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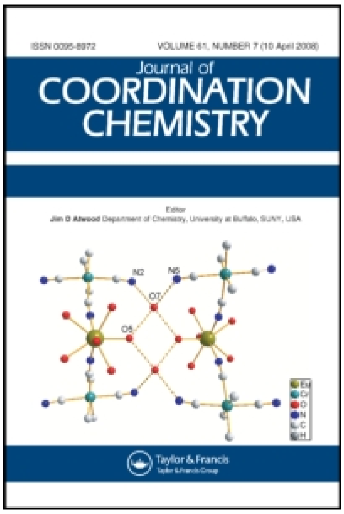

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Nahid Nishat[a]; M. Zulfequar[b]; Asma[a]; Sumaiya Hasnain[a]
[a] Department of Chemistry, Jamia Millia Islamia University, New Delhi 110025, India [b] Department of Physics, Jamia Millia Islamia University, New Delhi 110025, India

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Synthesis, spectral, and antibacterial screening studies of chelating polymers of bisphenol-A–formaldehyde resin bearing barbituric acid

NAHID NISHAT*†, M. ZULFEQUAR‡, ASMA† and SUMAIYA HASNAIN†

†Department of Chemistry, Jamia Millia Islamia University, New Delhi 110025, India
‡Department of Physics, Jamia Millia Islamia University, New Delhi 110025, India

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A new polymeric ligand was synthesized by the reaction of bisphenol-A and formaldehyde in the basic medium, followed by condensation polymerization with barbituric acid in the acidic medium. Polymer metal complexes were prepared by reaction of this resin with Mn(II), Co(II), Ni(II), Cu(II), and Zn(II). The polymeric resin and its metal polychelates were characterized by elemental analysis, FT-IR, ^{13}C-NMR, and ^{1}H-NMR spectra. The geometry of the polymer metal complexes was evaluated by electronic spectra (UV-Vis) and magnetic moment measurement. Thermal stabilities show an increased thermal stability of the metal polychelates compared to the ligand. The antibacterial activities of all the synthesized polymers were investigated against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, showing good antibacterial activities against these bacteria. Cu(II) polychelate showed highest biocidal activity.

Keywords: Barbituric acid; Polymer metal complexes; Thermogravimetric analysis

1. Introduction

Metal containing polymers show many potential applications as functional materials with desirable chemical and physical properties, catalytic activities [1, 2], and models for bioinorganic systems [3]. After the discovery of barbituric acid by Adolf von Baeyer in 1864, it was concluded that barbituric acid is not pharmacologically active, but its derivatives could be used as drugs, valuable in medicine as soporifics, hypnotics, sedatives, and anesthetics [4]. Barbituric acid is nontoxic and thermally stable [5]. The presence of potential donors such as two amine nitrogens and three carbonyl oxygens makes barbiturates interesting and polyfunctional ligands [6], suitable candidates for pharmacologically active and useful ligands in coordination and polymer chemistry. This article reports the synthesis and characterization of bisphenol-A–formaldehyde resin according to the reported method, and its condensation polymerization to obtain the polymeric ligand (BFB) containing barbituric acid moiety. Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) were added to BFB to obtain metal polychelates.

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*Corresponding author. Email: nishat_nchem08@yahoo.com

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The spectral, magnetic, thermal properties, the type of chelation of the ligand, and the geometry around the metal center are discussed. In addition, the antimicrobial activities are tested against several bacteria, *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative).

2. Experimental

2.1. *Chemicals*

The following materials were used as received: bisphenol-A (G.S. Chemicals), formaldehyde (37–41%) (S.D. Fine Chemicals), barbituric acid, sodium hydroxide pellets (Merck India), and metal(II) acetate (Qualigens Fine Chemicals, India). Solvents such as dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) (Qualigens Fine Chemicals) were of analytical grade and used without purification.

2.2. *Preparation of bacterial strains*

The antibacterial activities of the ligand and metal polychelates were screened *in vitro* against various bacteria in DMSO. The sample concentration was $20\,\mu g\,mL^{-1}$. Antibacterial activity was screened against *B. subtilis*, *S. aureus* (Gram positive) and *E. coli* (Gram negative) bacteria by the shaking flask method [7] and the number of viable cells was counted utilizing the spread plate method [8]. One loop full of bacteria was inoculated in 10 mL of a nutrient broth (peptone, $5\,g\,L^{-1}$; pH 6.8) and incubated at 37°C for 28 h in a test-tube shaker at 100 rpm. The nutrient agar (20 mL) was poured into sterile Petri plates and allowed to solidify at room temperature. After solidification, 0.1 mL of the bacterial culture was spread on the agar. A circular well was made at the center of each Petri plate with a sterilized steel borer. The test samples were prepared in DMSO at $20\,\mu g\,mL^{-1}$, and 0.1 mL of each test solution was added to the well and incubated at 37°C for 24 h. After incubation, the zones of inhibition were measured in millimeters and represented as inactive, mildly active, moderately active, and highly active depending on the diameter and clarity of the zone of inhibition. Kanamycin was used as positive control and DMSO as negative control.

2.3. *Instrumentations*

Elemental analyses (C, H, and N) were carried out on an elemental analyzer system GmbH Vario ELIII with the percentage of metal obtained by complexometric titration with EDTA after decomposing with conc. HNO_3 at room temperature, the results are given in table 1. Magnetic susceptibility measurements were recorded on a Vibrating Sample Magnetometer model 155 at room temperature. FT-IR spectra were obtained on a Perkin Elmer IR spectrophotometer model 621 using KBR pellets. Proton NMR (^{1}H-NMR) and carbon (^{13}C-NMR) nuclear magnetic resonance spectra were recorded on a JEOL-GSX 300 MHz FX 1000 FT-NMR spectrometer using DMSO-d_6 and tetramethylsilane (TMS) as an internal standard. UV-Visible spectra were carried out on a Perkin Elmer Lambda EZ-201 spectrophotometer in DMSO.

Table 1. Elemental analysis data for BFB and BFB–M.

Compound abbreviation	Empirical formula	Yield (%)	Elemental analysis (%)[a]			
			Carbon	Hydrogen	Nitrogen	Metal
BFB	$[C_{21}H_{20}N_2O_5]_n$	68	65.22 (66.30)	5.90 (5.29)	7.55 (7.36)	–
BFB–Mn(II)	$[MnC_{21}H_{18}N_2O_5 \cdot 2H_2O]_n$	74	53.77 (53.74)	4.63 (4.72)	5.87 (5.96)	11.64 (11.70)
BFB–Co(II)	$[CoC_{21}H_{18}N_2O_5 \cdot 2H_2O]_n$	71	53.51 (53.28)	4.35 (4.68)	5.83 (5.91)	12.36 (12.45)
BFB–Ni(II)	$[NiC_{21}H_{18}N_2O_5 \cdot 2H_2O]_n$	72	53.51 (53.31)	4.57 (4.68)	5.74 (5.92)	12.31 (12.40)
BFB–Cu(II)	$[CuC_{21}H_{18}N_2O_5]_n$	68	57.29 (57.07)	4.00 (4.10)	6.26 (6.33)	14.34 (14.37)
BFB–Zn(II)	$[ZnC_{21}H_{18}N_2O_5]_n$	72	57.09 (56.83)	4.01 (4.08)	6.21 (6.31)	14.64 (14.73)

[a] Observed value (calculated).

Thermal behavior was determined using a Perkin Elmer (Pyris Diamond) Thermal Analyzer in nitrogen at 20°C min^{-1}. The solubilities of the polymeric ligand and polymer metal complexes were determined in both polar and non-polar solvents by the addition of 2 mg of sample to 10 mL of the solvent kept overnight.

2.4. *Synthesis of polymeric ligand*

Initially, salicyl alcohol was formed by the addition of bisphenol-A and formaldehyde in the molar ratio of 1 : 2 (2.28 g, 1.5 mL, respectively) in DMSO. Stirring and heating on a magnetic stirrer was continued in a three-necked round bottom flask equipped with a thermometer and a water condenser in the presence of NaOH at 60°C for 2.5 h. Thereafter, salicyl alcohol of bisphenol-A {4,4′propane-2,2-diylbis[(2-hydroxymethyl)-phenol]} was obtained in good yield.

Barbituric acid (barbH2) (0.01 mol, 1.28 g) was added to the formed salicyl alcohol in slightly acidic medium and stirred magnetically at 70°C giving a light orange sticky compound. It was precipitated in distilled water and washed several times with acetone and diethyl ether, giving an orange powdery material. After drying in an oven for 6–8 h at 40°C, polymeric ligand (BFB) was obtained. This polymeric ligand lost two protons, so it can be written as (barb). Anal. Calcd for BFB (%): C, 65.22; H, 5.90; N, 7.55. Found (%): C, 66.30; H, 5.29; N, 7.36.

2.5. *Synthesis of metal polychelates*

A series of polymer complexes of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) were prepared from BFB in 2 : 1 molar ratio. A typical procedure for preparation of BFB–Co(II) complex follows:

The polymeric ligand was dissolved in DMSO and heated at 60–70°C in a three-necked round bottom flask fitted to an ice-cooled condenser. Cobalt(II) acetate (1.24 g, 0.005 mol) dissolved in DMSO was added to this hot solution and maintained for 8–10 h at the same temperature. Stirring was continued until complete dissolution and a royal blue viscous solution was obtained. It was then precipitated with distilled H_2O, filtered and washed several times with distilled H_2O and diethyl ether, giving a dark blue precipitate which was oven dried at 45°C for 5–6 h to obtain the polychelate BFB–Co(II). Similar procedure was adopted for the preparation of polychelates of Mn(II), Ni(II), Cu(II), and Zn(II). The percentage yield of the ligand (BFB) and its metal polychelates (BFB–M) are listed in table 1.

3. Results and discussion

3.1. *Chemistry of polymeric ligand and polymer metal complex*

Transition metal polychelate and polymeric ligand were prepared according to the synthetic route, as shown in scheme 1. The reaction of phenol with formaldehyde is followed by condensation. Formation of phenoxide is the initial step. Then, formaldehyde in its hydrated form gives salicyl alcohol. Condensation of barbituric acid occurs by the removal of water to form the ligand.

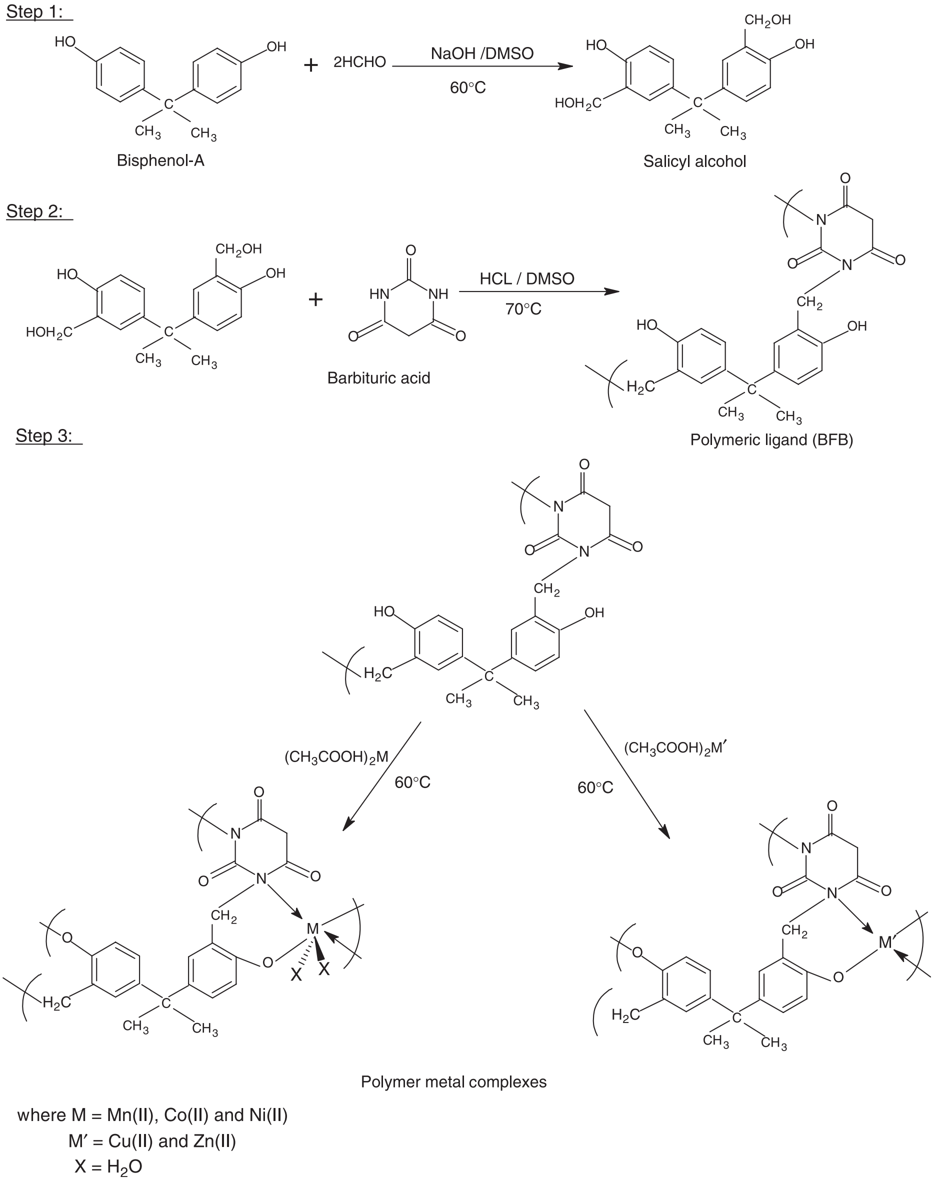
All polychelates including the ligand were colored and insoluble in distilled water, ethanol, methanol, benzene, and chloroform, but soluble in DMSO and DMF. Their insolubility indicates their polymeric behavior. Elemental analyses of all the polymers are in very good agreement with calculated values (table 1). The elemental analysis data also reveal that ligand-to-metal ratio is 2 : 1.

3.2. *FT-IR spectral studies*

Barbituric acid has two donors, nitrogen and oxygen, but the metals discussed here prefer nitrogen [9]. Similarity of FT-IR spectra of the complexes shows binding through deprotonated nitrogen for all the metals. The absorption at 3422 cm^{-1} from phenolic–OH stretching in the polymeric ligand (BFB) is a very broad band which disappears in the spectra of metal polychelates, suggesting the involvement of hydroxyl oxygen in bond formation [10]. In the ligand, a band at 1244 cm^{-1} corresponds to phenolic (C–OH) group vibration; in metal complexes, these bands shift to lower frequency by 10–14 cm^{-1}, indicating the coordination of metal to oxygen [11]. Sharp signal at 1718–1721 cm^{-1} is due to C=O; no change in the frequency of C=O in the ligand and metal polychelate shows that the oxygen of barbituric acid is not involved in bond formation. Aromatic ring has C=C stretching vibrations at 1654–1620 cm^{-1}. The isopropyl group of bisphenol-A shows peaks of almost equal intensity at 1363 cm^{-1} [12]. The peak at 1458–1452 cm^{-1} from C–N stretching is a strong evidence for the polymerization of bisphenol-A–formaldehyde resin with barbituric acid [13]. A band at 541–530 cm^{-1} indicates M–N bonding [14] while M–O appears at 618–610 cm^{-1} [15]. Medium-to-weak intensity bands are due to coordinated water at 952–950 cm^{-1} (rocking) and 774–770 cm^{-1} (wagging) in the spectra of Mn(II), Co(II), and Ni(II) polymer complexes but are not observed in the spectra of Cu(II) and Zn(II) polychelates. This difference suggests that water is present in Mn(II), Co(II), and Ni(II) polymer metal complexes.

3.3. *NMR spectrum analyses*

^{1}H-NMR spectra of BFB and its Zn(II) polychelate were carried out in DMSO at room temperature using TMS as internal standard (Supplementary material). Dimethyls of



Scheme 1. Proposed synthetic route to polymer metal complexes.

bisphenol-A are between 1.3 and 1.7 ppm in BFB and BFB–Zn(II) [16] while the resonance signals for the aromatic rings appear as multiplets at 6.6–6.9 ppm [17]. Phenolic OH is at 11.5 ppm [18] for BFB but is absent in the spectrum of BFB–Zn(II), indicating the coordination of phenolic oxygen. CH_2 at δ 4.1 and 4.4 ppm [19] in the ligand and metal polychelate, respectively, shows the polymerization of bisphenol-A–formaldehyde unit with barbituric acid.

The ^{13}C-NMR spectra of BFB and its Zn(II) polychelate show carbonyl carbons (Ca,Cc) at 154.87 and 164.51 ppm, respectively [20]. Methylene of barbituric acid (Cb) and formaldehyde (Cd) show peaks at 43.64 and 40.33 ppm, respectively [21]. Aromatic carbons show multiple overlapping peaks between 39.22 and 39.78 ppm, and at 114.56 and 127.29 ppm. The characteristic peak for the carbon to which hydroxyl is attached may have shifted to lower frequency after complexation but was not clearly visible in the spectrum due to several overlapped peaks. Methyl carbon of bisphenol-A (Cg) is at 31.88 ppm [22].

Thus, ^{1}H-NMR and ^{13}C-NMR spectra reveal that the metal is attached through the phenolic oxygen of bisphenol-A and nitrogen of barbituric acid.

3.4. *UV-Visible analysis*

Electronic spectra were recorded in DMSO and various parameters $10\,D_q$ (crystal field parameter), B (Racah interelectronic repulsion parameter), β (nephelauxetic ratio) and β° (covalency parameter) calculated (table 2). The nephelauxetic parameter, β, is obtained by using the relation $\beta = B_{\text{complex}}/B_{\text{free ion}}$, where $B_{\text{free ion}} = 960$, 1120, and 1080 for Mn(II), Co(II), and Ni(II), respectively.

The magnetic moment of BFB–Mn(II) is 5.72 B.M. corresponding to five unpaired electrons. The electronic spectrum has three absorptions at 15,562, 19,850, and 24,341 cm^{-1}, assigned to $^4T_{1g}(G) \leftarrow {}^6A_{1g}(F)$, $^4T_{2g}(G) \leftarrow {}^6A_{1g}(F)$, and $^4E_g(G) \leftarrow {}^6A_{1g}(F)$, respectively. The assignments obtained by fitting the observed spectrum to the Tanabe–Sugano diagram suggest octahedral geometry [23]. The 10 Dq and B are 4126 and 825 cm^{-1} while β and β° values are 0.85 and 0.15, respectively.

The magnetic moment of polymer Co(II) complex was 4.41 B.M. and electronic transitions at 9632, 14,670, and 18,980 cm^{-1} correspond to $^4T_{2g} \leftarrow {}^4T_{1g}$, $^4A_{2g}(P) \leftarrow {}^4T_{1g}$, and $^4T_{1g}(P) \leftarrow {}^4T_{1g}$, respectively. The positions of bands indicate the octahedral geometry [24]. The value of Dq was calculated from the Orgel energy diagram. The spectral parameters are $10\,\text{Dq} = 4694\,\text{cm}^{-1}$, $B = 670.6\,\text{cm}^{-1}$, $\beta = 0.59$, and $\beta^\circ = 0.41$, suggesting the covalent nature of the compound.

The magnetic moment of BFB–Ni(II) was 2.62 B.M. Bands at 8942, 16,880, and 21,443 cm^{-1} correspond to $^3T_{2g} \leftarrow {}^3A_{2g}$, $^3T_{1g} \leftarrow {}^3A_{2g}$, and $^3T_{1g}(P) \leftarrow {}^3A_{2g}$ transitions, respectively, suggesting octahedral complex [25]. Spectral parameters for this

Table 2. Magnetic susceptibility, electronic spectra, and their ligand field parameters.

Abbreviation	Magnetic moment (μ_{eff})	Electronic transitions (cm^{-1})	Assignment	Geometry	ε^a	B	$10D_q$	β	β°
BFB–Mn(II)	5.72	15,562	$^4T_{1g}(G) \leftarrow {}^6A_{1g}(F)$	Octahedral	11	825	4126	0.85	0.15
		19,850	$^4T_{2g}(G) \leftarrow {}^6A_{1g}(F)$		13				
		24,341	$^4E_g(G) \leftarrow {}^6A_{1g}(F)$		16				
BFB–Co(II)	4.41	9632	$^4T_{2g} \leftarrow {}^4T_{1g}$	Octahedral	10	670	4694	0.59	0.41
		14,670	$^4A_{2g}(P) \leftarrow {}^4T_{1g}$		12				
		18,980	$^4T_{1g}(P) \leftarrow {}^4T_{1g}$		14				
BFB–Ni(II)	2.62	8942	$^3T_{2g} \leftarrow {}^3A_{2g}$	Octahedral	15	794	9530	0.73	0.27
		16,880	$^3T_{1g} \leftarrow {}^3A_{2g}$		12				
		21,443	$^3T_{1g}(P) \leftarrow {}^3A_{2g}$		13				
BFB–Cu(II)	1.75	14,685	$^2A_{1g} \leftarrow {}^2B_{1g}(F)$	Square planar	14				
		21,872	Charge transfer		15				

μ_{eff}, effective magnetic moment in B.M.; ε^a, dm^3 mol^{-1} cm^{-1}.

polychelate are $10\,\mathrm{Dq}=9530.1\,\mathrm{cm}^{-1}$, $B=794.18\,\mathrm{cm}^{-1}$, $\beta=0.73$, and $\beta=0.27$, obtained from the Orgel diagram.

The BFB–Cu(II) has a magnetic moment of 1.75 B.M. and shows bands at 14,685 and $21{,}872\,\mathrm{cm}^{1}$ assigned to $^2A_{1g} \leftarrow {}^2B_{1g}$ and charge transfer indicative of square planar geometry. The BFB–Zn(II) is diamagnetic and no d–d transitions are observed.

$B_{\mathrm{free\ ion}}$ value in the metal complexes is reduced revealing that the complexation of the metal ion occurs. Thus, the electronic spectral study further supports the structure proposed for the polymeric ligand and its metal polychelates.

3.5. *Thermogravimetric analysis*

Degradation was carried out at a heating rate of $20^{\circ}\mathrm{C\,min}^{-1}$ in nitrogen to 850°C. The weight loss fractions of resin were calculated and the TGA thermograms of BFB and BFB–M are depicted in the "Supplementary material". The polymers start decomposing at 100°C with 2–8% weight loss due to the elimination of water/solvent. For Mn(II), Co(II), and Ni(II) polychelates, the curve showed 10–20% weight loss corresponding to two coordinated waters in the temperature range of 100–150°C, while Cu(II) and Zn(II) polychelates do not show weight loss in this temperature range, indicating the absence of coordinated water and thus favoring four coordinate geometry for Cu(II) and Zn(II) polychelates. Above 200°C, a gradual mass loss was observed, but the rate of decomposition was quite rapid between 300°C and 600°C, which can be attributed to the decomposition of the chelate polymer followed by the formation of metal oxides (MnO, CoO, NiO, CuO, and ZnO). Greater stabilities of the polymer metal complexes than the ligand may be attributed to the formation of stable six-membered rings in all the polymeric complexes. The thermal stability of polymeric chelates was $\mathrm{Cu} > \mathrm{Ni} > \mathrm{Zn} > \mathrm{Co} > \mathrm{Mn}$. The greater stability of BFB–Cu(II) compared with the other polychelates is in agreement with the spectrochemical series, where Cu(II) complexes are always more stable than other complexes.

3.6. *Antimicrobial effects*

Antibacterial activities show that the newly synthesized ligand and polymer metal complexes have biological activity (table 3 and figure 1). Polymer metal complexes of Cu(II) exhibited the highest activity against both Gram positive and Gram negative bacteria, the polymeric ligand was inactive to moderately active while other polychelate complexes were moderately active. Co(II) and Zn(II) polychelates were highly active against *S. aureus* but moderately active against *E. coli* and *B. subtilis*. Mn(II) polychelates were highly active against *E. coli* but moderately active against *B. subtilis* and *S. aureus*. Ni(II) polychelates exhibited high activity against *E. coli* while moderate activity against *S. aureus*. Metal containing polyesters with an antimicrobial activity have been published [26], and further increasing the biological activity including this work is still in progress.

The possible mode of biocidal action can be explained with the help of chelation theory [27, 28]. More activity of the Cu(II) polychelate can be attributed to its higher stability with N and O donors having strong interaction toward the central metal, by which its lipophilic nature was increased. The higher activity of Gram positive bacteria can be explained on the basis that there is a thick outer membrane made up of

Table 3. Antibacterial analysis (zones of inhibition in mm).

Compounds	*B. subtilis*	*S. aureus*	*E. coli*
BFB	3 ± 2	5 ± 1	4 ± 1
BFB–Mn(II)	14 ± 1	16 ± 1	20 ± 2
BFB–Co(II)	12 ± 1	19 ± 2	17 ± 1
BFB–Ni(II)	8 ± 2	12 ± 2	21 ± 2
BFB–Cu(II)	23 ± 2	16 ± 2	18 ± 3
BFB–Zn(II)	6 ± 3	22 ± 3	19 ± 2
Kanamycin	22 ± 1	23 ± 1	25 ± 2

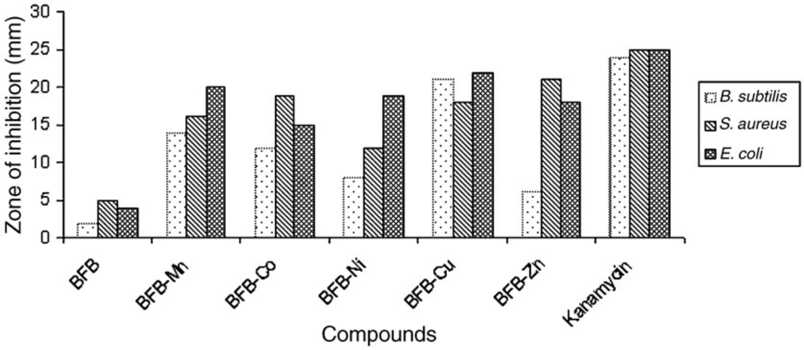


Figure 1. Antibacterial activity data.

lipopolysaccharide and protein in the Gram negative bacteria which inhibits or partially permits the penetration of polymer metal complexes into the bacterial cell, but such membrane is not present in the Gram positive bacteria; instead, a wall of peptidoglycan is present which easily permits the penetration of polymer metal complexes.

Various other biocidal activities of coordination polymers have been published [29, 30] with various mechanisms of the inhibition of bacteria. 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. As these polymers cause fewer allergic responses compared with the formaldehyde monomers, however, this issue should also be clarified before any internal use of these polymers.

4. Conclusions

The new polymeric ligand (BFB) has been synthesized by condensation polymerization and coordinated with several transition metals. The insolubility of the ligand and metal complexes indicate their polymeric nature. Cu(II) chelated polymer showed more antibacterial activity than other polymers. Further work will be done on some of the polychelates to enhance their properties and make them applicable in biomedical fields.

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