Synthesis, Spectral, Thermal and Antimicrobial Studies of Rare Earth Metal Complexes of 14-Membered Tetraaza [N_4] Macrocyclic Ligand

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Abstract: ***A series of rare earth metal complexes of Ce(III), Pr(III), Nd(III), Sm(III), Gd(III) and Eu(III) have been synthesized with newly synthesized biologically active macrocyclic ligand. The ligand was synthesized by condensation of β-diketone 1-(4-fluorophenyl)-3-(2-hydroxyphenyl)propane-1,3-dione and 4-chlorobenzene-1,2-diamine. These complexes were characterized by various physico-analytical techniques, elemental analysis, molar conductivity, magnetic susceptibility, thermal analysis, X-ray diffraction, IR, ^{1}H-NMR, electronic and mass spectroscopy. From the analytical data, stoichiometry of the complexes was found to be 1:1 (metal: ligand). Thermal behavior (TG/DTA) analysis suggests more ordered activated state in complex formation. All the complexes are of high spin type and seven coordinated. The antibacterial and antifungal activities of the ligand and its metal complexes, has been screened in vitro against Staphylococcus aures, P. aeruginosa, M. luteus and Aspergillus niger, C. abicans respectively. All the complexes were found to be the more active than their parent ligand and metal salts.***

Keywords: ***Macrocyclic ligand, Rare earth metal complexes, Thermal analysis, Powder X-ray diffraction, Biological activity.***

1. Introduction

Macrocyclic compounds have attracted increasing interest owing to their mixed soft-hard donor character, versatile coordination behavior and in the understanding of molecular processes. [1]. The intense interest in synthetic macrocycles and their metal complexes depends on the fact that they mimic naturally occurring macrocyclic molecules in their structural and functional features due to rich chemical properties [2]. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and medical applications [3]. Some macrocyclic complexes have been reported to exhibit potent antibacterial, antifungal and anti-HIV activities [4]. The stability of macrocyclic metal complexes depends upon a number of factors, including the number and types of donor atoms present in the ligand and their relative positions with in the macrocyclic skeleton, as well as the number and size of the chelate rings formed on complexation [5]. Macrocyclic complexes are thermodynamically more stable and more selective metal ion chelates than open chain analogue so the study of macrocyclic complexes is a growing class of research [6-8]. Lanthanides and actinides form complexes of higher coordination numbers ranging from 7 to 12 this is attributed to the large size of these metal ions together with the ionic nature of the metal-ligand bonding. Luminescent lanthanide complexes of chelating ligands have been suggested as markers in cytology and immunology and may serve as luminescent biomarkers. Lanthanide luminescent probes are presently extensively used for studying metal ion sites in macrocyclic complexes [9]. There is a continuous interest in synthesizing macrocyclic complexes because of their potential applications in fundamental and applied sciences and importance in the area of coordination chemistry [10-12]. Moreover coordination compounds of the lanthanides are frequently used as catalysts [13-15]. In view of the above applications, in the present paper we report the synthesis, characterization and antimicrobial studies of Ce(III), Pr(III), Nd(III), Sm(III) Gd(III) and Eu(III) complexes with orthophenyl diamine containing nitrogen donor [N_4] macrocyclic ligand having 14-membered backbone.

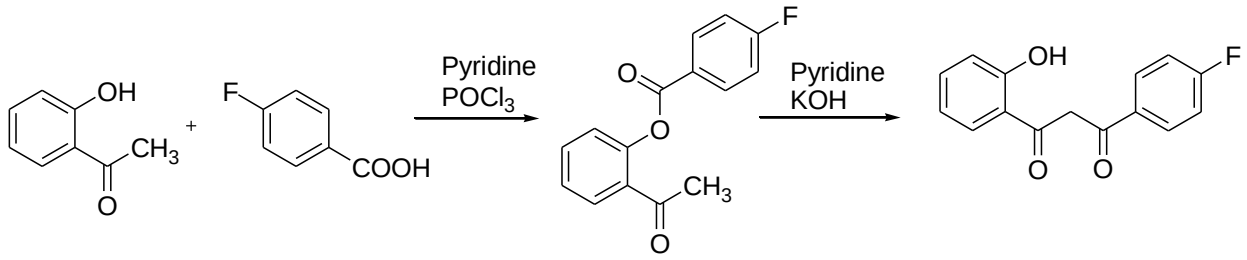
2 Experimental

All chemicals used were of the analytical grade (AR) and of highest purity. 4-Fluorobenzoic acid, ortho-hydroxy acetophenone and 4-chlorobenzene-1,2-diamine were used for ligand synthesis. AR grade metal nitrate were used for complex preparation. Spectroscopic grade solvents were used for spectral measurements. The carbon, hydrogen and nitrogen contents were determined on Perkin Elmer (2400) CHNS analyzer. IR spectra in the range of 4000-400 cm^{-1} were recorded on Jasco FT-IR-4100 spectrometer using KBr pellets. ^{1}H-NMR spectra of the ligand was recorded in DMSO using TMS as an internal standard. The TG/DTA analysis was recorded on Perkin Elmer TA/SDT-2960 and XRD were recorded on Perkin Elmer employing CuKα radiation λ= 1.541A^0 in the range 10-80^0. The UV-Vis spectra of the complexes were recorded on ShimadzuUV-1800 Spectrophotometer. Magnetic susceptibility measurements of the metal complexes were carried out on Gouy balance at room temperature using Hg[$Co(SCN)_4$] as calibrant. Molar conductance of complexes was measured on Elico CM-180 conductometer using 1mM solution in dimethyl sulphoxide.

2.1 synthesis of β-Diketone

Equimolar amount of 4-Fluorobenzoic acid and ortho-hydroxy acetophenone were dissolved in 50 mL dry pyridine. The reaction mixture was then cooled to 0°C. To this, phosphorus oxychloride (0.06mol) was added drop wise, maintaining temperature below 10°C. The reaction mixture was kept overnight at room temperature. It was then poured on crushed ice with vigorous stirring. The crimson colored solid (ester) was obtained which was filtered and washed several times with ice-cold water. Ester was then crystallized with distilled ethanol. Purity of the compound was checked by TLC. Ester was subjected to well known Baker-Venkatraman transformation. Ester (0.03mol) was dissolved in 15 mL dry pyridine. To this mixture, powdered KOH (1gm) was added and the reaction mixture was stirred on magnetic stirrer at room temperature

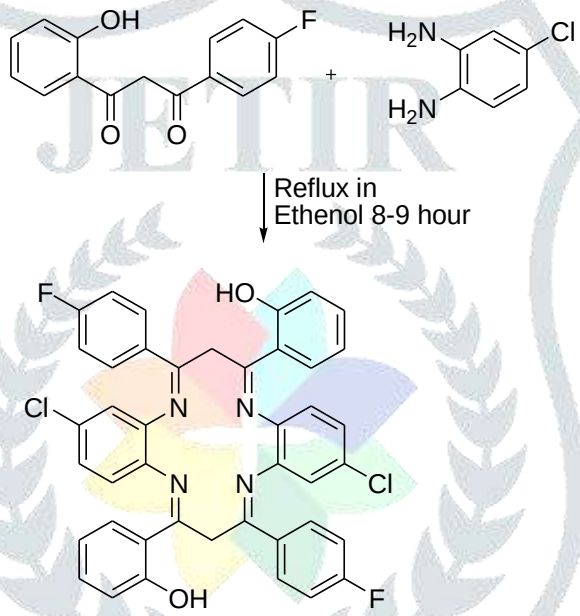
for 5 hours. Then it was poured over crushed ice and acidified with concentrated hydrochloric acid. Finally yellow colored product was obtained which was recrystallized from ethanol (Yield 71-73%). Purity of all synthesized β-diketones were checked by TLC using silica gel G and melting points.



Scheme1. synthesis of β-Diketone

2.2 synthesis of macrocyclic ligand

A hot ethanolic solution, 25 ml of 4-chlorobenzene-1,2-diamine 0.02M and an ethanolic solution 25 ml of β-Diketone 0.02M were mixed slowly under constant stirring. The resulting solution was refluxed for six hours in presence of 1-2 ml of concentrated HCl. On cooling, light yellowish crystals separated out were filtered, washed with ethanol and dried under vacuum.



Scheme 2. synthesis of macrocyclic ligand.

2.3 synthesis of metal complexes

A hot ethanolic solution, 25 ml of ligand (0.001M) and a hot ethanolic solution, 25 ml of required metal salt (0.001M) were mixed together under constant stirring. The mixture was refluxed for 8-9 hours. On cooling, a colored solid precipitate formed was filtered, washed with cold ethanol, chloroform and dried under vacuum (fig 1).

3 *Results and Discussion*

All the complexes were colored solids, air stable and soluble in polar solvents like DMF and DMSO. The elemental analysis show 1:1 (metal: ligand) stoichiometry for all the complexes. Micro analytical data and molar conductance values are given in (Table 1). The metal contents in complexes were estimated by gravimetric analysis [16]. All the complexes show low conductance which indicates their non-electrolytic nature. All the complexes indicating their paramagnetic nature. (Table 1).

Table .1

Physical characterization, analytical and molar conductance data of ligand and its metal complexes.

Ligand/ Complexes	F. W.	M.P. ^{0}C	Magnetic moment μ_{eff} (B.M.)	Molar conduc. Mho cm^2 mol^{-1}	% Found (Calculated) C	H	N	M
(HL)	728.15	185	----	----	70.12 (69.28)	3.95 (3.87)	7.50 (7.69)	_
[CeL(H_2O)$_2$$NO_3$]	964.28	214	2.63	25.00	52.58 (52.31)	3.48 (3.34)	7.71 (7.26)	14.45 (14.53)
[PrL(H_2O)$_2$$NO_3$]	965.07	210	3.54	24.04	52.82	3.49	7.50	14.72

					(52.27)	(3.34)	(7.25)	(14.60)
[NdL $(H_2O)_2NO_3$]	968.40	207	3.65	19.06	52.29 (52.09)	3.45 (3.33)	7.63 (7.23)	14.97 (14.89)
[SmL $(H_2O)_2NO_3$]	974.52	200	1.52	17.04	51.95 (51.76)	3.34 (3.30)	7.49 (7.18)	15.32 (15.42)
[EuL$(H_2O)_2NO_3$]	976.12	206	-	14.06	51.78 (51.68)	3.40 (3.30)	7.58 (7.17)	15.70 (15.56)
[GdL$(H_2O)_2NO_3$]	981.41	219	7.82	11.04	51.59 (51.40)	3.11 (3.28)	7.54 (7.13)	16.20 (16.02)

3.1 ^{1}H-NMR spectra of ligand

The ^{1}H NMR spectra of the ligand was recorded in DMSO. It shows following signals at 1.22δ, (s,4H -CH_2), 5.56 δ (s,2H,-OH), 6.81-8.25δ corresponding to phenyl ring protons (m,24H).

3.2 Mass Spectra of the ligand

Mass spectral data confirmed the structure of ligand HL as indicated by the peaks corresponding to the molecular mass.

3.3 FTIR spectra

The FT-IR spectra of ligand (Table 2) do not show any band at 1700 cm^{-1} (υ C=O) 3380 cm^{-1} (υas NH_2) and 3250 cm^{-1} (υs NH_2) corresponding to carbonyl groups and free amine [17]. There are two main features in the infrared spectrum of the macrocyclic ligand. The first feature is the disappearance of two characteristic bands between the primary amine group –NH_2 of the diamine and >C=O of the diketone. It also confirmed the elimination of a water molecule and complete condensation [23]. A band corresponding to the (υ C=N) (azomethine linkage) appears at 1641-1658 cm^{-1} in the spectra. The structure suggested to the ligand is shown in (scheme 2). The position of this band is shifted to lower frequency in the complexes as compared to free macrocyclic ligand suggesting that the coordination takes place through the nitrogen of (υ C=N) group [24].

Table.2 FTIR Spectra of the ligand (HL) and its macrocyclic metal complexes S (cm^{-1}).

Ligand/Complexes	(OH)	(C=N)	(C-N)	(C-O)	(M-N)
HL	3408	1625	1020	1245	
[CeL$(H_2O)_2$]	3365	1623	1027	1268	460
[Pr L$(H_2O)_2$]	3364	1621	1020	1269	455
[NdL$(H_2O)_2$]	3361	1607	1027	1268	460
[SmL$(H_2O)_2$]	3350	1620	1027	1269	460
[GdL$(H_2O)_2$]	3346	1621	1027	1269	458
[EuL$(H_2O)_2$]	3342	1647	1027	1268	411

3.4 Electronic absorption spectra and magnetic measurements

The electronic spectra of Ce(III), Pr(III), Nd(III), Sm(III), Gd(III) and Eu(III) complexes were recorded in DMSO. The data indicates that the energy of f-f transitions in the complexes is slightly reduced compared to the corresponding aquo ions either because of the slight covalent interaction of the 4f orbitals with vacant ligand orbitals, leading to some delocalization with consequent reduction in interelectronic repulsion [25]. All the complexes are characterized by intense charge transfer bands and most of the absorption arising from weak f-f transition are observed in the UV region. However all the complexes have almost similar spectra and they exhibit two transitions in the range 350-380 nm due to charge transfer transition and 400-430 nm due to f-f transition [26].

3.5 Thermal analysis.

The simultaneous TG/DT analysis of some representative metal complexes was done from ambient temperature to 800 ºC in nitrogen atmosphere using α-Al_2O_3 as reference. The thermogram curve of The Ce(III), and Pr(III) complexes were chosen for thermal study. On the TG curves of Ce(III) complex, the first step shows a steep slope between 140–220°C with a mass loss of 5.98 % (calculated 5.82%), indicating the removal of two molecules of coordinated water. An endothermic peak in the range 140–205 °C (ΔTmax = 140°C) on the DTA curve corresponds to the dehydration step. The anhydrous complex first show slow decomposition from 200-300°C, with 10.22% mass loss, (calc. 10.10%) and a broad exotherm (ΔTmax = 300°C) in DTA may be attributed to removal of coordinated nitrate part of the complex [27]. The second step decomposition is from 400 to 600°C with 20.10% mass loss (calc. 19.04%) which corresponds to decomposition of coordinated part of ligand. A broad endotherm in DTA is observed for this step. The mass of the final residue corresponds to stable oxide, 56.09% (calc.. 55.82%). The TG curve of Pr(III) complex shows first mass loss 5.96% (calc.. 5.76%) in the range 120-225°C and an endothermic peak in this region (ΔTmax = 179°C), which indicates removal of two coordinate water molecules. The anhydrous complex show slow decomposition from 220-325°C with 10.20% (calc.. 10.92%) mass loss. A broad exotherm (ΔTmax =272°C) in DTA may be attributed to the removal of coordinated nitrate part of the complex. The second step decomposition from 400 to 650°C with 22.90% mass loss (calc. 22.40%) corresponds to decomposition of coordinated part of ligand. A broad endotherm in DTA is observed for this step. The mass of final residue corresponds to stable Pr_2O_3, 56.10% (calc. 56.85%).

3.6 Powder X-ray diffraction analysis

The X-ray powder diffractogram of the metal complexes were used for the structural characterization and determination of lattice dimensions. The observed data of complexes under investigation was compared with other literature data having analogous cell and subsequently indexed to similar geometry. The X-ray diffractogram of metal complexes was scanned in the range 20–80 at wavelength 1.540A˚. The diffractogram and associated data depict 2θ values for each peak, relative intensity and interplanar spacing (d-values). The X-ray diffraction pattern of these complexes with respect to major peaks having relative intensity greater than 10% have been indexed by using computer program. The diffractogram of Ce(III) complex shows 26 reflections with maxima at 2θ =14.77 corresponding to d value 5.99A˚. The values of lattice constants, a= 14.00A˚, b = 813.50A˚, c = 12.00A˚ and α =β =γ = 90^o satisfy the condition a≠ b≠c and α =β =γ = 90^o required for the compound to be Orthorombic lattice type. The diffractogram of Pr(III) complex shows 16 reflections with maxima at 2θ =25.95 corresponding to d value 3.43A˚ and observed values of lattice constants, a= 14.55A˚, b = 14.18A˚, c = 13.64A˚ and α =β =γ = 90^o satisfy the condition a≠ b≠c and α =β =γ = 90^o required for the compound to be Orthorombic lattice type. The diffractogram of Nd(III) complex shows 11 reflections with maxima at 2θ =24.29 corresponding to d value 3.66A˚ and observed values of lattice constants, a= 11.21A˚, b = 11.33A˚, c = 11.00A˚ and α =β =γ = 90^o satisfy the condition a≠ b≠c and α=β=γ = 90^o required for the compound be orthorhombic lattice type. The diffractogram of Sm(III) complex shows 12 reflections with maxima at 2θ =63.10 corresponding to d value 1.47A˚ The values of lattice constants, a= 9.45A˚, b = 9.45A˚, c = 8.76A˚. These values satisfy the condition a=b≠ c and α =β =γ = 90^o required for the compound to be tetragonal lattice type. The diffractogram of Eu(III) complex shows 10 reflections with maxima at 2θ =14.75 corresponding to d value 5.99A˚ and observed values of lattice constants, a= 9.40A˚, b = 10.00A˚, c = 8.30A˚ and α = γ = 90^o≠β satisfy the condition a≠ b≠c and α =γ = 90^o≠β required for the compound to be Monoclinic lattice type. The diffractogram of Gd(III) complex shows 07 reflections with maxima at 2θ =25.998 corresponding to d value 3.42A˚ and observed values of lattice constants, a= 9.80A˚, b = 9.60A˚, c = 9.40A˚ and α =β =γ = 90^o satisfy the condition a≠ b≠c and α =β =γ = 90^o required for the compound to be Orthorombic lattice type.

3.7 Antimicrobial activity

Antibacterial activity

The antibacterial activity of the compounds was performed by enumerating the viable number of cells upon in the nutrient broth containing various concentrations of compounds. The viable number is represented by colony count method. The test organisms used on which the antibacterial activity was performed were *Bacillus subtilis* (NCIM-2063), *Pseudomonas aeruginosa* (NCIM-2036) and *Staphylococcus aureus* (NCIM-2901). In this method, the cells of test organisms were grown in nutrient broth till mid log phase and used as an inoculums for performing antimicrobial test. An approximately, 1 x 10^6 cells/mL test organisms were each inoculated with 0 to 500 ug/mL concentration of different compounds, separately, and each incubated for 16 to 18 h at 37^o C. During this incubation, cells tend to grow and multiply in number. However, if the compounds interfere with growth of cells, the numbers of cells decrease. After 16 to 18 h, viable numbers of cells were recorded by spreading an aliquot from the broth inoculated with test organisms and compounds as colony forming units per milliliter (CFU/mL). Minimum inhibitory concentration (MIC) was determined using standard agar method. Dimethyl sulfoxide was used as solvent control. Ciprofloxacin and Ampicillin were used as standards for the comparison of antibacterial activity.

Antifungal Activity

The antifungal activity was evaluated against different fungal strains such as C*andida albicans* (NCIM3471), *Fusarium oxysporum* (NCIM1332), *Aspergillus flavus (*NCIM539) *Aspergillus niger* (NCIM1196) and *Cryptococcus neoformans* (NCIM576). Minimum inhibitory concentration (MIC) values were determined using standard agar method

MIC Detgermination:

MIC was determined by standard agar method as per CLSI (formerly, NCCLS) guidelines (Approved Standard M7-A6, vol. 23. 2003)

The standards used in the study were dissolved in a suitable solvent. The primary solutions were further diluted to the final strength using test medium

Medium:

The medium yeast nitrogen base (Himedia, India) was dissolved in Phosphate buffer pH 7 and it was autoclaved at 110^0 C for 10 minutes. The suitable concentration of standards was incorporated in the medium. With each set a growth control without the antifungal agent and solvent control DMSO were included.

Preparation of standard inoculum:

The fungal strains were freshly subcultered on to Sabouraud dextrose agar (SDA) and incubated at 25^0 C for 72 hrs. The fungal cells were suspended in sterile distilled water and diluted to get n 10^5 cells/mL. Ten microlitre of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25^0 C for 48 hours. The readings were taken at the end of 48 hours and 72 hours.

Measu[rement of MIC:

The MIC was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible growth on the drug free control plates

Sterile filter paper discs (6mm diameter) were moistened with the test compound solution in Dimethyl sulfoxide of specific concentration 100 µg/disc were carefully placed on the agar cultures plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the results were recorded for antibacterial activity after 14 h

Table 2 In vitro antimicrobial activities of compounds

Compounds	Antibacterial activity[a]			Antifungal activity[a]	
	P. aeruginosa	*S. aureus*	*M. luteus*	*C. albicans*	*A. niger*
HL	150	225	250	200	150
[CeL(H_2O)$_2$]	250	100	150	*	*
[Pr L(H_2O)$_2$]	175	350	*	150	175
[NdL(H_2O)$_2$]	150	175	150	162.5	200
[SmL(H_2O)$_2$]	237.5	162.5	150	*	150
[GdL(H_2O)$_2$]	*	350	275	*	200
[EuL(H_2O)$_2$]	150	225	250	200	150
Ciprofloxacin	50	25	50	NA	NA
Ampicilin	250	100	250	NA	NA
Miconazole	NA	NA	NA	25	12.5
Fluconazole	NA	NA	NA	12.5	6.25

[a] Zone of inhibition (Mean three replicate ± standard deviation), NA- Not Applicable.

4.Conclusion

Metal complexes of Ce(III), Pr(III), Nd(III), Sm(III) and Eu(III) with novel macrocyclic ligand were synthesized. The structures of macrocyclic ligand have been proposed on the basis of IR, ^{1}H NMR mass spectra which acts as a tetradentade ligand by coordinating through four azomethine nitrogens. The elemental analysis, magnetic measurements, IR and electronic spectra revealed the formation of monomeric complexes. The analysis of x-ray diffraction study showed that the Nd(III), complexes have orthorhombic Ce(III), Pr(III), Nd(III), and Eu(III) complexes have monoclinic and Sm(III) complexes have tetragonal crystal structures. All these complexes were found to have enhanced antibacterial and antifungal activities than their parent ligand.

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