Synthesis, characterization, and study of biological activities of Ni (II) and Ag(I) bimetallic complex prepared by using reagents N,N'-bis(o-vanillinidene) ethylenediamine and 1-nitroso-2-naphthol

Jaiba Shaikh*, Dilip Yadav, Suhas Janwadkar, Shrutika Raut, Ramnath Andhale, Bhakti Borude
Department of Chemistry, Sonopant Dandekar College, Kharekuran Road, Palghar-401404, Maharashtra, India

Abstract: **The ligand N, N'-Bis(o-vanillinidene) ethylenediamine (o-VEDH$_2$) is prepared by using Schiff-base reaction and it is characterized by FT-IR, ^{1}H NMR, ^{13}C NMR. This ligand is used to prepare a monometallic complex with Ni (II). This monometallic complex is combined with another one, which is synthesized using reagent 1-nitroso-2-naphthol with Ag (I) to synthesize a heterobimetallic complex. This synthesized heterobimetallic complex is characterized using FT-IR,^{1}H NMR elemental analysis and P-XRD. Different bacterial strains such as *E. coli, S. aureus, B. subtilus* and *P.aeroginosa* are used to check the antibacterial activities of bimetallic complex. The biological activity data showed that the heterobimetallic complex exhibits good antibacterial and antifungal activities. Comparison of the inhibition values of the monometallic complexes and the heterobimetallic complex indicate that the heterobimetallic complex exhibits higher antimicrobial activity.**

Keywords: **(o-VEDH$_2$), heterobimetallic complex, antibacterial activities, and antifungal activities.**

1. INTRODUCTION

The chemistry of Schiff bases and their metal complexes became a subject of great interest due to their diversified application. Schiff bases are generated when primary amines condense with carbonyl molecules [1]. Chelating ligands containing O and N donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions [2,3]. Metal ions are architected with Schiff base ligands to form metal complexes, which enhance the biological activity of metals that have been the focus of metal coordination chemistry [4].Further, transition metal complexes of Schiff's bases have been synthesized and tested for their biological activity because they exhibited antibacterial [5-7] and anti-inflammatory activities[8]. Their metal chelates have been essential in developing chemical industries through catalysis, substrate carrier and dyes [9].

Metal complexes are categorized based on the number of metals in coordination spheres as monometallic, bimetallic, and polymetallic complexes.The bimetallic complexes are either homobimetallic when both the metals involved in the complex are the same or heterobimetallic when both the metals are different. Due to the presence of two metals, the activities of bimetallic complexes are enhanced due to the contribution of both metals [10]. Over the last several decades, much work has been done in the synthesis, characterization, and biological activities of bimetallic complexes. Since these bimetallic complexes have a wide range of applications, research on the synthesis and characterization of new bimetallic complexes is growing rapidly. It has been observed that bimetallic complexes are utilized for a variety of industrial and agricultural applications, including pesticides, fungicides and antifouling agents [11].

A metal complex containing an azomethine moiety possesses a wide range of pharmacological activities, including antimicrobial, antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, herbicidal, and anthelmintic properties [12,13]. Dinuclear Ni-Fe complexes with significant catalytic activity for the hydrogen evolution reaction have been reported [14]. Ogo and his coworkers reported the Ni-Ru based, H_2O coordinated bis (μ-thiolato) $Ni^{II}Ru^{II}$ complex, which successfully reacted with H_2 in water to give a hydrido bridged bis (μ-thiolato) $Ni^{II}Ru^{II}$ complex [15].Afterwards, the Ogo group reported several other Ni-Ru-based complexes by altering the organoruthenium moieties, such as the aromatic rings, in order to shed light on the structure-function relationships in this class of catalysts and to gain more insight into the reaction activity of the dinuclear complexes with H_2[16-18].

The synthesis and characterization of a bimetallic complex of Ni(II)-Zn (II) with a salen-type Schiff base was reported [19].

Frederic Pelletier et al. investigated the anti-cancer activity of bimetallic titanium-ruthenium complexes against human ovarian cancer cells [20].

The bimetallic complex of Ru (II)-Fe (III) has been used to detect signal amplifying and degrade oxalate, an industrial pollutant [21].

The hetero-bimetallic complex and the study of the electronic effect of aluminum-containing ligands in Ir-Al and Rh-Al were also reported [22].

In this work, we wish to report the synthesis and characterization of a Ni (II)-Ag (I) bimetallic complex with the reagents N, N'-Bis(o-vanillinidene) ethylenediamine (o-$VEDH_2$), and 1-nitroso-2-naphthol and their biological activities.

To the level of our best knowledge, Ni (II)-Ag (I) bimetallic complex with reagents N, N'-Bis(o-vanillinidene) ethylenediamine (o-$VEDH_2$) and 1-nitroso-2-naphthol was not reported.

2. EXPERIMENTAL

2.1 Materials

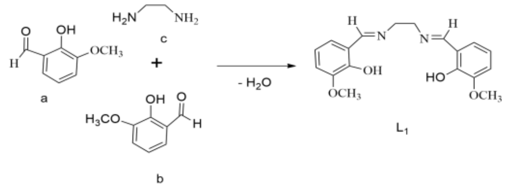
Without further purification, commercially available reagent-grade o-vanillin, ethylenediamine, Nickel chloride, 1-Nitroso-2-naphthol, silver nitrate, and ethanol were used.

2.2 Physical measurements

FT-IR spectra were recorded using FT-IR.8300 Shimadzu in the range of (4000-200) $cm^{-1.}$ The measurements were recorded using a concentration of (10^{-3}M) of the complex in ethanol. NMR spectroscopic studies were recorded on a Bruker 400 MHz and 75.45 MHz. FT-NMR spectrometer using tetramethyl silane (TMS) as the internal standard and DMSO solvent. Elemental and the Mass spectrum was analyzed using PerkinElmer 2400 CHN elemental analyzer and MALDI-TOF: Bruker Auto flex max LRF. PXRD measurement was carried out by BRUKER AXS, GERMANY X-ray diffractometer model using Cu K α-1 radiation.

2.3 Preparation of Schiff base ligand L_1 [N, N'-Bis(o-vanillinidene) ethylenediamine]

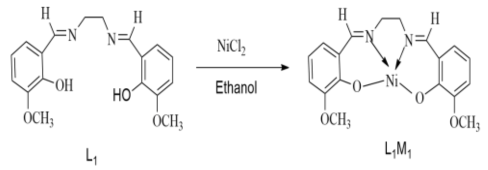
N, N'-Bis(o-vanillinidene) ethylenediamine (o-$VEDH_2$) Schiff base ligand has been prepared by the reaction of o-vanillin with ethylenediamine. The o-vanillin and ethylenediamine were stirred in a 2:1 ratio for 20 mins. After constant stirring, a yellow-colored solid was obtained. It was recrystallized using ethanol, which gave satisfactory IR, 1H-NMR data, and Elemental analysis.



Scheme 1: Synthesis of Schiff base ligand N, N'-Bis(o-vanillinidene) ethylenediamine L_1

2.4 Preparation of monometallic complex L_1M_1 [N,N'-Bis(o-vanillinidene) ethylenediamine] Ni(II)

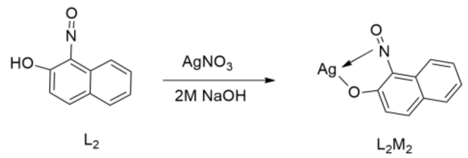
The ligand N, N'-Bis(o-vanillinidene) ethylenediamine was used to form a complex with Ni(II) to get the L1M1 complex. An ethanolic solution of Nickel chloride was added in a 1:1 ratio to the ethanolic solution of ligand (L_1) and stirred for 30 minutes at room temperature. The orange coloured precipitate was obtained. The product was filtered off and then dried under a vacuum.



Scheme 2: Synthesis of monometallic complex L_1M_1 [N,N'-Bis(o-vanillinidene) ethylenediamine] Ni(II)

2.5 Preparation of monometallic complex L_2M_2 [1-Nitroso-2-Naphthol] Ag(I)

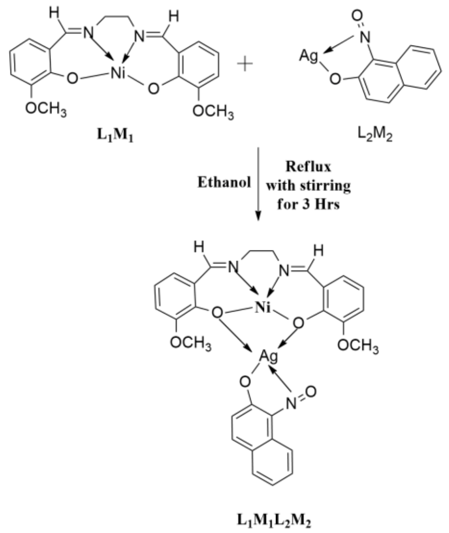
1-Nitroso-2-Naphthol(3.11 g, 18 mmol) was dissolved in 15 mL of distilled water. The Ph of the solution was brought to 11 with 2M sodium hydroxide solution. This solution was added drop by drop to 9 mL of 2 M silver nitrate solution. The brown-coloured complex(L_2M_2) was filtered and washed thoroughly with water and diethyl ether then dried at room temperature [23].



Scheme 3: Synthesis of monometallic complex L_2M_2 [1-Nitroso-2-Naphthol] Ag(I)

2.6 Preparation of hetero-bimetallic complex $L_1M_1L_2M_2$ [Ni(L_1,L_2)Ag]

The L_1M_1 complex was then combined with the complex prepared using the reagent 1-nitroso-2-naphthol. The ethanolic solution of (L_1M_1) was added to the ethanolic solution of (L_2M_2). The resulting mixture was then refluxed with stirring for 3 hours. The product that separated out on hot was filtered off and washed with ethanol.



Scheme 4: Synthesis of hetero-bimetallic complex [Ni(L_1, L_2) Ag] $L_1M_1L_2M_2$

3 RESULTS AND DISCUSSION

INFRARED SPECTROSCOPIC STUDY

The mode of binding between the ligands and the metal salts in complexes was investigated by comparing the IR spectrum of the free ligand and those of the complexes.

The FT-IR spectra of L_1 was characterized by bands at(1407),(3102) , (1295,1078), (1632),(1450) and (3322) cm^{-1}.These can be assigned to (υArC-C), (υAr C-H), (υCH2N), (υC=N), (υC-O) and (υO-H) respectively. In the L_1M_1complex a negative shift with reduced intensity were observed for (C=N) and (CH_2N) stretching bands. The characteristic phenolic υ(OH), due to the presence of a hydroxy group at the o-position was observed in L_1 at 3322 cm–1, and a band at 1450 cm–1 due to υ(C–O) of the phenolic group was also observed in ligand L_1. The sharp band due to the ligand's phenolic (OH) is absent in the complex L_1M_1 and bimetallic complex $L_1M_1L_2M_2$ to indicate the coordination of the phenolic oxygen to the metal ion after deprotonation. Such coordination is also supported by shifting the phenolic υ(C–O) band to lower wave numbers at 1440 cm–1 in the complex L_1M_1 and at 1435 in the bimetallic complex $L_1M_1L_2M_2$. The coordination of the azomethine nitrogen and phenolic oxygen in bimetallic complex $L_1M_1L_2M_2$ is further supported by the appearance of two non-ligand bands at 510–600 and 400–460 cm–1 due to υ(M–O) and υ(M–N), respectively.

Complex/ Ligand	υ Ar C=C	υ Ar C-H	υ CH2-N	υ C=N	υ N=O	υ C-O	υ M-O	υ M-N	υ O-H
(L_1)	1407.78 (s)	3102.2 (m)	1295.93, 1078.98 (s)	1632.45 (s)	-	1450.09 (s)	-	-	
(L_1M_1)	1451.17 (s)	3102.2 (m)	1280,1066 (s)	1617.02 (s)	-	1440.83 (s)	573.71 (m)		3322.5 (m)
(L_2M_2)	1405.47 (s)	971.74 (s)	1304.04, 1011.10 (ms)		1538.83, 1342.96 (s)				-
($L_1M_1L_2M_2$)	1451.17 (s)	3102.2 (m)	1249.65, 1171.54 (s)	1617.02 (s)	-	1435.2(S)	573.71 (m)	450.61 (m)	-

NMR SPECTROSCOPY

The ^1H NMR spectra of the metal complex (L_1M_1) demonstrated characteristic azomethine (CH=N) protons at 4.89 ppm as a singlet. As a singlet, the (CH_2) protons in the metal complex (L_1M_1) were observed at 3.05–3.98 ppm.

POWDER XRD

The powder XRD pattern of the Ni-Ag bimetallic complex recorded in the range (2θ = 0-80°). XRD pattern of the bimetallic complex shows the sharp crystalline peaks indicating its crystalline phase. The average crystallite size (d_{XRD}) of the complex was calculated using Scherer's formula. The bimetallic complex has an average crystallite size of 85 nm.

PHYSICAL CHARACTERISTICS AND ELEMENTAL ANALYSIS

COMPLEX / Ligand	MW(g mol^{-1})	COLOUR	%YIELD	% C	% H	% O	%N	% Ni	% Ag
				Found (Calculated)					
(L_1)	328.36	yellow	75	64.77 (65.84)	5.94 (6.14)	18.90 (19.49)	7.94 (8.53)	--	--
(L_1M_1)	400.07	Orange	65	56.84 (57.04)	5.05 (5.29)	15.36 (16.00)	6.49 (7.00)	14.12 (14.67)	--
(L_2M_2)	280.08	Brown	86	41.35 (42.85)	2.04 (2.14)	10.97 (11.42)	4.84 (5.00)	--	37.94 (38.21)
($L_1M_1L_2M_2$)	663.69	buff	54	50.08 (50.62)	3.32 (3.61)	14.09 (14.46)	5.91 (6.32)	7.65 (8.84)	15.85 (16.12)

ANTI-BACTERIAL

Procedure:

Antibacterial activity against *E. coli*, *Bacillus subtilis*, *S. Aureus*, *P. aeruginosa* bacteria by healthy diffusion method. The inoculums of the microorganism were prepared from the bacterial cultures. 15 ml of nutrient agar (Hi media) medium was poured into clean sterilized Petri plates and allowed to cool and solidify. 100 µl of broth of bacterial strain was pipetted out and spread over the medium evenly with a spreading rod till it dried properly. Once the agar was hardened, the sample slides were placed on the plate in the manner, and the plates were incubated at 370^0C for 24 h. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) [24].

Antibacterial Activities of samples against *E.coli*, *Bacillus subtilis*, *S Aureus*, and *P.aeroginosa*

Sr. No	SAMPLES	CONCENTRATION (mg/ml)	ZONE IN DIAMETER (mm) *E.coli*	ZONE IN DIAMETER (mm) Bacillus subtilis	ZONE IN DIAMET ER (mm) Staphylococcus Aureus	ZONE IN DIAME TER (mm) Pseudomonas aeruginosa
1	Standard (streptomycin)	1 mg	24	25	20	26
2	L_1M_1	5 mg	3	5	4	5
3	L_2M_2	5 mg	2	6	4	6
4	$L_1M_1L_2M_2$ Complex	5 mg	10	14	15	12

ANTI-FUNGAL

Procedure: Antifungal activity against Candida Albicans and A. Niger

(Agar healthy plate diffusion Method)

Difussion method for the determination of zone of inhibition:

Antifungal activity Stock solution for antifungal activity: For the antifungal study, sample concentrations of 5 mg was stored in a refrigerator till further use. The antifungal activities of the sample were evaluated using an agar well diffusion assay. The assay was carried out according to the method of (Hufford et al., 1975). Sabouraud dextrose agar (Hi media) was used to grow fungus. Media with acidic pH (pH 5.5 to 5.6) containing a relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121^0C for 15 minutes. Twenty-five ml of molten (45^0C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish. For counting spore (fungi) were suspended in normal saline to make volume up to 1 ml and then counted with help of haemocytometer. Once the agar was hardened, 6 mm wells were bored using a sterile cork borer. Then 0.1 ml (100µl) from the stock solution of the sample having a final concentration of 5 mg was placed in each well, and the plates were incubated for 72 hours at 29ºC. The antifungal activity was measured as the diameter (mm) of the clear zone of growth inhibition. (Umadevi et al., 2003) [25,26,27].

Antifungal Activity of heterobimetalliccomplex against Candida Albicans and A. Niger

Sr. No	SAMPLES	CONCERN TRACTION (mg/ml)	ZONE IN DIAMETER (mm) against Candida albicans	DIAMETER (mm) against Candida albicans ZONE IN DIAMETER(mm) against A. Niger
1	STD	1 mg	20	22
2	$L_1M_1L_2M_2$ Complex	5 mg	14	16

4. CONCLUSION

The heterobimetallic complex was synthesized. This synthesized heterobimetallic complex is characterized with FT-IR, ^{1}H NMR, elemental analysis, and P-XRD. Different bacterial strains such as *E. coli, S. aureus, B. subtilus* and *P.aeroginosa* are used to check the antibacterial activities of bimetallic complex. The biological activity data showed that the heterobimetallic complex exhibits good antibacterial and antifungal activities. Comparison of the inhibition values of the monometallic complexes and the heterobimetallic complex indicate that the heterobimetallic complex exhibits higher antimicrobial activity.

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