Synthesis, Characterization, Spectral and Antimicrobial Studies of Macrocyclic Complexes of Mg(II), Ca(II), Sr(II) and Ba(II)

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Abstract: ***Oxaaza-mixed macrocyclic complexes of alkaline earth metals such as Mg(II), Ca(II), Sr(II) and Ba(II) synthesized by using macrocyclic ligand, which is obtained by condensation of L^1 and L^2 (where L^1 = 1,16-diamino-4,7,10,13-tetraoxahexadecane and L^2 = 2,3-pentanedione). These resulting complexes have been characterized by elemental analyses, IR, ^{1}H-NMR, Mass spectra, molar conductance, and magnetic moments. All the synthesized complexes have been screened for their antifungal and antibacterial activities.***

Keywords: Macrocyclic complexes, Mass spectra, IR, ^{1}H-NMR, antibacterial and antifungal activities.

1. Introduction

Macrocycles contain active subunits, which have ability both to sense bound guest species and control the coordination environment of the host[1]. Oxa-aza mixed donor macrocycle have strong complexation ability with both alkaline earth metals and transition metals. Therefore these N-O donor macrocycles have received much attention as receptors for a range of metal ions and other cations[2]. There are many macrocyclic systems studied which play vital role for the capability to actively transport the metal ion across membrane[3]. Some macrocyclic complexes have been reported to exhibit antibacterial and antifungal activities[4-5]. Hence, the investigations on macrocyclic complexes have been carried out in our laboratories. These investigations will help to understand the role of metal ions in metalloenzymes, studying the toxic effect of metal ions, detoxification mechanisms and drug designing i.e. in pharmacological properties.

Large number of reported papers are available on synthesis and characterization of macrocyclic complexes using alkaline earth metals Mg(II), Ca(II), Sr(II), and Ba(II) as central metal ion. Wankhede *et al*[6] have been reported Macrocyclic complexes of alkaline earth metals Mg(II), Ca(II) and Ba(II) derived from condensing diethyl malonate with 1,2-diaminoethane and 1,3-diaminopropane. Prasad *et al*[7] have reported a series of large ring tetraaza-macrocyclic complexes of Ca(II) obtained by the reaction of α-diketones and diaminoalkanes. Reaction of 2,6-diacetylpyridine with diethylenetriamine in the presence of Ba^{+2}, Sr^{+2} or Ca^{+2} in refluxing methanol gives complexes of the macrocyclic ligand[8]. Prasad *et al*[9] have been synthesized the 12-membered macrocyclic ring by the 2+2 cyclocondensation of 1,7-diaminoheptane with α-diketones viz. 2,3-butanedione, 3,4-hexanedione or 4,4′-dimethylbenzil in the presence of Mg(II), Ca(II), Sr(II) and Ba(II) ions as templates yield a series of complexes of the type [ML(X_2)] (where L= N_4 macrocycles having a 12-membered ring and X= Cl or NCS). Chandra *et al*[10] have been synthesized a novel tetradentate nitrogen donor [N_4] Macrocyclic ligand and its complexes with first row transition metals, which gives an important information about spectral studies and biological approaches of macrocycles.

In the present work we have synthesized, macrocyclic complexes of the type [ML^1L^2].Cl_2 (M= Mg(II), Ca(II), Sr(II) and Ba(II)) (L^1 = 1,16-diamino-4,7,10,13-tetraoxahexadecane and L^2 = 2,3-pentanedione) are reported. These complexes are made up of by cyclocondensation reaction in butanolic solution. These complexes are characterized by using spectral techniques viz. IR, proton-NMR, Mass spectrometry and by *in vitro* antifungal and antibacterial activities.

2. Experimental

2.1 Material

2,3-pentanedione, ethanol, methanol and solvent butanol were purified by distillation and amine i.e. 1,16-diamino-4,7,10,13-tetraoxahexadecane was synthesized in our laboratory by using 2-methyl propane2-ol, 3-amino-1-propanol, benzene (distillation), ditosyl ester, KOH pallets, sodium sulphate and potassium metal.

2.2 Synthesis of Macrocyclic complexes of Mg(II), Ca(II), Sr(II) and Ba(II)

We used a convenient method for the preparation of diamine i.e. 1,16- diamino-4,7,10,13-tetraoxahexadecane, which is made by the reaction of the ditosylate ester with 3-amino-1-propanol in the presence of potassium and potassium hydroxide solution in benzene as a solvent[11]. Ditosylate ester was synthesized by the reaction of triethylene glycol, pyridine and p-toluene ptosylayl chloride in 1:1:1 molar ratios and recrystallized with pet-ether[12]. To a butanolic solution of Mg(II), Ca(II), Sr(II) and Ba(II) chloride (about 0.350≈ 0.450g. in 20 ml butanol) a butanolic solution of 2,3-pentanedione (≈0.170g. in 12ml) and 1,16-diamino-4,7,10,13-tetrahexadecane (0.450g.in 15ml butanol) were added with constant stirring. This reaction mixture was stirred for 5-6 hours. pH of the solution was measured by pH paper and the reaction mixture was neutral. The solid

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obtained was filtered, washed with n-butanol and dried under reduced pressure.

2.3 Physical Measurements

The mass spectra were recorded on a MSD1100VL mass spectrometery/ data system. Infra-red spectra of KBr pellets of the complexes were recorded in the region 4000-200cm⁻ on a Nicolet Magna- 550 FTIR spectrometer. ^1H NMR spectra were recorded in DMSO-d_6 on JEOL FX 90 QFT NMR Spectrometer at 90 MHz using TMS as a reference. Magnesium, calcium and strontium were determined volumetrically by EDTA and barium was determined gravimetrically. Elemental analysis was also done by the help of analyzer. Molar conductance measured at room temperature in DMSO solutions using a glass cell having cell constant 1.0. Magnetic moment measurements were taken on a model 155 vibrating sample magnetometer.

2.4 Anti-microbial Screening

2.4.1 Anti-Fungal Studies

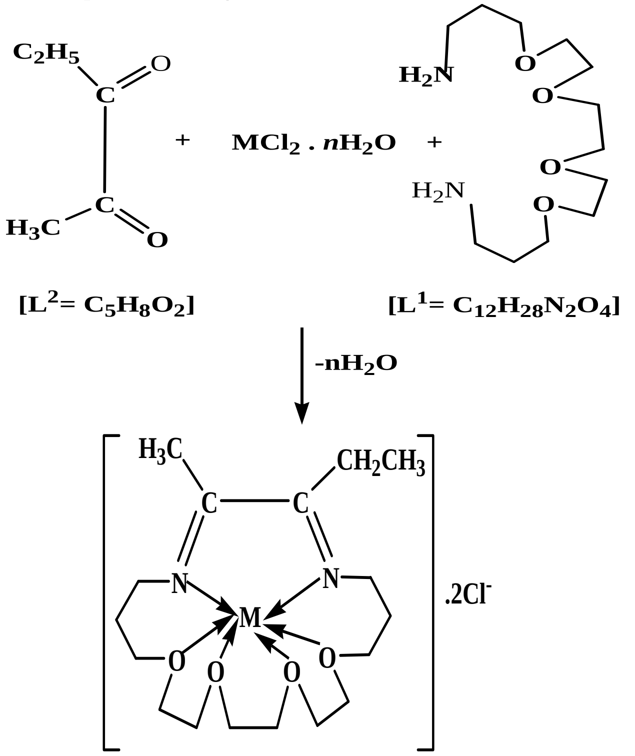
Antifungal screening of the synthesized compounds were checked *in vitro*. *Microsporum gypseum* ATCC 2819, *Trichophyton rubrum* ATCC 296, *Trichophyton tonsurans* ATCC 8475 and *Microsporum fulvum* ATCC 2837, four pathogenic fungal species were used as the test organism for the purpose of antifungal screening by agar plate technique[13]. The potato dextrose agar (PDA) medium was prepared in the laboratory to maintain fungal growth. Potato Dextrose Agar (PDA), medium was prepared by mixing 1000 ml of potato infusion to 20g each of agar and dextrose followed by autoclaving as usual. Solutions of the test compounds in methanol at 100 and 200 ppm were prepared and then mixed with the medium. The medium was then poured into Petri plates and spores of fungi were placed on the medium with the help of inoculum's needle. These Petri plates were placed in an incubator at 25±2℃. The activity was determined after 4 days (96 h) of incubation at room temperature (25℃).

2.4.2 Anti-bacterial Studies

In vitro antibacterial screening is performed by disc diffusion method[14]. *Escherchia coli*(gram-negative) ATCC 25922, *Bacillus subtilis*(gram-positive) ATCC 6633, *Pseudomonas putida*(gram-negative) ATCC 2633 pathogenic species of bacteria were used for screening of newly synthesized complexes. The nutrient agar medium having the composition agar-agar 20g, NaCl 5g, beef extract 5g, peptone 5g and distilled water 1000 mL was prepared by cooling the molten agar to 38℃ then add the 10 mL of bacterial suspension. The complexes were dissolved in methanol in 500 and 1000 ppm concentrations[15]. Paper discs of Whatmann No. 1 filter paper measuring diameter of 5mm were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with organisms in petri plates at suitable distance. The Petri plates were stored in an incubator at 36±2℃ for 1 Day.

3. Results and Discussion

The cyclocondensation reactions of hydrated metal chlorides with 1,16-diamino-4,7,10,13-tetraoxahexadecane and 2,3-pentanedione have been carried out in butanolic solution and can be represented as given in **scheme-1**.



Scheme-1. M(II) complexes of 20-membered N,O-donor macrocycles
M = Mg(II), Ca(II), Sr(II) or Ba(II)

The resulting macrocyclic complexes have been obtained as light colored solids which are quite stable and exhibit solubility in methanol, DMF and DMSO. Their molecular weights, yields, analyses and temperature of decomposition are recorded in **Table-1**. Magnetic moment results show that these complexes are diamagnetic in nature. Same kind of results about the magnetic susceptibility of these metals [M = Mg(II), Ca(II), Sr(II) and Ba(II)] were reported[6]. Molar conductance measured in DMSO found to be very low (16-24 ohm^{-} cm^2 mol^{-}), showing the non electrolytic behavior of metal complexes.

Table 1: Physicochemical Characteristics of Macrocyclic Complexes $[C_{17}H_{32}N_2O_4M]$
{Where M = Mg(II), Ca(II), Sr(II) & Ba(II)}

S. No	*Complexes Molecular formula*	*Formula Weight/g*	*Colour and Decomposition temp. (°C)*	*Yield (%)*	*Molar Conductance ($\Omega^{-}cm^2mol^{-}$)*
1	$C_{12}H_{28}N_2O_4$(Ligand[1])	264.36g	Light yellow -	65	-
2	$C_5H_8O_2$(Ligand[2])	100.66g	Light yellow -	-	-
3	$C_{17}H_{32}N_2O_4MgCl_2$	423.66g	Light brown 222	32	23.7
4	$C_{17}H_{32}N_2O_4CaCl_2$	439.44g	Dark brown 258	27	21.2
5	$C_{17}H_{32}N_2O_4SrCl_2$	486.98g	Dark brown 234	23	16.7
6	$C_{17}H_{32}N_2O_4BaCl_2$	536.69g	Dark brown 228	26	18.3

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4. IR Spectra

The IR spectra of all macrocyclic metal complexes are compared with those of the free ligands. In the infra-red spectrum of the ligand 2,3-pentanedione, the strong absorption band recorded at 1724 cm^{-1} & 1710 cm^{-1} (stretching frequencies) due to (CH_3-C=O) & (CH_3-CH_2-C=O) respectively and IR band at 3375 (medium) and 3310 cm^{-} due to -NH_2 (weak) in 1,16-diamino-4,7,10,13-tetraoxahexadecane.

In the infra-red spectra of metal complexes, the υ(C=N) stretching vibrations are observed at around ~1482 cm^{-1}, are due to the coordination of nitrogen (from C=N) to the metal whereas free ligand shows strong peak at 1520 cm^{-1}. Weak to medium intensity bands observed in the complexes in the region 400-500 cm^{-1}, which are absent in the free ligands may be attributed to υ(M-O) vibrations. A broad band appears at 2590-2765 cm^{-1}, which may be attributed to C-H* str. Specific C-O-C etheric peaks were observed at 1080-1205 cm^{-1} in the IR spectra of the macrocyclic complexes where as bands observed at 1175-1225 cm^{-} in free C-O-C etheric diamine. Ugras *et al*[16] studied a newly synthesized macrocyclic schiff base, which also have specific etheric peak due to the presence of etheric group. IR spectrum of the macrocyclic complex [$SrC_{17}H_{32}N_2O_4$].Cl_2, reported in **fig-1.**

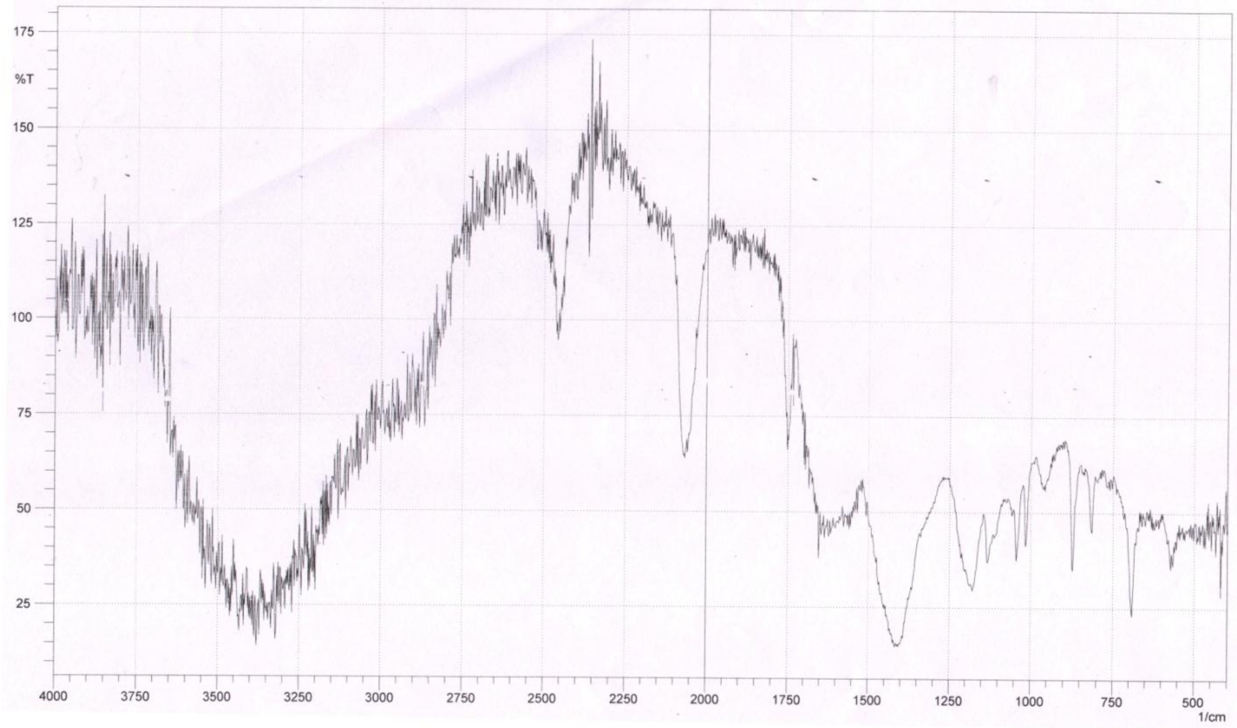

Figure 1: IR spectrum of macrocyclic complex [$SrC_{17}H_{32}N_2O_4$].Cl_2

^{1}H-NMR Spectra

The ^{1}H NMR spectral data of the ligands and their corresponding metal complexes have been recorded in DMSO-d_6 by using TMS as internal Standard. The NMR of 2,3-pentanedione gives a singlet in the region δ 2.68 ppm for 3H of methyl group, a triplet in the region 1.98 ppm for 3H and a quartet in the region 2.56 ppm for 2H (CH_3-CH_2-). The ^{1}H NMR spectra data for 1,16-diamine-4,7,10,13-tetraoxahexadecane are as follows: δ 1.59 ppm (s, H^a, 4H, -NH_2), 2.69 (m, H^b, 4H, NH_2-$\underline{CH_2\text{-}}$), 2.08 (m, H^c, 4H, CH_2-$\underline{CH_2}$-CH_2-), 3.26 (m, H^d, 4H), 3.43 (m, H^e, 4H), 3.47 (m, H^f, 4H) and 3.53 (m, H^g, 4H). Similar kinds of result have been reported for the synthesis of α, ω-diaminosubstituted oligo(oxyethylene)s. In the corresponding metal complexes ^{1}H NMR peaks have been recorded as follows: δ 2.59 ppm (s, 3H), 2.39 (m, 2H), 1.32 (q, 3H), 3.28 (q, 4H, =N-CH_2-), 2.12 (m, 4H, N-CH_2-$\underline{CH_2}$-CH_2-), 3.43 (m, H^d, 4H), 3.49 (m, H^e, 4H), 3.53 (m, H^f, 4H) and 3.55 (m, H^g, 4H).

5. Mass Spectra

EI-mass spectra for all complexes [$M(C_5H_8O_2)(C_{12}H_{28}N_2O_4)$]$Cl_2$ (where M = Mg(II), Ca(II), Sr(II) or Ba(II) and $C_{12}H_{28}N_2O_4$ = 1,16-diamino-4,7,10,13-tetraoxahexadecane) have been recorded and spectrum of Mg(II) complex is reproduced in **fig-2.** Spectrum exhibit a peak at 425 m/z with very low abundance (5.23%), which is its molecular ion/parental ion peak (calculated atomic mass [$C_{17}H_{32}N_2O_4Mg$].Cl_2, 423.66). The peak observed at m/z 355.3 due to the species [$Mg(C_5H_8O_2)(C_{12}H_{28}N_2O_4)$]$^+$ which is formed by the loss of 2 Chloride ions, has 100% abundance, hence it is known as base peak. Peaks are observed at 100.1 (25%) due to ($C_5H_8O_2$)$^+$, 208.2 (60%) due to[$MgC_5H_8O_2$]$^+$, 264.2 (30%) due to ($C_{12}H_{28}N_2O_4$)$^+$, 282.2 (80%) due to (Mg- $C_{12}H_{28}N_2O_4$)$^+$. By the loss of MgH^-, 397.3 (25%) due to ($C_{17}H_{31}N_2O_4Cl_2$)$^+$, by the loss of MgH_2^- 396.3 (50%) due to ($C_{17}H_{30}N_2O_4Cl_2$)$^+$ and many other peaks such as 340.3, 283.2, 282.2, 211.2, 208.2, attributable to different fragments of the complex. These data suggests that 2 + 2 cyclocondensation reaction of 2,3-pentanedione with 1,16-diamino-4,7,10,13-tetraoxahexadecane occur in presence of Mg(II) chloride.

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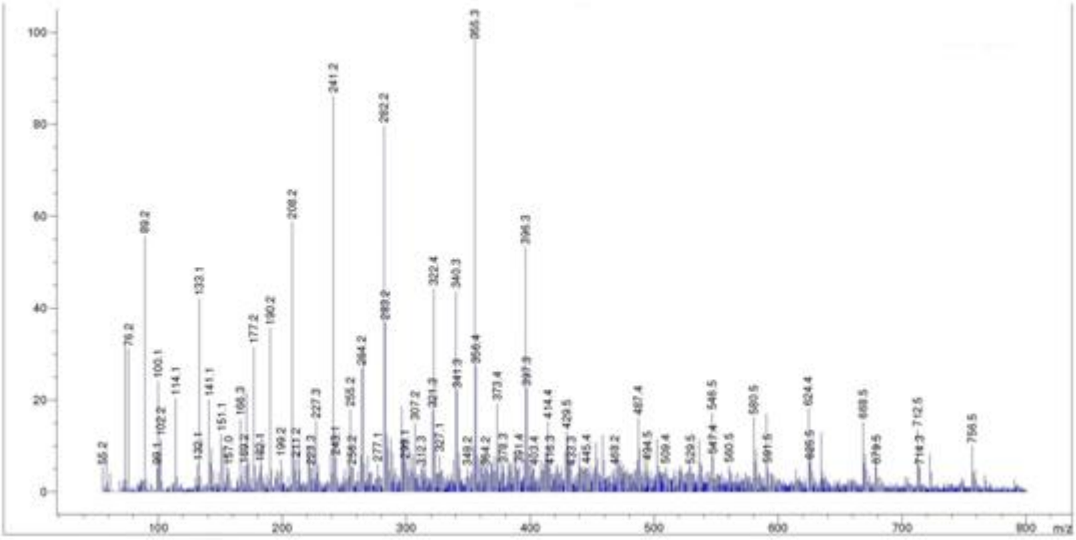

Figure 2: Mass spectra of the macrocyclic complex $[C_{17}H_{32}N_2O_4Mg].Cl_2$

6. Antibacterial and Antifungal Activities

The antimicrobial screening shows that the metal complexes exhibit antimicrobial properties against both fungal and bacterial strains. It is important to note that the metal chelate exhibits higher inhibitory effects towards both of the bacterial as well as fungal strains than the parent ligand. The increased activities of the metal chelates as compare to ligand can be explained on the basis of chelation theory. According to chelation theory[17], chelation reduces the positive charge of metal ion by the partially sharing of its positive charge with donor atoms of the ligand.

Table 2: Antibacterial activities of Macrocyclic Complexes

Complexes	Zone of inhibition in MM(Conc. in ppm)					
	Bacterial pathogens					
	E. coli (gm-ve)		*B.subtilis* (gm+ve)		*P. putida* (gm-ve)	
	500	1000	500	1000	500	1000
$C_{17}H_{32}N_2O_4MgCl_2$	++	+++	+	++	+	++
$C_{17}H_{32}N_2O_4CaCl_2$	-	++	+	++	+	++
$C_{17}H_{32}N_2O_4SrCl_2$	++	+++	--	++	-	++
$C_{17}H_{32}N_2O_4BaCl_2$	++	+++	++	+++	+	++
Ciprofloxacin	++	+++	++	+++	++	++

Table 3: Antifungal activities of Macrocyclic Complexes

Complexes	Zone of inhibition in MM(Conc. in ppm)							
	Fungal pathogens							
	M. gypseum		*M.fulvum*		*T. rubrum*		*T. tonsurans*	
	100	200	100	200	100	200	100	200
$C_{17}H_{32}N_2O_4MgCl$	76	82	72	81.4	65	66.7	68	69.3
$C_{17}H_{32}N_2O_4CaCl_2$	-	32.7	13	33.2	61	62.5	66	67.8
$C_{17}H_{32}N_2O_4SrCl_2$	21.6	24.3	27.	28.5	-	12.8	11.3	18.8
$C_{17}H_{32}N_2O_4BaCl_2$	36.7	36.8	43	48.7	51.	51.6	52.1	52.9
Fluconazole	92±1.	97±.	93	94±1.	92	95±1.	92±0.	97±1.

All macrocyclic metal complexes were compared by using standard antibiotic Fluconazole for fungi and Ciprofloxacin for bacteria. These complexes show better antimicrobial activities but less active as compared to the Fluconazole and Ciprofloxacin as standard drugs. The results indicate that Mg(II) and Ba(II) complexes showed more effective and better antimicrobial activity than Ca(II) and Sr(II) complexes. **Table 2 & 3** representing antibacterial and antifungal activities for the complexes respectively.

7. Conclusion

On the basis of the physicochemical studies and spectral analysis, it can be proposed that all complexes are nonelectrolytic, diamagnetic in nature and exhibit an octahedral geometry. The IR spectra data reveal that a broad band appears at 1615-1665 cm^{-1} due to the presence of υ(C=N), which confirmed the cyclocondensation reaction of the both ligands in presence of metal salt. Mass spectral study further confirms the proposed structure of the complexes. All complexes are biological active and exhibit antimicrobial activities. The enhancement in biological activity upon coordination may be elucidated on the basis of overtone's concept and chelation theory.

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References

[1] J. W. Sibert, G. R. Hudnt, A. L. Sargent and V. Lynch (2005). "Wurster's crownophanes: an alternate topology for *para*-phenylenediamine-basesd macrocycles," *Tetrahedron* 61:12350-12357.

[2] B. Zhao, Y. J. Wu and J. C. Tao (1995). "The synthesis of new nitrogen-oxygen donor macrocycles and liquid membrane transport of alkali cations," *polyhedron* 14:3557-3561.

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[3] R. G. Hosssein and S. K. Raziyeh (2001). "A thermodynamic study of complex formation between 15-crown-5 with Mg^{+2}, Ca^{+2}, Sr^{+2} and Ba^{+2} in acetonitrile methanol binary mixtures using conductometric method," *Iran. J. Chem & Chem. Eng* 20:82-89.

[4] A. Chaudhary, N. Bansal, A. Gajraj and R.V. Singh (2003). **"**Antifertility, antibacterial, antifungal and percent disease incidence aspects of macrocyclic complexes of manganese(II)," 96:393-400.

[5] D. Singh and R. Kumar (2007). "Trivalent metal ion directed synthesis and characterization of Macrocyclic complexes," *J. Serb. Chem. Soc* 72:1069:1074.

[6] D. S. Wankhede, P. B. Wagh and O. Shankarrao (2012). " Synthesis, characterization and biological activities of Macrocyclic complexes of alkaline earth metals such as Mg(II), Ca(II) and Ba(II)," *Asian. J. Bio. Pharma. Res* 2:126-134.

[7] R. N. Prasad, M. Agrawal and S. Malhotra (2004). **"**Ca(II) complexes of tetraazamacrocycles derived from 3, 4-hexenedione and diaminoalkanes," *J. Serb. Chem. Soc* 69:661-668.

[8] M. G. B. Drew, J. Nelson and S. M. Nelson (1981). "Metal-ion-controlled transamination in the synthesis of macrocyclic Schiff-base ligands. Part 2. Stepwise synthesis, ring expansion/contraction, and the crystal and molecular structure of a ten-co-ordinate barium(II) complex," *J. Chem. Soc., Dalton Trans.*, 1678-1684.

[9] R. N. Prasad and S. Gupta (2002). "Synthesis and characterization of 2,3,13,14-tetramethyl (ethyl or *p*-tolyl)-1,4,12,15-tetraazacyclodocosa-1,3,12,14-tetraene complexes of Mg(II), Ca(II), Sr(II) and Ba(II)" *J.Serb.Chem.Soc*. *67*(7):523–530.

[10] S**.** Chandra and L.K. Gupta (2007). "Synthesis spectroscopic and biological approach in the characterization of novel [N_4] Macrocyclic ligand and its transition metal complexes," *Trans. Met. Chem*. 32:558-563.

[11] W. Kern, S. Iwabuchi, H. Sato and V. Bohmer (1979). "A convenient synthesis of α-ω- diamino substituted oligo(oxyethyene)s," *Makromol, Chem* 180:2539:2542.

[12] V. C. Sekera and C. S. Marvel (1933). "Higher alkyl sulfonates," *Urbana,illionis* 55:345-349.

[13] J. B. Chauhan, R. B. Subramanian and P. K. Sanyal (2002). "Influence of heavy metals and a fungiside on growth profiles of nematophagous fungus, Arthrodotrys musiformis A potential biocontrol agent against animal parasitic nematodes," *Indian j. Environ. Toxicol.*12:22.

[14] T. D. Wilkins, L.V. Holdeman, I. J. Abramson and W. E. C. Moore (1972). "Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria," *Antimicrob. Agents.Chemothe*. 6:451.

[15] M. S. Rafat, S. Chandra and M. Tyagi (2010). "Spectroscopic, thermal and biocidal studies on Mn(II), Co(II), Ni(II) and Cu(II) complexes of tridentate ligand havingsemicarbazone moieties," Journal of thermal analysis and calorimetry, 61:261-267.

[16] H. I. Ugras, I. Basaran, T. Kilic and U. Cakir (2006). "Synthesis, complexation and antifungal, antibacterial activity studies of a new Macrocyclic Schiff base,"*J Heterocyclic Chem* 43:1679.

[17] M. EI-Behery and H. EI-Twigry (2007). "Synthesis, magnetic, spectral and antimicrobial studies of Cu(II), Ni(II), Co(II), Fe(III) and UO_2(II) complexes of a new Schiff base hydrazone derived from 7-chloro-4-hydrazinoquinoline," *Spectrochimica Acta A* 66:28-36.

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