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Research Article

Biological Investigations of Novel Terpolymer Ligand and Its Polychelates

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Abstract

A novel terpolymer ligand (PUF) was synthesized through the condensation polymerization of *p*-phenylenediamine and urea with formaldehyde in the presence of basic medium. Terpolymer-metal complexes were prepared using the PUF ligand with some transition metal ions such as Cu(II) and Ni(II) in 2:1 ligand:metal molar ratio. The terpolymer ligand and its metal complexes were intended to spectral characterizations *viz.* FTIR, UV-vis, ^{1}H NMR and ^{13}C NMR to establish the structure. The molecular weight of the terpolymer ligand was determined by gel permeation chromatography. The empirical formula of the repeating unit for both the terpolymer ligand and its metal complexes were justified by elemental analysis. Scanning electron microscopy (SEM) was used to establish the surface morphology of the terpolymer ligand. In addition, the terpolymer ligand and its metal complexes were screened against the growth of few microbes and their inhibitions were measured by disc diffusion technique.

Keywords: Novel Terpolymer; Elemental analysis; Antimicrobial; Polychelates

This paper is dedicated to the memory of Dr. A. Burkanudeen, who had a road accident and sadly died on December 28, 2012. In the grace of almighty, May his soul rest in peace.

1. Introduction

The synthesis of new polymers with reactive functional groups has evoked considerable interest in recent years. By incorporating biologically active organic moieties into the polymer backbone, the activities can be introduced. In terms of their biological activity, these polymers are more effective than their monomers. Such polymers are known for their biocidial activity against some bacterial, fungal and viral strains [1,2]. 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 [3,4]. 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 [5-8]. Polychelates of urea–formaldehyde resin with Cr (III), Mn(II), Co(II), Ni(II), and Zn(II) metal ions were prepared. 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 antibacterial activities of these polychelates were also found to be reasonably good compared with standard drugs, namely *ciprofloxacin*, *ampicillin and kanamycin*. Ahamad et al. reported a new class of metal chelated polyurea for its excellent antimicrobial activity against *S. aureus*, *E. coli*, *B. subtilis*, and *S. typhi* [9,10]. Recently our research group synthesized terpolymers involving anthranilic acid-urea-formaldehyde and anthranilic acid-salicylic acid-formaldehyde resins and reported their excellent and antimicrobial activity. The antimicrobial activity was due to the donor atoms present in the lattice [11,12].

This article describes the synthesis and characterization of a novel terpolymeric ligand and its complexes with Cu^{2+} and Ni^{2+} metal ions. The surface morphology of the terpolymer ligand was analyzed using SEM. The antimicrobial activity of the synthesized ligand and its metal complexes were tested against several microbes.

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2. Experimental Procedure

2.1. Materials

p-Phenylenediamine and urea were procured from Merck, India and purified by rectified spirit. Formaldehyde (37%) were of AR grade, Merck and used as received. Double distilled water was used for all the experiments. All other chemicals were of analytical grade and used without further purification.

2.2. Synthesis of terpolymer ligand

The terpolymer ligand involving *p*-phenylenediamine and urea with formaldehyde was synthesized by condensation polymerization technique using in the presence of dimethylformamide as a reaction medium in 1:1:2 mole ratio at 140 ± 2 °C for 6 h. The reaction mixture was then cooled, poured into crushed ice with constant stirring and left overnight. The brown colored product was separated out and washed with warm water and methanol. It was then filtered off to remove unreacted monomers. The dried resin sample was dissolved in 1:1(v/v) HCl/water and regenerated using 10 % NaOH and then filtered off, and cured in an air oven at 75 °C for 24 h. The reaction process to obtain the *p*-phenylenediamine – urea – formaldehyde (PUF) terpolymer is shown in scheme 1.

Preparation of metal complexes

The terpolymer metal complexes have been prepared using the synthesized terpolymer ligand with Cu^{2+} and Ni^{2+} metal ions. The PUF terpolymer (2 g) was taken in a round-bottomed flask and immersed for 2h in ethanol solution for swelling. The cupric nitrate (1 g) was dissolved in ethanol solution and then poured into round bottomed flask equipped with mechanical stirrer and a reflux condenser. The reaction mixture was refluxed at 60 °C for 3 h. The obtained colloidal precipitate in the flask was separated out. The product was then filtered off and washed with ether and ethanol to remove the impurities. This process has been repeated several times to separate the purified product. The resultant purified sample was air dried, powdered, and kept in a vacuum desiccator with silica gel. The same procedure was also followed for the preparation of PUF complexes with Ni^{2+} metal ion in the form of its nitrate salt. The reaction process is shown in scheme 2.

2.3. Spectral and elemental analyses

Elemental analysis of the terpolymeric ligand and its metal complexes was performed with an Elementar instrument (Vario EL III,Germany). The molecular weights of the terpolymer were determined using a Shimadzu gel permeation chromatograph (Japan). FTIR spectra were recorded with a Shimadzu spectrophotometer (Japan). UV-visible spectra were obtained in the range 200-800 nm with a Shimadzu UV-visible spectrophotometer (1601 PC, Japan). ^{1}H and ^{13}C NMR spectra of the terpolymer ligand were recorded using deuterated dimethylsulfoxide (DMSO-d_6) as a solvent with a Bruker 400 MHz spectrometer (USA). The morphology of the terpolymer ligand was examined using SEM (S-3000H, Hitachi, Japan).

2.4. Antimicrobial activity

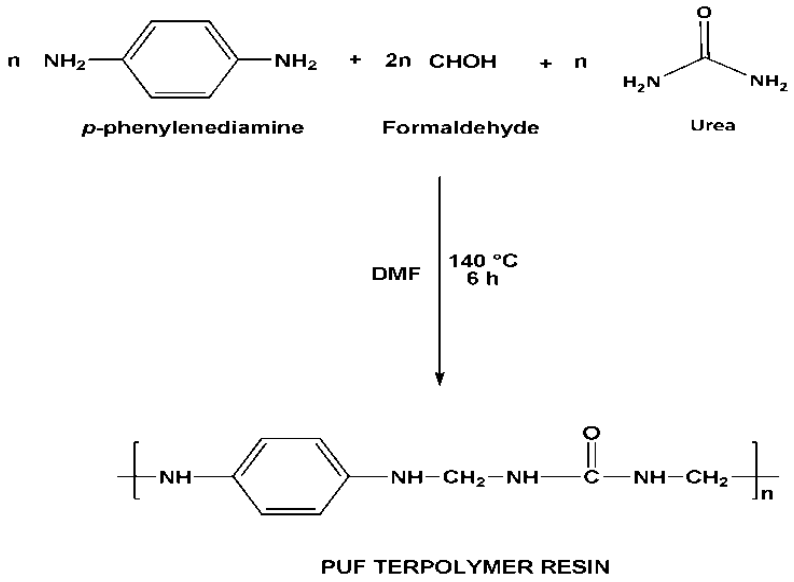
Antimicrobial activity was tested by the filter paper disc diffusion technique involving the cultures of the selected organisms for 24 h. [13] Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The test solutions of the terpolymer and its metal complexes were prepared in sterile DMSO solvent for the study. The synthesized terpolymer and its metal complexes were tested at different concentrations ranging from 50 to l000 ppm to find out the minimum concentration of the terpolymer ligand and its metal complexes required for inhibiting the bacterial growth.

Ciprofloxacin (100 µg/mL) was taken as the standard for antibacterial activity. The organisms were seeded into sterile nutrient agar medium by mixing 1 mL of inoculum with 20 mL sterile melted nutrient agar kept at 48–50 °C in a sterile petri dish. The medium was allowed to solidify first. Then the test solutions, the standard drugs as well as the blank were impregnated in whatman filter paper discs and placed on the solidified medium in the petri dish and left undisturbed for 2 h at room temperature. The petri dishes were then incubated at 37 °C for 24 h and the zone of inhibition for the test samples, standard and the control (DMSO) was measured.

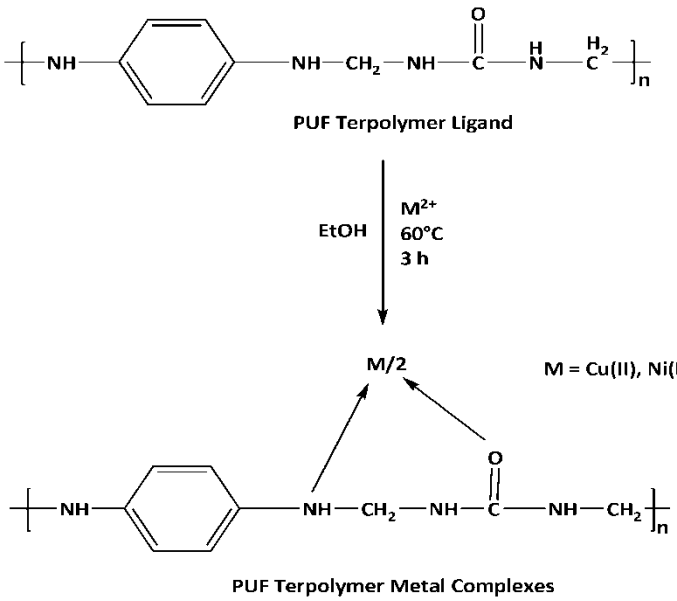
Sterile yeast nitrogen base (HI Media) with 2% agar was inoculated by a rotating swab (soaked in standard inoculums suspension) over the surface of the media. Nystatin (100 mg/ml) was taken as the standard for antifungal activity. The test solution impregnated discs were placed on the agar and incubated at 37 °C for 18 h. The zone of inhibition was measured by measuring the minimum dimension of the zone of fungal growth around the filter paper disc.

3. Results and Discussion

The *p*-phenylenediamine and urea with formaldehyde terpolymeric ligand was soluble in solvents like tetrahydrofuran (THF), dimethylsulfoxide (DMSO), hydrochloric acid, sulphuric acid where as the ligand insoluble in benzene, acetone, ether, toluene, water and aqueous NaOH and KOH solutions. The elements, such as carbon (%C), hydrogen (%H) and nitrogen (%N) content were analyzed for the PUF terpolymer and its metal complexes are presented in table 1. Based on the empirical formula of the repeating unit for the PUF terpolymer and its metal complexes are found to be $C_9H_{12}N_4O$, $C_{18}H_{24}N_8O_2$ Cu.$2H_2O$ and $C_{18}H_{24}N_8O_2$ Ni.$2H_2O$ respectively.



Scheme 1: Synthesis route of the PUF terpolymeric ligand



Scheme 2: Synthesis route of the PUF terpolymeric metal complexes

3.1. Molecular weight measurements

The average molecular weight of the PUF terpolymer was determined by GPC. The weight average ($\overline{Mw}$) and number average ($\overline{Mn}$) molecular weight of the terpolymer were found to be 1830 and 1785, respectively. The polydispersity index $(\overline{Mw} / \overline{Mn})$ was found to be 1.0251.

Table 1: Elemental data of PUF terpolymer ligand and its metal complexes

Compound	Empirical Formula of the single repeating unit	Formula mass	Elemental Analysis (%)			
			C (calculated)	**H** (calculated)	**N** (calculated)	**M** (calculated)
PUF	$C_9H_{12}N_4O$	192.22	56.24 (56.36)	6.29 (6.37)	29.15 (29.28)	-
PUF-Cu	$C_{18}H_{24}N_8O_2$ $Cu.2H_2O$	484.01	44.67 (44.69)	5.83 (5.86)	23.15 (23.18)	13.13 (13.17)
PUF-Ni	$C_{18}H_{24}N_8O_2$ $Ni.2H_2O$	479.16	45.12 (45.18)	5.89 (5.93)	23.39 (23.46)	12.25 (12.32)

3.2. Spectral Studies

3.2.1. FTIR spectra

FTIR spectroscopy was used for the analysis of the polymeric ligand and its polymer-metal complexes, depicted in figure 1. The band frequencies and the groups assigned for the terpolymer ligand and its metal complexes are based on the earlier literature [14,15]. The ligand spectrum showed that a broad band appeared in the region of 3456.7 cm^{-1} is assigned to the –NH group present in the aromatic ring. A peak appeared at 2854.7 cm^{-1} is assigned to aromatic ring stretching modes. The 1,4-disubstitution of aromatic benzene ring was confirmed by sharp, medium/weak absorption bands appeared between 1022.5 and 874.3 cm^{-1}. The band appeared at 1615.0 cm^{-1} is due to –C=O stretching vibrations [16]. A weak band appeared in the region 2922.6 cm^{-1} is attributed to -CH_2 linkage present in the terpolymer [17]. In the spectra of the PUF terpolymer–metal complexes, the bands are slightly broadened compared to those of the terpolymeric ligand due to complex formation. The band appeared in region of 3,583.5 - 3,453.3 cm^{-1} may be due to the coordination of the ligand with the metal ion through the lone pair of nitrogen atom present in Ar-NH. The bands appeared in the region of 1,128.4 - 1,022.6 cm^{-1} is assigned to C-O-M stretching modes. The bands in the range 1274.8 - 1212.1 cm^{-1} are due to C-N stretching vibrations of the terpolymer–metal complexes which are shifted to lower frequency compared to the ligand [18-20]. This is clear evidence for the involvement of nitrogen atom in the chelate formation. The bands appearing in the region 749.6–747.8 cm^{-1} are assigned to metal–oxygen bonding in the respective polychelates. The bands appearing in the region 520.5 - 515.0 cm^{-1} are attributed to metal–nitrogen bonding in the terpolymer–metal complexes [24].

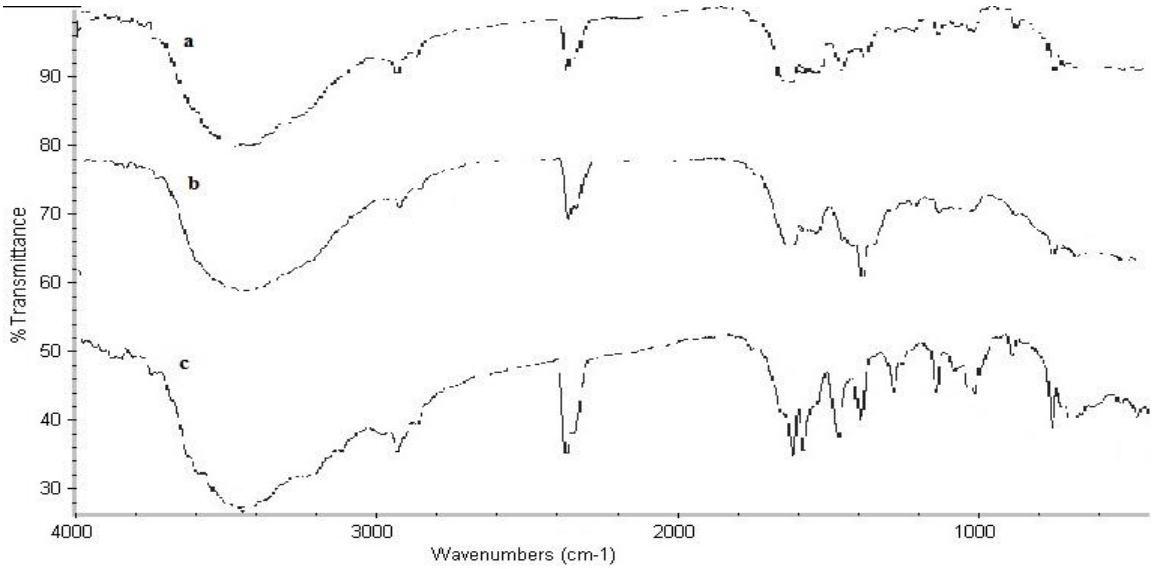


Figure 1: FTIR spectra of (a) PUF ligand, and (b) PUF-Cu, (c) PUF-Ni complexes

3.2.2. UV-Visible spectra

The UV-Vis spectra provide more information about the electronic structure of the ligand and polychelates. Clear evidence is observed from the electronic absorption spectra of the PUF terpolymer ligand and its metal complexes in figure 2. The PUF terpolymer ligand shows absorption bands at 267 and 321 nm which is attributed to

$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. These transitions were affected by the metal chelation and shifted to the longer wavelength clearly indicates the formation of complex takes place through the lone pair of electrons present in the nitrogen of -NH group. The band appearing in the range of 288 to 242 nm is assigned to polychelates, which clearly establishes the metal ions coordination with the nitrogen atom present in the *p*-phenylenediamine ring.

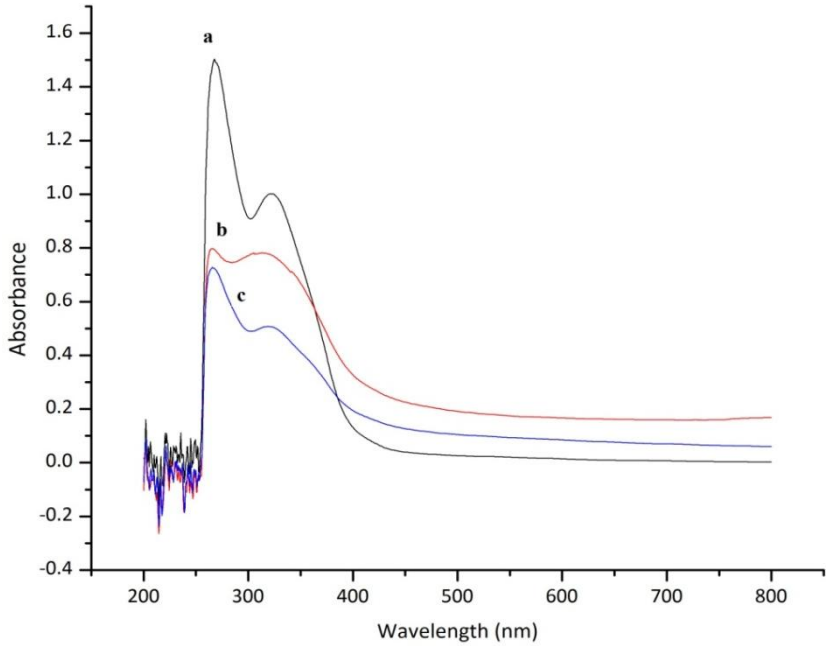


Figure 2: Electronic spectra of (a) PUF ligand, and (b) PUF-Cu, (c) PUF-Ni complexes

3.2.3. NMR spectra

The ^{1}H NMR spectrum of the PUF terpolymer is shown in figure 4. The signals obtained for the terpolymer was interpreted on the basis of the literature [19-21]. The signal in the region of 7.43 (δ) ppm is assigned to all the protons of the aromatic ring. The signal appeared in the region at 6.96 (δ) ppm is due to the –NH bridge present in the terpolymer. The signal obtained at 2.49 (δ) ppm is attributed to CH_2 moiety of aromatic ring. A singlet observed in the region 3.74 (δ) ppm is due to the methylene proton of Ar-CH_2-N moiety.

The ^{13}C NMR spectrum provides useful information about the nature of the carbon present in the synthesized ligand. The observed chemical shifts are assigned on the basis of the literature [20-22]. The spectrum shows the corresponding peaks at 115.42, 145.42, 126.19, 136.39, 125.39 and 132.72(δ) ppm with respect to C_1 to C_6 of the aromatic ring *p*-phenylenediamine. The peak appeared at 39.89 (δ) ppm is assigned to the Ar-CH_2 in the terpolymer resin. The peak appeared at 196.81 ppm may be due to the –C=O of amide moiety.

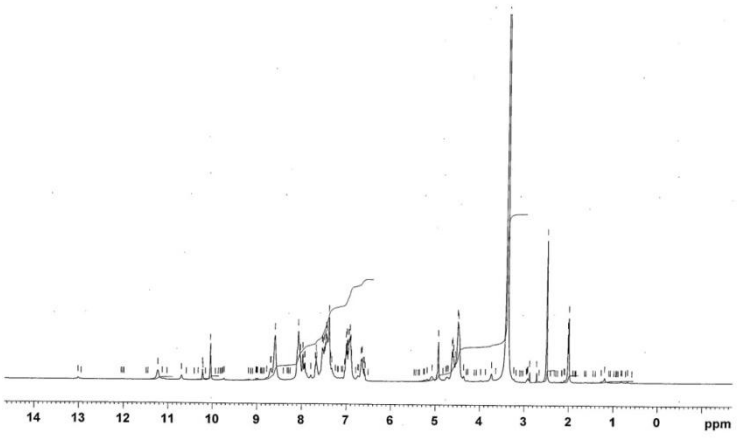


Figure 3: ^{1}H NMR spectrum of PUF terpolymer

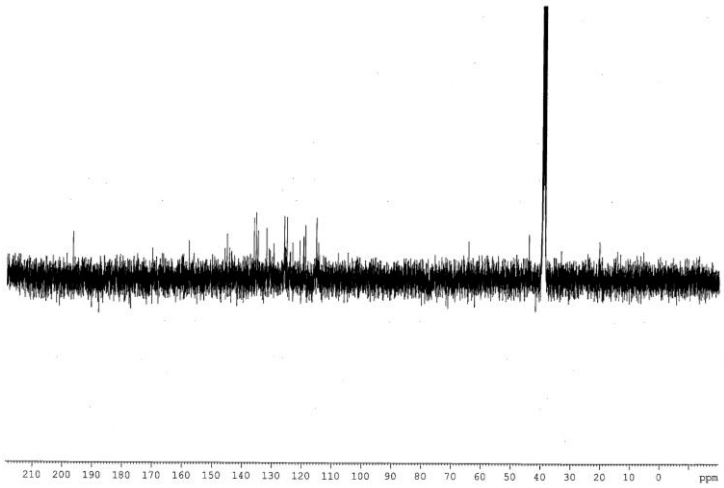


Figure 4: ^{13}C NMR spectrum of PUF terpolymer

3.2.4. Scanning Electron Microscopy

Surface analysis was carried out for the PUF terpolymeric ligand and its metal complexes using SEM. SEM micrographs of the ligand and its complexes are depicted in figure 6. The photograph clearly shows that the terpolymer has deep shallow pits with more amorphous character and less close packed surface. The SEM images of the metal complexes show very closely packed surface with stiff morphology and observed as crushed ice-like structure which is absent in the image of the ligand. Compared to the ligand, deep pits or voids are absent in the images of the metal complexes. This is clear evidence for confirming that effective chelation takes place between the PUF terpolymeric ligand and the corresponding metal ions.

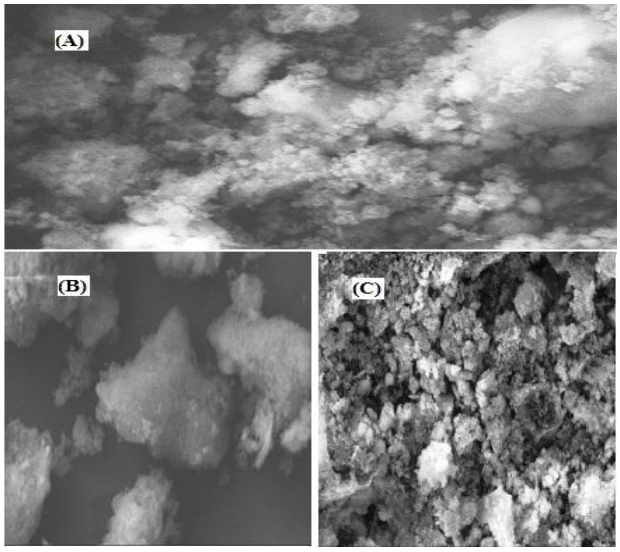


Figure 5: SEM Images of (A) PUF ligand and (B) PUF-Cu, (C) PUF-Ni complexes

3.2.5. Antimicrobial activity

The microbial screening results of the PUF terpolymer ligand and its metal complexes are presented in table 2. Both the ligand and polychelates have good inhibition against the growth of *Gram-negative* bacteria which induces tumor. Hence the polychelates and ligand may possess antitumor activity. The *Gram-positive* bacteria are both pathogenic and invasive. The metal complexes have good inhibition characteristics against the growth of this pathogen. *Staphylococcus aureus* is a *Gram-positive* and spherical bacteria, leads to life-threatening diseases like pneumonia, osteomyelitis, endocarditis and toxic shock sindrom. Toxic shock sindrom is characterized by the sudden onset, high fever, vomiting, diarrhea and muscle aches, followed by low blood pressure, which can lead to shock and death. There may be a rash resembling sunburn with pealing of skin. The activity of PUF ligand and its metal complexes showed a moderate activity against *S.aereus* species. *E.coli* is an aerobic, Gram-negative, rod shaped bacteria. Infection of *E.coli* can lead to the bloody diarrhea and kidney failure. The synthesized PUF

terpolymer ligand and its complexes seize a good activity against the *E.coli* growth. *Aspergillus niger* causes aspergillosis, the growth of the fungus is controlled by the terpolymer chelate to some extent.

Compared to the ligand, the metal complexes show higher activity is due to the metal ions shared with the donor atoms of the ligand and the π-electron delocalisation over the whole chelate ring. This effect increases the lipophilic character of the metal ion, which favours the permeation through the lipoid layers of the bacterial and fungal membranes. It is perceived that the factors such as solubility, conductivity, dipole moment and cell permeability mechanism may be alternative reasons for the increased activity of the metal complexes [23,24]. The PUF terpolymer ligand was found to have good antifungal activity compared to the complexes. The PUF terpolymer ligand and its metal complexes may be used to control the growth of bacterial and fungal strains. The results obtained are in varying on close agreement with the standard (Ciprofloxacin, Nystatin).

Table 2: Antimicrobial studies of PUF terpolymer ligand and its metal complexes

Name of the Compound	Zone of inhibition (mm)		
	Staphylococcus aureus	*E.coli*	*Aspergillus niger*
PUF	20	18	20
PUF-Cu	25	20	15
PUF-Ni	22	19	13
Standard	35	38	30

Standard - Ciprofloxacin for Bacteria, Nystatin for Fungi; Control: DMSO

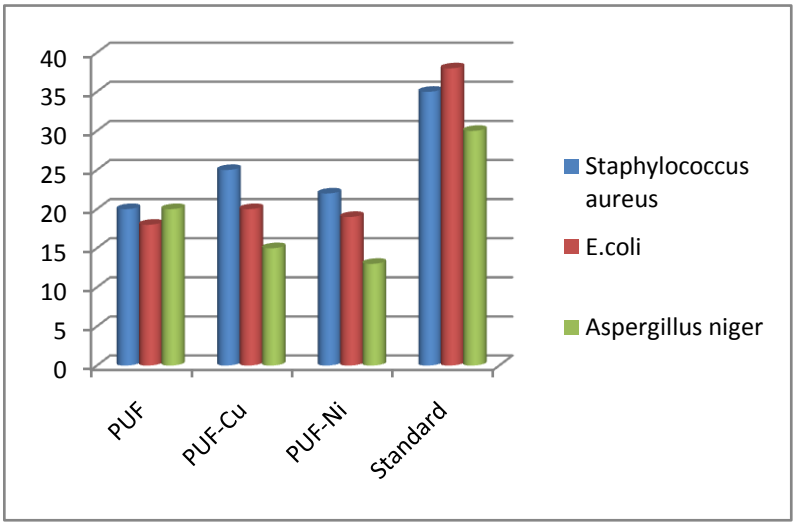


Figure 6: Antimicrobial studies of (A) PUF ligand and (B) PUF-Cu, (C) PUF-Ni complexes

4. Conclusion

The formation of terpolymer from *p*-phenylenediamine and urea with formaldehyde in DMF medium by condensation technique. The polymeric ligand also coordinated with Cu (II) and Ni (II) to give polymer-metal complexes. The formation of terpolymer has been established by elemental analysis, FTIR, UV-Visible and NMR (^{1}H & ^{13}C NMR) spectral studies. The molecular weight of the terpolymer was measured by gel permeation chromatography. The surface morphology of the terpolymer was investigated by scanning electron microscopy. The antimicrobial studies revealed that the complex possess better inhibition against the growth of pathogenic bacteria. From the results it has been proved that Cu (II) complex had excellent antibacterial activity compared to the ligand. Owing to their excellent activity, they can successfully be used as antibacterial agents. The terpolymer ligand had excellent antifungal activity compared to the complexes. Hence this ligand and its metal complexes can be used as antibacterial and antifungal agents.

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