

ANTIBACTERIAL AND ANTIALGAL STUDIES
OF SOME LANTHANIDE SCHIFF BASE COMPLEXES

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ABSTRACT: A new series of rare earth complexes of the Schiff base 4-hydroxybenzilidene-2-aminophenol having the general formula $[Ln(HL)_2(H_2O)_3]NO_3$ have been synthesised and characterized by elemental analysis, conductance studies, infrared and electronic spectral analysis and magnetic studies. The Schiff base and the complexes were screened for their biological activity against the bacteria, *E.coli*, *B.subtilis*, *S.aureus* and *P.aeruginosa* and the algae, *Oscillaforia subrivis* and *Chlorella pyrenoclosa*. All the complexes were highly active against the bacterial species; Schiff base being less active comparatively. Algal growth was effectively inhibited by the ligand and the complexes, exhibiting total inhibition against the unicellular green algae, *Chlorella pyrenoclosa*.

Key words: Schiff base, Lanthanide, Complex, Antibacterial studies, Antialgal studies

INTRODUCTION

Rare earth complexes with Schiff base ligands have been widely investigated due to their versatile structures, interesting coordination and wide pharmacological applications (Niu, L.E. et al, 2009; Nair, M.K.M. et al, 1996; Song, Y.S., 2007). Many research works in this field have proved the antibacterial, antifungal, anti-inflammatory and even anticancer activities of various Schiff base complexes. Recently we have reported a few metal complexes with Schiff bases containing hydroxyl substitution (Seema Varghese, Nair, M.K.M., 2009; Seema Varghese, Nair, M.K.M., 2010) which exhibited remarkable antibacterial properties. As a continuation of our work, we report herein the synthesis, characterization and antibacterial and antialgal studies of the Schiff base, 4-hydroxy benzilidene-2-aminophenol and its rare earth complexes with La, Ce, Sm, Tb and Er.

EXPERIMENTAL

All chemicals used in this work were of analytical reagent grade. 4-hydroxy benzaldehyde and 2-aminophenol were purchased from Loba Chemie. Rare earth nitrates $\text{Ln}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ were prepared from Ln_2O_3 (>99.95%) by the standard method (Nair, M.K.M. et al, 1995).

Synthesis of the Schiff base: 4-hydroxybenzilidene-2-aminophenol (H_2L)

2-aminophenol (0.109 g, 1 mM) was dissolved in 20 ml ethanol and was mixed with 4-hydroxybenzaldehyde (0.122 g, 1 mM) in 20 ml ethyl acetate. The mixture was refluxed on a boiling water bath for 3 hours, concentrated by slow evaporation in air. Yellowish brown solid formed which was recrystallized from ethanol and dried over anhydrous CaCl_2 .

Synthesis of Lanthanide Complexes

The ligand (0.11 g, 0.5 mM) was dissolved in 20 ml ethanol and refluxed on a boiling water bath. To the boiling solution, 0.5 mM solution of the lanthanide nitrate $\text{M}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (where M = La, Ce, Sm, Tb and Er) was added drop by drop, refluxed for four hours, concentrated and kept in ice. The complex separated was washed with 50% ethanol and then with ether and kept over anhydrous CaCl_2 . Finally recrystallized from ethanol.

Physico-Chemical Analysis

The melting points were determined on a Thoshiniwal melting point apparatus. The Schiff base and the complex were analyzed for carbon, hydrogen and nitrogen on a Heracus CHN rapid analyzer. The metals were determined gravimetrically as oxides. Molar conductance in DMF and ethanol in (10^{-3}M) solutions were measured at room temperature using an Elico CM-180 conductivity meter with a dip type platinum electrodes (Hossain, M.E., 1996) of cell constant 0.986cm^{-1} . The IR spectra of the Schiff base and complexes were recorded in the range $400\text{--}4000\text{cm}^{-1}$ on a Shimadzu IR-470 spectrophotometer in KBr discs. The electronic spectra in ethanol solution (10^{-3}M) were recorded in the range $200\text{--}900\text{nm}$ on a Shimadzu UV-160A spectrometer. The magnetic susceptibility measurements were carried out on a Sherwood Scientific Gouy Balance.

Antibacterial Studies: Agar Diffusion Method

Laboratory cultures of the bacteria *E.coli*, *B.subtilis*, *S.aureus* and *P.aeruginosa* were used for inoculation. Nutrient agar medium was prepared in sterilized petry dishes. The surface of the agar plates was uniformly inoculated with bacterial stain using a platinum wire loop. The sample solution was prepared by dissolving 0.3 mg/ml of each compound in DMSO and was added to a 5mm well, bore onto the agar medium (Osowole, A.A. et al, 2009). The plates were allowed to stand on bench for 30 minutes. Then these were transferred to an incubator and kept for 24 hours. The inhibitory zones in mm were taken as a measure of antibacterial activity.

Algal Defacement Test

The test was carried out according to ASTM D 5589–97. A filter paper of 1.5 mm diameter was dipped in solution (0.3 mg/ ml), dried and used as the substrate for algal growth. Allen's agar medium was prepared in a petry dish. The test specimen was placed over the solidified Allen's agar. Two algal species *Oscillatoria subtrivis* (unicellular green algae) and *Chlorella pyrenoidosa* (filamentous blue green algae) were used for the present study. Algal inoculums were transferred from the flask using a sterilized sprayer and applied a thin coat of algae suspension to each specimen making sure that the surface was equally covered (Hector, R.B. 1996). The inoculated plates were kept under light at temperature 29°C and at humidity of >85%. The samples were examined weekly for growth up to 4 weeks.

RESULTS AND DISCUSSION

The physico-chemical data of the Schiff base and the complexes are given in Table 1. The ligand is soluble in all common solvents while the complexes are soluble only in ethanol, acetonitrile, DMF and DMSO. Table 2 provides molar conductance data which suggests 1:1 electrolytic nature (Geary, W.J., 1971) for the complexes. Magnetic moment data are also given in Table 2.

TABLE 1: ANALYTICAL DATA OF THE COMPOUNDS

Molecular Formula	Molecular Weight	Colour	Melting Point (°C)	Yield	% found/ calculated			Metal %
					C	H	N	
H ₂ L	213.0	Brown	269	85%	61.83/ 61.97	5.26/ 5.16	6.54/ 6.57	–
[La(HL) ₂ (H ₂ O) ₃]NO ₃	678.9	Yellowish Brown	>300	78%	38.63/3 8.89	3.98/ 3.83	6.27/ 6.19	20.32/2 0.46
[Ce(HL) ₂ (H ₂ O) ₃]NO ₃	680.1	Yellow	280	82%	38.51/3 8.82	3.95/ 3.82	6.04/ 6.18	20.53/2 0.60
[Sm(HL) ₂ (H ₂ O) ₃]NO ₃	690.4	Brown	>300	75%	38.16/3 8.24	3.85/ 3.77	5.96/ 6.08	21.69/2 1.78
[Tb(HL) ₂ (H ₂ O) ₃]NO ₃	698.9	Brown	>300	80%	37.65/3 7.77	3.81/ 3.72	5.92/ 6.01	22.65/2 2.74
[Er(HL) ₂ (H ₂ O) ₃]NO ₃	707.3	Brown	>300	82%	37.21/3 7.33	3.72/ 3.68	5.87/ 5.94	23.56/2 3.64

TABLE 2: CONDUCTANCE AND MAGNETIC MOMENT DATA

Molecular Formula	Molar Conductance Λ_m ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)		Magnetic Moment μ (B. M.)
	DMF	Acetonitrile	
H ₂ L			
[La(HL) ₂ (H ₂ O) ₃]NO ₃	81	127	
[Ce(HL) ₂ (H ₂ O) ₃]NO ₃	79	135	2.79
[Sm(HL) ₂ (H ₂ O) ₃]NO ₃	85	155	1.87
[Tb(HL) ₂ (H ₂ O) ₃]NO ₃	89	164	9.02
[Er(HL) ₂ (H ₂ O) ₃]NO ₃	92	172	9.34

IR Spectra

The relevant infrared data are given in Table 3. The assignments of the bands were made by comparison of spectral data of analogous compounds (Hankare, P.P., 2003; Giri, V., 1983). The presence of a strong, sharp peak at 1626cm^{-1} in the ligand spectrum corresponds to C=N vibrations and confirms the formation of the Schiff base. In complexes, this band suffers a bathochromic shift to $1570\text{--}1590\text{cm}^{-1}$ region indicating the involvement of imine nitrogen atom in coordination to the metal ion. There are two $\nu(\text{OH})$ bands at 3410cm^{-1} and 3302cm^{-1} in the ligand. The disappearance of one of the bands and appearance of strong $\nu(\text{CO})$ band near 1200cm^{-1} in complexes suggests coordination through phenolic oxygen. The peak at 3400cm^{-1} is retained in complexes indicating the presence of one uncoordinated $-\text{OH}$ group. In complexes a new broad band near 3500cm^{-1} is assigned to coordinated water. A very strong band at 1380cm^{-1} and a medium band 825cm^{-1} are due to ν_3 and ν_2 vibrations respectively of coordinated nitrate ion of D_{3h} symmetry (Nakamoto, K., 1986). The bands due to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$ were observed at $456\text{--}410\text{cm}^{-1}$ and $552\text{--}520\text{cm}^{-1}$ respectively in complexes, supporting coordination through azomethine nitrogen and phenolic oxygen donor atoms of the ligand.

TABLE 3: IR SPECTRAL DATA OF THE COMPOUNDS

Compound	$\nu(\text{OH})$ (cm^{-1})	$\nu(\text{CH}=\text{N})$ (cm^{-1})	$\nu(\text{C}-\text{O})$ (cm^{-1})	$\nu(\text{M}-\text{N})$ (cm^{-1})	$\nu(\text{M}-\text{O})$ (cm^{-1})	$\nu(\text{NO}_3)$ (cm^{-1})
H ₂ L	3302 _m 3410 _m	1604 _s	1278 _s	–	–	–
[La(HL) ₂ (H ₂ O) ₃] ₂ NO ₃	3401 _m 3492 _b	1582 _s	1263 _s	546 _w	402 _w	1382 _s
[Ce(HL) ₂ (H ₂ O) ₃] ₂ NO ₃	3404 _m 3482 _b	1580 _s	1261 _s	546 _w	409 _w	1382 _s
[Sm(HL) ₂ (H ₂ O) ₃] ₂ NO ₃	3398 _m 3491 _b	1579 _s	1255 _s	549 _w	405 _w	1382 _s
[Tb(HL) ₂ (H ₂ O) ₃] ₂ NO ₃	3402 _m 3478 _b	1580 _s	1255 _s	551 _w	405 _w	1382 _s
[Er(HL) ₂ (H ₂ O) ₃] ₂ NO ₃	3405 _m 3487 _b	1579 _s	1247 _s	559 _w	410 _w	1382 _s

Intensity of IR bands are indicated as *s* = strong, *m* = medium, *w* = weak and *b* = broad.

Electronic Spectra

The spectra of the complexes exhibit alterations in intensity and shift in positions of the absorption bands relative to the ligand. The shift has been attributed to the effect on the crystal field of the metal ion, interelectronic repulsion between the 4f electrons and is related to the covalent character of the metal–ligand bond. The electronic spectrum of the ligand shows two maxima, one at 329nm corresponding to $n \rightarrow \pi^*$ and the other at 232nm corresponding to $\pi \rightarrow \pi^*$ transition. In complexes, the $n \rightarrow \pi^*$ band is red-shifted to 325–322nm while the $\pi \rightarrow \pi^*$ band is blue-shifted to 244–239nm (Singh, K.N. et al, 2001). The f–f transitions of the complexes are characteristic of the lanthanide and are not influenced by the ligand. Intensity of the peaks also varies according to the metal ion. The f–f bands are sharp and line like. This is because of the effective shielding of the 4f orbital by the 5s, 5p octet and consequently minimum ligand field perturbation of the electronic energy levels in lanthanides. La³⁺ has no observable visible spectra and Ce³⁺ has no transition in this region. The observed f–f transitions in 500–700nm region and the tentative assignments are given in Table 4.

TABLE 4: ELECTRONIC SPECTRAL DATA

Complex	Wave length (nm)	Tentative Assignment
[La(HL) ₂ (H ₂ O) ₃]NO ₃	–	–
[Ce(HL) ₂ (H ₂ O) ₃]NO ₃	–	–
[Sm(HL) ₂ (H ₂ O) ₃]NO ₃	445	⁶ H _{5/2} → ⁴ G _{9/2}
	402	⁶ H _{5/2} → ⁶ P _{3/2}
[Tb(HL) ₂ (H ₂ O) ₃]NO ₃	545	⁵ D ₄ → ⁷ F ₅
	617	⁵ D ₄ → ⁷ F ₃
	675	⁵ D ₄ → ⁷ F ₂
[Er(HL) ₂ (H ₂ O) ₃]NO ₃	509	⁵ I _{15/2} → ² H _{11/2}
	531	⁵ I _{15/2} → ³ S _{3/2}
	612	⁵ I _{11/2} → ⁴ F _{9/2}

Antibacterial Activity

The results of antibacterial activity are presented in Table 5. The ligand was sensitive to all the organisms with inhibitory zones within 7–11mm except for *S.aureus* which was found to be resistant. All the metal complexes exhibited prominent activity in the range 14–32mm inhibition zones. The higher activity of the complexes compared to free ligand may be attributed to chelation (Osowole, A.A., 2008) which reduces polarity of the metal ion by partial sharing of the positive charge with donor atoms of the ligand. This increases the lipophilic character, favouring the permeation through lipid layers of the bacterial membrane. Higher activity observed against the gram negative bacteria *E.coli* can be explained by considering the effect on lipo-polysaccharide (LPS), a major component of the surface of gram negative bacteria (Islam, M.S., 2002). LPS is an important entity in determining the outer membrane barrier function and the virulence of gram negative pathogens. The Schiff base can penetrate the bacterial cell membrane by coordination of metal ion through oxygen or nitrogen donor atom to LPS which leads to the damage of outer cell membrane and consequently inhibits growth of the bacteria.

TABLE 5: ANTIBACTERIAL ACTIVITY RESULTS

Compound	<i>E.coli</i>	<i>B.subtilis</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>
DMSO	–	–	–	–
H ₂ L	++	+	–	+
[La(HL) ₂ (H ₂ O) ₃]NO ₃	++++	+++	++	++++
[Ce(HL) ₂ (H ₂ O) ₃]NO ₃	++++	+++	+++	+++
[Sm(HL) ₂ (H ₂ O) ₃]NO ₃	++++	+++	+++	+++
[Tb(HL) ₂ (H ₂ O) ₃]NO ₃	+++	++	+	++
[Er(HL) ₂ (H ₂ O) ₃]NO ₃	+++	++	++	++

Inhibition Zone: Below 5mm = (–), 5mm–10mm = (+), 10mm–15mm = (++) , 15mm–20mm = (+++) and >20mm = (++++)

Antialgal Activity

The observations of antialgal studies after week 1 and week 4 are reproduced in Table 6. Both of the algal species, *Chlorella pyrenoidosa* and *Oscillatoria subviridis* showed trace growth over the Schiff base. But all complexes showed total inhibition of growth against the algae, *Chlorella pyrenoidosa*. La and Ce complexes were highly effective against the filamentous algae, *Oscillatoria subviridis*, while other complexes exhibited trace growth.

TABLE 6: RESULTS OF ALGAL DEFACEMENT TEST

Sample	<i>Oscillatoria subviridis</i>				<i>Chlorella pyrenoidosa</i>			
	Week 1		Week 4		Week 1		Week 4	
	OG	Rating	OG	Rating	OG	Rating	OG	Rating
DMSO	T	1	M	3	T	1	M	3
H ₂ L	T	1	L	2	T	1	T	1
[La(HL) ₂ (H ₂ O) ₃] ₃ NO ₃	T	1	N	0	N	0	N	0
[Ce(HL) ₂ (H ₂ O) ₃] ₃ NO ₃	T	1	N	0	N	0	N	0
[Sm(HL) ₂ (H ₂ O) ₃] ₃ NO ₃	T	1	T	1	N	0	N	0
[Tb(HL) ₂ (H ₂ O) ₃] ₃ NO ₃	T	1	L	2	N	0	N	0
[Er(HL) ₂ (H ₂ O) ₃] ₃ NO ₃	T	1	T	1	N	0	N	0

OG indicates the Observed Growth on Samples explained as N = Nil, T = Traces, L = Light, M = Moderate and H = Heavy. Standard Ratings are given as 1–10% = 1, 10–30% = 2, 30–60% = 3 and 60–100% = 4.

CONCLUSION

We have successfully synthesized the Schiff base, 4-hydroxybenzilidene-2-aminophenol and its rare earth complexes with La, Ce, Sm, Tb and Er. All the compounds were characterized by spectroscopic, magnetic and conductance studies. The ligand and the complexes were analyzed for their antibacterial and antialgal activities. The Schiff base showed considerable activity against all the bacteria except *S.aureus* while all the complexes showed high antibacterial activity. Comparatively high activity of complexes over the corresponding ligand is explained based on the theory of chelation. High inhibition shown by the complexes against the gram negative bacteria *E.coli* is explained in terms of the effect on LPS found in the cell membrane of such bacteria. The compounds exhibited remarkable antialgal activity against both the species, *Oscillatoria subviridis* and *Chlorella pyrenoidosa*. Total inhibition was observed in the case of La and Ce complexes against both the species.

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