Antimicrobial Screening of Polymeric Resins derived from 2,2'Dihydoxybiphenyl, Formaldehyde and Biuret

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***Abstract*--Novel chelating polymeric resin has been synthesized from 2,2' dihydoxybiphenyl, biuret and formaldehyde in dimethyl formamide medium with varying mole proportions(4:2:7) The polymer was characterized by FTIR, Proton NMR, ^{13}C NMR spectroscopy, Gel permeation chromatography (GPC) and scanning electron microscopy (SEM). The thermal decomposition pattern and the kinetics of thermal decomposition of the copolymer resins investigated by thermogravimetric analysis (TGA) in a static nitrogen atmosphere at a heating rate of 10 ^{0}C/min. Freenman-Carroll and Sharp Wentworth methods have been adopted to evaluate the kinetic and thermodynamic parameters such as thermal activation energies (Ea), order of the reaction (n), entropy change (S), free energy change, apparent entropy and frequency factor (Z). The thermal decomposition model for the copolymer resin was also proposed using Phadnis-Deshpande method. The synthesized copolymer resins were screened for antimicrobial activity against pathodenic bacteria and fungi. The resins show potent inhibition against bacteria suchasEschericida coli, Klebsiella Staphylococcus aureus, and Pseudomonusaeruginosa and fungi viz. Aspergillusflavus, Aspergillusniger, Penicillium species, Candida albicans, Cryptococcasneoformans and mucor species.**

***Keywords*-- Synthesis, ion-exchanger, distribution coefficient, resin, polycondensation, Antimicrobial screening.**

I. INTRODUCTION

Antimicrobial screening commercialized in a wide range of formulations with different characterization and have now a large practical applicability in various industrial processes, such as chemicals, nuclear industry for treatment of liquid waste, pharmaceuticals, food industries etc [1-5]. For their versatile properties, the copolymer resins are used both in the ion exchange area and in the heterogeneous catalysis field [6-8]. Organic ion exchange resins are the most important class of ion exchangers. Several workers [9] have investigated the role played by the polymer structure on ion exchange equilibrium and physical properties of the system. Donarum L G, Tomic E A and DeGieso R C developed the evaluation procedure for determining the selectivity of chelating polymer prepared from salicylic acid and formaldehyde. M. RiswanAhamed[10] et. al. reported the properties of ion exchangers containing chelate groups derived from anthranillic acid and formaldehyde. Ion exchange resins have been employed for many new organic and inorganic separations.

Few of the synthesized copolymer derivatives possess potent antibacterial activity and some of the compounds were reported for its cytotoxic activity by Chaudhary et al. [11]. A series of cyano derivatives of N-alkyl and N-aryl piperazine were synthesized and their antimicrobial activities were evaluated against Gram-positive and Gram-negative strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *S.epidermidis*,*Escherichia coli* and antifungal activities against *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. Biological evaluation of novel nitrogen containing aniline-formaldehyde resin has been studied and the compounds were reported as a potent antifungal and antibacterial agent by Parveen et al. [12]. Long chain aliphatic esters as well as organic and ferrocene containing Schiff bases were synthesized and reported to have good antitumor, anticancer and antioxidant agents [13]. Poly[(2-hydroxy-4-methoxybenzophenone) ethylene] resin and its polychelates with lanthanides(III) were screened for antibacterial activity and the metal chelated compounds maintain better activity compared to the ligand [14].

Hence keeping in mind of all these properties of these monomers, we have planned to synthesize such polymers, which have combined properties of these monomers. The synthesized copolymer was characterized by elemental analysis, UV-VIS, FT-IR, ^{1}H NMR, ^{13}C NMR, intrinsic viscosity and number average molecular weight. One of the important applications of chelating and functional copolymer is their capability to recover metal ions from waste solutions. Hence, the chelating ion-exchange properties of the 2,4-DBBF copolymer resin were also reported for specific metal ions.

The purpose ofthe selection of these monomers for present study, is to explore the adsorption behavior of seven metal ions Fe^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} on the newly synthesized copolymer resins p-HBBF at different pH values, different concentrations of different electrolytes and at different shaking time intervals. The adsorption behavior of these metal ions are based on the affinity differences towards the chelating resins as functions of pH, electrolyte concentrations and shaking time. One of the important applications of chelating and functional polymers is their capability to recover metal ions from waste solution. Hence the chelating ion exchange property of the 2,4-DBBFcopolymer resin was also reported for specific metal ions. Pollution by toxic heavy metals due to their toxicities in relatively low concentration and tendency to bioaccumulation in the ecosystem, agriculture and human body has received wide spread attention in recent years. Various approaches such as ion-exchange, reverse osmosis, electro dialysis, precipitation and adsorption techniques have been developed for the removal and recovery of the metal ions from sewage and industrial wastewater.

Among these techniques, many research works have focused on metal ions removal by adsorption on chelating polymers, because they are reusable, easily separable, and with higher adsorption capacity and selectivity having physical and chemical stabilities [15]. Some commercially available ion-exchange resins are given in Table 1.

II. EXPERIMENTAL

The important chemicals (starting materials) like p-hydroxybenzaldehyde, biuret and formaldehyde used in the preparation of new 2,4-DBBFcopolymer resin were procured from the market and were of chemically pure grade, and wherever necessary the purity was tested and confirmed by thin layer chromatography.

Synthesis of p-HBBF copolymer resin

The 2,4-DBBF copolymer resin was prepared by the condensation polymerization of 2,4-dihydroxybiphenyl (0.4mol) and biuret (0.2mol) with formaldehyde (0.7mol) in acidic hydrochloric acid medium at 130 ± 2 °C in an oil bath for 5 hrs. The solid product obtained was immediately removed from the flask as soon as the reaction period was over. It was washed with cold water, dried and powdered. The powder was repeatedly washed with hot water to remove excess of 2,4-dihydroxybiphenyl - formaldehyde copolymer, which might be present along with the 2,4-DBBF copolymer. The dried resin was further purified by dissolving in 8% NaOH and regenerated in 1:1(v/v) HCl/water. The purified copolymer resin was finally ground well to pass through a 300 mesh size sieve and kept in a vacuum over silica gel. The yields of these copolymer resins found to be 81.5% and the melting point is between 450 - 455^0K. The sieved resin was used for further characterization.

Characterization of 2,4-DBBFcopolymer resin

The copolymer resin was subject to micro analysis for C, H and N on an Elementary Vario EL III Carlo Erba 1108 elemental analyzer. The number average molecular weight $\overline{M}n$ was determined by conductometric titration in DMSO medium using ethanolic KOH as the titrant by using 25 mg of sample. A plot of the specific conductance against the milliequivalants of potassium hydroxide required for neutralization of 100 gm of copolymer was made. Inspection of such a plot revealed that there were many breaks in the plot. From this plot the first break and the last break were noted. The calculation of $\overline{M}n$ by this method is based on the following consideration [15] : (1) the first break corresponds to the stage at which the first acidic phenolic hydroxyl group is neutralized, and (2) the last break observed beyond the first break represents the stage at which phenolic hydroxyl group of all the repeating units are neutralized.On the basis of the average degree of polymerization, $\overline{(DP)}$ the average molecular weight has to be determined by following eq. (1)…

$$\overline{DP} = \frac{(Total\ milliequivalents\ of\ base\ required\ for\ complete\ neutrilization)}{(Milliequivalents\ of\ base\ required\ for\ smallest\ interval)} \quad - - - (1)$$

$$\overline{Mn} = \overline{DP}\ \times molecular\ weight\ of\ the\ repeating\ unit$$

The intrinsic viscosity was determined using a Tuan-Fuoss viscometer [16] at six different concentrations ranging from 0.3 wt % to 0.05 wt % of resin in DMSO at 30ºC. Intrinsic viscosity (η) was calculated by the Huggin’s eq.(2) [17] and Kraemer’s eq.(3) [18].

$$\text{In}\,\square\ \eta_{sp}/C = [\eta] + K_{1.}\,[\eta]^2.C \text{ -------------(2)}$$

$$\text{In } \eta_{rel}/C = [\eta] - K_{2.}\,[\eta]^2.C \text{ -------------(3)}$$

Spectral Analysis

Electronic (UV-visible) absorption spectra of the copolymer in DMSO was recorded with a double beam spectrophotometer UV-1800 fitted with an automatic pen chart recorder on themosensitive paper in the range of 200 – 850 nm at Kamla Nehru Mahavidyalaya Nagpur.Infrared spectra of 2,4-DBBF copolymer resin was recorded in najol mull with IR Affinity-I FT-IR spectrophotometer in KBr pallets in the range of 4000 – 500 cm^{-1} at Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh. Proton NMR and ^{13}C NMR spectra was recorded with Bruker Adanve – II 400 NMR spectrophotometer using DMSO-d_6 as a solvent, at Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh. The surface analysis was performed using scanning electron microscope at different magnifications. SEM has been scnanned by JEOL JSM-6380A Analytical Scanning Electron Microscope at VNIT, Nagpur.

Antimicrobial study by disc diffusion technique

Antibacterial activity was tested by the filter paper disc diffusion technique involving the cultures of the selected organisms for 24 h at P. G. and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli 620 020, Tamil Nadu, India. Mueller Hinton agar no.2 (Hi Media, India) was used as the bacteriological medium. The test solutions of the synthesized copolymers and their metal complexes were prepared in sterile dimethyl sulphoxide solvent for the study. The synthesized copolymers and their metal complexes were tested at different concentrations ranging from 50 to l000 ppm to find out the minimum concentration of the copolymer ligands and their metal complexes required to inhibit the bacterial growth. Amoxicillin (100 µg/mL) was taken as the standard for antibacterial activity. The organism were seeded into sterile nutrient agar medium by mixing one mL of inoculums with 20 mL of 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 Whatmann 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) were measured.

Antifungal activity was tested by the filter paper disc diffusion technique involving the cultures of the selected organisms for 24 h at P. G. and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli 620 020, Tamil Nadu, India. 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. Fluconazole (100 mg/ml) was taken as the standard for antifungal activity. The test solution impregnated discs were placed on the agar and incubated at 37ºC 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.

III. RESULTS AND DISCUSSION

The resin sample was light pink in color, insoluble in commonly used organic solvents, but was soluble in dimethyl formamide, dimethyl sulphoxide, tetrahydrofuran, pyridine and concentrated H_2SO_4. The resin synthesized do not show sharp melting point but undergo decomposition between 450-455 K**.** Based on the analytical data, the empirical formula of the copolymer resin is found to be $C_{19}H_{20}N_3O_{7,}$ which is in good agreement with the calculated values of C, H, N and O. The resin was analyzed for carbon, hydrogen, and nitrogen content C = 56.73% (Cal) and 56.26% (F); H = 4.90% (Cal) and 4.66% (Cal); N = 10.45% (Cal) and 10.41% (F).

Molecular weight determination

The number average molecular weight ($\overline{M}n$) could be obtained by multiplying the $\overline{DP}$ by the formulaweight of the repeating unit [19]. The calculated molecular weight for 2,4-DBBF resin is 12752.Viscometric measurement was carried out in DMSO at 30ºC. The intrinsic viscosity was determined by the Huggin's eq. (2) and Kraemer's, eq. (3) which is 0.27 and 0.25 respectively. In accordance with the above relations, the plot of ηsp/c and ηrel/c against C was linear giving as slopes K_1 and K_2 (0.52)respectively. The intercept on the axis of viscosity function gave the (η) value in both the plots [19]. The values of (η) obtained from both relations were in good agreement with each other.

Spectral Analysis

The UV-visible spectram of 2,4-DBBF copolymer resin shown in Fig.1. UV-visible spectra of p-HBBF resin has been recorded in pure DMSO in the region of 200 – 800 nm at a scanning rate of 100 nm min^{-1} and at a chart speed of 5 cm min^{-1}. The spectra of these copolymers exhibit two absorption maxima in the region 280 nm and 320 nm. These observed positions of the absorption bands indicate the presence of carbonyl group (ketonic) processing double bond, which is in conjugation with the aromatic nucleus [20]. The appearance of former band (more intense) can be accounted for $\pi \rightarrow \pi^*$ transition while the later band (less intense) may be due to $n \rightarrow \pi^*$ electronic transition. The shift from the basic value (viz. 240 nm and 310 nm respectively) may be due to conjugation effect, and presence of phenolic hydroxyl group (auxochromes) is responsible for hyper chromic effecti.e. Σ_{max} higher values.

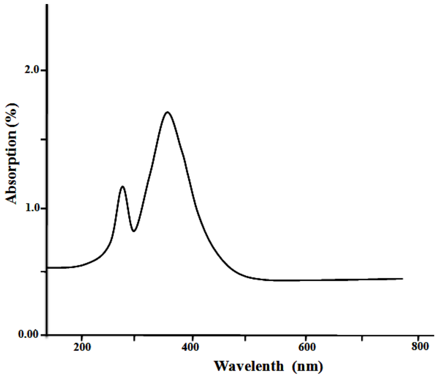

Fig. 1. Electronic spectra of 2,4-DBBFcopolymer resin

The IR spectrum of 2,4-DBBFcopolymer resin is presented in Fig. 2 and IR data are specified in Table 2. [21]. A broad band appeared in the region 3500–3250 cm^{-1} may be assigned to the stretching vibration of the phenolic hydroxyl groups exhibiting intermolecular hydrogen bonding. The sharp band displayed at 1615–1640 cm^{-1} may be due to the stretching vibration of carbonyl group of both, ketonic (CHO) as well as biuret moiety. The presence of -NH- in biuret moiety may be ascribed to aromatic skeletal ring.

The bond obtained at 1374, 1273, and 815 cm-^{1} suggest the presence of methylene bridges in the polymer chain.1, 2, 3, 4, 5 pentasubstitution of aromatic ring is recognized from the bonds appearing at 1273, 1163, 1085, 992, and 813 cm^{-1}, respectively.

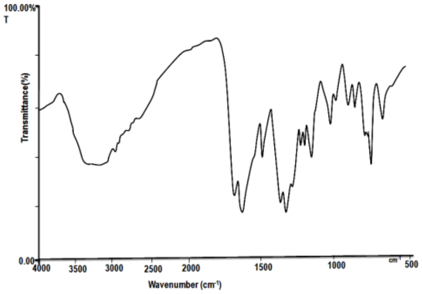

Fig.2. Infrared spectrum of 2,4-DBBFcopolymer resin

H^{1} NMR spectrum of 2,4-DBBFcopolymer is shown in Fig. 3 and Proton NMR data are specified in Table 3 [22]. H^{1} NMR spectra of p-HBBF copolymer shows a weak multiplicity signals (unsymmetrical pattern) in the region 7.52 to7.88 (δ) ppm may be due to the aromatic protons. A weak signal appeared in the region of 6.86 – 7.14 shows the presence of imido proton of – CO-NH-CO- linkage. The weak multiply signals appearing at 5.15 – 5.45 (δ) ppm may due to the amido –CH_2 -NH-CO linkage. A signal appeared in the region 4.07 – 4.98 (δ) ppm may be due to proton of methelenic bridges (Ar – CH_2 – N) of polymer chain. The signal in the range of 3.62 – 3.90 (δ) ppm is attributed to phenolic -OH proton.

A ^{13}C NMR spectrum of 2,4-DBBFcopolymer resin is shown in Fig. 4. The peaks appeared at 121.14, 127.71, 129.09, 131.88 and 155.94 ppm may be corresponded to carbons present in aromatic benzophenone ring. The peak appeared at 162.11 ppm of may be correspond to carbonyl group of biuret moiety. The medium peak appeared at 115.33 ppm may be confirmed the presence of –C-NH group of copolymer resin. The peak appeared at 65.56 ppm may be due to the presence of –C-OH group in aromatic benzophenone group [22]. The peaks appeared at 38.91 to 40.17 ppm may be due to the –CH_2 – bridge in copolymer resin. All these peaks confirmed that the p-HBBF copolymer resin must have linear structure.

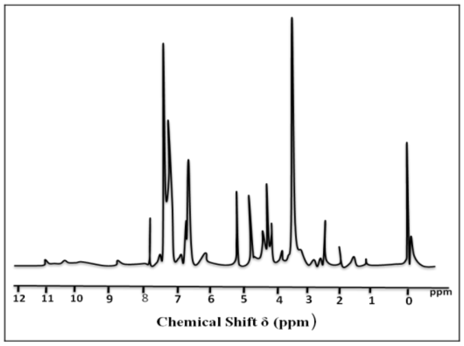

Fig 3.Proton NMR spectrum of 2,4-DBBFcopolymer resin

Scanning electron micrographs

Surface analysis has found great use in understanding the surface features of the materials. The morphology of the reported resin sample was investigated by scanning electron micrographs at different magnification, which is shown in Fig 4. It gives the information of surface topology and defect in the structure. The resin appeared to be dark drawn in color. The morphology of copolymer resin shown spherule and fringed model. The spherules are complex polycrystalline formation having as good as smooth surface. This indicates the crystalline nature of p-HBBF copolymer resin sample. The morphology of resin polymer shows also a fringes model of the crystalline amorphous structure. The extent of crystalline character depends on the acidic nature of the monomer. But the photograph shows the fringed and scatted nature having shallow pits represent the transition between crystalline and amorphous. The resin exhibits more amorphous characters with closed packed surface having deep pits, which is shown Fig.6. Due to the deep pits, resin exhibits higher exchange capacity for Pb^{2+}ions (as well as other many metal ions). This could be the reason of bigger nitrated Pd^{2+} ions, which can easily penetrate in to the deep pits. Thus by SEM micrographs morphology of the resin shows the transition between crystalline and amorphous nature. When compare to the other resin [23], the 2,4-DBBFcopolymer resin is more crystalline-amorphous in nature, hence shows higher metal ion exchange capacity.

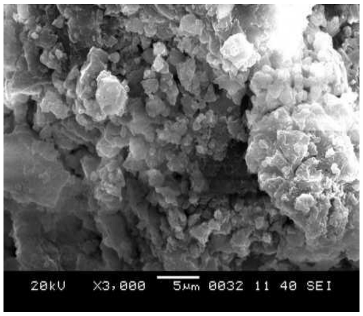

Fig 4. SEM Micrographs of 2,4-DBBFcopolymer resin at 3000x magnification.

Antimicrobial testing

The results of microbial screening of the 2,4-DBBFcopolymer resin is presented in Table 4 for antibacterial and antifungal studies.

Antibacterial activity

An admirable result was obtained for 2,4-DBBFcopolymer resins against *E. coli* bacterium which is markedly higher than the standard. E. coli, a gram negative rod-shaped bacterium affects the urinary tracts in humans. *Klebsiella* is a genus of non-motile, gram-negative bacterium that causes a wide range of disease states, notably pneumonia, septicemia and soft tissue infections. The results showed a good activity against Klebsiella. *P. aeruginosa* is a common bacterium which can cause diseases like pulmonary tract and urinary tract burns and wounds in animals and human beings for which the 2,4-DBBFresins had moderate activity.

Antifungal activity

The antifungal activities of the 2,4-DBBFcopolymer resins also provide appealing results against the chosen fungal strains. *Penicillium* sp. cause infection in humans and the resulting disease is known generically as penicilliosis. The resins had moderate activity against *Penicillium* sp. *C. albicans* is a diploid fungus and a causal agent of opportunistic oral and genital infections in humans and also emerged as an important cause of morbidity and mortality in immune compromised patients. The 2,4-DBBFcopolymer resin exhibits a very good activity against *Penicillium* sp. *C. neoformans* is an encapsulated yeast-like fungus that can live in both plants and animals cause lung infection. The resins had good activity against this fungal strain. The antimicrobial activity of the copolymer resins may be due to the presence of $–NH_2$ and –OH groups. It is perceived that the factors, such as solubility, conductivity, dipole moment and cell permeability may also contribute to the increased antimicrobial activity against the chosen microbes [24].

Table 1.Antibacterial and antifungal activity of the 2,4-DBBF copolymer resin

Compound (Copolymer resin)	Zone of inhibition (mm)				
	Bacteria			Fungi	
	E. Coli	*Klebseilla*	*P. Aeruginosa*	*C. Albicans*	*Pencillium species*
2,4-DBBF	29	19	19	21	16
Standard	28	26	25	20	22
Control (DMSO)	-	-	-	-	-

IV. CONCLUSIONS

2,4-DBBFCopolymer resin was prepared from 2,4-dihydroxybiphenyl and biuret with formaldehyde in hydrochloric acid medium by condensation technique. The spectral characterizations of the copolymer confirm the linear structure. The semi crystalline - amorphous nature of the 2,4-DBBFcopolymer resin was confirmed by the SEM studies. The 2,4-DBBFcopolymer resin was found to have excellent antimicrobial activity against the chosen bacteria and fungi. Further, in the case of *C. albicans* and *E. coli*, the copolymer resin showed enhanced activity than the standard drug.

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