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Research Article

SYNTHESIS, CHARACTERIZATION AND DNA INTERACTION OF LEAD (II) COMPLEX OF SCHIFF BASE DERIVED FROM 2-(METHYLTHIO)ANILINE

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ABSTRACT

A new Pb(II) complex, [Pb(PMTPM)Cl_2], where L = 2-pyridyl-N-(2'methylthiophenyl)methyleneimine,(pmtpm) has been synthesized and characterized by elemental analysis, UV, IR, ^{1}H NMR and mass spectroscopy. The interaction of this complex with calf-thymus DNA (CT-DNA) has been investigated by electronic absorption, fluorescence, and viscosity measurements. The experimental results suggest that the complex binds to DNA in an intercalation mode.

Keywords: Lead (II) complex, Synthesis, Characterisation, DNA binding study

1. INTRODUCTION

Schiff base is a compound that contains azomethine group (>C=N-) connected to an aryl or alkyl group but not hydrogen. Recently, a large number of metal Schiff base complexes have been synthesized and characterized [1-4]. Metal Schiff-base complexes have sustained to play the role of one of the most important stereo chemical models in main group and transition metal coordination chemistry due to their preparative accessibility, diversity, and structural variability. Now a days there has been a growing interest in metal complexes bearing Schiff's base ligands due to their diverse biological activities such as antifungal, analgesic, anti-inflammatory, antibacterial, antioxidant, antitumor, local anesthetic, and antimicrobial activities [5-9]. On the other hand, radicals retard the progress of many chronic diseases such as vascular diseases, oxidative stress responsible for DNA, protein and membrane damage, and some forms of cancer [10, 11]. Pb(II) ion, having a large metal centre, can adopt many different ligands and form compounds with flexible coordination numbers as well as novel structures [12-16].

Considering the above facts, I describe here the synthesis of one mononuclear complex of lead(II) [Pb(PMTPM)Cl_2] containing Schiff base ligand (L) obtained in situ from pyridine-2-carboxaldehyde and 2-methylthioaniline (Scheme 1). The complex has been characterized by elemental analysis, conductivity, IR, NMR and electronic spectra. The binding affinity of this Pb(II) complex with calf thymus DNA has also been studied by UV-Vis absorption, fluorescence and carrying out viscosity measurements.

2. EXPERIMENTAL

2.1. Materials and physical measurement

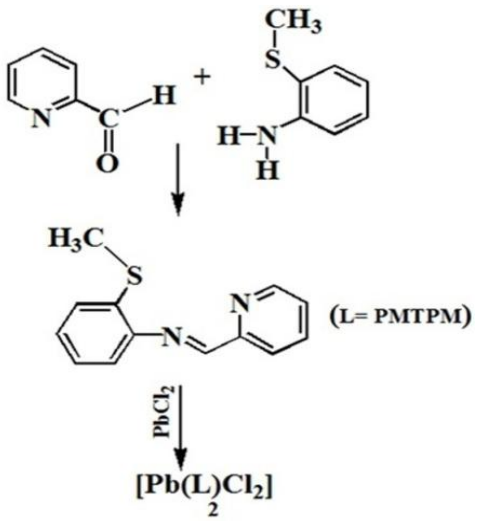
All experiments were carried out using standard apparatus. The chemicals such as Pyridine-2-carboxaldehyde, 2-(methylthio)aniline were purchased from Aldrich and used without further purification. The other chemicals used were of analytical grade and obtained from commercial sources. Solvents were distilled off from an appropriate drying agent before use. Ultrapure water (resistivity=18.2 MΩcm^{-1}, Sartorius stedim biotech, arium®61316) was used for the preparation of all the solution.

The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. IR spectra of complexes were recorded on a Perkin Elmer FTIR model RX1 spectrometer (KBr disc, 4000-400 cm^{-1}). NMR spectrometer (JNM ECX-500, Jeol India) and mass spectrometer (Impact HD, M/S Bruker Daltonik GmbH) were used to obtain NMR and MS of metal complexes. Thermal behaviours were examined with Okay 1200 TGA–DSC at the heating rate of 10°C/min in nitrogen atmosphere.

2.2. Synthesis of 2-pyridyl-N-(2'-methylthio -phenyl)methyleneimine(PMTPM)

The ligand PMTPM has been prepared following a reported synthetic procedure [17, 18]. To a methanol

solution of pyridine-2-carboxaldehyde (10 mmol, 1.076 g), 2-(methylthioaniline) (10 mmol, 1.392 g) in methanol was added at stirring condition. The reaction mixture was then arefluxed under nitrogen for 6 h. After cooling the reaction mixture to room temperature, the solvent was removed completely using a rotary evaporator to yield thick yellow oil. The oil was then dissolved in$CHCl_3$ and successively washed with distilled water, brine solution, and finally with distilled water. The organic layer separated out was then dried over Na_2SO_4 for 1 h and filtered, and the solvent was completely removed. The yellow oil kept at 4°C overnight to get a yellow solid that was recrystallized from $CHCl_3$/hexane to get needle shaped crystals (yield 80-85 %). **$C_{13}H_{14}N_2S$**: Anal. Found: C, 68.31; H, 5.37; N, 12.16 Calc.: C, 68.39; H, 5.30; N, 12.27; % IR (KBr, ν_{max}/cm^{-1}, selected peaks): 1622vs (C=N), 693 m (C–S).^{1}**H NMR (300 MHz, $CDCl_3$):** δ 8.70 (1H, d, pyridine proton adjacentto N), 8.57 (1H, s, HC=N), 8.33 (1H, d, pyridine proton), 7.81 (1H, t,pyridine proton), 7.37 (1H, t, pyridine proton), 7.28-7.16 (3H, m,phenyl ring proton), 7.08 (1H, d, phenyl ring proton), 2.47 (3H, t,methyl group of S-Me).



Scheme 1: Reaction scheme

2.3.Synthesis of [Pb(PMTPM)Cl_2] Schiff based complex

Lead chloride, $PbCl_2$ (100mg, 0.359mmol) was dissolved in 10 ml of hot DI water. To this solution was added an ethanol solution (5 mL) of 2-pyridyl-N-(2'-methylthiophenyl)methyleneimine(PMTPM) (82.1mg, 0.3596 mmol). Upon addition of the ligand, a bright yellow precipitate was formed immediately. The reaction mixture was stirred further for 6 hours to complete precipitation of the yellow solid which was then filtered, washed with ether and dried.

Complex: [Pb(L)Cl_2]: Yield: 71%; $C_{13}H_{12}PbCl_2N_2S$: Anal. Found: C, 29.87; H, 2.23; N, 5.65 Calc.: C, 30.83.18; H, 2.39; N, 5.53. IR (KBr,ν_{max}/cm^{-1}, selected peaks): 1585vs (C=N), 695 m (C–S).^{1}**H NMR** (500 MHz, $CDCl_3$): δ 8.71 (1H, d, pyridine ring proton adjacent to N), 8.57(1H, s, HC=N), 8.34(1H, d, pyridine ring proton),7.83(1H, t, pyridine ring proton), 7.39 -7.37(1H, m, pyridine ring proton), 7.3-7.26(1H, m, (1H,d, phenyl ring proton merged with $CHCl_3$ peak), 7.24 -7.21(1H, m, phenyl ring proton), 7.2-7.16(1H, m, phenyl ring proton), 7.08(1H, d, phenyl ring proton), 2.79(3H, s, -SMe)(Fig. 2). ESI-MS: $[M + H]^+$, m/z, 507.23; where M = molecular weight of complex and L= molecular weight of ligand]; Molar conductance (DMF, 298 K) = 12 ohm^{-1} cm^2 mol^{-1}

2.4. DNA binding experiments

In all type CT-DNA experiments, Tris-HCl buffer solution (pH 7.2) used which was prepared by using deionized and sonicated HPLC grade water (Merck). The CT-DNA used in the experiments was checked to be sufficiently free from protein as the ratio of UV absorbance of DNA solution in tris-HCl at 260 and 280 nm (A_{260}/A_{280}) was around 1.9 [19]. The concentration of DNA was estimated by using the extinction coefficient (6600 $M^{-1}cm^{-1}$) at 261 nm [20] and stock solution of DNA was always stored at 4°C. The interaction with CT-DNA of Pb(II) complex was studied by dissolving each complex in 2 ml of DMSO and diluting with tris-HCl buffer to get the required concentration for all the experiments. Absorption spectral titration experiment was performed by maintaining the constant complex concentration and varying the CT-DNA concentration. To eliminate the absorbance of DNA itself, equal amount of CT-DNA was added to the reference solution.

In the ethidium bromide (EB) fluorescence displacement experiment, 5.0 µL of the EB Tris-HCl solution (1.0 mmol L^{-1}) was added to 1.0 mL of DNA solution (at saturated binding levels), stored in the dark for 2.0 h. Then the solution of the complex was titrated into the DNA/EB mixture and diluted with tris-HCl buffer to 5.0 ml to get the appropriate complex/CT-DNA mole ratio in solution. Before measurements, the mixture was shaken up and incubated at room temperature for 30 min. The fluorescence spectra of EB bound to DNA were obtained in the fluorimeter with λ_{ex} of 522 nm. To adjudge the binding mode (groove/intercalative) of

Pb(II) complex with DNA, the viscosity measurement method was used by employing the Ostwald's viscometer. Titrations were carried out in the viscometer by adding complex (0.5-3.5 μM) to the CT-DNA solution (5.0 μM). The viscosity values of the solutions were calculated from the observed flow time of CT-DNA-containing solution corrected from the flow time of buffer alone (t_0), $\eta = t\text{-}t_0$. The obtained data were used to plot the $(\eta / \eta_0)^{1/3}$ versus the ratio of the concentration of complex and CT-DNA, where η is the viscosity of the CT-DNA solution in presence of complex and η_0 is the viscosity of the CT-DNA solution only.

3. RESULT AND DISCUSSION

3.1. Spectral properties

The infrared spectra of the metal complex provide information about their formation in respect to the presence and coordination of the ligand. In FT-IR spectra of Pb(II) complex (Fig. 1), the position of ligand band due to $\nu_{C=N}$ at 1622 cm^{-1} shifted towards lower side i.e. 1585 cm^{-1} compared to the uncoordinated ligand, indicating its involvement in coordination of imine nitrogen [17, 21]. The $\nu_{C\text{-}S}$ stretching frequency observed at 694 cm^{-1}, in Pb(II) complex similar to peak of free ligand indicating no coordination of S atom. Furthermore presence of intense, sharp multiple peaks assignable to ν(pyridyl) fall in the range 1400 to 1600 cm^{-1} supports the existence of the nitrogeneous heterocyclic ring.

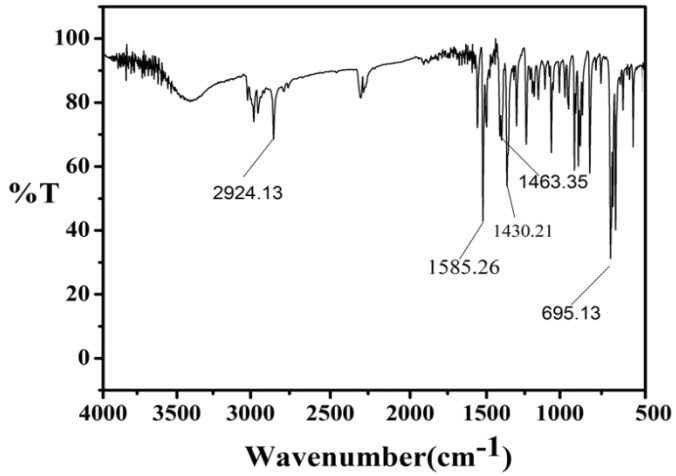


Fig. 1: FTIR spectrum of [Pb(PMTPM)Cl_2] complex

On the other hand The ESI positive mass spectra of the complex (Fig. 3)was recorded in MeCN solution that display the 100% molecular ion peak at m/z, 507.23 that corresponds to [{(pmtpm)$PbCl_2$}]H^+.

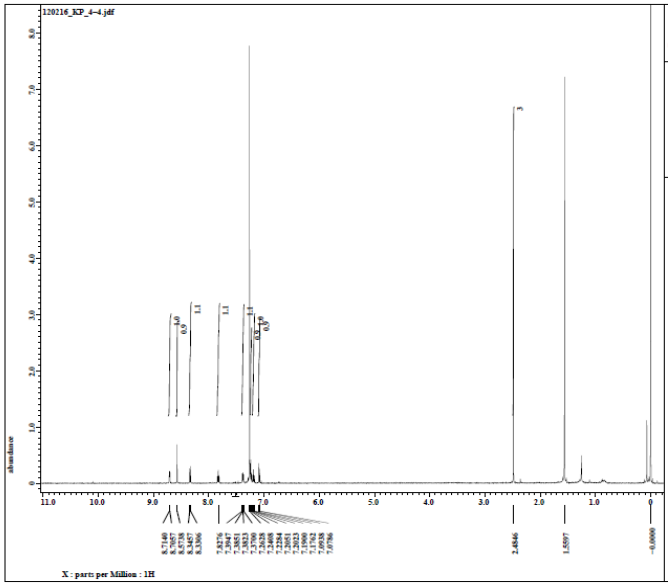


Fig. 2: ^{1}H NMR spectrum of [Pb(PMTPM)Cl_2] complex in Chloroform-D

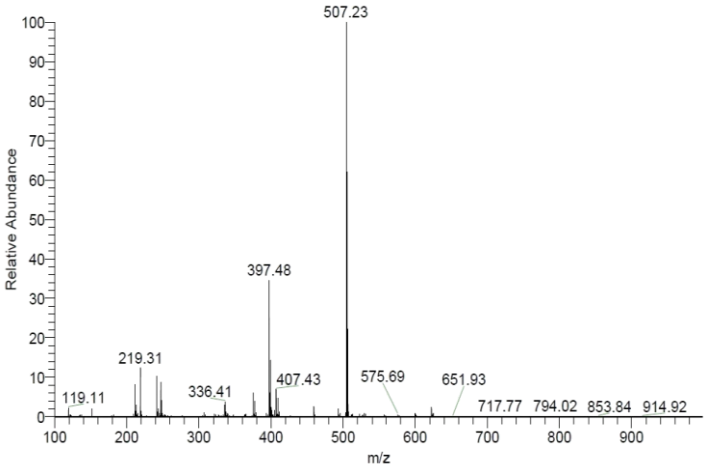


Fig. 3: ESI-MS spectrum of [Pb(PMTPM)Cl2] complex

The molar conductance value of the presently prepared complex is very small. The low conductance values of the prepared complex in DMF solutions indicate their non-electrolytic nature [22]. Being a d^{10} system, the present Pb(II) complexshow no d-d transition in the visible region, and are coloured only through their intense charge transfer absorptions tailing in from the ultraviolet. Besides, the other bands are due to intraligand transitions. The peaks observed below 373 & 269 nm (Fig. 4) are tentatively assumed as due to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions. Considering to all results, the following structural formula of these chelate complex may be proposed in Fig.5.

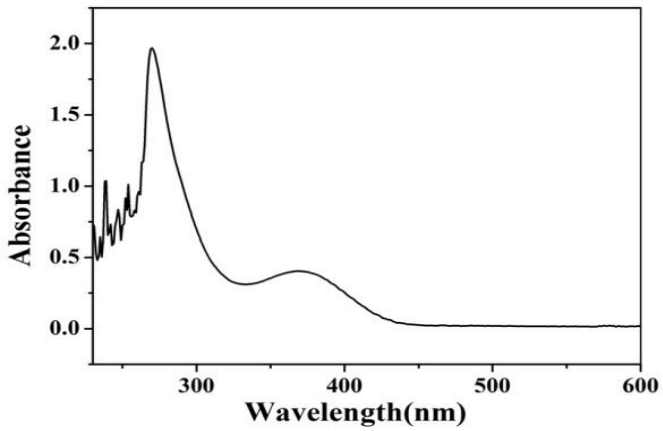


Fig. 4: UV-Vis spectrum of [Pb(PMTPM)Cl2] complex

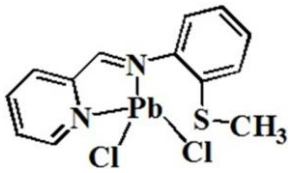


Fig. 5: Probable structure of [Pb(PMTPM)Cl_2] complex

3.2. DNA-binding studies

The mode of interaction of the Pb(II) complex with calf thymus DNA (CT-DNA) is in the same fashion and the investigation by using absorption and emission spectra. Electronic absorption spectroscopy is an effective method to examine the binding modes of metal complexes with DNA. In general, binding of the lead (II) complex to the CT-DNA helix is examined by an increase of the absorption band (c.a. 264 nm) of lead (II) complex. This increasing absorbance indicates that there is the involvement of strong interactions between complex and the base pairs of DNA [23]. The absorption spectra of the Pb (II) complex in the absence and presence of CT-DNA are given in Fig. 6. The extent of the hyper-chromism in the charge transfer band is generally consistent with the strength of interaction [24-27].

In order to further illustrate the binding strength of the Pb(II) complexwith CT-DNA, the intrinsic binding constant K_b was determined from the spectral titration data using the following equation[28]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/[K_b(\varepsilon_b - \varepsilon_f)]$$

where [DNA] is the concentration of DNA, ε_f , ε_a and ε_b correspond to the extinction coefficient, respectively, for the free Pb(II) complex, for each addition of DNA to the lead(II) complex and for the Pb(II) complex in the fully bound form. A plot of [DNA]/(ε_a-ε_f) versus [DNA], gives K_b, the intrinsic binding constant as the ratio of slope to the intercept. From the [DNA]/(ε_a-ε_f) versus[DNA] plot (Fig. 7), the binding constant K_b for the lead(II) complex was estimated to be 1.07 x 10^5 M^{-1} for (R = 97022, n = 5 points) [29].

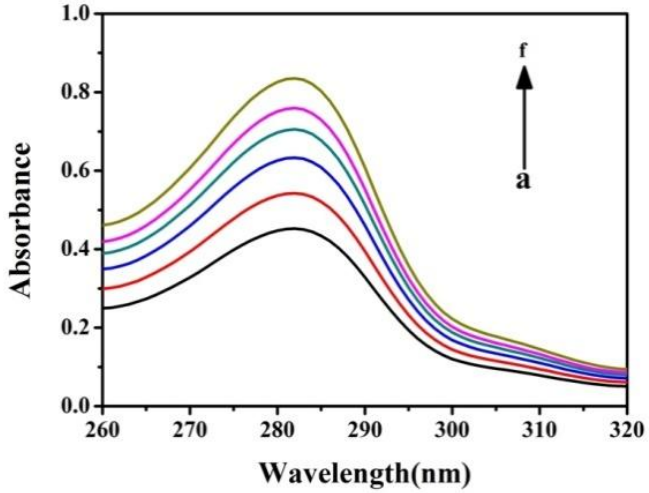


Fig. 6: Electronic spectral titration of Pb(II)complex with CT-DNA at 270 nm in tris-HCl buffer. [Complex]=2.51 ×10^{-5} mol L^{-1}; [DNA]: (a) 0.0, (b) 1.31×10^{-6}, (c) 2.48×10^{-6}, (d) 3.55×10^{-6}, (e) 4.40×10^{-6} and (f) 5.60×10^{-6} mol L^{-1}. The increase of DNA concentration is indicated by an arrow

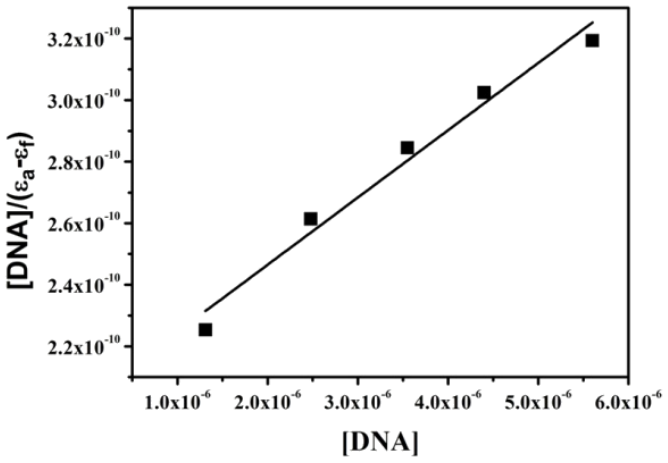


Fig. 7: Plot of [DNA]/(ε_a-ε_f) *vs* .[DNA] for the absorption titration of CT-DNA with Pb(II) complex in tris-HCl buffer (R = 0.97022 for five points)

Fluorescence intensity of EB bound to DNA at 522 nm shows a decreasing trend with the increasing concentration of the lead(II)complex (Fig. 8). Pink colored spectrum indicates the maximum binding with

lead(II) complex replacing EB. The quenching of EB bound to DNA by the Pb(II) complex is in agreement with the linear Stern–Volmer equation[30] :

$$I_0/I = 1 + K_{sv}[Q]$$

where I_0 and I represent the fluorescence intensities in the absence and presence of quencher, respectively. K_{sv} is a linear Stern-Volmer quenching constant, Q is the concentration of quencher. In the quenching plot (Fig. 9) of I_0/I versus [complex], K_{sv} value is given by the ratio of the slope to intercept. The Ksv value for the lead(II) complex is 5.1 $\times 10^4$ (R = 0.97587for five points), suggesting a strong affinity of the lead(II) complex to CT-DNA.

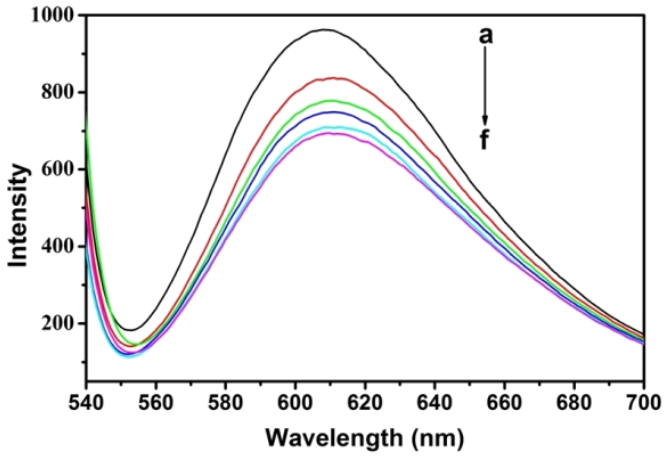


Fig. 8: Emission spectra of the CT-DNA–EB system in tris–HCl buffer during the titration of Pb(II)complex (λ_{ex} = 522 nm). [EB] =1.03×10^{-6} mol L^{-1}, [DNA] = 1.23×10^{-6}; [Complex]: (a) 0.0, (b) 1.22×10^{-6}, (c)2.44×10^{-6}, (d) 3.66 ×10^{-6}, (e) 4.89×10^{-6} (f) 5.73X10^{-6} mol L^{-1} The arrow shows the intensity change by increasing the complex concentration.

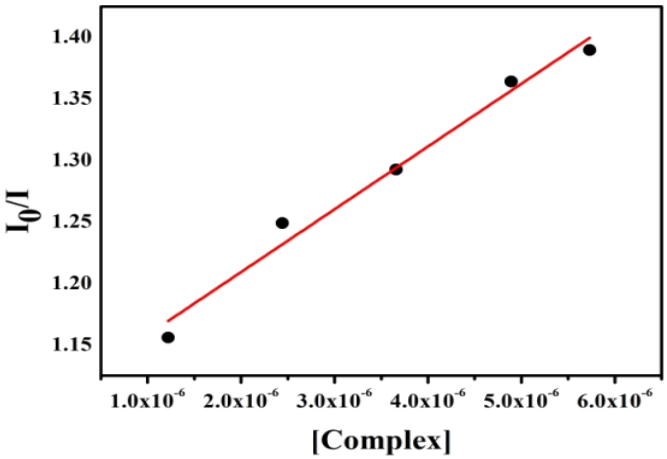


Fig. 9: Plot of I_0/I *vs.* [complex] for the titration of Pb(II) complex with CT-DNA-EB system in tris-HCl buffer.

Furthermore, the interactions between the complexes and DNA were investigated by viscosity measurements. The viscosity measurement is regarded as the most effective means to study intercalative binding mode of DNA in solution [30, 31]. A classical intercalative mode causes significant increase in viscosity of the DNA solution due to an increase in separation of base pairs at the intercalation sites and hence an increase in overall DNA length. In contrast, a partial, non-classical intercalation or groove binding of the complex could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity [32, 33]. As seen in (Fig. 9), the viscosity of complex increases with increase in the ratio of complexes to CT- DNA. These results support that the Pb(II) complex binds to CT-DNA by intercalation. This result also resembles the binding mode of EB to CT DNA.

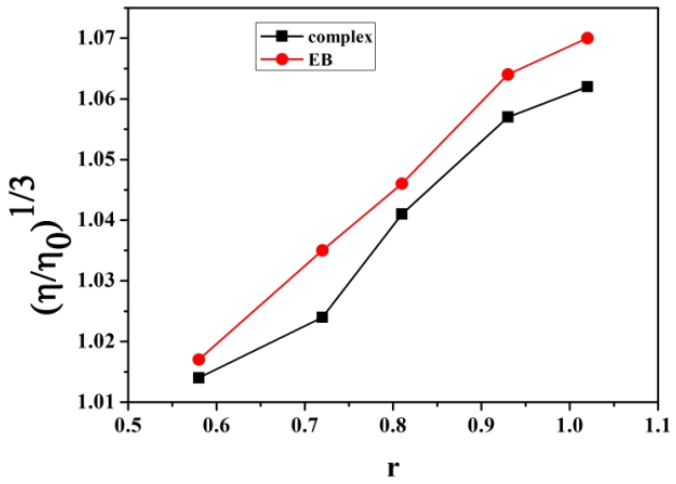


Fig. 10: Change in the relative viscosity $(\eta/\eta_o)^{1/3}$ of CT DNA as a function of r, the molar ratio of the compound to the DNA base pairs. The effect of increasing amounts of the concentration of the EB (●)and complex (■) on the relative viscosities of CT–DNA.

4. CONCLUSION

In this work, I have reported the synthesis and characterization of a lead(II) complex bearing a Schiff base derived from pyridine-2-carboxaldehyde and 2-methylthioaniline. The DNA binding properties of the complex were examined by UV-vis absorption spectrum and fluorescence ethidium bromide displacement experiment as well as by viscosity measurements. The intrinsic binding constant is calculated as 1.07x10^5 M^{-1} and the linear Stern-Volmer quenching constant of EB bound to DNA by the complex is 5.1x10^4. The results indicate that the complex can bind to DNA in an

intercalation mode. This result may be valuable for evaluating and understanding those factors that determine the DNA-binding modes of lead complexes.

5. ACKNOWLEDGEMENT

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