Research Article

Synthesis, Characterization, Antimicrobial and Antioxidant Properties of Sm(III) Complexes

Rajesh Kumar[1], Poonam[2], S. P. Khatkar[2], V.B.Taxak[2*]
[1]Department of Applied Science, UIET, Maharshi Dayanand University, Rohtak
[2]Department of Chemistry, Maharshi Dayanand University, Rohtak – 124001, India
***Corresponding author's E-mail:** v_taxak@yahoo.com

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ABSTRACT

New ternary Sm (III) ion complexes, Sm $(HDMPE)_3$.DMPHEN(C1) and Sm $(HDMPE)_3$.dmphen (C2), where HDMPE=1-(2-hydroxy-4, 6-dimethoxyphenyl)ethan-1-one, DMPHEN=2,9-Dimethyl-1,10-phenanthroline and dmphen=5, 6-dimethyl-1,10-phenanthroline,were synthesized by adopting solution precipitation method. The synthesized complexes were identified on the basis of various techniques like elemental analysis, ^{1}H-NMR and FT-IR spectroscopy. We studied the antimicrobial and antioxidant properties of the ligand (HDMPE) and their Sm (III) ion complexes. The *in vitro* antibacterial activities were studied by using Gram-positive bacteria: *B.subtilis, S.aureus* and gram-negative bacterium: *Escherichia coli.* The antifungal activities were studied by using fungi *C. albicans* and *A.niger.* The antibacterial activities of ligand (HDMPE) are poor but excellent of Sm (III) ion complexes. The antioxidant activities of the synthesized complex were determined by using DPPH method. The ligand (HDMPE) and its corresponding Sm (III) complexes have poor antioxidant activities.

Keywords: Sm (III) complex, antimicrobial, antioxidant properties.

INTRODUCTION

The developing rate of microbial resistance to currently utilized antibiotics represents a serious therapeutic issue and has dramatically increased in developing countries due to non-availability of desired medicines and emergence of widespread microbial resistance[1, 2]. Various clinical reports in the United States and worldwide have independently depicted the development of vancomycin resistance in methicillin-resistance Staphylococcus *aureus* (MRSA) segregates and other human pathogen Gram-negative isolates[3-5]. A lot of research on new antimicrobial agent makes it a growing and thrust area for researchers. Infections caused by these microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to a search for potential antibacterial agents. Subsequently, there is a critical need to develop new classes of therapeutic agents to treat microbial diseases[6-8].

Moreover, free radicals in diet, chemicals and even in living frameworks are created by an oxidation procedure assuming an essential part in procedures of food decay, organ degeneration, materials debasement and numerous different types of deactivation[9,10]. Oxidation is fundamental in many living structures for the generation of vitality to fuel organic procedures. Regardless, the uncontrolled generation of oxygen-inferred free radicals is included in the onset of numerous infections[11, 12]. Continuous generation of free radical leads to many pathological diseases like rheumatoid joint pain, cirrhosis and arteriosclerosis also as in degenerative procedures related with maturing[13-16]. It also has been linked to many cardiovascular and inflammatory disorders. Many natural and synthetic derivatives has been evaluated for their antioxidant potential and found to be successful to an extent[17-19]. Further, to prevent oxidation, several kinds of antioxidants are in use for the last few decades and mainly include organic compounds and other metal complexes[20-23]. They can inhibit oxidizing chain reactions in several ways, direct extinguishing of receptive oxygen species, inhibition of oxidative enzymes, and chelating of metal ions (Fe2+, Cu+). Considering these facts and the growing need for new antimicrobial agent in this research article new ternary Sm(III) complexes were explored for their antimicrobial and antioxidant potential.

MATERIALS AND METHODS

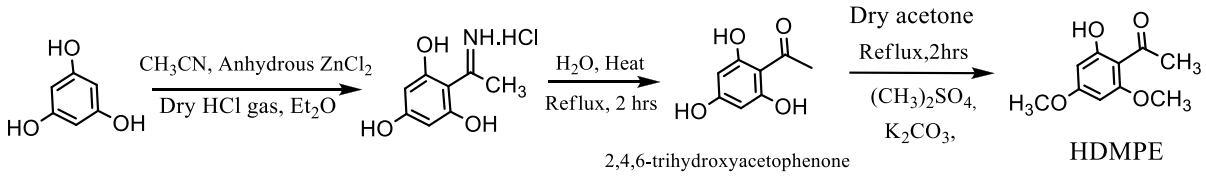
2,9-Dimethyl-1,10-phenanthroline, 5,6-dimethyl-1,10-phenanthroline, Sm $(NO_3)_3 6.H_2O$ (99.9), benzene-1,3-5-triol, dimethyl sulphate, potassium carbonate, xylenol orange and 1,1-Diphenyl-2-picrylhydrazylradical (DPPH) were purchased from Sigma-Aldrich and used as received without additional purification. The microorganisms used in antimicrobial activities were purchased from Institute of Microbial Technology, Sector 39-A, Chandigarh, India. Nutrient broth medium, nutrient agar medium, subourand dextrose agar medium and subourand dextrose broth medium were purchased from Hi-Media Pvt Ltd. The synthesized ligand HDMPE was recrystalized three times with ethanol before synthesis of complexes. The elemental analysis was performed using thermo scientific flash 2000 elemental analyzer. The percentage of Sm (III) was estimated by complexometric titration with EDTA. The ^{1}H-NMR spectra were measured on Bruker Avance II 400 spectrometer using tetramethyl

silane (TMS) as an internal reference (chemical shift in δ ppm). Infrared spectra were recorded (Perkin Elmer spectrum 400) from 4000–400 cm^{-1} in KBr pellets. Antimicrobial and Antioxidant activities were determined by tube dilution method and DPPH method respectively. All measurements were made at room temperature unless otherwise stated.

Synthesis

Synthesis of ligand HDMPE

The ligand HDMPE was synthesized by adopting conventional method as per literature[24] and is given in Scheme 1 as follow:



Scheme 1. The synthetic route of ligand HDMPE.

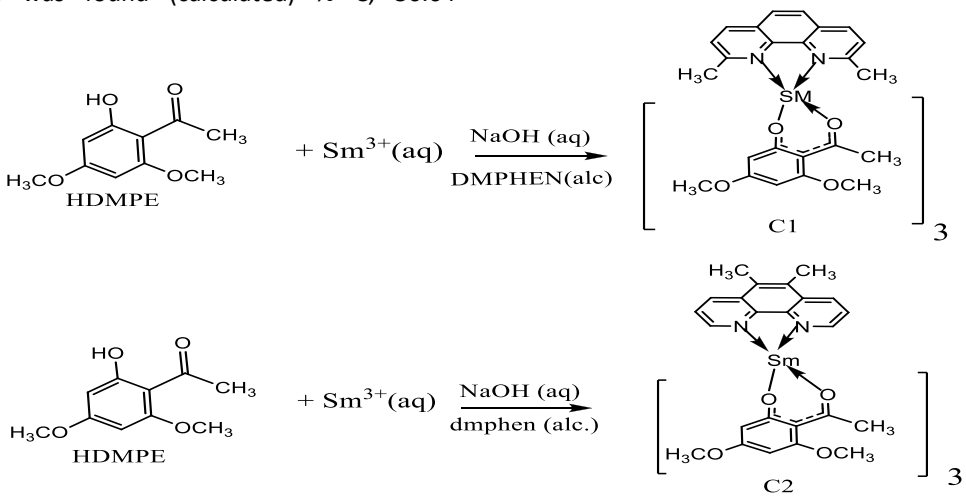
The product was obtained as white powder with 85% yield. The elemental analysis data for HDMPE ($C_{10}H_{12}O_4$) were found (calculated) % C, 60.87 (61.22); H, 6.14 (6.16); O, 32.19 (32.61) IR (KBr) cm^{-1} 3430 (b), 3099 (m), 3005 (w), 2943 (w), 2847 (w), 1640 (s), 1538 (s), 1457 (m), 1366 (s), 1324 (m), 1270(s), 1221 (s), 1207 (s), 1112 (m), 1079 (m), 1045 (w), 896 (m), 835 (m), 658 (m), 594 (s). ^{1}HNMR (400 MHz, DMSO): d 2.52 (s, 3H, CH3), 3.83 (s, 6H, OCH3), 6.02 (s, 2H, Ar-H), 13.84 (s, 1H, OH).

Synthesis of complexes

The complex Sm $(HDMPE)_3$.DMPHEN (C1) was synthesized by mixing ethanolic solution of 3 mmol HDMPE ligand and 1 mmol DMPHEN with ethanolic solution of 1 mmol $Sm(NO_3)_3.6H_2O$. Afterwards the pH of mixture was adjusted to 7, using NaOH (0.05 M) solution with constant stirring. This resulted into formation of white precipitates. These precipitates were stirred for 3 h at about 35°C and then allowed to digest for 1 h. Finally, a suction filter was used to filter precipitates, washed with doubly distilled water and then with ethanol, dried in vacuum oven at 50 °C. Sm$(HDMPE)_3$.DMPHEN was obtained as white powder with 79 % yield. The powdered the complex was stored in sample tube in vacuum desiccator.

The elemental analysis data for Sm (HDMPE) 3.DMPHEN ($C_{44}H_{45}O_{12}N_2Sm$) was found (calculated) % C, 56.04 (55.97); H, 4.92 (4.80); N, 3.01 (2.96); O, 20.29 (20.33); Sm, 15.86 (15.92). IR (KBr) cm^{-1}: 3008 (m), 2961 (m), 1618 (s), 1570 (s), 1540 (s), 1453 (m) 1406 (m), 1386 (s), 1362 (m), 1260 (s), 1225 (s), 1202 (s), 1160 (s), 1026 (s), 1079 (s), 966 (m), 853 (s), 832 (s), 695 (m), 592 (m), 535 (m), 442 (w); ^{1}H-NMR (400MHz, DMSO): δ 2.59 (s, 9H, CH_3), 2.69 (s, 9H, DMPHENCH_3), 3.28 (s, 18H, OCH_3), 5.94 (s, 6H, Ar-H), 7.64 (d, 2H, DMPHEN), 8.17 (d, 2H, DMPHEN), 8.78 (d, 2H, DMPHEN).

The complex Sm $(HDMPE)_3$.dmphen (C2) was synthesized by same procedure as C1, except mixing 1 mmoldmphen instead of DMPHEN as shown in Scheme 2. The obtained complex was white powder with 90 % yield. The powdered the complex was stored in sample tube in vacuum desiccator. The elemental analysis data for Sm $(HDMPE)_3$.dmphen ($C_{44}H_{45}O_{12}N_2Sm$) was found (calculated) % C, 56.06 (55.97); H, 4.84 (4.80); N, 2.74 (2.96); O, 20.41 (20.33); Sm, 15.78 (15.92). IR (KBr) cm^{-1}: 2931 (w), 2832 (w), 2717 (m), 1616 (s), 1590 (s), 1532 (s), 1420 (m), 1365 (s), 1261 (s), 1225 (s), 1209 (s), 1158 (s), 1121 (m), 1076 (m), 963 (m), 865 (m), 834 (m), 776 (m), 712 (m), 595 (m), 534 (w), 472 (w), 428 (w); ^{1}H-NMR (400MHz, DMSO): δ 2.62 (s, 9H, CH_3), 2.71 (s, 9H, dmphCH_3), 3.82 (s, 18H, OCH_3), 6.03 (s, 6H, Ar-H), 7.71 (d, 2H, dmph), 8.52 (d, 2H, dmph), 8.68 (d, 2H, dmph).



Scheme 2. The synthetic route of Sm(III) complexes C1-C2 with HDMPE.

Biological activity

Antimicrobial activity

The assay was carried out on the synthesized ligand HDMPE and their corresponding Sm (III) ion complexes using tube dilution method[25]. The following bacteria were used for in vitro antibacterial activities, Gram-positive bacteria: *B. subtilis, S. aureus* and gram-negative bacterium: *Escherichia coli.* The following fungi were used for antifungal activity *C. albicans* and *A. niger.* The standard drugs ciprofloxacin and fluconazole[26] have also tested for their antibacterial and antifungal activity at the same concentration under the same condition as that of the tested HDMPE and Sm (III) ion complexes. The dilutions of synthesized complexes as well as standard drugs have been prepared in double strength nutrient broth I.P and sabouraud dextrose broth I.P media for bacteria and fungi respectively[27]. The standard, ligand and Sm(III) ion complexes were dissolved in DMSO to give concentration of 100µg/mL. The incubation period for HDMPE, C1 and C2 were 24 h at 37 °C for bacteria, 48 h at 37°C for *C. albicans* and 7 days at 25 °C for *A. niger* respectively. The zone of inhibitions of the antimicrobial activity has been recorded in terms of minimum inhibitory concentration (MIC).

Antioxidant activity

The antioxidant activities of the synthesized ligand HDMPE and Sm(III) ion complexes were determined by using DPPH method[28]. When DPPH reacts with antioxidant HDMPE and Sm (III) ion complexes, it shows a significant absorption decrease at 517 nm. Methanol was used as a solvent for preparation of various concentrations (25, 50, 75, and 100) µg/ml of the materials. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Tests were carried out in triplicate and ascorbic acid was used as a standard antioxidant. The DPPH scavenging activity is expressed as IC_{50}, whose concentration is sufficient to obtain 50% of maximum scavenging activity. Standard curve is plotted for different concentration of ascorbic acid, HDMPE, C1 and C2. Scavenging of DPPH free radical was calculated as:

DPPH scavenging activity (%) = [(Ac-At) / Ac] ✕ 100

Where, Ac is the absorbance of the control reaction and at is the absorbance of the test sample.

RESULTS AND DISCUSSION

Elemental analysis and solubility

The elemental analytical data for the HDMPE, C1 and C2 presented in Table 1 reveals that the stoichiometry of the ternary Sm(III) ion complexes to be 1: 3:1 (Sm: HDMPE: ancillary ligand). Both these complexes were stable under atmospheric condition and were found to be soluble in dimethylsulfoxide, dimethylformamide, chloroform and acetone, sparingly soluble in methanol and ethanol but insoluble in benzene and hexane.

Table 1: Elemental analysis data for ligand HDMPE and complexes C1-C2.

Complexes	C (%) Found (calc.)	H (%) Found (calc.)	N (%) Found (calc.)	O (%) Found (calc.)	Sm (%) Found (calc.)
HDMPE	60.84 (61.22)	6.14 (6.16)	---	32.58 (32.62)	---
C1	56.04 (55.97)	4.92 (4.80)	C101 (2.96)	20.29 (20.33)	15.86 (15.92)
C2	60.64 (60.71)	4.53 (4.62)	2.59 (2.62)	18.01 (17.98)	1C195 (14.07)

^{1}H-NMR and IR spectra

The ^{1}H-NMR spectrum of the ligand HDMPE showed singlet at δ 13.84 due to phenolic proton which disappeared in the complexes C1 and C2 was indicating that HDMPE is coordinated with Sm (III) ion through the oxygen atom of phenolic OH group. The FT-IR spectra of HDMPE, C1 and C2 in KBr pellets were recorded with Perkin Elmer spectrum 400. The main IR bands are presented in Table 2.

Table 2: The characteristic IR bands (cm^{-1}) of the free ligand HDMPE and its corresponding Sm(III) complexes C1-C2 .

Compound	ν(O-H)	ν(C=O)	ν(C=N)	ν(C=C)	ν(Ph-O)	ν(Sm-N)	ν(Sm-O)
HDMPE	3430	1640	---	1538	1270	---	---
C1	---	1618	1570	1537	1260	592	449
C2	---	1616	1584	1532	1261	595	428

HDMPE exhibits abroad absorption band at 3430 cm^{-1} assigned to ν (O-H) stretching vibration[29-31] which disappeared in the IR spectra of C1 and C2. The HDMPE also displays the intense C=O stretching vibration band at 1640 cm^{-1}, which was red shifted 22-24 cm^{-1} in complexes C1-C2, indicating that phenolic and carbonyl group of HDMPE participated in coordination with Sm (III) ion[32]. The strong absorption band at 1570 cm^{-1} in complex C1

and at 1584 in complex C2 assigned to C=N stretching vibration, provided good evidence that the nitrogen atoms of ancillary ligands were coordinating with the Sm(III) ion [33, 34]. The peak for Ph-O vibration of the ligand HDMPE present at 1270 cm^{-1} showed a redshift of 10-11 cm^{-1}in the complexes C1-C2 , indicating that the phenolic group is included in coordination with the Sm (III) ion. The appearance of absorption bands at 592 cm^{-1}(C1), 595 cm^{-1}(C2) and at 449 cm^{-1} (C1), 428 cm^{-1} (C2) are assigned to ν(Sm-N) and ν(Sm-O)[33, 35] respectively, which affirms that the nitrogen atoms of the ancillary ligands and oxygen atoms of the ligand HDMPE participated in coordination with the Sm(III) ion. Finally, it can be concluded from the FT-IR and ^{1}H-NMR spectra of the HDMPE and its corresponding Sm (III) complexes C1-C2, that the coordination of Sm (III) was through the oxygen atoms of phenolic and carbonyl group of HDMPE and nitrogen atoms of the respective ancillary ligand.

Antimicrobial activity

The synthesized ligand HDMPE and complexes C1-C2 were evaluated for their in vitro antimicrobial activity as tabulated in Table 3 and presented as bar diagram in Figure 1. The antimicrobial activity has been investigated by taking ciprofloxacin and fluconazole[26] as standard drugs for antibacterial and antifungal activity respectively. The results revealed that the ligand HDMPE was having insignificant antimicrobial activity against bacterial and fungal strains, while complexes C1-C2 showed moderate to good activity compared to the standard antibiotics and showed excellent activity against *S. aureus.* Further it was noticed that complexes was excellently active in case of *C. albicans, while* moderately active in case of *A. niger.* Moreover, it was interesting to note that complexes C1-C2 proved to be better than ciprofloxacin against *S. aurius* and better than fluconazole against *C. albicans*.The increase in antimicrobial activity of the complexes may be due to the presence of Sm (III) ion coordinated with the donor atom of the ligand which leads to the π- electron delocalization over the chelate rings[36].

Table 3: Minimum inhibitory concentration of HDMPE and complexes C1, C2. The bold values indicate highest values of the respective properties.

Compound	Minimum Inhibitory Concentration (µM/mL)				
	B. subtillis	***S.aureus***	***E.coli***	***C.albicans***	***A.niger***
HDMPE	31.8	31.8	31.8	31.8	63.7
C1	13.2	6.61	13.2	6.61	13.2
C2	13.2	6.61	13.2	6.61	13.2
Standard	8.71[a]	8.71[a]	8.71[a]	10.09[b]	10.09[b]

[a]Ciprofloxacin[b] Fluconazole

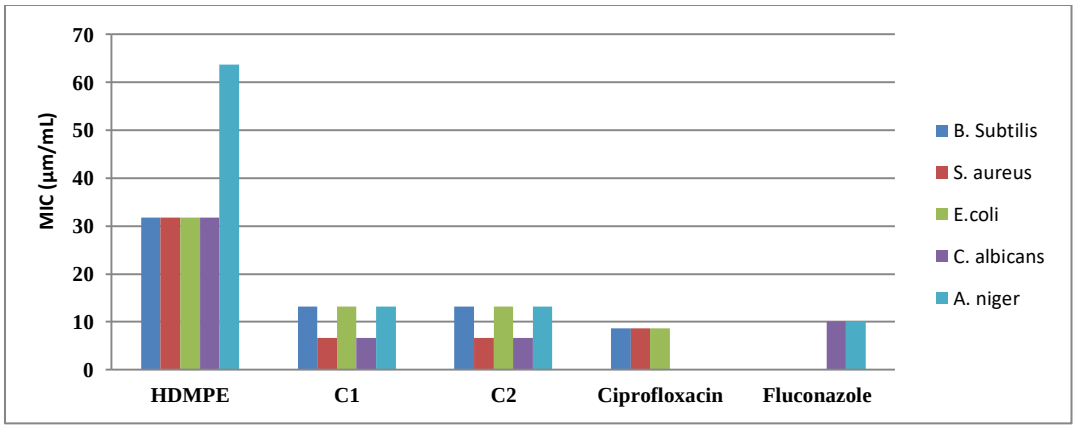


Figure 1: Bar diagram showing the antimicrobial activities of HDMPE and complexes C1-C2with respect to standard drugs.

Antioxidant activities

In DPPH free radical scavenging activity, antioxidant reacting with stable free radical 2, 2-diphenyl-2-picrylhydrazyl (DPPH) produced colorless 2, 2-diphenyl-2-picrylhydrazyl. The absorbance decreased, after receiving hydrogen radical. The DPPH scavenging activity is expressed as IC_{50}. The IC_{50} value of ligand and complexes C1-C2 were calculated from the graph plotted as inhibition percentage against concentration of HDMPE and complexes C1-C2 as shown in Table 4and Figure 2. The results show that ligand HDMPE and complexes showed poor activity, when compared with standard ascorbic acid (IC_{50}= 43.78µg/ml).

Table 4: Percentage inhibition and IC_{50} values of DPPH radical scavenging activity of synthesized HDMPE and complexes C1-C2.

Compound	Concentration (µg/mL)				
	25	50	75	100	IC_{50}
HDMPE	23.12	43.02	60.08	80.83	60.42
C1	23.56	42.56	65.98	89.41	56.43
C2	23.34	46.67	67.34	91.72	54.46
Ascorbic acid	34.02	56.22	76.12	92.01	43.78

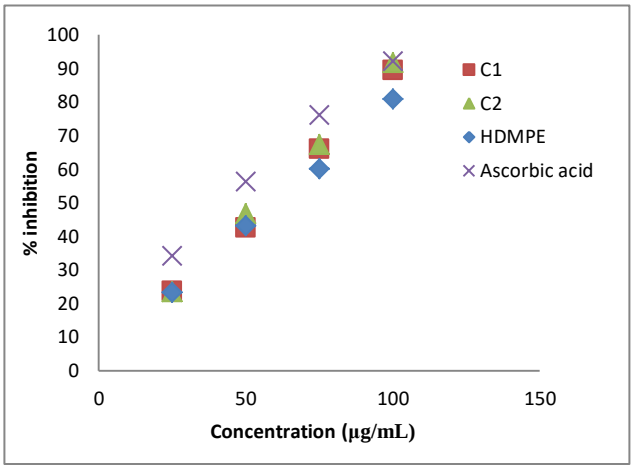


Figure 2: Percentage inhibition of HDMPE and complexes C1-C2 with respect to standard ascorbic acid.

CONCLUSION

In this work, two new ternary Sm (III) complexes C1-C2 have been synthesized. The complexes was characterized by elemental analysis, FT-IR, ^{1}H-NMR and photoluminescence spectroscopy. Difference in the FT-IR and NMR spectra of the free ligand (HDMPE) and the complexes, indicated that the oxygen atom of phenolic as well as carbonyl group of the ligand and nitrogen atoms of the ancillary ligands (DMPHEN or dmphen) were coordinated to Sm(III) ion effectively. The synthesized complexes C1-C2 exhibited excellent *in vitro* antimicrobial against *S. aureus* and *C. albicans* and proved to be better than ciprofloxacin fluconazole but poor antioxidant profile as compared to standard ascorbic acid (IC_{50}= 43.78µg/ml).

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