2850, 1625, 1500, 1265, 748, 620, 540. Anal. Calcd [($C_{18}H_{18}N_2O_4$)Cu(II)]%: C, 55.50; H, 4.66; N, 7.78; Cu, 17.73. Found: C, 55.48; H, 4.65; N, 7.80; Cu, 17.72.

2.2.3. Synthesis of metal chelated polyester. Adipoyl chloride (3.6 g, 0.2 mol) was added dropwise slowly through a dropping funnel to 0.2 mol of metal chelated diol [(Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] in 100 mL DMF in a three-necked round bottom flask with a water-cooled condenser. The reaction mixture was continuously stirred at 40°C for 36 h and progress was monitored by thin layer chromatography (TLC). The reaction mixture was precipitated with excess methanol and the precipitated metal polyester was filtered, washed thoroughly with water and then methanol, and dried in vacuum at 60°C for 5 h.

2.3. *Antimicrobial assessment*

The antimicrobial activities of the metal chelated polyesters were tested against different microorganisms in DMSO. The sample concentrations were 50 and 100 $\mu g\,mL^{-1}$ for anti-bacterial and anti-fungal studies, respectively. Bacterial strains were nourished in nutrient broth (Difco) and yeasts in malt extract broth (Difco) and incubated for 24 and 48 h, respectively. According to agar diffusion method, bacteria were incubated on Mueller-Hinton Agar and yeast on Sabouraud dextrose agar. Wells were dug in the media with a sterile steel borer and 0.1 mL of each sample was introduced in the corresponding well. Other wells were supplemented with solvent (DMSO) for positive control and standard drug *viz Kanamycin* (anti-bacterial) and *Miconazol* (anti-fungal) for negative control. The resulting zones of inhibition on the plates were measured in mm.

2.4. *Measurements*

Microanalyses of metal chelated polyesters were carried out on a Perkin Elmer Model-2400 elemental analyzer (CDRI, Lucknow). The metal contents were determined by complexometric titration [15] against EDTA after decomposing with a mixture of perchloric acid, sulfuric acid and concentrated nitric acid (HNO_3) [1 : 1.5 : 2.5]. FT-IR spectra were recorded over the 4000–500 cm^{-1} range on a Perkin Elmer infrared spectrophotometer model 621 using KBr pellets. UV–Visible spectra were carried out on a Perkin Elmer Lambda EZ-201 spectrophotometer using DMSO. Proton and carbon-13 nuclear magnetic resonance spectra (^1H NMR and ^{13}C NMR) were recorded on a JEOL–GSX 300-MHz FX–1000 FT NMR spectrometer using DMSO; tetramethylsilane (TMS) was an internal standard. Thermal behaviors of the polyesters {thermogravimetric analysis (TGA) and differential scanning calorimetric analysis (DSC)} were carried out on a TG analyzer 2000 in nitrogen with heating rates of 20°C/min and 10°C/min. The solubility of metal chelated polyesters was tested in various solvents at room temperature. Antimicrobial tests of synthesized metal chelated polyesters were carried out in the Department of Microbiology, JNU, New Delhi.

3. Results and discussion

3.1. *Chemistry and composition*

The chelated polyesters were synthesized by condensation polymerization of a diol, and carboxylic acid or carboxylic acid chlorides (scheme 1). The chelated polyesters were colored solids, soluble in DMSO and DMF but insoluble in common organic solvents. The molecular weight of the chelated polyester could not be determined by Gel Permeation Chromatography (GPC) due to their insolubility in common organic solvents. Analytical data of the metal chelated polyesters are given in table 1, indicating

Table 1. Elemental analysis of chelated polyesters.

		Elemental analysis			
Abbreviations	Yield in (%)	Carbon	Hydrogen	Nitrogen	Metal
PSB-Mn(II)	75	58.66 (58.65)	4.92 (4.90)	5.70 (5.72)	11.18 (11.20)
PSB-Co(II)	75	56.37 (56.36)	4.73 (4.74)	5.48 (5.47)	11.52 (11.51)
PSB-Ni(II)	74	56.39 (56.37)	4.73 (4.75)	5.48 (5.50)	11.48 (11.51)
PSB-Cu(II)	73	55.86 (55.87)	4.69 (4.66)	4.53 (4.52)	12.32 (12.33)
PSB-Zn(II)	75	55.66 (55.65)	4.67 (4.70)	5.41 (5.43)	12.63 (12.60)

Calculated, (found) values.

Table 2. IR spectral bands and their assignments of chelated polyesters.

	Assignments								
Compounds	νN–H	νAr–CH	-CH (sym-asym)	νC=O	νC=N	νC=C	νC–N	νM–O	νM–N
PSB-Mn(II)	3300,3210(b)	3050(m)	2930–2860(m)	1710(s)	1600(s)	1560(m)	1540(m)	625(s)	520(s)
PSB-Co(II)	3300,3220(b)	3055(m)	2930–2865(m)	1710(s)	1630(s)	1550(m)	1050(s)	640(m)	525(s)
PSB-Ni(II)	3300,3215(b)	3055(m)	2930–2860(m)	1710(s)	1605(s)	1545(m)	1055(s)	630(s)	520(s)
PSB-Cu(II)	3305,3210(b)	3052(b)	2930–2865(m)	1710(s)	1630(s)	1565(s)	1050(m)	645(s)	520(m)
PSB-Zn(II)	3302,3220(b)	3055(m)	2932–2860(m)	1710(s)	1600(s)	1565(m)	1060(m)	635(m)	530(s)

s, strong; b, broad; m, medium; w, weak; sym, symmetric; asym, asymmetric.

1 : 1 molar ratio (metal chelated diols to adipoyl chloride). The analytical data are supported by FT-IR and TGA data.

3.2. *Characterization*

3.2.1. FTIR spectra. Significant IR bands of the chelated polyesters with their assignments are given in table 2. The presence of methylene in all the metal chelated polyesters was confirmed by two strong bands at 2930 and 2860 cm^{-1} due to νCH_2 symmetric and asymmetric stretching and a band at 1422 cm^{-1} due to δCH_2 bending. Bands at 1630–1600 cm^{-1} due to the azomethine group of Schiff base indicate participation of azomethine in coordination with metal ions [16]. In all the synthesized polyesters absorption bands around 1275 and 1160 cm^{-1} are due to the polyester [17]. The appearance of these two bands confirmed the polymerization of chelated diols with adipoyl chloride. The band at 890–785 cm^{-1} can be attributed to substituted phenyl. νM–O and νM–N stretching vibrations at 645–625 cm^{-1} and 530–520 cm^{-1}, respectively [18], further support participation of oxygen and nitrogen.

3.2.2. ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectra of Zn(II) chelated polyester in DMSO-d^6 is shown in Supplemental Material. The spectra show a peak for the

Table 3. Magnetic moments and electronic spectral data of chelated polyesters.

Abbreviations	Magnetic moment in B.M.[a]	Electronic spectral data		Geometry
		Electronic transition (cm^{-1})	Assignment	
		19980	$^4T_1(\mathrm{G}) \leftarrow {}^6A_1$	
PSB-Mn(II)		21170	$^4T_2(\mathrm{G}) \leftarrow {}^6A_1$	Tetrahedral
	5.10	23408	$^4A_1(\mathrm{G}) \leftarrow {}^6A_1$	
PSB-Co(II)		9035	$^4T_2(\mathrm{F}) \leftarrow {}^3A_2$	Tetrahedral
	3.46	16120	$^4T_1(\mathrm{P}) \leftarrow {}^3A_2$	
PSB-Ni(II)	Diamagnetic	16984	$^1A_{2g} \leftarrow {}^1A_{1g}$	Square planar
		23065	$^1B_{1g} \leftarrow {}^1A_{1g}$	
PSB-Cu(II)	1.82	17675	$^2A_{1g} \leftarrow {}^2B_{1g}$	Square planar
		19595	$^2E_g \leftarrow {}^2B_{1g}$	
		25236	Charge-transfer	
PSB-Zn(II)	Diamagnetic			Tetrahedral

[a] Bohr megneton.

azomethine group (CH=N) at 8.12 ppm. Resonances at 1.65 and 2.2 ppm were attributed to methylene protons of adipoyl, while methylene protons of N–CH_2–CH_2- and Ar–CH_2–O- are at 3.71 ppm and 5.25 ppm, respectively. Resonances at 6.8 to 7.45 ppm are due to aromatic protons. The number of protons from integration and from CHN analyses were in good agreement.

The ^{13}C NMR spectra of Zn(II) chelated polyester (shown in Supplemental Material) show resonances at 164 ppm and 174 ppm due to the carbonyl of azomethine and ester, respectively. The methylene carbon of Ar–CH_2–O was at 68.3 ppm. The methylene carbons of adipoyl showed signals from 24–33.6 ppm. Aromatic carbons produced a very broad signal in the region 119.1–162.4 ppm [19]. A resonance at 61.8 ppm was assigned to methylene carbon of N–CH_2–CH_2- group.

3.2.3. Magnetic moments and electronic spectra. The electronic spectra of 10^{-3} M solution of the chelated polyesters were recorded in DMSO. Magnetic susceptibility measurements of these polyesters are given in table 3. The magnetic moment of PSB-Mn(II) complexes was 5.10 B.M., suggesting five unpaired electrons. The electronic spectra of PSB-Mn(II) showed bands at 19980, 21170 and 23408 cm^{-1} due to $^4T_1(\mathrm{G}) \leftarrow {}^6A_1$, $^4T_2(\mathrm{G}) \leftarrow {}^6A_1$ and $^4A_1(\mathrm{G}) \leftarrow {}^6A_1$ transitions, respectively, consistent with tetrahedral environment around Mn(II) [20]. The magnetic moment of PSB-Co(II) complex was 3.46 B.M., indicating three unpaired electrons in a tetrahedral environment [21]. The electronic spectra of the PSB-Co(II) complex showed bands at 16120 and 9035 cm^{-1}, assigned to $^4T_1(P) \leftarrow {}^3A_2$ and $^4T_2(F) \leftarrow {}^3A_2$ transitions, respectively, of a tetrahedral cobalt [22]. The diamagnetic PSB-Ni(II) complex exhibited bands at 16984 and 23065 cm^{-1} due to $^1A_{2g} \leftarrow {}^1A_{1g}$ and $^1B_{1g} \leftarrow {}^1A_{1g}$ transitions, respectively, of a square-planar Ni(II) [23]. The magnetic moment of PSB-Cu(II) complex was 1.82 B.M., consistent with square planar geometry. Electronic spectra of PSB-Cu(II) showed two lower energy weak bands at 17675 and 19595 cm^{-1} and a strong high energy band at 25236 cm^{-1}. The low energy bands were expected for square planar configuration and may be assigned to $^2A_{1g} \leftarrow {}^2B_{1g}$ and $^2E_g \leftarrow {}^2B_{1g}$ transitions, respectively, the high energy band assigned to charge transfer spectra.

Table 4. Thermal behaviors of chelated polyesters.

Materials	Tg (°C)	Temperature (°C) corresponding to weight loss of					Char. (%) weight at 800 °C
		100	200	300	400	600	
PSB-Mn(II)	198	98	96	86	75	62	30%
PSB-Co(II)	208	97	84	80	72	60	32%
PSB-Ni(II)	210	98	90	87	74	58	30%
PSB-Cu (II)	235	97	92	86	75	64	38%
PSB-Zn(II)	212	98	90	84	73	62	34%

Tg (°C) – glass transition temperature.

3.2.4. Thermal properties. Thermal decompositions (Supplementary Material and table 4) show two steps. The first decomposition can be correlated to the decomposition of the non-coordinated part of the polymer while the second decomposition is the coordinated polymer [24]. The TGA trace of PSB-Cu(II) reveal initial decomposition temperature at 275°C. About 27% weight loss was observed corresponding to aliphatic, non-coordinated part such as CH_2–CH–CH_2 [25]. Continued mass loss was observed up to 670°C due to volatilization of the aromatic part into low molecular weight fractions such as CH_4, N_2 and H_2O.

The thermal analysis results revealed a reduced mass of 30.2% and 32.6% (Calcd 31.01% and 31.97%) for all the coordination polymers at 800°C, indicating formation of metal oxides. The reduced mass of the polyesters was more than the calculated value due to the formation of other compounds during thermal reaction. The order of stability on the basis of TGA results is PSB-Zn(II)– < PSB-Cu(II)– > PSB-Co(II) > PSB-Ni(II) > PSB-Mn(II), matching with Irving-Williams order of stability of the complexes of divalent metal ions [26]. The PSB-Cu(II) complex was more stable than the other chelated polyesters due to higher stability constant of Cu(II).

The glass transition temperature of all the synthesized polyesters was 198–235. All the polymers show a single Tg value due to the absence of any homo polymers, block polymers, or heterogeneous impurities in the polymers.

3.3. *Anti-microbial activity*

The antimicrobial activities of the synthesized polymers were screened against selected bacteria and fungi by agar well diffusion methods and their inhibition zones are given in tables 5 and 6. All the synthesized chelated polyesters showed promising antibacterial activity against all the bacteria. The PSB-Cu(II) complex showed zones of inhibition 20, 22, 25 and 19 mm against *S. aureus*, *S. typhi*, *P. aeruginosa* and *S. boydii.*

The maximum zone of inhibition was 21 mm for PSB-Zn(II) complex against *P. aeruginosa* and *S. typhi*, while PSB-Mn(II) showed lowest antibacterial activity. PSB-Cu(II) showed higher zones of inhibition than other metal complexes.

The antifungal activity of all the synthesized polyesters were tested against seven fungi *C. albicans*, *T. species*, *A. flavus*, *A. niger*, *F. species*, *M. species*, *P. species* with the highest inhibitory zones i.e. 21 and 22 mm for PSB-Cu(II) against *F. species* and *M. species*, respectively. The zones of inhibition were 18 and 17 mm, respectively, for PSB-Zn(II) complex against *A. flavus* and *A. niger*. For *C. albicans*, *F. species* and *P. species* the inhibition zones of 18, 20 and 18 mm were found for PSB-Mn(II).

Table 5. Antibacterial activity of chelated polyesters.

	Zone of inhibition[a] (mm) 50 μg/disk						
Abbreviation	*E. coli*	*B. subtelillis*	*B. megaterium*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*S. boydii*
PSB-Mn(II)	17	17	19	17	19	22	17
PSB-Co(II)	17	17	17	19	21	23	17
PSB-Ni(II)	17	19	20	19	22	24	17
PSB-Cu(II)	19	17	19	20	22	25	19
PSB-Zn(II)	19	19	20	19	21	21	17
Kanamycin[a]	15	16	18	17	20	20	18
DMSO[b]	–	–	–	–	–	–	–

[a] Standard drug (positive control).
[b] Solvent (negative control) antifungal activity.

Table 6. Antifungal activity of chelated polyesters.

	Zone of inhibition[a] (mm) 50 μg/disk						
Abbreviations	*C. albicans*	*T. species*	*A. flavus*	*A. niger*	*F. species*	*M. species*	*P. species*
PSB-Mn(II)	18	21	21	27	20	21	18
PSB-Co(II)	20	18	18	20	18	18	21
PSB-Ni(II)	18	20	20	22	20	20	21
PSB-Cu(II)	21	21	21	18	21	22	22
PSB-Zn(II)	18	18	18	17	18	19	20
Miconazol[a]	15	16	18	17	20	20	18
DMSO[b]	–	–	–	–	–	–	–

[a] Standard drug (positive control).
[b] Solvent (negative control).

The synthesized chelated polyesters showed effective antimicrobial activity. Polymer-containing reactive groups cause membrane linkage by interfering with the change of the phosphate on phospholipids of the membranes. The antimicrobial activity of all the compounds increased after metal chelation [27]. This process increased the lipophilic nature of central metal ion, which in turn favored its permeation to the lipid layer of the membrane. Other factors viz. stability constant, solubility and magnetic moment may also be responsible for increasing the antimicrobial activity of the complexes [28].

4. Conclusion

Chelated polyesters were synthesized by reaction of bifunctional metal chelated diols with adipoyl chloride in 1 : 1 molar ratio and characterized on the basis of analytical, magnetic, spectral and thermal data. The PSB-Cu(II) complex was more thermally stable than the other metal chelated polyesters. All the synthesized polyesters exhibited a single Tg value confirming the absence of any homopolymers, block polymers or other heterogeneous impurities in the polymers. PSB-Cu(II) showed a wide effectiveness in antibacterial and antifungal activity. Since all the chelated polyesters were relatively stable at high temperatures, they can be safely used for biomaterial applications as thermal sterilizers.

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