A NOVEL ANTIBACTERIAL STUDY OF SCHIFF'S BASES AND THEIR METAL COMPLEXES ON SRB

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***Abstract:** Schiff bases viz. benzene-1,2-diyldimethylylidenedicarbamic acid, N,N'-benzene-1,2-diylbis[1-(pyrazin-2-yl)methanimine], (E)-2-((3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methyleneamino)phenol and their metal complexes were prepared. The complexes were screened for their antibacterial character against strains of SRB's using anaerobic vial culture by MPN method. Various parameters such as effect of turbidity, pH and dry weight using UV-VIS spectroscopy were determined at different intervals. (E)-2-((3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methyleneamino) phenol (Ligand L3) was found to be highly effective at optimized dose of 500 ppm. The antibacterial activity of the ligands was found to be in order: L3>L1>L2 and the metal complexes of Schiff base ligands shows enhancement in their antibacterial activity. The copper complex with ligand L3 was found to be highly active and follows the order: Cu-L3>Co-L3>Ni-L3≈Fe-L3> Zn-L3.*

***Key words:** SRB, MPN, ligand, antibacterial activity.*

I. INTRODUCTION

Schiff bases are diversified organic compounds which are prepared between carbonyl and amine compound due to the condensation reaction. The presence of N, O, S atoms in their molecules allow them to behave as donor molecules or ligands to the central metal ion. The linkage of these molecules with various metals ions through coordinate bonds can widespread their study in the development of coordination complex compounds and also finds numerous application in catalytic, optical, pharmaceutical and bio-organic/bio-inorganic chemistry. The incorporation of metal ions or binding components makes them to exhibit a significant role in various bio-chemical reactions [1-4].

Chelation of metals with chelating ligands shows an exceptional enhancement of lipophilic character of organic moiety [5]. Antimicrobial properties of these compounds are further enhanced when treated with various metals due to the formation of metal-ligand chelating complexes and thus these can be broadly used for biological activities. They are extensively studied for their antimicrobial properties due to the presence of imine or azomethine groups that is intermediate of various biochemical reactions. Their correlations with various biochemical reactions formulate an encouraging research for the development of novel pharmacological methodologies towards the antimicrobial resistance [6-9].

Desulfovibrio desulfuvicans is a sulphate reducing bacteria (SRB) that exclusively belongs to the most troublesome assemblage of microbes against entire microorganisms. These are obligate unicellular anaerobes which reduces sulphates to sulphides. They attain energy for their growth from the oxidation of organic compounds using sulphates as external e^- acceptor as shown in Fig. 1 and involved in microbial induced corrosion (MIC) of steel and other metals especially in the oil-gas and shipping industry [10, 11]. Costerton and Boivin assessed that their occurrence poses severe threat to oil and gas pipelines used for transportation and storage tanks. They also deteriorate quality of petroleum products, enhance the refinery costs and causes environmental pollution through hydrogen sulphide (H_2S) generation [12]. Consequently, existence of SRB rapidly accelerates microbial induced corrosion in crude oil and gas industry particularly where sea-water injection system is used for reservoir's pressure maintenance.

It is revealed that MIC is a significant area of research for laboratory and industrial studies and various investigations on antimicrobial character of Schiff bases against SRB's were reported [13]. In the present study, three Schiff base ligand and their metal complexes were synthesized. The synthesized ligand and metal-complexes were characterized and used for the study of antibacterial properties against SRB's using anaerobic vials experiments.

II. EXPERIMENTAL

2.1 Chemicals and Instruments

Various organic chemicals that were used are for synthesis are carbamic acid, benzene-1,2-dicarbaldehyde, pyrazine-2-carbaldehyde, benzene-1,2-diamine, 4-amino antipyrine, thiosemicarbazide, and 2-methoxy-4-methyl benzaldehyde. These chemicals were purchased from Sigma-Aldrich while acetone, absolute ethanol, and other metal salts other chemicals were obtained from Fluka. All these chemicals and reagents were used as such without any additional purification. Double distilled water was used for making all sorts of aqueous solutions.

The absorbance was determined by Hitachi UV-VIS U 1900 spectrometer in the range of 1100–190 nm. The ^{1}H-NMR and ^{13}C-NMR spectra were determined in deuterated chloroform ($CDCl_3$) on a Bruker Avance 300 MHz spectrometer. Turbidity measurements were determined using Equiptronics EQ 811 turbidity meter. The pH determinations were recorded by using digital pH meter (Beckman Coulter PHI 450). The FTIR spectra of the ligand and complex were determined by using Labtronics FTIR spectrometer in the range of 4000–350 cm^{-1}. The structure of compound was proved by ^{1}H-NMR and ^{13}C-NMR studies.

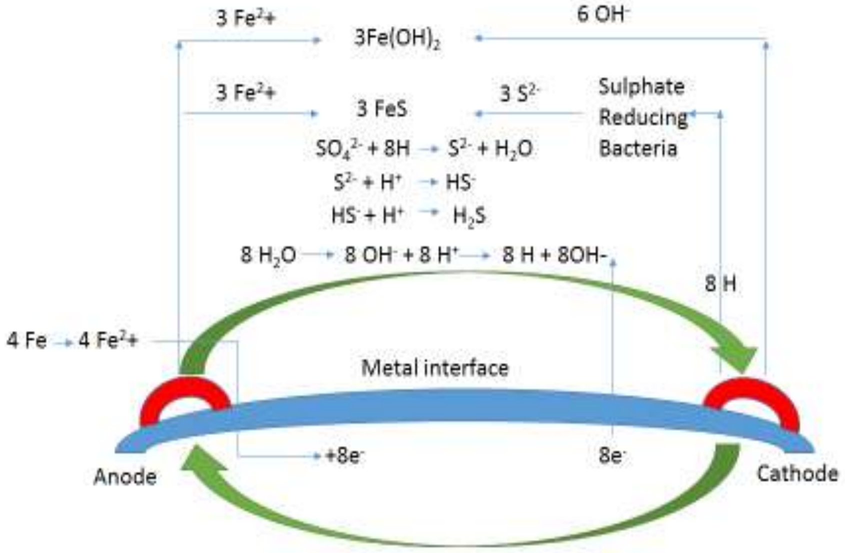


Fig. 1: Mechanism of metal corrosion due to SRB activity

2.2 Synthesis of Schiff base ligands and their Metal complexes:

Three different types of Schiff base ligands i.e. benzene-1,2-diyldimethylylidenedicarbamic acid (L1), *N,N'*-benzene-1,2-diylbis[1-(pyrazin-2-yl)methanimine] (L2) & (*E*)-2-((3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyleneamino)phenol (L3) were synthesized.

2.2.1(a) Synthesis of L1: 1 mmol of benzene 1,2-dicarbaldehyde dissolved in 8 ml of DMSO was added with 1 mmol of carbamic acid in 5ml DMSO. The resultant mixture was then allowed to react in reflux condition under nitrogen atmosphere for 100 minutes at 80ºC, resulting in a red color precipitated product. The precipitated product was further filtered and dissolved in absolute ethanol at room temperature. The ethanol was then allowed to evaporate in vacuum till the micro crystals of the Schiff base ligand were obtained (Fig. 2).

The physical properties and analytical data of the L1 ligand is given below:

Empirical formula: $C_{10}H_8N_2O_4$, formula weight: 220.168 gmol^{-1}, yield: 74%, melting point: 68ºC.

^{1}**H-NMR** (ppm): 8.24 (s, 2H, aromatic), 7.63 (d, 2H, aromatic), 7.29 (s, 4H, iminic), 7.19 (s, 2H, carboxylic acid).

^{13}**C-NMR** (ppm): (6C aromatic); 182.7, 181.3, 180.8, 178.9, 178.2, 176.6, 145.8, 145.5, 105.9, 105.6.

IR data- The IR spectrum of the free Schiff base ligand shows bands of intensity at 3148-3154, 1615-1640, 1720-1730, 1555-1565, 1680-1700, 1755-1765 which corresponds to frequency ν(C-H), ν(C=N), ν(C=O), ν(COO$^-$), ν(C=C) & ν(C-N) respectively.

Schiff base Complex: 3145-3150 ν(C-H), 1600-1625 ν(C=N), 1550-1565 ν(C=O), 1550-1560 ν(COO$^-$), 1670-1680 ν(C=C), 1751-1763 ν(C-N) cm^{-1}.

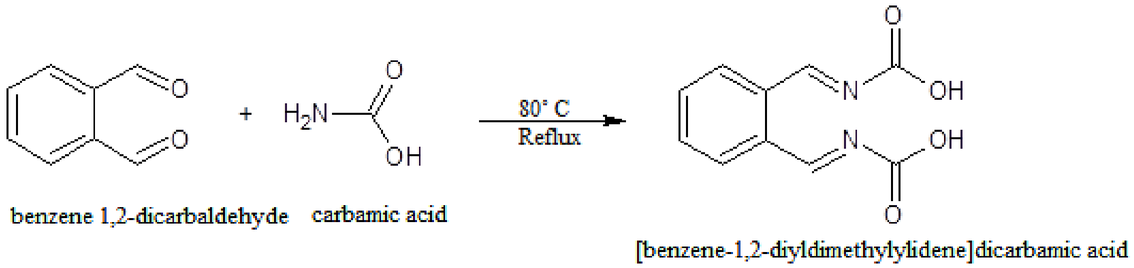


Fig. 2: Schematic diagram of preparation of L1

2.2.1(b) Synthesis of Metal-L1 complex: M(II) complexes of Ni, Co, Pd, Mn and Zn were synthesized with the synthesized Schiff base ligand L1 by the procedure available in the literature[14]**.** A solution of $NiCl_2$ (3 mmol in DMSO) was taken and drop wise added into the flask containing solution of Schiff base ligand (3 mmol in 10 mL DMSO) in the presence of ammonium hydroxide solution. The mixture was continuously stirred and refluxed at 80°C under inert atmosphere of nitrogen till the red colored precipitate was obtained. The red colored solid product was then separated by vacuum filtration, washed with cold acetone solution and dried in vacuum desiccator at room temperature (Fig. 3).

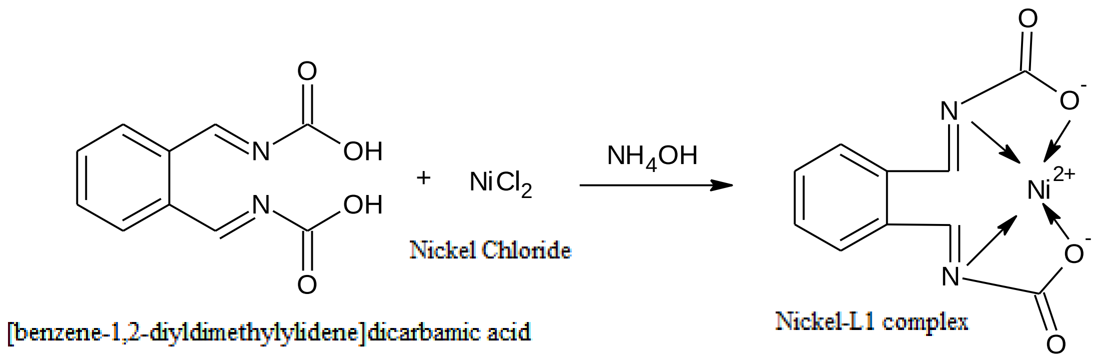


Fig. 3: Schematic diagram of preparation of Nickel complex of L1

The bonding between the free ligand molecules and metal ions significantly affects the stretching frequencies, therefore the complexation of ligand with Ni(II) ion was examined by analyzing the IR spectra of free ligand and complex compound. The IR spectral data indicates that the C=N stretching frequency changes from 1615–1640 cm^{-1} in the free ligands to lower values of 1600-1625 cm^{-1} in the Ni(II) complexes, which indicates that azomethine nitrogen is coordinated with the metal ion in the complex. The COO^{-} stretching frequency also drops from 1555–1565 cm^{-1} to 1550–1560 cm^{-1}. This is due to the coordination of oxygen with metal ion.

2.2.2(a) Synthesis of L2: Equimolar proportions (1 mmol) each of pyrazine-2-carbaldehyde and benzene-1,2-diamine were dissolved in 10 ml of absolute ethanol. The resultant mixture was constantly stirred and reflux under inert atmosphere of nitrogen for 70 minutes at 80ºC. The resultant pale yellow solution of the ligand was kept at room temperature and ethanol was allowed to evaporate in vacuum till the micro crystals of the Schiff base ligand, N,N'-benzene-1,2-diylbis[1-(pyrazin-2-yl)methanimine] was formed (Fig. 4). The micro crystals of the ligand were further filtered with ethanol and then recrystallized from ethanol-/chloroform (2:1, v/v).

The physical properties and analytical data of the L2 ligand is given below:

Empirical formula: $C_{16}H_{12}N_6$, formula weight: 288.306 gmol^{-1}, yield: 73%, melting point: 138ºC.

^{1}**H-NMR** (ppm): 8.55 (s, 2H, pyridinic), 8.30 (s, 2H, iminic), 7.64 (d, 4H, pyridinic), 7.36-7.22 (d, 2H, aromatic), 6.84 (t, 2H, aromatic).

^{13}**C-NMR** (ppm): (16 C aromatic); 176.8, 173.4, 173.1, 172.5, 164.3, 161.2, 159.4, 149.3, 136.2, 134.8, 133.5, 124.1, 121.9, 119.4, 110.2, 109.5.

IR data- The IR spectrum of the free Schiff base ligand shows bands of intensity as:

Schiff base free ligand: 1630–1640 ν(C = N), 1454 - 1461 ν(C = N), 1543-1455 ν(C = C ar.) cm^{-1}.

Schiff base Complex: 1603 - 1609 ν(C = N), 1426–1439 ν(C = N), 1534-1468 ν(C = C ar.) cm^{-1}.

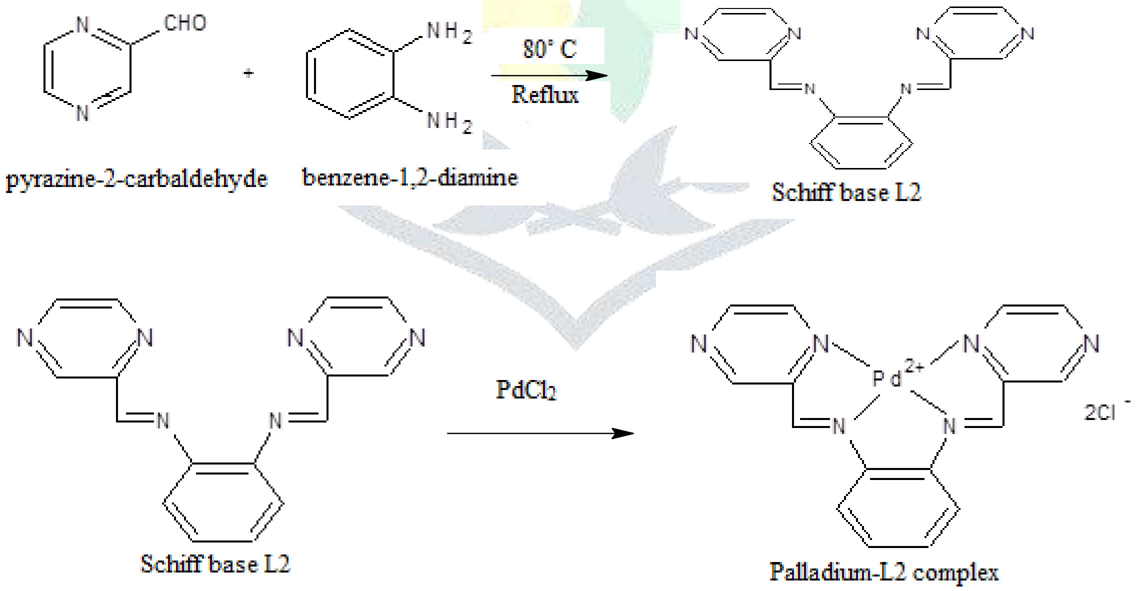


Fig. 4: Schematic diagram of preparation of L2 and palladium complex

2.2.2(b) Synthesis of Metal-L2 complex: M(II) complexes of Pd, Ni, Co, Cd and Zn were synthesized with the synthesized Schiff base ligand L2 by the procedure available in the literature [15]. 3 mmol each of Palladium Chloride and Schiff base ligand L2 were separately dissolved in 10 ml of acetone. Both the solutions were then added in a flask and the mixture constantly stirred and refluxed under inert atmosphere of nitrogen for 20 minutes at 85°C which results in the formation of solid product. The solid product was further filtered by vacuum filtration and washed with cold acetone solution followed by drying in a vacuum desiccator at room temperature.

The FTIR analysis of the Schiff base ligand (L2) and its complex with Pd(II) shows significant variations in IR stretching frequencies. Substantial change was observed in stretching frequencies of C=N from 1630–1640 cm^{-1} in the free ligands to lower values of 1603-1609 cm^{-1} in the Pd(II) complex. It indicates the co-ordination linkage of azomethine (nitrogen atoms) to the central palladium metal ion.

This substantial variation of C=N stretching frequencies occurs as the electron pairs on a nitrogen atom is involved in bond formation with the palladium ion. The C=N stretching frequencies of pyrazine falls from 1454–1461 cm^{-1} to 1426–1439 cm^{-1} due to the coordination linkage of lone pair of nitrogen with Pd(II) ion.

2.2.3(a) Synthesis of L3: The Schiff base ligand L3 was prepared by the condensation reaction between equimolar proportions of 3-(3-nitrophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (0.01 mol) with *o*-amino phenol (0.01 M) in 30 ml of ethanol. The mixture was then refluxed for 8hrs at 70-80ºC under inert atmosphere of nitrogen and the resultant product was poured over crushed ice. The solid product was separated and filtered. It was further washed with cold saturated solution of sodium bisulphate and recrystallized with ethanol. The orange color product of (*E*)-2-((3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyleneamino) phenol was obtained (Fig. 5).
The physical properties and analytical data of the L3 ligand is given below:
Empirical formula: $C_{22}H_{16}N_4O_3$, formula weight: 384.388 $gmol^{-1}$, yield: 88%, melting point: 142ºC.
^{1}**H-NMR** (ppm): 9.23 (s, 1H, Phenolic), 8.71 (s, 1H, azomethinic).
^{13}**C-NMR** (ppm): (22 C); 196(C=N), 157(Phenolic C), 152.23, 150.95, 150.48, 139.06, 137.17, 130.15, 129.78, 129.33, 126.95, 126.68, 122.75, 121.80, 120.05, 119.94, 119.06, 118.50, 118.23, 115.55, 115.30, 114.32.
IR data- The IR spectrum of the free Schiff base ligand shows bands of intensity as:
Schiff base free ligand: 3415-3423 ν (OH), 1653–1658 ν(C = N), 1045-1055 ν (N - N) cm^{-1}.
Schiff base Complex: 3290-3350 ν (H_2O), 1588–1598 ν(C = N), 1065-1090 ν (N - N), 490–540 ν (M-N), 420-428 ν (M-O) cm^{-1}.

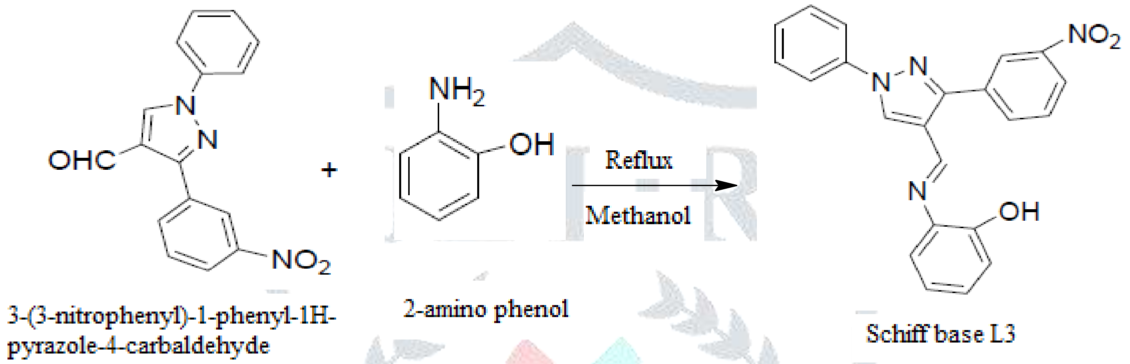


Fig. 5: Schematic diagram of preparation of L3

2.2.3(b) Synthesis of Metal-L3 complex: Metal complexes of Fe, , Ni, Co, Cu and Zn were synthesized with the synthesized Schiff base ligand L3 using the following methodology. Precisely weigh 0.01 mol of metal salt and added drop wise to the 0.02 mol of Schiff base (L3) in 20 ml of ethanol. The mixture was kept at 70-80ºC under inert atmosphere of nitrogen and refluxed for 48 hours. The solid product formed was allowed to cooled, filtered and washed several times with warm ethanol until washing becomes colourless. The product was further dried in air and kept in desiccator over anhydrous $CaCl_2$. The coloured metal complexes, thus formed were found to be stable in air and moisture (Fig. 6).

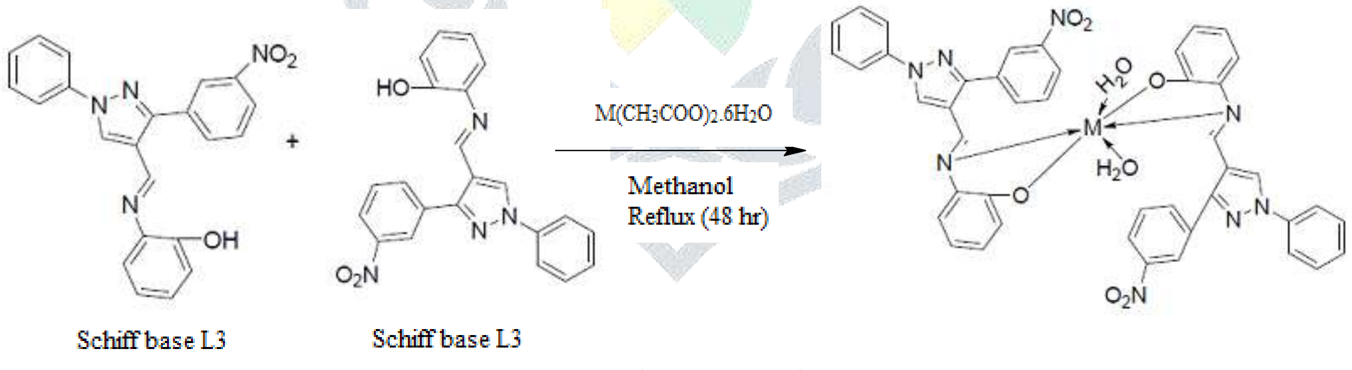


Fig. 6: Schematic diagram of preparation of Metal complex with L3

The FTIR analysis of the Schiff base ligand (L3) showed strong IR stretching frequencies at 1653-1658 cm^{-1} and 3415-3423 cm^{-1} which corresponds to the specific band of azomethine (-HC=N-) and phenolic (-OH) group respectively. Substantial decrease was observed in stretching frequencies of C=N from 1653-1658 cm^{-1} to 1588–1598 cm^{-1} in the spectral data of metal complexes suggesting the linkage of nitrogen atom of the azomethine group to the central metal ion through coordinate bond. All the metal complexes shows a characteristic broad band in the region of 3250 to 3450 cm^{-1} that was attributed to the ν (-OH) that specifies the existence of lattice or coordinated water molecules. The data also reveals that the metal was linked to the Schiff base ligand molecule through imino nitrogen 490–540 ν (M-N), and phenolic oxygen 420-428 ν (M-O) cm^{-1}.

2.3. Preparation of Test Matrix:

Test medium API-RP 38 (API) was used is a strictly anaerobic bacterial culture that was recommended by API to use as standard for monitoring SRB's in oil and water systems. It contains nutrients such as Sodium lactate USP (4 ml), Yeast Extract (1.0 g), Ascorbic acid (0.1 g), $MgSO_4 * 7H_2O$ (0.2 g), anhydrous K_2HPO_4 (0.01 g), NaCl (25 g), ferrous ammonium sulphate $Fe(NH_4)_2(SO_4)_2$ (0.2 g per 100 ml)), Distilled Water (1000 ml) exclusively articulated to facilitate the growth of SRB [16, 17].

2.4. Antibacterial activity:

The antibacterial activity of the synthesized ligands (L1, L2, and L3) and their complexes (ML1, ML2, ML3) was determined against strains of SRB bacteria at 200-1000 ppm by most probable number (MPN) method [18-20]. It was observed that the most probable number (MPN) is far more sensitive and consistent enumeration procedure than SRB colony counting using plate cultures for the determination of extremely low cell counts (Xu and his co-workers) The MPN method is a standard procedure in oil and gas fields to determine the presence of anaerobic bacteria like SRB that are comparatively slow growing in comparison to the aerobic heterotrophs. In comparison to the colony count technique, the MPN method has been shown to be more fast, reliable and accurate in assessing SRB counts.

The used SRB contaminated water was obtained from pigging sample of well fluid line on Mumbai high asset of ONGC (India). The test had been conducted using 10 ml of clean serum bottles that was filled with 9 ml of hot broth (test medium). One ml of pigging sample (that contains SRB) was taken in pre-sterilized disposable syringe and inoculated in serum bottle. Precaution were taken to avoid any ingress of air and the inoculated bottle turned upside down 3-4 times for mixing the contents for first dilution (10^{-1}). Another sterile syringe was used to withdraw 1 ml contents form the bottle and injected to the next dilution bottle for second dilution (10^{-2}). Similar repetition of inoculation was carried out for the third dilution (10^{-3}). The serum bottles of various dilutions were kept in incubator for 28 days for SRB growth and temperature was varied from 20 to 80^0 Celsius. The same set of experiments was repeated in the presence of Schiff base ligand and their metal complexes at 200, 500, 750, and 1000 ppm. Effect of parameters such as pH and turbidity were also examined at various intervals.

III. RESULTS AND DISCUSSIONS

The synthesized Schiff bases and their metal complexes were examined for their antibacterial behavior. Anaerobic serum vials containing API-RP 38 medium was used for SRB culture. In the present study, effect of turbidity, pH and optimized dose of Schiff base ligand and their metal complexes required as antibacterial agent were studied. UV-VIS spectroscopy was used to determine the dry weight of SRB bacteria.

Figure 7 displays the monitoring of SRB growth in vials. The occurrence of black colour in the various diluted vials confirms the presence of SRB. The black color corresponds to the iron sulphide that develops after the generation of H_2S gas due to the SRB activity.

Fig. 7a: Monitoring of SRB growth kept in incubator

Fig. 7b: Shows the occurrence of SRB in third dilution in vials in control experiment.

Effect of Turbidity:

Turbidity is a measurement of the degree of transparency of a solution. It provides an indication about the presence of suspended particulates and growth of microbes. Turbidity measurements were carried out on Turbidity meter Equiptronics EQ 811. The sample was diluted prior to turbidity determination. 10 ml of test sample was filled in a clean glass cell and diluted with 30 ml of distilled water and shaken the contents for mixing. Precaution was taken to avoid formation of air bubbles as it may lead to errors. The glass cell was inserted in the turbidity meter and turbidity of various samples was measured in NTU (Table 1).

Table 1: Turbidity measurements at different temperatures vs incubation period

Incubation Period (in days)	Turbidity at temperature (in NTU)			
	20^0C	37^0C	60^0C	80^0C
7	2.32	3.22	0.75	0.55
14	1.93	3.68	0.52	0.48
21	2.15	2.35	0.58	0.38
28	1.98	2.58	0.62	0.39

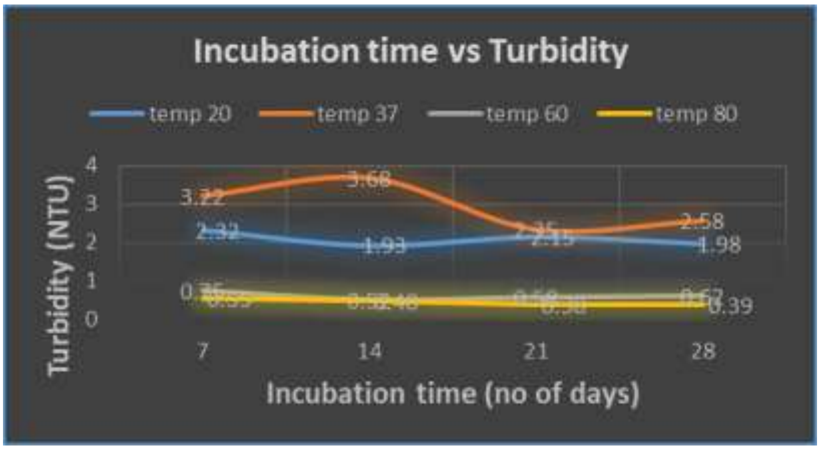
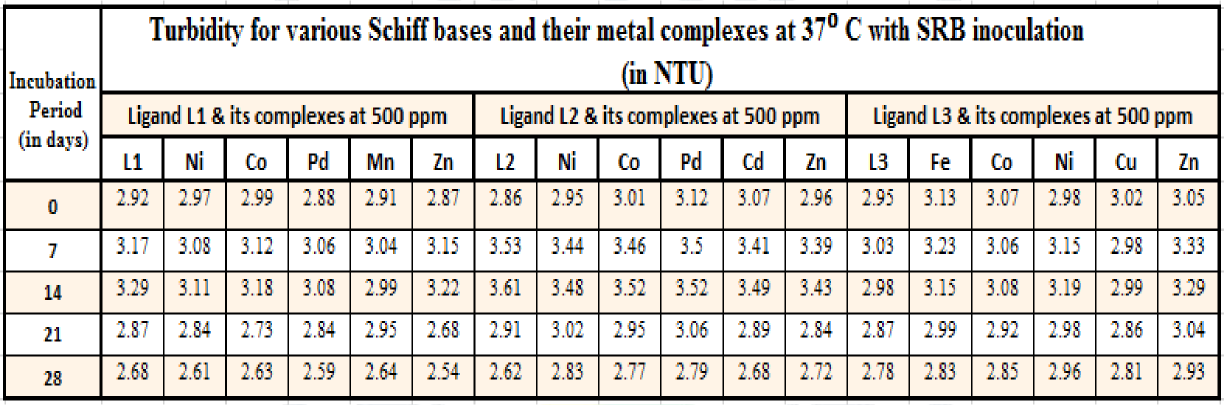


Fig. 8: Graph showing Turbidity measurements with incubation time at different temperatures.

The graph in fig. 8 was plotted against the data in table 1 and shows that the turbidity of the samples that were kept at 37^0 C was found to be high. This suggested that SRB growth rate was also high at 37^0 C corresponding to the elevated turbidity. The turbidity was also measured for ligands and their complexes at constant temperature of 37^0 C at various intervals of 0, 7, 14, 21 and 28 days and are summarized in the Table 2:

Table 2: Turbidity for Ligands and M-complexes at various intervals after SRB inoculation



Incubation Period (in days)	Turbidity for various Schiff bases and their metal complexes at 37^0 C with SRB inoculation (in NTU)																	
	Ligand L1 & its complexes at 500 ppm						Ligand L2 & its complexes at 500 ppm						Ligand L3 & its complexes at 500 ppm					
	L1	Ni	Co	Pd	Mn	Zn	L2	Ni	Co	Pd	Cd	Zn	L3	Fe	Co	Ni	Cu	Zn
0	2.92	2.97	2.99	2.88	2.91	2.87	2.86	2.95	3.01	3.12	3.07	2.96	2.95	3.13	3.07	2.98	3.02	3.05
7	3.17	3.08	3.12	3.06	3.04	3.15	3.53	3.44	3.46	3.5	3.41	3.39	3.03	3.23	3.06	3.15	2.98	3.33
14	3.29	3.11	3.18	3.08	2.99	3.22	3.61	3.48	3.52	3.52	3.49	3.43	2.98	3.15	3.08	3.19	2.99	3.29
21	2.87	2.84	2.73	2.84	2.95	2.68	2.91	3.02	2.95	3.06	2.89	2.84	2.87	2.99	2.92	2.98	2.86	3.04
28	2.68	2.61	2.63	2.59	2.64	2.54	2.62	2.83	2.77	2.79	2.68	2.72	2.78	2.83	2.85	2.96	2.81	2.93

The graphical representation of the data in Table 2 illustrates that the turbidity initially increases slightly up to 14 days and then decreases. This can be attributed towards the early growth of SRB as shown in Figure 8a and 8b. The increase in turbidity is not as high due to the presence of Schiff base or their metal complex which functions as antibacterial agent and slows down or inhibits the growth of the SRB's. However, the turbidity seems to be almost remains constant as shown in Figure 8c where ligand L3 and their complexes were used. The blue line corresponding to Cu-L3 founds to be a straight line that indicates that the presence of this compound even does not allow to developing any growth of SRB microbes.

The above experiment aims to determine the antibacterial activity of synthesized Schiff base ligand and their metal complexes against SRB strains. The optimum concentration of various ligands and their metal complexes required as bactericide was determined by anaerobic vial culture test and by using Hitachi UV-VIS U 1900 spectrometer in the range of 1100–190 nm.

Dry weight of SRB growth was logged by spectrophotometric measurements at 420 nm at the time of inoculation and subsequently at an interval of every 7 days for a period of 28 days. Sterile serum bottle was used as control for the spectrophotometric determination. The dry weight of bacteria (SRB) was calculated using the formula [21, 22].

$$\text{Dry weight of bacteria (g/l)} = -0.02 + 0.5 \times \text{Absorbance at 420 nm}$$

Existence of SRB were tested by anaerobic vials tests in various diluted serum bottles (10^{-1}, 10^{-2}, and 10^{-3}) at 0, 200, 500, 750 and 1000 ppm of synthesized ligands i.e. L1, L2, & L3 for optimized dose. The test data was recorded in Table 3. It was observed that all Schiff bases shows antibacterial activity. The effective dose rate of Schiff base ligand L1 & L2 was found to be above 750 ppm, while the Schiff base ligand L3 found to be very effective at dose rate above 500 ppm.

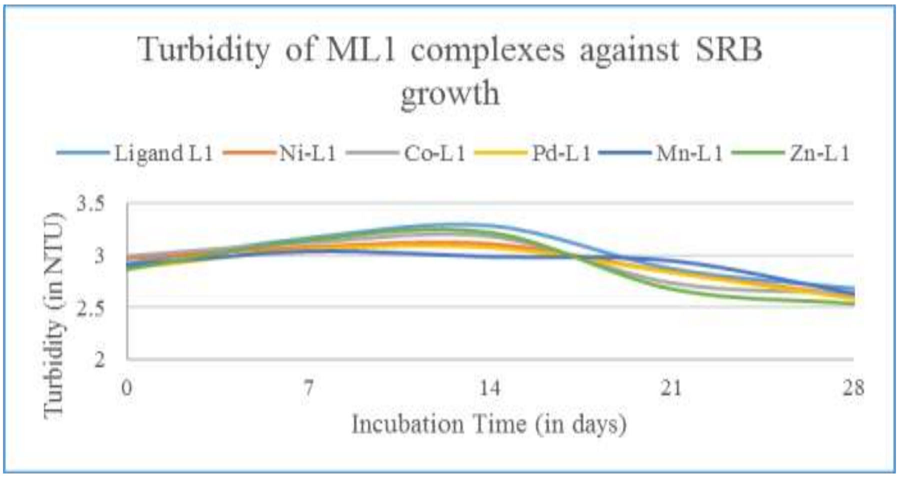


Fig. 8a: Graph showing Turbidity measurements at various intervals for Ligand L1 and their complexes against SRB growth

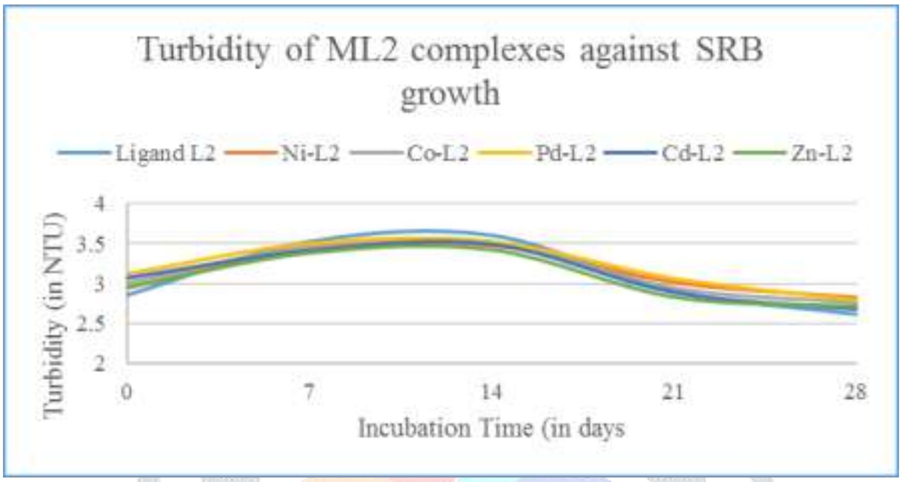


Fig. 8b: Graph showing Turbidity measurements at various intervals for Ligand L2 and their complexes against SRB growth

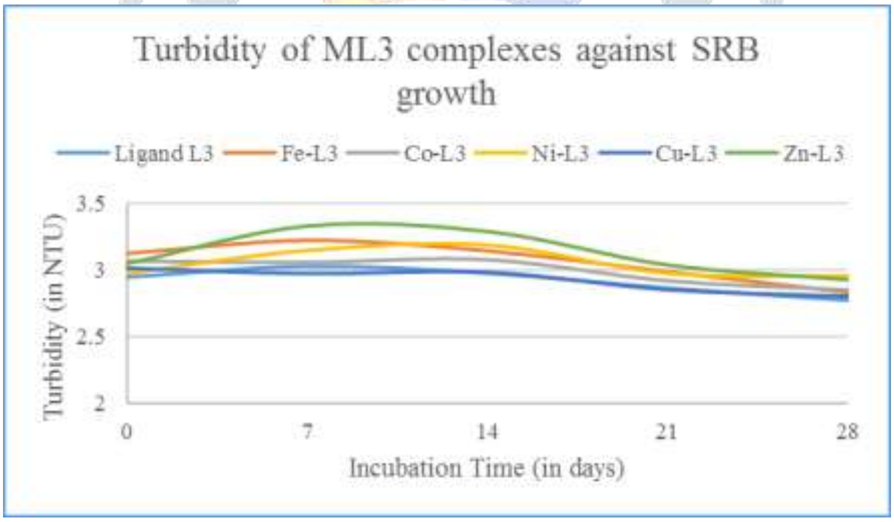


Fig. 8c: Graph showing Turbidity measurements at various intervals for Ligand L3 and their complexes against SRB growth

Table 3: SRB anaerobic vial test with Ligands L1, L2 & L3 at various concentrations at 37^0 C

Parameters (concentration in ppm)	SRB VIAL TEST WITH LIGAND L1 (*1000/ml)				SRB VIAL TEST WITH LIGAND L2 (*1000/ml)				SRB VIAL TEST WITH LIGAND L3 (*1000/ml)			
	10^{-1}	10^{-2}	10^{-3}	MPN	10^{-1}	10^{-2}	10^{-3}	MPN	10^{-1}	10^{-2}	10^{-3}	MPN
0	+VE	+VE	+VE	25	+VE	+VE	+VE	25	+VE	+VE	+VE	25
200	+VE	+VE	+VE	17	+VE	+VE	+VE	17	+VE	+VE	-VE	15
500	+VE	+VE	-VE	9	+VE	+VE	-VE	11	+VE	-VE	-VE	7

750	+VE	-VE	-VE	3	+VE	-VE	-VE	5	-VE	-VE	-VE	<1
1000	-VE	-VE	-VE	<1	-VE	-VE	-VE	<1	-VE	-VE	-VE	<1

Determination of absorbance of the liquid growth medium has been used earlier to observe the SRB growth. The SRB growth was measured at peak on 7th day and started diminishing after 14 days of inoculation (Table 4).

Table 4: Weight determination of SRB at 420 nm

Parameters (@500 ppm)	**Dry weight of SRB after inoculation g/ltr (in days)**				
	0	**7**	**14**	**21**	**28**
Control	0.0097	0.614	0.635	0.429	0.258
Ligand L3	0.0095	0.325	0.311	0.174	0.131
Fe-L3	0.0091	0.295	0.286	0.155	0.126
Ni-L3	0.0086	0.293	0.275	0.161	0.118
Co-L3	0.0069	0.285	0.263	0.175	0.119
Cu-L3	0.0058	0.192	0.097	0.084	0.064
Zn-L3	0.0089	0.312	0.293	0.167	0.128

This occurrence was may be due to the nutrient restrictive environments arrived after 14 days of inoculation and also attributed due to the inhibitory effect of the metabolites produced by bacterial in the growth media.

In the present study, it was observed that the dry weight of bacteria was reduce to 0.258 g/L during the growth period of 28 days after attaining a peak value of 0.635 g/L on 7th day in control experiment. The incorporation of the Schiff base L3 at 500 ppm reduces the growth of SRB's and hence dry weight was only limited to 0.325 g/ml on 7th day. However the SRB growth was further diminutive when metal ligand complex with Schiff base L3 was used. The Schiff base complex with copper (Cu-L3) was found to be most effective at 500 ppm that limits the peak growth of SRB to 0.192 g/ml.

Effect of pH:

Literature reveals that SRB's can easily grow in wide range of pH from 5.5-10 [23]. The same set of experiments were carried out by adjusting the pH from 5.5-10 to determine the effectiveness of the Schiff base and their complexes against pH variation. Figure 9 displays the monitoring of antibacterial effectiveness of Schiff base and their metal complexes against variation in pH. It was observed that all the compounds were found to possess good antibacterial character from pH 5.5-10 where optimum growth of SRB was recorded. However, there is decline in the dry weight of SRB at 7.5 pH which indicates that the Schiff base behaves as an excellent antibacterial agent at this pH. Similar curve showing decline in dry weight of SRB was obtained for Copper complex with L3 ligand. This decline in dry weight for this curve is attributed towards exceptional antibacterial character for Cu-L3 complex.

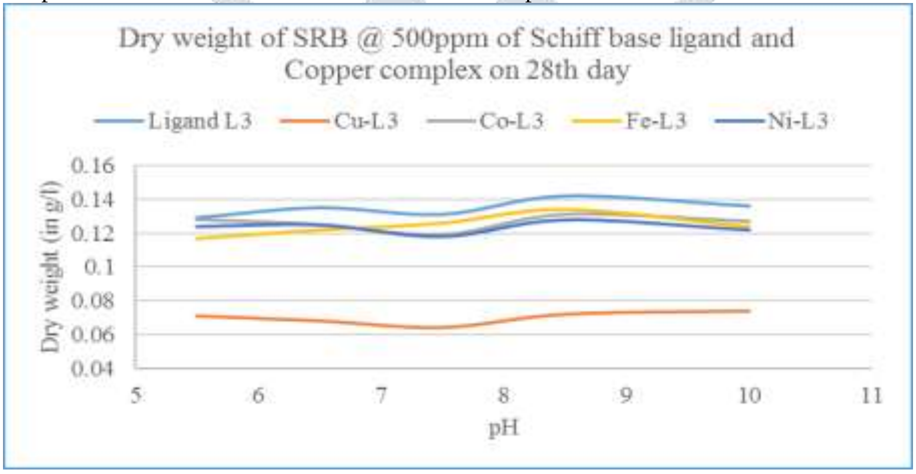


Fig.9: Monitoring of effectiveness of Schiff base and their complexes against pH

IV. CONCLUSION:

Schiff base ligands (L1, L2, and L3) and their metal complexes were synthesized and analyzed for their antibacterial behavior against strains of sulphate reducing bacteria. The maximum growth rate of SRB was observed at 37^0 C in control experiment using API-RP 38 as growth medium. All the synthesized ligands possesses antibacterial activity. The antibacterial activity of the ligands was found to be in order: L3>L1>L2 and the metal complexes of Schiff base ligands shows enhancement in their antibacterial activity. The copper complex with ligand L3 was found to be highly active and follows the order: Cu-L3>Co-L3>Ni-L3≈Fe-L3> Zn-L3. The variation can be attributed to the nature of metal ion and the complexity of the microbes (SRB). The advanced bioactivity of the metal complexes as compared to that of the Schiff base ligands was due to the reduction in the charge localization of the metal ion that leads to more fat solubility across cell membrane. The enhanced antibacterial activity of Cu complexes was due to the chelate formation with Schiff base ligand L3. This can reduce the polarity of Cu ion to a greater extent due to the overlapping of ligand orbitals along with partial sharing of positive charge on the metal ion. It was also concluded that all the compounds were exceptional antibacterial agent for a wide range of pH 5.5-10 where optimum growth of SRB was recorded.

V. ACKNOWLEDGEMENT

The authors would like to offer his gratitude towards Oil and Natural Gas Corporation Ltd for granting permission to carry out the research work and to support necessary requirements needed for this research work.

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