Synthesis and characterization of Mn and Co (II) complexes with tetradentate N_2O_2 donor Schiff Base: interaction with human serum albumin (HSA) with Mn(II) complex and antibacterial activity

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ABSTRACT

A tetra coordinated manganese(II) and cobalt (II) complexes formulated as [Mn(L] (**1**) and [Co(L)](**2**) H_2**L** = 2,2'-(4-nitro-1,2-phenylene)bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)bis(4-nitrophenol)were synthesized and characterized by elemental, physico-chemical and spectroscopic methods. The four coordination spheres of metal (II) ions have been satisfied by two imine nitrogen- N and two phenolate oxygen-O donors of organic moiety (**HL**).The interactions of manganese (II) complex towards human serum albumin (HSA) was examined with the help of absorption spectroscopic tools. Antibacterial activity of complexes (**1** and **2**) studied by agar disc diffusion method with some pathogenic bacteria namely *Escherichia coli, Vibrio cholerae, Streptococcus pneumonia and Bacillus cereus.*

Key words: Transition metal complexes; Schiff base; binding with HSA; antibacterial activity.

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1. Introduction

Tetradentate Schiff bases with a N_2O_2 donor atom set are well known to coordinate with various metal ions, and this has attracted the interest of many authors [1, 2]. Complexes of Schiff base ligand have been studied for their dioxygen untaken [3] and oxidative catalysis [4]. Schiff bases and their metal complexes have been prepared because of their interesting and important properties, e.g., ability to bind toxic and heavy metal atoms, undergo tautomerism [4], exhibit catalytic reduction and photochromism. The basic strategy to design such materials is to organize paramagnetic centres into poly nuclear aggregates or polymeric networks by use of bridging ligands that can efficiently propagate magnetic super exchange. Several studies have also shown that diazo compounds exhibit properties similar to those of Schiff bases [5]. Napthylamine-derived azo-linked Schiff bases and their metal complexes have additional applications, especially in the dye industry. The role of the metal– Schiff base complexes in such applications is related to molecular structure. Thus, it is quite important to have a good understanding of the structure of such metal complexes [6].

Among bio macromolecules, the serum albuminsare the major soluble protein constituent of the circulatory system; they have many physiologicalfunctions. Human serum albumin is the most important soluble protein in the circulatory system and secreted from liver cells [7]. It is composed of three structurally homologous domains (I, II and III), each subdomains containing A and B. Each domain contains 10 helices; 1-6 helices form the respective subdomains A, and helices 7-10 comprise subdomains B. Aromatic and heterocyclic ligands are found to bind within two hydrophobic pockets in subdomains IIA and IIIA, described as site I and site II [8]. It plays an important role in plasma as well as in interstitial fluids and has many physiological functions, such as maintaining the osmotic pressure, pH of blood and transportation of various number of endogenous and exogenous compounds including fatty acids, amino acids, steroids, drugs, and pharmaceuticals[9].

In this respect, present study aimed to investigate the reaction of tetra dentate Schiff bases derived from the condensation of salicylaldehyde with 4-nitro 1,2-diamino benzene. The complexes are prepared by the

equimolar ratio of ligand with metal (II) acetate. The prepared ligands and complexes were characterized by elemental analysis, molar conductivity measurements, and infrared and electronic spectral data. The interactions of manganese (II) complex towards human serum albumin (HSA) were examined with absorption spectroscopic tools.

2. Experimental

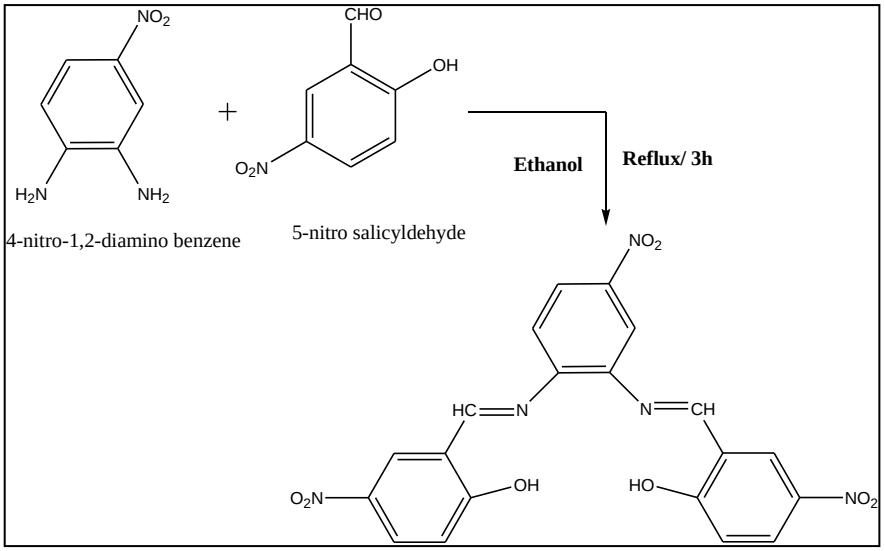
2.1. Materials and Physical measurements

All chemicals and reagents were obtained from commercial sources and used as received, unless otherwise stated. Solvents were distilled from an appropriate drying agent. The organic moieties were synthesized following the procedure. The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. Electronic absorption spectra were recorded on a SHIMADZU UV-1800 spectrophotometer. IR spectra (KBr discs, 4000–400 cm^{-1}) were recorded using a Perkin-Elmer FTIR model RX1 spectrometer. Molar conductance's (Λ_M) were measured in a systronics conductivity meter 304 model using ~10^{-3} $mol.L^{-1}$ solutions in appropriate organic solvents. The stock solutions of protein (1.00×10^{-4}mol L^{-1}) was prepared by dissolving the solid HSA in 0.05 M phosphate buffer at pH 7.4 and stored at 0−4 °C in the dark.

2.2. Preparation of the ligand (HL)

The ligand **H_2L** was Synthesize by adding 0.688 g (4.0 mmol) of 5-nitro Salicylaldehyde and then 0.206 g (2.0 mmol) of 4-nitro 1,2-diamino benzene with 10 mL of ethanol in a round bottom flask with magnetic stirring given in **Scheme 1**. Stir the solution for 3 hour and then reflux up to 2 hour and kept overnight to get the precipitate of the orange solid ligand. The precipitate was filtered by using vacuum pump and washed several times using ethanol to remove any unreacted materials, finally crude product was collected by recrystallization in ethanol and dried the solid product as much as possible, Yields >80%.

$C_{20}H_{13}N_5O_8$: Anal. Found: C, 65.46; H, 2.12; N, 11.45; Calc.: C, 65.24; H, 2.04; N, 11.26, m.p. 236 ± 1 ^oC; IR (KBr, cm^{-1}):$\nu_{O-H,}$ 3442, ν_{NO2}, 1342,$\nu_{CH=N}$, 1618.



Scheme 1: Synthetic procedure for the preparation of Ligand (**H_2L**)

2.3. Preparation of [Mn(L)] (1), [[Co(L)] (2) and [Ni(L)] (3)

To prepare these complexes (**1**and **2**) a common procedure was followed as described below, using manganese acetate for complex (**1**) and cobalt acetate for complex (**2**) and the organic ligand (**H_2L**) in equimolar ratio (1:1). A methanolic solution of **H_2L** was mixed with 1.0 mmol of metal acetate in methanolic solution with stirring condition, and the mixture was refluxed for 4 h.The product was collected by filtration and washing with cold methanol and water; and dried in vacuo.

Complex **1:** $C_{20}H_{11}N_5O_6Mn$: Yield 80-85%; Anal.Found; C,60.24; H, 1.65; N, 10.54; Mn, 8.89; Calc: C, 60.14; H, 1,56; N, 10.48; Mn, 8.40. IR (cm^{-1}): $\nu_{CH=N}$, 1614; ν_{NO2}, 1340. m.p. 218 ± 1 ^oC. Conductivity (Λo, ohm^{-1} cm^2 mol^{-1}) in DMF: 74.

Complex **2:** $C_{20}H_{11}N_5O_6Co$: Yield 75-80%; Anal.Found; C, 59.88; H, 1.64; N, 10.47; Cu, 8.82; Calc: C, 59.74; H, 1.58; N, 10.36; Cu, 8.78. IR

(cm^{-1}): $\nu_{CH=N}$, 1612; ν_{NO2}, 1340. m.p. 224 ± 1 °C. Conductivity (Λo, ohm^{-1} cm^2 mol^{-1}) in DMF: 78.

2.4. Protein (HSA) binding experiments

The quantitative analyses of the interaction between Mn(II) complex and human cerum albumin were performed by absorption spectroscopic titration[10]. A 3.0 mL portion of aqueous solution of protein was titrated by gradual addition of the appropriate concentration of Mn (II) complex solution (to give a final concentration of 3.8×10^{-6}mol L^{-1}). For every addition, the solution mixture was shaken and allowed to stand for 10 minute, and then the absorption spectra were measured.

2.5. Antimicrobial Screening

The antibacterial activities of the ligand (H_2L) and its metal (II) complexes have been studied by agar plate diffusion method [11, 12]. The antibacterial activities were done at 100 μg/mL concentrations of different compounds in DMF solvent by using four pathogenic gram negative bacteria (*Escherichia coli, Vibrio cholerae, Streptococcus pneumonia)* and one gram positive pathogenic bacteria *(Bacillus cereus).* The solution of ligand and its metal (II) complexes were added to the agar plates. The DMF solvent was used as a negative control. Incubation of the plates was done at 37°C for 24 hours, inhibition of the organisms was measured and used to calculate mean of inhibition zones in millimetres.

3. Results and Discussion

3.1. Synthesis and characterization

The organic ligand **H_2L** was synthesized by the reaction of the respective of 5-nitro Salicylaldehyde and then 4-nitro 1,2-diamino benzene (2:1) in presence of Ethanol.

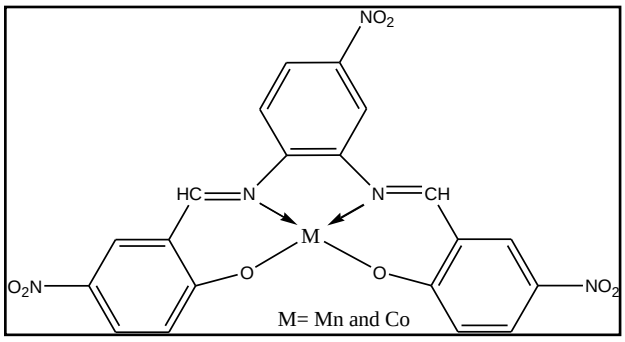


Fig 1. Probable structure of ML complexes

The complexes were obtained in good yield from the reaction of metal acetate with equimolar amount of respective organic moiety **H_2L** in the methanol medium. In these complexes the organic molecule **L** act as tetradentate ligand through N_2O_2- donor centres.The complexes conductivity measurement in dimethylformamide for **1** and **2** suggest that complexes exist in solution as non-electrilytes.These complexes are air-stable, non-hygroscopic, coloured solids, partly soluble in ethanol and methanol, and soluble in DMSO and DMF and are monomeric in nature. The elemental analysis data of the Schiff base and their complexes are consistent with the calculated results from the empirical formula of each compound. The probable structure of complexes is given in **Fig. 1**.

3.2. Infrared spectra

Infrared spectral data of the Schiff base (**Fig. 2**) shows several bands at 3442, 1342, and 1618 cm^{-1} due to phenolic O-H group, nitro group and imine CH=N stretching vibrations

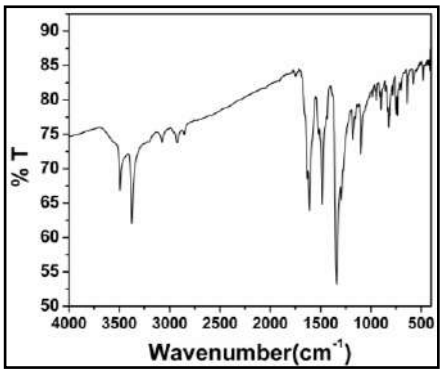


Fig 2. IR spectrum of ligand H_2L

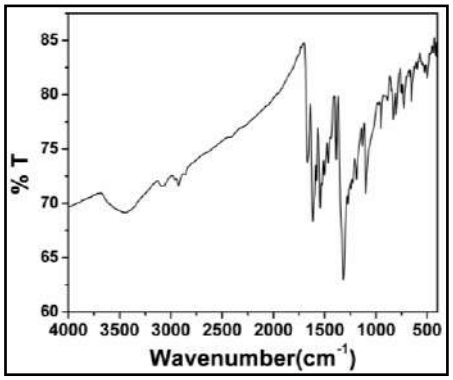


Fig 3. IR spectrum of MnL complex

in the solid state. These bands are shifted to lower frequency on complexation with Mn(II) and Co(II) ions. New vibrations at 412 and 514 cm^{-1} which are not present in the free Schiff base are attributed to the existence of ν (M-O) and ν (M-N)**.** The appearance of these vibrations confirmed the involvement of nitrogen and oxygen atoms (**Fig. 3**) in chelation with metal ions[13]**.** All the IR data suggest that the metal ions are bonded to the Schiff base through the phenolic oxygen and the imino-nitrogen.

3.3. Electronic absorption Spectral Study

The electronic spectra of the ligand and its complexes were recorded in DMF at room temperature. The spectra of the Schiff base H_2L exhibit three main peaks: at 296.0, 342.0 and 426.0 nm. The first and second peaks were attributed to benzene $\pi\rightarrow\pi^*$ and imino $\pi\rightarrow\pi^*$ transitions, again an absorption band at 426.0 nm due to intra ligand charge transfer transition. All the spectra of complexes shows lower bands than 400 nm are due to intramolecular $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions for the aromatic ring. The Mn-complex shows only high energy bands at 290 and 332 nm, which are due to intraligand transitions and lower energy band at 420 nm attributed to the L→M charge transfer transition.

In cobalt (II) complex, three spin allowed transitions are expected from the energy level diagram for d^7 ion due to $^4A_2\rightarrow{}^4T_1$ (P), $^4A_2\rightarrow{}^4T_1$(F), $^4A_2\rightarrow{}^4T_2$ transitions, which are observed at low to high wavelengths respectively. For cobalt complex bands at 424 nm and 524 nm, which may be assigned to $^4A_2\rightarrow{}^4T_1$(P) and $^4A_2\rightarrow{}^4T_1$(F) transitionrespectively. Again the intensity of the peak at around 612 nm observed due to the $^4A_2\rightarrow{}^4T_2$ transition [14].

3.4. Absorption characteristics of HSA –Mn(II) complex

The absorption spectra of HSA in the absence and presence of Mn(II) complex was studied at different concentrations. From this study we observed that absorption of increases regularly upon increasing the concentration of the complex. The absorption spectral data of manganese complex with HSA shown in **Fig. 4**. It is may be due to the adsorption of HSA on the surface of the complex [15]. From these data the apparent association

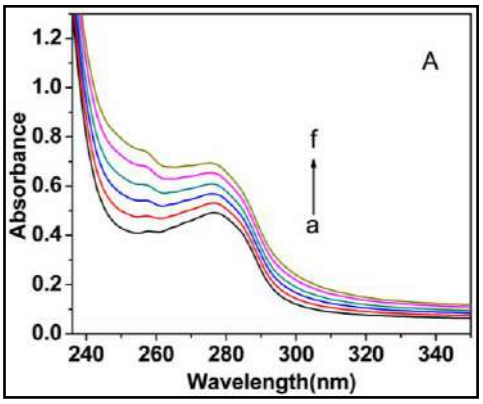


Fig 4.The Electronic spectral titration of complex 1with HSA in phosphate buffer. Arrow indicates increase the concentration of HSA.

constant (K_{app}) determined of the complex with HSA has been determined using the Benesi-Hildebrand equation[16].

$1/(A_{obs} – A_0) = 1/(A_c – A_0) + 1/ K_{app}(A_c – A_0)[comp]$

Where, A_{obs} is the observed absorbance of the solution containing different concentrations of the complex at 280 nm, A_0 and Ac are the absorbances of HSA and the complex at 280 nm, respectively, with a concentration of complex and K_{app} represents the apparent association constant. The enhancement of absorbance at 280 nm was due to adsorption of the surface complex, based on the linear relationship between $1/(A_{obs}- A_0)$ *vs* reciprocal concentration of the complex with a slope equal to $1/K_{app}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$. The value of the apparent association constant (K_{app}) of HSA is 4.28 $\times 10^{-4}$ (R = 0.99897) determined from this plot (**Fig.5**) and represent a good linear relationship [17].

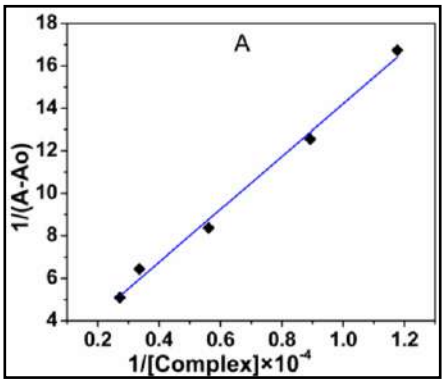


Fig5. Plot of 1/(A-Ao) vs 1/[Complex 1] resulting from the electronic spectral titration with HSAin phosphate buffer

3.5. Antibacterial activity

Compared the biological activity of the synthesized ligand and its compounds with standard antibiotic Levofloxacingiven in **Fig.6**. From the antibacterial studies it is inferred that, all complexes have higher activity than ligand. The increased activity of the metal chelates can be explained by

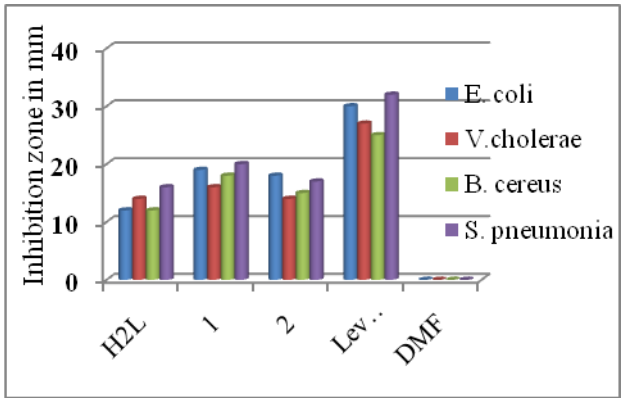


Fig 6.Comparison antibacterial studies of the ligand (H_2L) and its complex 1 and 2 with standard antibiotics.

overtone concept and the Tweedy chelation theory. The variation in the activity of different complexes against some different organisms depend either on the impermeability of the cells of the microbes or difference in ribosome of microbial cells [18]. In a complex, increases the delocalization of π-electrons over the whole chelate ring and increase the lipophilic character of the metal complexes. This increased lipophilicity also helps the penetration of the bacterial cell membranesand blocks the metal binding sites in enzymes of microorganisms and restricts further growth of the microorganisms [19].

4. Conclusion

The synthesis and characterization of two mononuclear Mn and Co (II) complexes with a N_2O_2- donor set have been performed. The ligand **H_2L** behaves as a N_2O_2-donors. On the basis of the physical and spectral data of the Schiff base and the complexes discussed above, one can assume that the metal ions are bonded to the Schiff base via the phenolic oxygen and the imino nitrogen and all the complexes are distorted Square planar geometry.The interactions of manganese (II) complexes towards HSA were examined with the absorption spectroscopic tools. The absorption spectral titration indicated that Mn(II) ion strongly bind with HSA protein.

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