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Research Article

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Resin III: Preparative, structural and antibacterial screening of terpolymer resin derived from 2, 2'-biphenol, ethylene diamine, and formaldehyde

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ABSTRACT

Terpolymer (BPEDF-III) has been synthesized using the monomers 2, 2'-biphenol, ethylene diamine and formaldehyde in 3:1:4 proportion in the presence of 2M HCl as a catalyst. The number average molecular weight of this terpolymer has been determined by conductometric titration in nonaqueous medium. Viscometric measurement in dimethylformamide has been carried out with a view to ascertain the characteristic functions and constants. Elemental analysis confirms the stoichiometry of the BPEDF-III terpolymer whereas the structure has been elucidated on the basis of IR, NMR, UV-Visible spectral studies. The antibacterial activities of the terpolymer were screened on various bacteria. The synthesized polymer has shown excellent antibacterial activities as compared to the standard Ciprofloxacin.

Keywords: Synthesis, BPEDF-III Terpolymer, Elemental Analysis, Spectral Studies, Antibacterial Activities.

INTRODUCTION

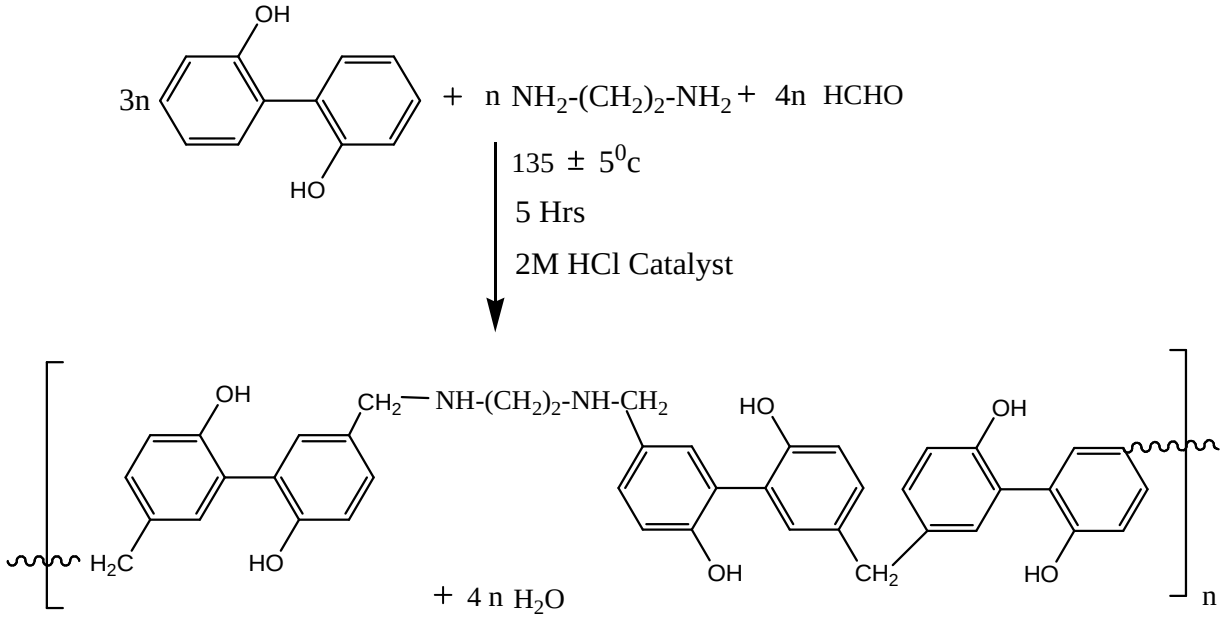
Now a day, various workers have pressing demand to synthesize eco-friendly polymers having some biological activities like antibacterial. The invasion of polymers by fungi, bacteria and other organism is manifested by loss of mechanical properties, surface degradation, discoloration, staining and other deteriorations [1-4]. Terpolymers have been prepared from o- & m-hydroxyacetophenones, m- aminoacetophenones, p-chlorobenzoic acid, 4-hydroxylphenacyl bromide with substituted aromatic compound and formaldehyde which shows pronounce bactericidal and antifungal activity [5-7]. Perusal of literature reveals that biological activities of p-hydroxybenzaldehyde oxime, Substituted Acetophenone Based Terpolymers, and p-hydroxybenzaldehyde oxime based terpolymers, Phenolic Resin with Lanthanides (III) and Poly [(2-Hydroxy-4-Methoxy Benzophenone) Ethylene] Resin have been studied by various coworkers [8-11]. Synthesis, characterisation and thermal degradation studies of terpolymers containing 8-hydroxyquinoline with substituted amino compound and formaldehyde have been studied [12, 13]. Dhanraj Masram and coworkers have synthesized terpolymers by the condensation of salicylic acid, salicylaldehyde and p-hydroxy benzoic acid with various amines viz. ethylenediamine, butylenediamine, p-phenylenediamine and 2, 4-naphthalene diamine with formaldehyde [14-18]. M. M. Jadhao and coworkers have prepared terpolymer resins containing 2, 2'- biphenol with urea, thiourea, guanidine and formaldehyde and reported their structure, thermal degradation studies, and ion exchange properties [19-23]. However, the literature survey revealed that no terpolymer has been studied using the monomers 2, 2' biphenol, ethylene diamine and formaldehyde. Therefore, in this present communication we report synthesis, characterization and antibacterial activities of a 2, 2' - biphenol – ethylenediamine - formaldehyde (BPEDF - III) terpolymer. The conductometric titration and viscometric measurements have been carried out for molecular weight determination and to find out the characteristic functions and constants respectively. The elemental analysis and spectral studies have been used to characterize the complete structure of the BPEDF-III terpolymer.

EXPERIMENTAL SECTION

All the chemicals used were of analytical grade or pure grade. 2, 2'-biphenol (Aldrich chem.), ethylene diamine, formaldehyde, N, N'-dimethyl formamide, dimethylsulphoxide (all from Merk) were used.

SYNTHESIS OF BPEDF-III TERPOLYMER

A mixture of 2, 2'-biphenol (0.3 mol), ethylene diamine (0.1 mol), and formaldehyde (0.4 mol) in presence of 2M HCl was heated in an oil bath at $135^0c \pm 5^0c$ for 5 hr with occasional shaking. The separated resinous product was washed with hot water to remove unreacted monomers. It is then thoroughly washed with methanol to remove copolymers which might be present along with terpolymer. It was purified by dissolving in 8m NaOH and reprecipitated by drop wise addition of 1:1 (v/v) HCl with constant stirring. The regenerated product was washed with hot water, dried in a vacuum desicator over anhydrous calcium chloride. The yield of this terpolymer was found to be 73%. The reaction taking place in the synthesis of BPEDF-III terpolymer is as shown in Scheme 1.



Scheme I: Synthesis of BPEDF-III Terpolymer.

ANTI-BACTERIAL ANALYSIS

Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa were analysed for antibacterial activities. Pure culture of these pathogenic bacteria was taken for analysis. Agar diffusion method was used for antibacterial studies. Nutrient agar medium was used for culture of the bacteria. The composition was peptone (10.0 g), sodium chloride (10.0 g), yeast extract (5.0 g) and agar (20.0 g) in 1000 ml of distilled water. 20 mg sample was dissolved in 1 ml of N, N'- dimethylformamide solvent and Ciprofloxacin Standard Antibiotic of known concentration was used for analysis.

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hrs. Old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control wells with Ciprofloxacin were also prepared. All the plates were incubated at 37ºC for 24 hrs and the diameter of inhibition zone were noted. Concentrations of sample for antibacterial activity were taken as 50 µg / ml, 100 µg / ml, and 200 µg / ml. The antibacterial activities of the terpolymer were screened on various bacteria at these concentrations. The synthesized polymer has shown excellent antibacterial activities as compared to the standard Ciprofloxacin [24-27].

CHARACTERISATION OF TERPOLYMER

The number average molecular weight (Mn) was determined by conductometric titration in DMF using KOH in alcohol as a titrant. The viscosity measurement study was done by using a Tuan-Fuoss viscometer at six different concentrations (3.0% to 0.5%) in DMF at 30^0c. The elemental analysis of the terpolymer was carried out for C, H, and N by using a ThermoFinnigan CHNSO Analyser (Italy), FLASHEA 1112 series and Infrared spectrum was recorded in the region of 600-4000 cm^{-1} on Nicolet Instruments corporations (USA), Model MAGNA 550, at SAIF, IIT, Powai, Mumbai. The UV-Visible study was carried out on a spectrophotometer in the range of 200-1200 nm at

RTM Nagpur university, Nagpur. NMR studies in dimethylsulphoxide as a solvent, was carried out on BRUKER AVANCE II 400 NMR spectrometer, SAIF Panjab University, Chandigarh.

RESULTS AND DISCUSSION

The BPEDF-III terpolymer was found to be light pink in colour and insoluble in commonly used organic solvents but was partly soluble in DMF and DMSO. This synthetic terpolymer does not show a sharp melting point but undergo decomposition above 320°C. Synthesized terpolymer was analysed for carbon, hydrogen and nitrogen content and the composition of the terpolymer obtained on the basis of the elemental analysis for carbon, hydrogen and nitrogen content was found to be in good correlation to that of the calculated value.

Calculated value for BPEDF-III Terpolymer: - C: 68.29%; H: 6.23%; N: 3.79%
Observed value for BPEDF-III Terpolymer: - C: 68.43%; H: 6.17%; N: 3.73%
The molecular weight (Mn) of the terpolymer was determined by non aqueous conductometric titration in DMF against KOH in alcohol using 100 mg of resin sample. A plot of specific conductance against the milliequivalents of potassium hydroxide required for neutralization of 100 mg of terpolymer was made, as shown in figure 1. Inspection of such a plot revealed that there are many breaks in the plot. From this plot the first and the last break were noted.

The calculation of (Mn) by this method is based on the following considerations [28, 29].

(1) The first break corresponds to neutralization of the more acidic phenolic hydroxy group of all of the repeating units; and
(2) The break in the plot beyond which a continuous increase in conductance is observed represents the stage at which the phenolic hydroxy group of all repeating units is neutralized.

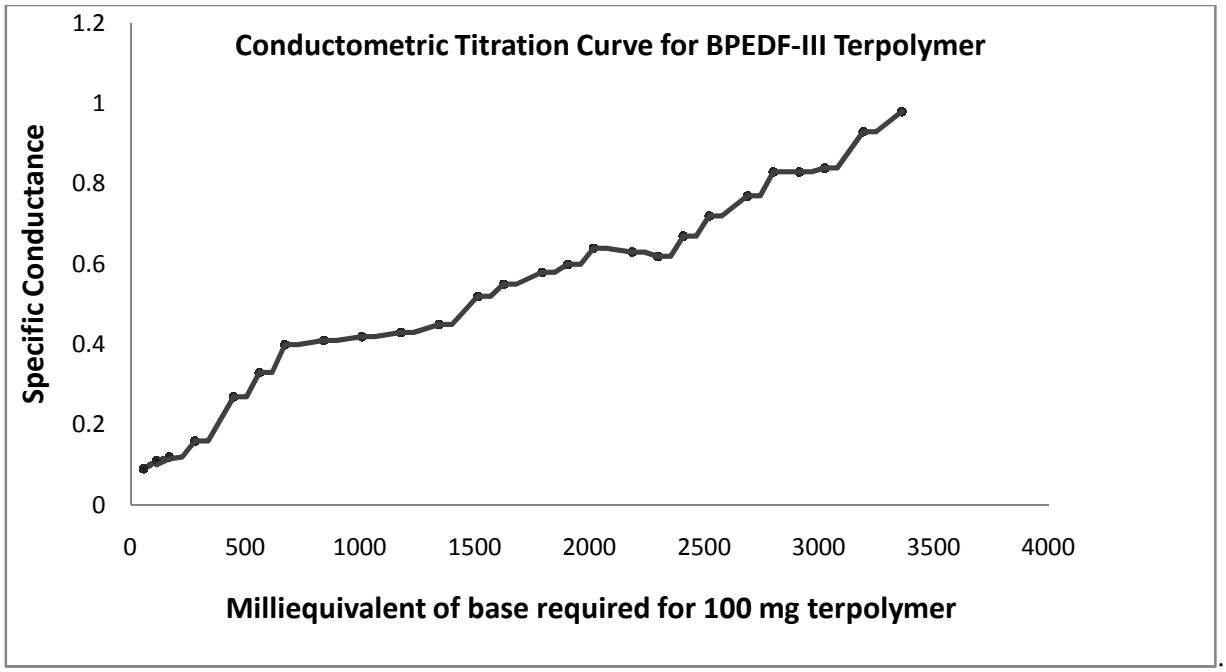


Figure 1:- Conductometric Titration Curve of BPEDF-III Terpolymer.

The average degree of polymerization ($\overline{Dp}$) is given by relation.

$$\overline{Dp} = \frac{total\ milliequivalents\ of\ base\ required\ for\ complete\ neutralisation\ i.e.\ last\ break}{milliequivalents\ of\ base\ required\ for\ smallest\ interval\ i.e.first\ break}$$

The number average molecular weight (Mn) could be obtained by multiplying the Dp by the formula weight of the repeating unit [30]. The average degree of polymerization is found to be 20. The number average molecular weight (Mn) is 14,760.

Viscometric measurements were carried out in DMF at 30°C. The intrinsic viscosity was determined by the Huggins [31] equation and the Kraemers [32] equation.

$\eta_{sp} / C = [\eta] + k_1 [\eta]^2 C$ (1)

$\ln \eta rel / C = [\eta] - k_2 [\eta]^2 C$ (2)

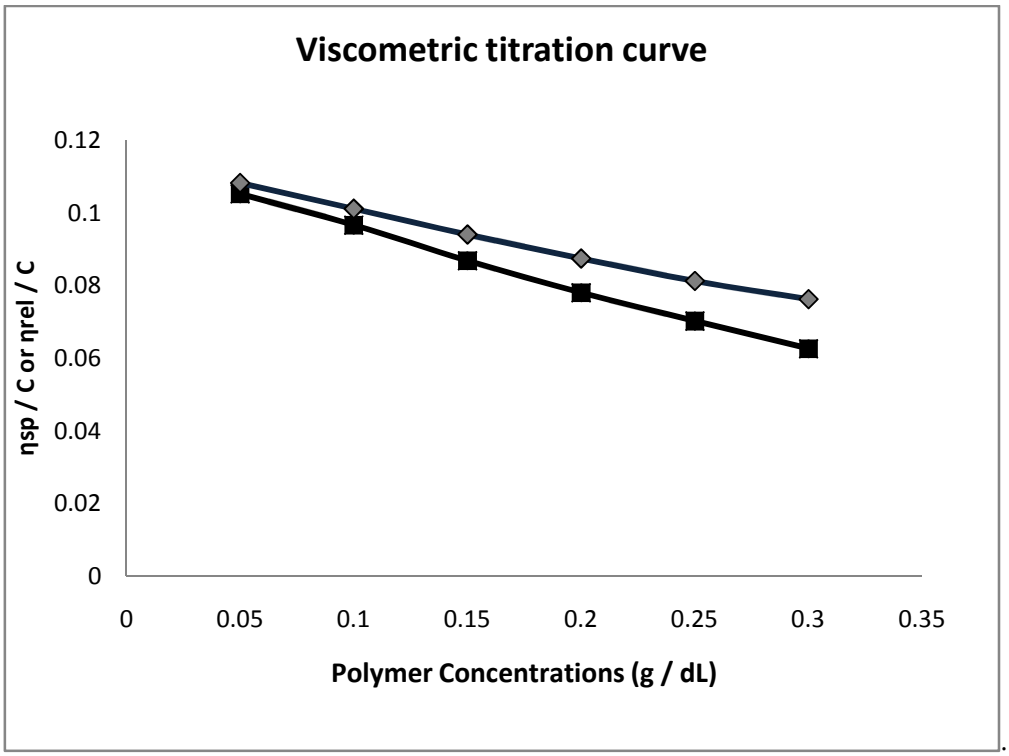


Figure 2: Viscometric Plot of BPEDF-III Terpolymer.

From the Huggins and Kraemers equation, ηsp / C and ηrel / C against C were plotted, shown in figure 2 which is found to be linear, giving slopes k_1 and k_2 respectively. The intercept on the axis of viscosity function gave the value of [η] in both the plots. The calculated values of constants k_1 and k_2 in most cases satisfy the relation k1 + k2 = 0.5 favourably [33]. The intrinsic viscosity is found to be 0.11343. The values of [η] obtained from eq^{ns}. (1) and (2) were in close agreement with each other.

Spectral studies

The electronic spectrum of terpolymer is depicted in figure 3. The spectra show three absorption maxima in the region 230 - 420 nm. The intense band at 230 - 250 nm is due to phenolic – OH groups in repeated units of the terpolymer and is assigned to (n→σ*) transition [34]. The intense band at 270-300 nm is due to (π→π*) allowed transition of biphenyl moiety, which readily attain coplanarity [34-38], while the later intense band at 350-420 may be attributed to n→π*transitions for the presence of the phenolic hydroxyl group (auxochrome) [35-38]. This observation is in good agreement with the proposed probable structure for the BPEDF terpolymer. The electronic spectrum of this terpolymer resin is depicted in figure 3.

The IR spectrum of terpolymer is shown in figure 4. A broad band appearing in the region 35150.11cm^{-1} may be assigned to stretching vibrations of phenolic –OH groups [34, 35-37]. The band at 2923.04 cm^{-1}, 1495.96 cm-1, and 630.58 cm-1are assignable to –NH- stretching, bending and deformation out of plane respectively [34, 35]. The band at 1602.29 cm^{-1} may be ascribed to an aromatic skeletal ring [34]. The presence of methylene bridges (-CH2-) in the polymeric chain may be assigned to the presence of a band at 1460, 1281.36 – 1370 and 751.91 cm^{-1} [-CH2- bending, wagging and rocking] [34-38]. The band at 1219.01 cm^{-1} may be due to >C-O stretch of the polymeric phenol [35, 38-40]. The bands obtained in the range 937 cm^{-1}, 1074.46 cm^{-1} and 1123.45 cm^{-1} confirms the 1,2,3,5 substituted aromatic ring [35, 36]. The 1,2,3,5 – substitution of the benzene ring is also confirmed by the presence of a band at 887.63 cm-^{1} and 818.02 cm-^{1} for a tetrasubstituted benzene ring [34-36].

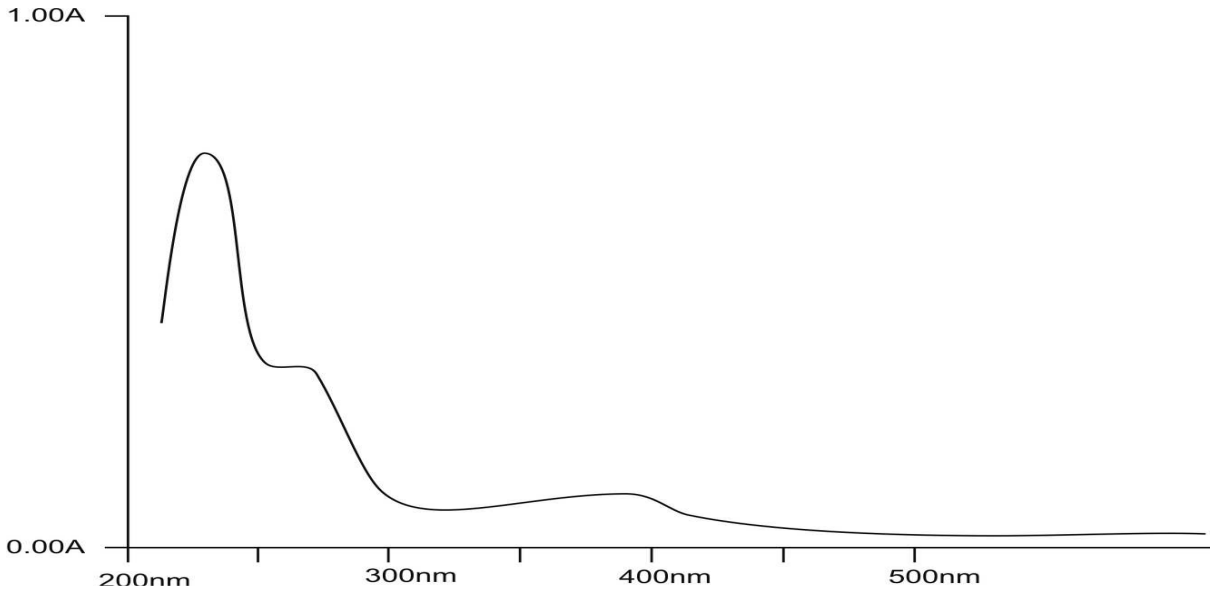


Figure 3: Electronic spectra of BPEDF-III terpolymer.

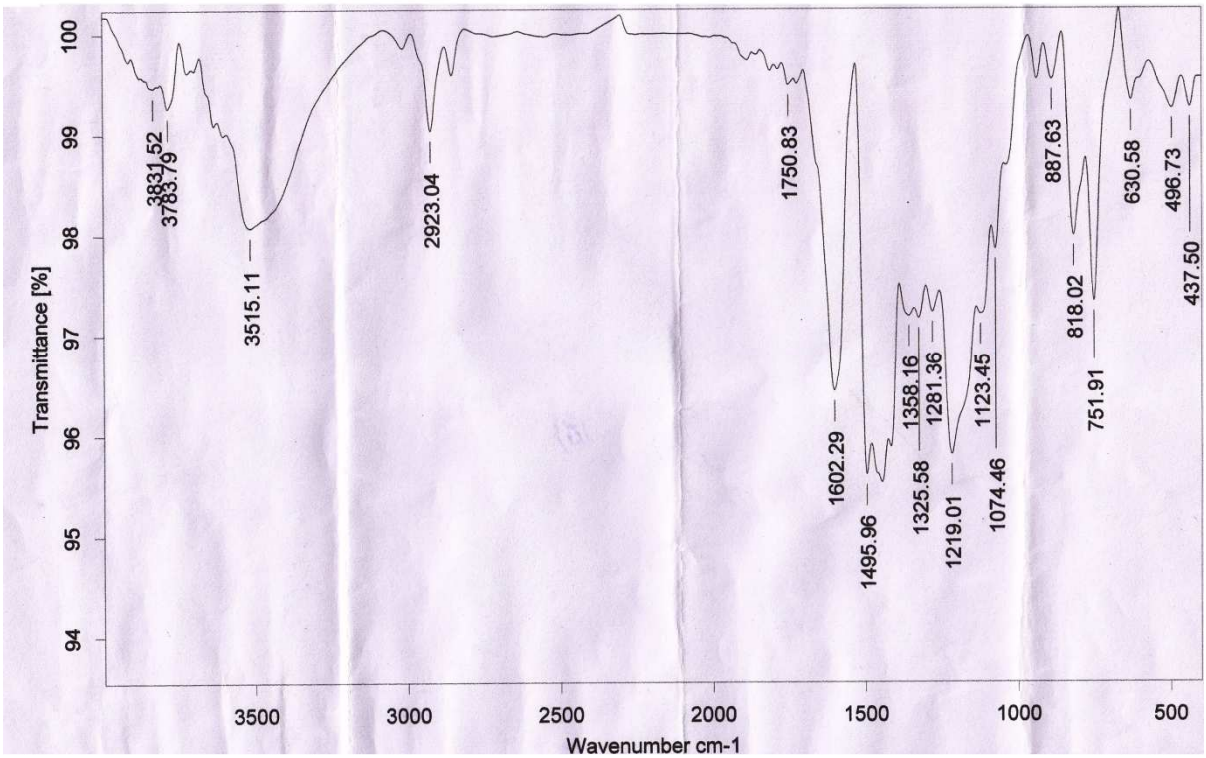


Figure 4:- Infrared Spectra of BPEDF-III Terpolymer.

^{1}H NMR spectra of terpolymers are presented in figure 5 and NMR spectral data is shown in table 5. These spectra show a multiple signal (asymmetrical pattern) in the region 6.7 to 7.3(∂) ppm, which are due to aromatic protons [35, 36]. A doublet signal appearing in the region 8.9-9.2 (∂) ppm can be assigned to the proton of the phenolic –OH group involved in hydrogen bonding [35, 37, 39]. A broad signal at 9.2(∂) ppm shows intramolecular hydrogen bonding of the $–NHCH_2-$ group or intermediate proton exchange reaction of both phenolic –OH groups [35, 41]. A weak signal at 7.8-8.0 (∂) ppm may be due to protons of the –NH- bridges [39-41]. A signal at 3.2-4.0 (∂) ppm may be assigned to ethylenic protons of an $Ar-CH_2-NH-CH_2$ moiety [34-38,41]. A medium signal in the range of 3.7-4.0 (∂) ppm is attributed to the presence of –NH- bridging [41].

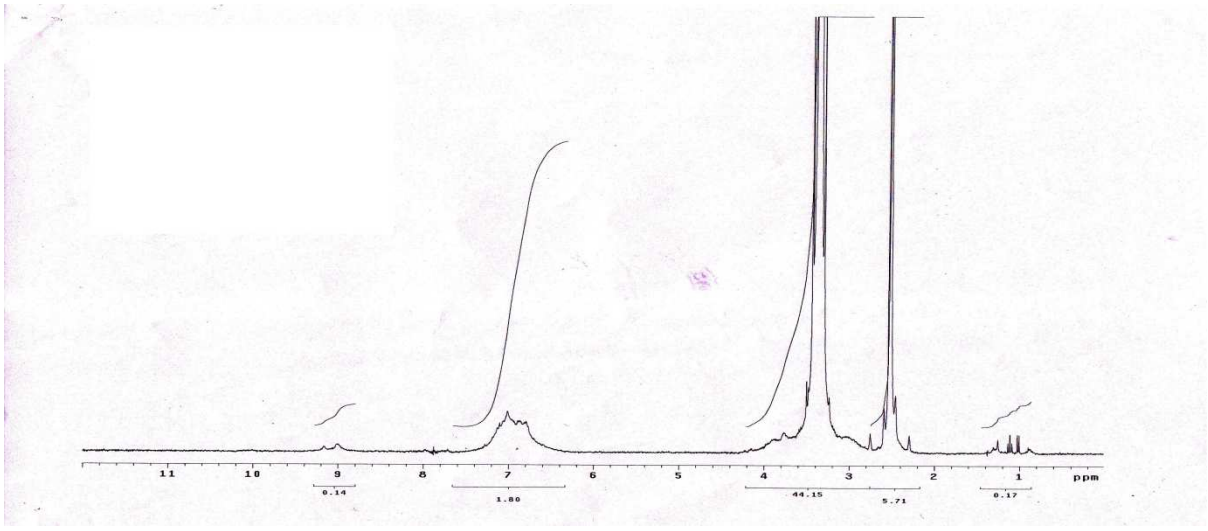


Figure 5: Nuclear Magnetic Resonance Spectra of BPEDF Terpolymer.

Antibacterial activity

In order to explore antibacterial activity, synthesised terpolymer has been tested for their antibacterial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Zone of inhibition with respect to standard antibacterial drug (ciprofloxacin) were measured (Table 1).

Table 1: - Relative antibacterial activity of BPEDF-III terpolymer (50 µg/ml, 100 µg/ml, 200 µg/ml)

Sr.no	terpolymers	Concentrations screened (µg/ml)	Diameter of inhibition zones in cm (activity index)			
			B. subtilis	*P. aeruginosa*	*E. coli*	*S. aureus*
1	BPEDF-III	50	0.9 (0.69)	0.7 (0.30)	0.6 (O.42)	0(0)
		100	1.3 (0.86)	1.1 (0.39)	1.4 (0.87)	0.6 (0.37)
		200	3.3 (1.73)	2.3 (0.71)	2.9 (1.38)	1.6 (0.8)
2	Ciprofloxacin (Standard)	50	1.3 (1)	2.3 (1)	1.4 (1)	1.3 (1)
		100	1.5 (1)	2.8 (1)	1.6 (1)	1.6 (1)
		200	1.9 (1)	3.2 (1)	2.1 (1)	2.0 (1)

The synthesized resin show good antibacterial activity against all bacteria. The graphical representation is shown in figure 6. From the graph, as the concentrations of the terpolymer sample increases diameter of inhibition zone in cm increases. Among all the concentrations, at 200 µg / ml terpolymer show good antibacterial activity.

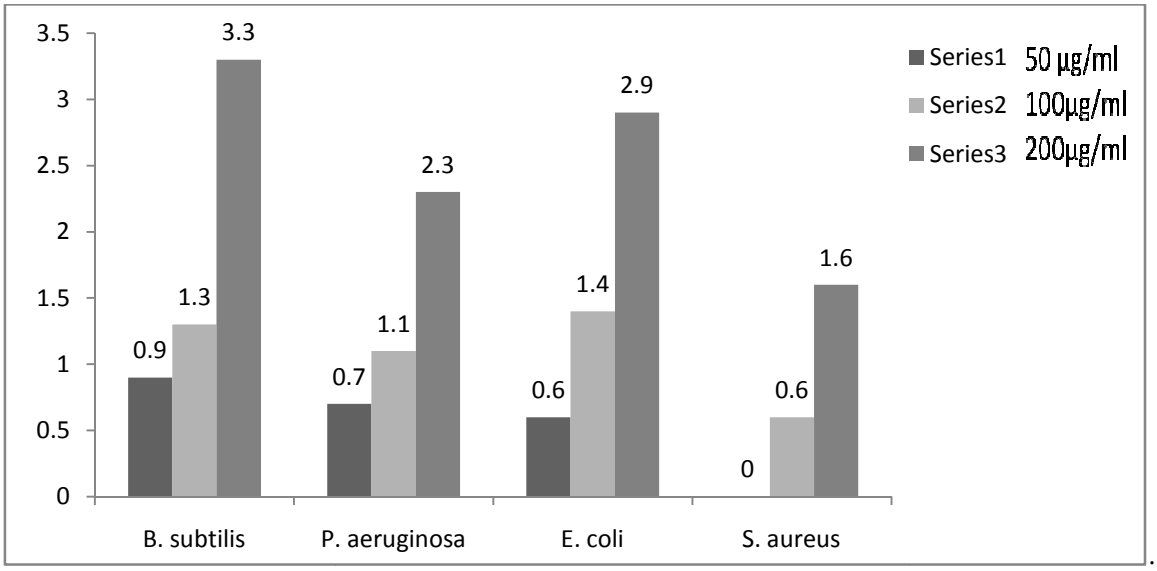


Figure 6: Comparative Antibacterial Activity of BPEDF-III Terpolymer and Standard Drug Ciprofloxacin against Bacteria Viz. *S. Aureus, P. Aeruginosa, E .Coli And B. Subtilis*.

CONCLUSION

The terpolymer is light pink in colour and partly soluble in DMF, DMSO and insoluble in commonly used organic solvents and concentrated acids. On the basis the nature and reactive positions of the monomers, molecular weight, elemental analysis, electronic, IR, and NMR spectra, the structure of terpolymer is determine. The terpolymer show excellent antimicrobial activities as compared to standard drugs. The synthesized terpolymer may find variety of applications in the field of material science.

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