INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-2, April-June-2012 Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Received: 25th Feb-2012 *Revised: 06th Mar-2012* *Accepted: 10th Mar-2012*

Research Article

FACILE SYNTHESIS OF CHELATING BIS LIGANDS: SPECTROSCOPIC, PHYSICOCHEMICAL AND BIOLOGICAL STUDIES

Ratiram G. Chaudhari*, Mangesh P. Gharpure[1] and Harjeet D. Juneja[1]

*Department of Chemistry, S. K. Porwal College Kamptee, Maharashtra, India
[1]Department of Chemistry, Mahatma Jyotiba Phule Educational Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440 033, Maharashtra, India

Email: chaudhary_rati@yahoo.com, hd_juneja@yahoo.com

ABSTRACT: Seven bis-ligands of carbamides were synthesized and characterized on the basis of elemental analysis, Infrared spectroscopy, ^{1}H NMR, mass spectra and UV-Visible studies. These bis-ligands were synthesized by condensation method, of acidic dichloride with 4-methoxyphenylcarbamide (4-MPC) in stoichiometric ratio of 1:2 respectively. It were found that, the behaviour of bis-ligands acting as chelating agents (chelators), therefore these were comparatively interpreted on the basis of physicochemical properties such as viscosity, surface tension, conductivity, pH and solubility. The antimicrobial activities of chelating ligands also have been studied. The results were revealing that, the activities of newly synthesized chelating ligands were found to be higher than its parent's compound. The aromatic chelating compounds shows more activity in all physicochemical properties as compare to aliphatic chelators. The nanoparticle sizes of chelating ligands were significantly identified by scanning electron microscopy (SEM) and morphological result of each chelating compound were found totally different.

Keywords: Chelating agents, antimicrobial, conductivity, carbamides, physicochemical properties, Nanoparticle, SEM and chelators

INTRODUCTION

Aromatic chelating amides are well aware for high performance as they possess good thermal stability and heat resistance with excellent mechanical properties as compare to aliphatic chelating compounds. The chelating or chelation therapy is the process of removing the undesirable ionic material by the influence of organic compounds which has suitable properties. Because of these properties they are of major commercial and industrial important. The chelating therapy is widely use for the treatment of chronic degenerative disease. These chelating compounds show more metals gripping potential (Pande *et al*., 1987; Patel and Patel, 2010) so it forms better thermal stability; also they have good antibiological properties (Bankova *et al.,* 1996), selective complexation and separation of toxic and carcinogenic metal ions (Singh and Shrinivasan, 2002; Beauvais and Alexandratos, 1998) and ligand exchange chromatography (Hruby *et al*., 2004) due to carbamide moiety.

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Recently there has been valuable interest in the synthesis and use of functionalized compound having chelating ability due to their practical convenience, operational flexibility and formation of coordination with high metal to polymer bond energy. The large numbers of chelating resins have been reported (Shamal *et al.,* 1999; Radia *et al.,* 1986). These chelating compounds acting as macrocyclic ligand because they are good host for metal anion therefore template reaction were largely used for preparation of macrocyclic complexes (Singh *et al.,* 2010). Here we reported facile method for the preparation of several stable chelating agents which are chemically and thermally stable and have high binding affinity towards a large variety of transition metals, furthermore, its application have been found in various fields like synthetic fibre, antimicrobial and to remove highly toxic heavy metal ions from aqueous solution (Ansari and Raofie, 2006; 1998; Kadirvelu *et al.,* 2001). These chelating agents increased the removal efficiency of heavy metal ions from aqueous solution. These also show paramagnetic behaviour, conductivity and resistance to high energy. Therefore, they can be used to prepare composites with resistance to high temperature (Persak and Fleming *et al*., 1986). In this work, we have investigated the different physicochemical parameter viz. colour, melting point, various viscosity, surface tension, pH and conductivity. Also we have investigated antibacterial and antifungal activities of chelating bis ligands. Our main aim of production and synthesis of antimicrobial compounds is to inhibit the causal microbe without any side effect. The amide containing aromatic structure melt at high decomposing temperature and it have solubility in some organic solvents. The melting point of compounds depends on its molecular weights and it can be determined by viscosity method trough viscous solution. The coloured amide compounds have not received attention much more in recent year; therefore it is interestingly decided to explore the field of coloured amide compounds. A present article we characterize through IR, UV-visible, ^{1}H NMR spectroscopy, SEM technique, and solubility, density, various viscosities, surface tension, pH, conductivity, antimicrobial activities in order to discuss comparatively study of chelating ligands.

MATERIALS AND METHODS

The acid dichlorides were prepared for synthesis of chelating ligands: (1a) Isophthaoyl dichloride (1b) Phthaoyl dichloride, (1c) Terphthaoyl dichloride, (1d) Adipoyl dichloride, (1e) Suberoyl dichloride, (1f) Azeloyl dichloride, (1g) Sebacoyl dichloride. All these acid dichlorides were prepared in laboratory by standard procedure (Ukey and Juneja, 2005). Fresh double distilled water was used for the preparation of 4-methoxyphenylcarbamide. It was also prepared by slight modification of a method reported in literature. The solvents were double distilled before used. Other chemicals were Analytical grade and used without further purification. ^{1}H NMR spectra of chelating ligands were recorded on Bruker Advance Model II 400 MHz NMR spectrometer in DMSO with TMS as internal standard. The infrared (IR) spectra of all the samples were scanned in solid state on Shimadzu Spectrophotometer FTIR-8101A using KBr pallets in the range of 400-4000 cm^{-1}. UV- Visible spectra of bis-ligand were recorded on Shimadzu double beam UV-Visible spectrophotometer at National Environmental Engineering Research Institute (NEERI), Nagpur. The density of all chelating ligands has been determined by pyknometrically at room temperature using variable concentration.

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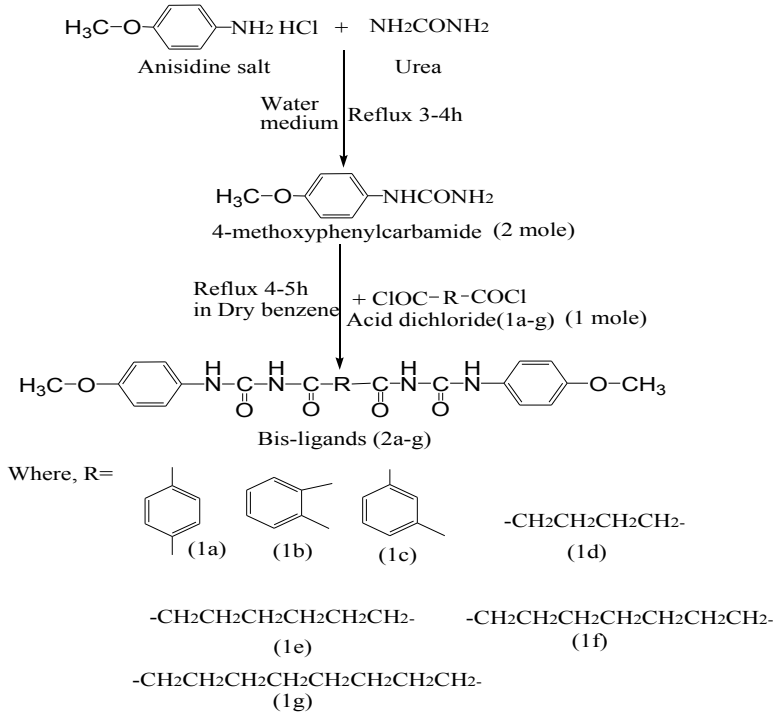
The surface tensions of chelating ligands were determined by drop number method using stalagmometer while the relative viscosity obtained by Oswald's viscometer at variable concentration in room temperature by using water as reference medium. Conductivity measurements carried out at room temperature on Systronics conductivity meter 306, whereas pH measurements carried out on Systronics digital pH meter 335.

Synthesis of bis-ligands (1a-g)

Seven amide chelating ligands were synthesized, out of seven chelating ligands; three were reported in previously communication. Four chelating ligands such as phthaoyl 1, 2 bis (4-methoxyphenylcarbamide) (PB4-MPC), terphthaoyl 1, 3 bis (4-methoxyphenylcarbamide) (TB4-MPC), isophthaoyl 1, 4 bis (4-methoxyphenylcarbamide) (IB4-MPC) and suberoyl 1, 6 bis (4-methoxyphenylcarbamide) (SUB4-MPC) were newly synthesized by modification of condensation method reported in literature (Juneja *et al.,* 2006)14. The bis-ligands were synthesized by the condensation reaction of acid dichloride (**1a-g**) (0.1M) and 4-methoxyphenylcarbamide (0.2M) in benzene medium. The solution of acid dichloride (**1a-g**) were mixed drop-wise into the solution of 4-methoxyphenylcarbamide with rigorously stirring at room temperature. The solutions were refluxed for 4-5 h. The sticky solid product obtained and triturated with alcohol. Products were repeatedly washed with hot ethanol and dried in oven. After recrystallization in ethanol: dimethylformamide mixture yields were obtained in range of 80-85%. The resultant chelating ligands have shown in scheme 1.



Scheme1. Synthesis of bis ligands (**1a-g**)

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RESULTS AND DISCUSSION

Spectral analysis

The infrared spectra of all newly synthesized bis-ligands exhibited several common absorption frequencies. The formation of chelating ligands was confirmed from the typical absorption band observed around 1690-1741 cm^{-1} which may be assigned to the stretching frequency of the >C=O vibration (Pizarro *et al.,* 2009; Bulmus *et al.,* 2003; Rivas and Castro, 2003) and band observed around 3255-3365 cm^{-1} which is attributed to N-H stretching vibration (Cardenas *et al.,* 2000) of secondary amide. The band observed at 2926-2932 cm^{-1} and 2845-2860 cm^{-1} have assigned to asymmetric and symmetric stretching frequency of $–CH_2$ and C-H group , respectively. A strong band observed in chelating ligands around 1491-1512 cm^{-1} which may be attributed to the C=C group stretching frequency, it supports the presence of aromatic ring. The band observed around 870-920 cm^{-1} may be due to the C-H bending vibration of aromatic ring. Infrared spectra of chelating ligands containing band at around 1330-1370 cm^{-1} was confirming the presence of $–CH_3$ group. The band appeared at 1400 cm^{-1} which may be due to the C-N stretching vibration of amide group (Rosa *et al.,* 2003; Dubey and Bajpai, 2006). The UV-Visible spectral studies of 4-MPC and its derivative were carried out in DMF medium. The electronic spectral data of chelating ligands were found at 264, 288 nm and 290, 309 nm. The first peaks attributed to aromatic benzene ring π- π^* and second peak due to n-π^* transition, this transition is shifted to lower wavelength with high intensity. This shift is indicated the donation of lone pair of electron of nitrogen in ligands to central metal atom (Pagadala *et al.,* 2009). The high resolution ^1H NMR spectra of chelating ligands were scanned in dimethylformamide using an internal reference (TMS). ^1H NMR spectra of PB4-MPC, IB4-MPC and TB4-MPC shows multiplet at δ 6.7-7.8 ppm (Asundariya *et al.,* 2009) that may be due the presence of aromatic protons. 3H of methyl of -O-CH_3 of 4-methoy phenyl ring produce singlet at δ 3.6 ppm. Hydrogen of -CONH was produces a singlet at δ 9.9 ppm. ^1H NMR spectra of methylene proton shows multiplet for 6 H of methylene $(–CH_2)_6$ of SUB4-MPC at δ 1.9 - 2.9 ppm and methylene proton of AB4-MPC shows multiplet for 4 H at δ 1.8-3.4 ppm, similarly AZB4-MPC and SB4-MPC shows multiplet for 7 H and 8H at δ 2.2 -3.5 ppm.

Physiochemical parameter studies

The physiochemical properties of chelating ligands were discussed and interpreted in well definably in following points-

Yield and Colour

The percent yield of recrystalized chelating ligands were found to be varied from 76 to 89 % (Table1) whereas the percent yield of crude amide chelating ligands was higher as compare to recrystalized chelating ligands. It was observed that the yields of aromatic chelating ligands were found to be higher than non aromatic chelating agents. All aliphatic chelating agents are white colour powder form, whereas aromatic chelating ligands are variable colours powder form (Table 1).

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Table 1. Physical properties of chelating ligands

Chelating ligands	Colour	% Yield	Melting Point(^{0}C)
4-MPC	Brown	88	168
PB4-MPC	Yellow	78	345
TB4-MPC	Red	89	355
IB4-MPC	Pink	76	354
AB4-MPC	White	81	187
SUB4-MPC	Dull white	81	190
AZB4-MPC	White	79	245
SB4-MPC	White	88	250

Melting point and solubility

The melting point of 4-methoxyphenylcarbamide and its derivatives (Table1) were carried out on melting point apparatus at room temperature for powder form. The melting points of all ligands were found to be totally different. The high thermal stabilities are shown by aromatic chelating ligands due resonance effect involved while aliphatic bis-ligands are less stable. Due to high melting point of amide bis chelating ligands these can be used as thermally resistance material. The solubility of these ligands (Table2) was determined for powder forms at room temperature with concentration approximately 1 %(w/v). 4-MPC was soluble in almost all common organic solvents while bis ligands were soluble in aprotic solvents only and partly soluble in chloroform, dichloroethane. It was also noticed that the solubility increases suddenly when increase temperature and those ligands are partially soluble at 25 ^{0}C and get dissolved in higher temperature. The solubility of bis ligands were decreases as bulkiness of ligand increases.

Table 2. Solubility of bis-ligands in various solvents

Bis ligands	Solvents									
	DMF	DMSO	$CHCl_3$	CCl_4	C_6H_6	THF	DCE	C_2H_5OH	H_2SO_4	HCl
4-MPC	+	+	±	±	++	+	+	+	++	+
PB4-MPC	++	++	±	-	±	±	-	-	+	+
IB4-MPC	++	++	-	-	-	-	±	-	+	+
TB4-MPC	++	++	-	-	-	±	-	-	+	+
AB4-MPC	++	++	-	-	-	±	-	-	++	+
SUB4-MPC	++	++	-	-	-	±	-	-	++	+
AZB4-MPC	++	++ +	-	-	-	±	-	-	++	+
SB4-MPC	++	+	-	-	-	±	-	-	++	+

The symbol indicates the solubility of bis-ligands at room temperature. Where + = Soluble, ++ = Strong soluble, - = Insoluble, ± = Partly soluble

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Density

0.01M, 0.02M and 0.03M solution of all chelating ligands were prepared in dimethylformamide. The density of each seven chelating ligands at given concentration was measured at 30 ± 1^{0}C. All sample dissolved in hot dimethylformamide and remains in same state even after long time. The densities of all chelating ligands were determined in density bottle and different concentration was made in METLLER Balance. The results of all chelating ligands are shown in Table 3. The density of all chelating compounds varies from 0.9316 to 1.1025 g / cm^{-1}. The highest density was display by TB4-MPC and SB4-MPC while lowest density shown by 4-MPC and AB4-MPC. The density of all amide ligands were varied with increasing methyl group and ortho, meta, para substitution at benzene ring. The result reveals that as increases methyl group increase the density and vice versa.

Viscosity and surface tension Measurements

0.1, 0.2 and 0.3 mol g^{-1} solution were prepared in dimethylformamide (DMF) medium for measurements of relative viscosity and surface tension at temperature 25 ± 2 ^{0}C. The various viscosities such as intrinsic, specific, reduced and inherent viscosity of chelating ligands were measured at 0.4 % solution (Table 4). The relative viscosity and surface tension of bis bidentate ligand at different concentration are presented in table 3. Viscosity of a bis bidentate ligand solution depends on molecular weight and concentration of the dissolved ligand. The tabulated data reveal that SB4-MPC has higher viscosity of solution and hence the higher molecular weight among the all chelating ligands where as 4-MPC and AB4-MPC has lower value of viscosity hence these have lower molecular weight. It is used for determination of molecular weight introduced by Staudinger, is the one most commonly employed in research. Accurate measurements of absolute viscosity being difficult, it is convenient relative viscosity. Here we have measured various viscosities. Let η be the viscosity of solution and η_o is the viscosity of pure solvent and flow time for DMF solvent was 126 sec. By using the relation $\eta_r = \eta / \eta_o$ or $\eta_r = t / t_o$ relative viscosity can measure while the relative viscosity is related to some other quantities as follow: $\eta_{sp} = \eta_{rel} - 1$, $\eta_{red} = \eta_{sp}/c$, $[\eta] = \lim (\eta_{sp}/c)$ where η_{sp} is specific viscosity, η_{red} is reduced viscosity and $[\eta]$ is intrinsic viscosity is also called viscosity number or Staudinger index. In these equations, c is the concentration of the chelating ligands. The plots of η_{sp}/c and $l_n\eta_{red}/c$ versus c give straight lines which confirm to the following equation: Huggin equation: $\eta_{sp}/c=[\eta]+k'[\eta]^2c$ Kraemer equation: $\ln \eta_r / c = [\eta] + k'' [\eta]^2c$. Both these equations are applicable only in dilute solutions. For chelating ligands, k'=0.4 + 0.1 and k'=0.50 + 0.05. Similarly surface tensions also were carried out at room temperature by using Stalagmometer.

Conductivity and pH measurements

The molar conductivities and pH of 10^{-3} solution of 4-MPC and its derivatives in DMF were measured at temperature 25 ± 2 ^{0}C. By using the relation $\Lambda_m = K/C$, the molar conductance of chelating ligand can be measured and where C is concentration of ligand solution. The molar conductance and pH measurements (Table 3) were carried out at different dilution. It is concluded that ligands have molar conductance value, hence it found ionic nature. The data reveal that at 0.0347 mol g^{-1} shows good conductivity while 0.0119 and 0.0244 mol g^{-1} shows moderate conductivity.

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Morphology of chelating ligands

The surface morphologies of chelating bis ligands were investigated by SEM. The micrographs of all bis ligand (Fig. 1) were totally different from 4-MPC, as well as the micrograph of seven chelating bis-ligand were found to be totally different from each other. Scanning electron microscopy image were reveal that the particle size of all chelating ligands were found to be nanoparticle. The image of PB4-MPC (Fig. 1) were found globule like droplets structure while TB4-MPC and IB4-MPC compound shows round shape globules with smaller size droplets are attached on its surface, whereas in AZB4-MPC image shows flat rod-plane plate structure with end side broken. Other images of chelating ligands also seen somewhat similar structure and many particles were appeared in one micrometer area. The significant different morphological appearances were observed of chelating ligands from their parents' compound shows the evidence of formation of chelating ligands. The solution side of aromatic chelating ligands PB4-MPC, PB4-MPC and IB4-MPC (Fig. 1) shows globules like droplets structure are significantly are significantly different from aliphatic ligands which shows flat rod shape structure.

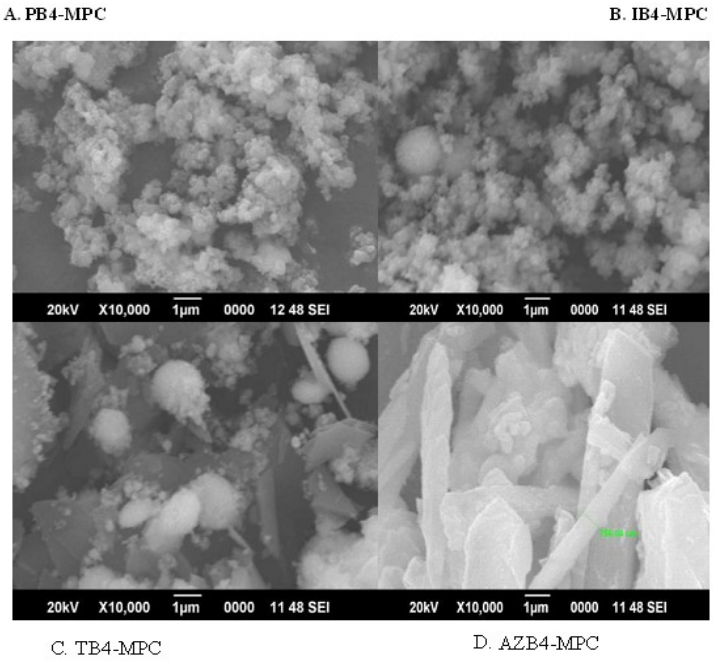

Fig.1 SEM image of chelating ligands A. PB4-MPC, B. IB4-MPC, C. TB4-MPC and D. AZB4-MPC

Microbial activities

The antimicrobial activity at higher concentration shows more toxic as compare to fungus. Amide ligand shows moderate activity as compare to standard bactericide and fungicide. It is also suggest that the percentage of bacteria and fungus inhibition is in the range of 50 to 72 % depending on the concentration of amide ligand.

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The paper disc method was used for antibacterial activity tested against E. coli. Solutions of 4-MPC and bis ligand were prepared by dissolving in DMF solvent of concentration 500 and 1000 ppm. The paper disc of whatman filter paper (**No. 42**) of diameter 2 cm were cut and sterilized in anautoclave. The paper disc soaked in 500 and 1000 ppm concentration of bis ligands solutions were placed in the aseptically in the petri dishes containing nutrient agar media seeded with E. Coli. The petri dishes were incubated at 35 ^{0}C and the inhibition zones were recorded after 24 h of incubation. The percentage activity index for 4-MPC and its derivatives were calculated. It is observed that antibacterial activity of derivatives compounds shows more effect than its parent's compounds. Similarly the antifungal activity of bis ligands were tested for their effect on the growth of microbial culture and studied for their interaction with Rhizopus nigricans using agar medium having the composition, 20 gm starch, 20 gm glucose, 20 gm agar-agar and distilled water 1000 mL. A requisite amount of substance was dissolved in DMF so as to get the certain concentrations of 100 and 200 ppm. The medium was taken in to the petri plates and the spores of fungi were placed on the medium with help of inoculum's needle. Then these petri plates wrapped in the polythene bags and were placed in an incubator 30 ^{0}C. After 72 h linear growth of fungus was seen by measuring the diameter of fungal colony. It was observed that the antifungal activities shown by parent's compounds are not more effective but its derivative compounds are so much powerful.

Table 3. Density, Surface tension, pH and conductivity of chelating ligands

Bis ligands	Molality $\left(\frac{m}{mol.g^{-1}}\right)$	Density $\left(\frac{\rho}{g.cm^{-3}}\right)$	Surface Tension $\left(\frac{\gamma}{dyne.cm^{-1}}\right)$	Relative Viscosity ηr = η /ηo	Conductivity $\left(\frac{conductivity}{\mu.Simen.cm^{-1}}\right)$	pH
4-MPC	0.110 0.201 0.310	0.783 0.872 0.899	25.2563 23.8790 21.6732	0.8732 0.8997 0.9788	17.97 16.76 14.88	7.88 7.55 8.08
PB4-MPC	0.101 0.199 0.310	0.989 0.998 1.205	30.2312 29.2381 28.1988	0.9987 1.2434 1.4122	22.12 22.07 24.13	7.71 8.23 8.99
IB4-MPC	0.102 0.201 0.311	0.998 1.023 1.296	28.2376 27.5543 26.3478	0.9870 1.3176 1.4001	23.67 22.31 26.45	7.72 8.75 8.97
TB4-MPC	0.108 0.206 0.304	0.9854 1.1250 1.3128	31.7525 29.8792 28,8235	1.0067 1.2032 1.2672	23.45 22.43 24.11	9.13 8.91 8.19
AB4-MPC	0.119 0.244 0.347	0.9357 0.9360 0.9366	29.1191 26.5970 26.0461	0.9589 0.9715 1.0326	18.60 20.71 21.30	8.06 7.92 7.66
SUB4MPC	0.101 0.213 0.317	0.9355 0.9376 0.9380	29.2378 27.8791 28.9873	1.1984 1.2190 1.2281	16.74 18.43 18.93	8.43 8.81 9.00.
AZB4MPC	0.113 0.215 0.319	0.9298 0.9364 0.9311	30.1145 29.3476 26.5634	0.9345 0.9945 1.1254	13.18 16.88 18.99	10.8 9.48 8.82
SB4-MPC	0.112 0.218 0.336	0.9316 0.9362 0.9382	29.3410 26.3151 25.8182	1.2279 1.2613 1.2781	11.40 12.11 19.30	8.93 9.03 9.09

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Table 4. various viscosity values at 0.4 % solution of all chelating ligands

Chelating ligands*	Flow time t sec	Intrinsic viscosity η	Relative viscosity ηr = t /to	Specific viscosity ηs = ηr-1	Reduced viscosity ηred=ηS/c	Inherent viscosity ηi = ln ηr/c	Huggin Constt. K'	Kremer Constt. K"
4-MPC	158	0.569	1.253	0.253	0.632	0.417	0.471	1.178
AZB4MPCS	217	1.503	1.722	0.722	1.805	0.633	0.334	0.963
B4-MPC	235	1.750	1.865	0.865	2.162	0.668	0.336	0.883
SUB4MPC	210	1.582	1.666	0.666	1.665	0.619	0.370	0.954
AB4-MPC	169	0.734	1.341	0.341	0.852	0.525	0.436	0.972
TB4-MPC	178	0.883	1.412	0.412	1.030	0.547	0.471	1.740
PB4-MPC	197	1.232	1.563	0.563	1.407	0.591	0.387	1.056
IB4-MPC	199	1.253	1.579	0.579	1.447	0.596	0.330	1.046

* Concentration of solution is 0.4 gram per decilitre of chelating ligands

CONCLUSIONS

The present paper describes facile synthesis of seven chelating ligands, also it deal with coloured chelating ligands. The resulting chelating ligands were shows good antimicrobial activity. Also it reveals that the physicochemical properties of chelating ligands were better as compare to its parent's moiety. The morphology of compounds is different from their reactants. The chelating ligands can be considered processable and thermally stable. The chelators show moderate conductivity at lower concentration, while better at higher concentration.

Acknowledgements: The authors are thanks to Head Department of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur for providing necessary research facilities. The authors are thankful to University Grants Commission, New Delhi for awarding Rajiv Gandhi National Fellowship for financial assistance.

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