

SYNTHESIS AND CHARACTERIZATION OF MANNICH BASE TRANSITION METAL COMPLEXES: DNA BINDING AND ANTI-BACTERIAL STUDIES

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ABSTRACT

Objective: Succinimide (pyrrolidine 2,5-dione) is a synthetically versatile substrate used for the synthesis of heterocyclic compounds and as a raw material for drug synthesis. Derivatives of succinimide are of important biological and pharmaceutical interest.

Methods: The novel mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-methoxyphenyl)methyl)thiourea succinimide, methoxy benzaldehyde and thiourea (SMBTU) has been synthesized in good yield by condensation of equimolar quantities of SMBTU, manganese (II), cobalt (II), nickel (II) and copper (II) complexes of the above ligand have also been synthesized.

Results: Structures of newly synthesized compounds were confirmed by elemental analysis, infrared, ultraviolet-visible and nuclear magnetic resonance. All the complexes adopt octahedral geometry around the metal ions. All the newly synthesized compounds were screened for their anti-microbial activity against *Escherichia coli* and *Bacillus subtilis* bacteria by minimal inhibitory concentration technique. The binding of the cobalt chloride complex of the ligand with calf thymus DNA has been investigated using absorption spectroscopy, fluorescence spectroscopy and viscosity measurements.

Conclusion: All the complexes exhibit octahedral geometry. The ligand and its metal complexes have shown significant antibacterial activity. The Co (II) metal complex showed efficient DNA binding ability and the binding constant value is consistent with other typical intercalators.

Keywords: Mannich base, Metal complexes, Anti-bacterial, DNA binding, Fluorescence and Viscosity measurements.

INTRODUCTION

Mannich reaction consists of amino alkylation of an acidic proton placed next to a carbonyl group with formaldehyde and ammonia or any primary or secondary amine. The final product is a β -amino carbonyl compound. Reactions between imides and aromatic aldehydes have also been considered as Mannich reactions. A review of the literature regarding Mannich reactions shows extensive volume on chemical, biological and toxicological feature of Mannich bases [1-6] with vast applications as polymers, dispersants in lubricating oil and pharmaceutical agents. It is well known that compounds containing amide moiety as functional group have been found to possess donor properties and exhibited a wide range of biological activities [7-13]. Transition metals are essential for normal functioning of living organisms and are, therefore, of great interest as potential drugs [14]. The coordination chemistry of nitrogen donor ligands is an interesting area of research. A great deal of attention in this area has been focused on the complexes formed by 3D metals with bidentate ligands using both the nitrogen atoms of the substrates. The study of structural and binding features of various Mannich base complexes can play an important role in better understanding of the complex biological processes. Several drugs showed increased activity as metal chelates rather than as organic compounds [15]. It has been reported in the literature survey that cobalt (II) complexes with octahedral geometry show remarkable intercalative binding affinity as well as DNA cleavage properties [16,17]. Further cobalt is an element of biological interest which is present in the active center of vitamin B₁₂, which regulates the synthesis of DNA indirectly. It is known that there are about eight cobalt dependent proteins [18]. Many cobalt complexes possess antitumor, anti-proliferative, antimicrobial and antifungal activity [19-26]. To the best of our knowledge, no work has been done on this class of metal complexes with the Mannich base ligand succinimide, methoxy benzaldehyde and thiourea (SMBTU). In the continuation of our research work, herein, we report the synthesis of a new Mannich base derived from SMBTU and the metal complexes with Mn (II),

Co (II), Ni (II) and Cu (II). The characterization studies of all the metal complexes have been done with appropriate methods. All the metal complexes were screened for antibacterial activities. The DNA binding studies of the cobalt complex containing the ligand SMBTU is reported.

METHODS

All the reagents and solvents used for the synthesis of ligand and the metal complexes were analar grade of highest available purity and used as such without further purification.

Elemental analysis were performed using Carlo Erba 1108 analyzer and Coleman N analyzer and were found within $\pm 0.5\%$. The molar conductivities of the metal complexes were measured in approximately 10^{-3} M ethanol solution using a systronics direct reading digital conductivity meter-304 with dip type conductivity cell. The infrared (IR) spectra was recorded as KBr pellets on Perkin-Elmer 1000 unit instrument. Absorbance in ultraviolet-visible (UV-VIS) region was recorded in dimethylformamide (DMF) solution using UV-VIS spectrometer. The ¹H and ¹³C nuclear magnetic resonance (NMR) of the ligand was recorded on a Bruker instrument employing tetramethylsilane (TMS) as internal reference and dimethyl sulfoxide (DMSO) - DMF as solvent. The mass spectral study of the ligand was carried out using liquid chromatography (LC) mass spectrometer. Magnetic susceptibility measurements at room temperature were made by using a Gouy magnetic balance. Anti-bacterial activity has been carried out using minimal inhibitory concentration (MIC) technique.

Anti-bacterial activity

The ligand SMBTU and the synthesized metal complexes were dissolved in DMSO, and the working concentrations of the above were taken in Milli-Q water for treatment. Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria were taken to analyze the anti-bacterial activity of metal complexes. Primarily, MIC was determined by spectrophotometer method. For this purpose, an equal number of

colonies (1×10^{10} CFU/ml) were in 0.7% of sterile saline, and the final concentrations of metal complexes were varied from 50 μg to 400 μg . 12 hrs incubation was given, and absorbance was taken at 600 nm. 50% of reduction was calculated as MIC. Afterward, the activity of the drug was visualized by well-diffusion assay, and the zone of inhibition was calculated.

Synthesis

Preparation of Mannich base 1-((2,5-dioxopyrrolidin-1-yl)(4-methoxyphenyl)methyl)thiourea

SMBTU were taken in 1:1:1 molar ratio. In aqueous solution of succinimide and thiourea, methoxy benzaldehyde was added drop wise, and the mixture was stirred in a magnetic stirrer at room temperature for 8-10 hrs. After a week a solid product formed was filtered, washed with distilled water, dried in an air oven at 60°C and recrystallized using ethanol and chloroform in 1:1 ratio (Fig. 1).

Mechanism

The reaction route for the synthesis of Mannich base (SMBTU) is illustrated in Fig. 2 which involves the condensation reaction of methoxy benzaldehyde with thiourea to form the imine product. This electron deficient imine is then attacked by imide to give the ligand SMBTU.

Synthesis of metal complexes

All the metal complexes of SMBTU were prepared by slow addition of hot methanolic solution of the metal salt with hot ethanolic solution of the ligand in 1:1 molar ratio. The insoluble metal complexes were formed after 2 weeks. It was washed with methanol and ethanol to remove unreacted metal salt and ligand. The products were then dried in an air oven at 60°C. The proposed structures of the metal complexes are shown in Fig 3.

RESULTS AND DISCUSSION

Physical properties and elemental analysis

The physical properties and elemental analysis of the prepared ligand and their metal complexes are described in Table 1. Structures have been

suggested according to these data together with obtained from spectral analysis. The structure of metal complexes was further confirmed by conductivity measurements and magnetic moment determinations. Most of the metal complexes have been found to possess high melting points.

UV-VIS spectroscopic studies

The electronic spectra of the metal complexes were recorded for their solution in DMSO in the range of 180-1800 nm (Table 2).

The electronic spectrum of manganese chloride complex exhibits four absorption bands at 18050/cm, 24985/cm, 29125/cm and 31272/cm for ${}^6A_{1g} \rightarrow {}^4T_{1g}$, ${}^6A_{1g} \rightarrow {}^4E_{2g}$, ${}^6A_{1g} \rightarrow {}^4E_{1g}$ and charge transfer transitions respectively. The μ_{eff} value of 4.85 B.M points to a high spin octahedral geometry [27-30].

The cobalt chloride complex shows four absorption bands at 6703/cm, 14,365/cm, 18,742/cm, 29,066/cm assigned for ${}^4T_{1g} \rightarrow {}^4T_{2g}$, ${}^4T_{1g} \rightarrow {}^4A_{2g}$, ${}^4A_{1g} \rightarrow {}^4T_{1g}$ and charge transfer transition. The μ_{eff} value was found to be 4.48 B.M which supports octahedral geometry.

The nickel chloride complex shows absorption bands at 10,525/cm, 15,780/cm and 24,890/cm and 35,235/cm for the transitions ${}^1A_{1g} \rightarrow {}^3T_{1g}$, ${}^1A_{1g} \rightarrow {}^3T_{2g}$, ${}^1A_{1g} \rightarrow {}^3T_{1g}$ and charge transfer transitions respectively. The μ_{eff} value was found to be 3.56 B.M suggestive of octahedral geometry [31].

The copper chloride complex registers absorption bands at 9275/cm, 10374/cm, 12557/cm due to ${}^2B_{1g} \rightarrow {}^2A_{1g}$, ${}^2B_{1g} \rightarrow {}^2B_{2g}$, ${}^2E_g \rightarrow {}^2T_{2g}$ transitions respectively. The charge transfer transition bands occur at 24330 and 28327/cm. The μ_{eff} value was found at 2.09 B.M suggesting octahedral geometry.

IR spectra

In order to study the binding mode of the ligand to metal in the complexes, the IR spectrum of the free ligand was compared with the corresponding metal complexes. Selected vibrational bands of the ligand and its metal complexes and their assignments are listed in Table 3. The IR spectrum the free ligand exhibited a strong band at 1690/cm

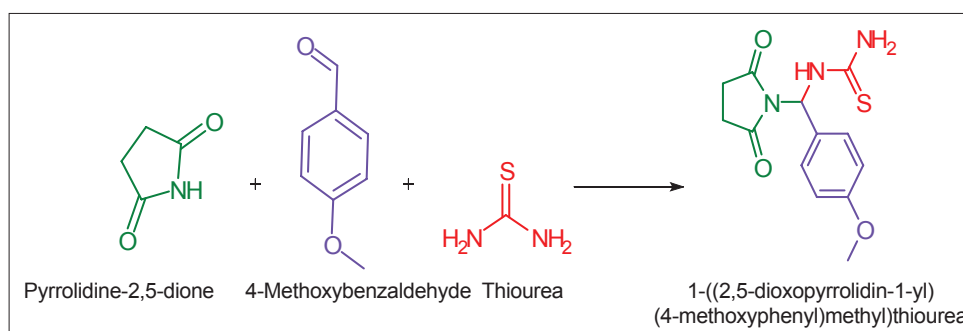


Fig. 1: Synthesis of the ligand SMBTU

Table 1: Analytical data of the ligand SMBTU and its metal complexes

Compound	M.Pt°C	Color	Yield %	Found (Calculated %)				
				C	H	N	O	M
SMBTU $C_{13}H_{15}N_3O_3S$	123	White	86	53.23 (53.17)	5.15 (5.08)	14.32 (14.26)	16.36 (16.31)	-
$MnCl_2 \cdot 2H_2O$	160	White	90	35.91 (35.86)	4.31 (4.29)	8.97 (8.95)	17.08 (17.01)	11.73 (11.65)
SMBTU $C_{14}H_{20}Cl_2MnN_3O_5S$								
$CoCl_2 \cdot 2H_2O$	164	Light blue	83	35.61 (35.56)	4.27 (4.22)	8.90 (8.88)	16.94 (16.90)	12.48 (12.33)
SMBTU $C_{14}H_{20}Cl_2CoN_3O_5S$								
$NiCl_2 \cdot 2H_2O$	170	Dull green	94	35.63 (35.58)	4.27 (4.20)	8.90 (8.86)	16.95 (16.89)	12.44 (12.39)
SMBTU $C_{14}H_{20}Cl_2NiN_3O_5S$								
$CuCl_2 \cdot 2H_2O$	175	Light green	85	35.26 (35.21)	4.23 (4.18)	8.81 (8.79)	16.78 (16.72)	13.33 (13.27)
SMBTU $C_{14}H_{20}Cl_2CuN_3O_5S$								

SMBTU: Succinimide, methoxy benzaldehyde and thiourea

which could be assigned to $\nu_{C=O}$ of the succinimide ring. A band around 3297/cm could be attributed to stretching vibration of ν_{N-H} bond [32]. A strong band observed around 1392/cm can be assignable to $\nu_{C=S}$ vibration mode. In the metal complexes, the band corresponding to $\nu_{C=O}$ of succinimide ring was shifted to lower frequency range suggesting the coordination of the carbonyl group with a metal ion. There is no shifting of bands at 1400/cm and 750/cm indicating the absence of coordination of C=S group with a metal ion. The N-C-N stretching frequency of the ligand at 1472/cm was shifted toward lower values in all the complexes, indicating the involvement of the nitrogen of thiourea in coordination to the central metal ion. The participation of oxygen and nitrogen in coordination with the metal ion is further supported by the new band appearance of ν_{M-N} around 420-425/cm in the far IR region [33,34].

1H NMR Data: (DMSO/TMS, 500.3MHz): δ 2.56 (s, 4H), 3.77 (s, 3H), 3.80 (s, 3H), 6.49 (bs, 2H), 6.93 (d, 1H, J=8.30 Hz), 7.06-7.08 (m, 1H), 7.42 (d, 1H, J=1.80 Hz), 7.75 (s, 1H), 10.09 (s, 1H), 11.06 (s, 1H), ppm.

^{13}C NMR Data: (DMSO/TMS, 125.7 MHz): δ 179.4, 156.9, 149.9, 139.5, 127.6, 120.9, 111.3, 108.2, 55.6, 40.0, 29.5.

LC mass data: Calculated for SMBTU $C_{13}H_{15}N_3O_3S$ m/z=293.08; Found 294.37 (M+1).

Anti-bacterial activity of Mannich base

The MIC of ligand SMBTU was found to be 300 μ g for *E. coli* and *B. subtilis*. The activity was a higher rate when the growth inhibition was observed at 600 nm. But low concentrations survival of bacteria was observed. The inhibitory effect was proved with well-diffusion method and cleared zone of inhibition was observed with Mannich base and its metal complexes that are listed in Table 4. The effect of metal complexes as anti-bacterial agents has also been discussed in the literature (Neves et al., 2009).

Table 2: Molar conductance (in dmf), magnetic moment, assigned transitions with λ_{max} and geometry of the metal complexes

Complex	$^{\circ}\Lambda_m$ ($ohm^{-1} cm^2 mol^{-1}$)	μ_{eff} (B.M)	λ_{max} (cm^{-1})	Transition assignment	Geometry
MnCl ₂ . 2H ₂ O. SMBTU	62	4.85	18050 24985 29125 31272	$^6A_{1g} \rightarrow ^4T_{1g}$ $^6A_{1g} \rightarrow ^4E_{2g}$ $^6A_{1g} \rightarrow ^4E_{1g}$ CT	High spin Octahedral
CoCl ₂ . 2H ₂ O. SMBTU	55.5	4.48	6703 14365 18742 29066	$^4T_{1g} \rightarrow ^4T_{1g}$ $^4T_{1g} \rightarrow ^4A_{2g}$ $^4A_{1g} \rightarrow ^4T_{1g}$ CT	Octahedral
NiCl ₂ . 2H ₂ O. SMBTU	61	3.56	10525 15780 24890 35235	$^1A_{1g} \rightarrow ^3T_{1g}$ $^1A_{1g} \rightarrow ^3T_{2g}$ $^1A_{1g} \rightarrow ^1T_{1g}$ CT	Octahedral
CuCl ₂ . 2H ₂ O. SMBTU	89	2.09	9275 10374 12557 24330 28327	$^2B_{1g} \rightarrow ^2A_{1g}$ $^2B_{1g} \rightarrow ^2B_{2g}$ $^2E_g \rightarrow ^2T_{2g}$ CT	Octahedral

SMBTU: Succinimide, methoxy benzaldehyde and thiourea

Table 3: Characteristic IR absorption frequencies (cm^{-1}) of SMBTU and its metal complexes

Compound	ν_{NH}	$\nu_{C=O}$	$\nu_{C=S}$	$\nu_{CH(st)}$	$\nu_{CH(b)}$	N-C-N	-OCH ₃	H ₂ O coordinates	M-X	M-S
SMBTU	3297	1690	1392	3174	814	1472	1272	-	-	-
MnCl ₂ . 2H ₂ O. SMBTU	3289	1699	1394	3170	811	1462	1271	3746, 1512, 728	422	-
CoCl ₂ . 2H ₂ O. SMBTU	3291	1684	1398	3170	808	1467	1271	3778, 1591, 728	425	-
NiCl ₂ . 2H ₂ O. SMBTU	3290	1683	1396	3170	808	1463	1271	3747, 1590, 728	423	-
CuCl ₂ . 2H ₂ O. SMBTU	3292	1687	1400	3169	808	1466	1268	3747, 1591, 727	424	-

IR: Infrared, SMBTU: Succinimide, methoxy benzaldehyde and thiourea

DNA binding

One of the most important approaches in the development of drugs and chemotherapy against some cancers, viral and parasitic diseases involve drugs that interact reversibly with DNA. Hence, syntheses of new metal complexes, which can bind with specificity to DNA and bring about its cleavage are of importance in the development of new antitumor agents [35].

Electronic absorption spectra

The binding of calf thymus (CT)-DNA with the synthesized Co (II) complexes was studied using UV absorption spectral method.

The concentration of CT-DNA per nucleotide was measured by using its known extinction coefficient at 260 nm (6600/m/cm). Tris HCl-buffer [5 mM Tris(hydroxymethyl) amino methane, pH 7.2] 50 mM NaCl was used for the absorption, viscosity and thermal denaturation experiments.

Absorption titration experiments were carried out by varying the DNA concentration (0-100 μ M) and maintaining the metal complex concentration constant. Absorption spectra were recorded after successive addition of DNA and equilibration (approximately

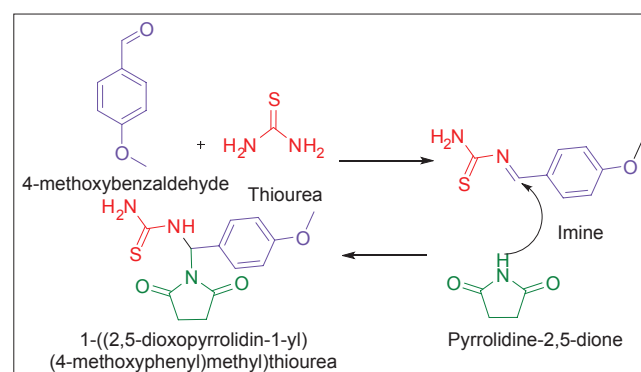


Fig. 2: Proposed mechanism

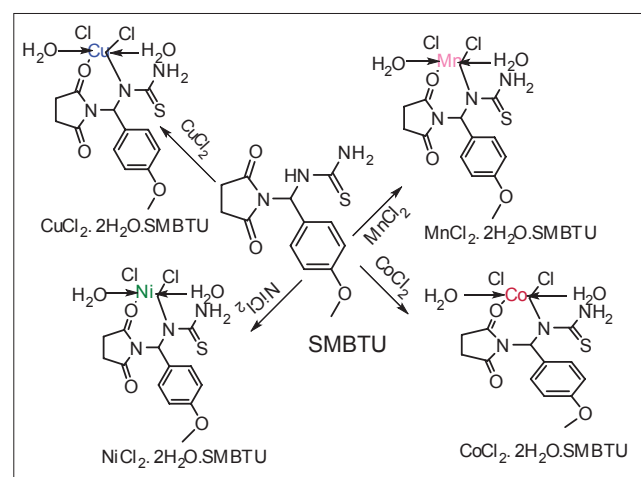


Fig. 3: Proposed structures of the metal complexes

10 minutes). Absorption titration experiments with CT-DNA had shown intense absorption peaks at 230 and 280 nm in the UV region of the complex due to inter ligand π - π^* transition of the coordinated groups in the complex. In addition of increasing amounts of DNA to the complex, both of the two characteristic peaks decreased gradually with the maximum hypochromicity of 15% and 20% respectively, suggesting the strong interaction between the complex and DNA. The observed data were then fitted into Eq. 1 to obtain the intrinsic binding constant K_b [36].

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_a - \epsilon_f) \quad (1)$$

where ϵ_a , ϵ_b , and ϵ_f are the apparent, bound, and free metal complex extinction coefficients, respectively, at 263 nm (Fig. 4). A plot of $[DNA]/(\epsilon_b - \epsilon_f)$ versus $[DNA]$ gave a slope of $1/(\epsilon_b - \epsilon_f)$ and intercept y equal to $1/K_b(\epsilon_b - \epsilon_f)$, where K_b is ratio of the slope to the y intercept (Fig. 5).

Fluorescence spectra

Fluorescence quenching experiments were performed with ethidium bromide (EB) bound DNA with increasing concentrations of metal

Table 4: Diameter of inhibition against bacteria in millimeter (mm) by SMBTU and its metal complexes

Compound	<i>E. coli</i>	<i>B. subtilis</i>
SMBTU	2.0±0.2	1.9±0.3
MnCl ₂ .2H ₂ O.SMBTU	2.5±0.1	2.3±0.2
CoCl ₂ .2H ₂ O.SMBTU	2.2±0.3	2.1±0.05
NiCl ₂ .2H ₂ O. SMBTU	1.4±0.2	1.3±0.2
CuCl ₂ .2H ₂ O.SMBTU	1.8±0.4	1.7±0.2

SMBTU: Succinimide, methoxy benzaldehyde and thiourea, *E. coli*: Escherichia coli, *B. subtilis*: Bacillus subtilis

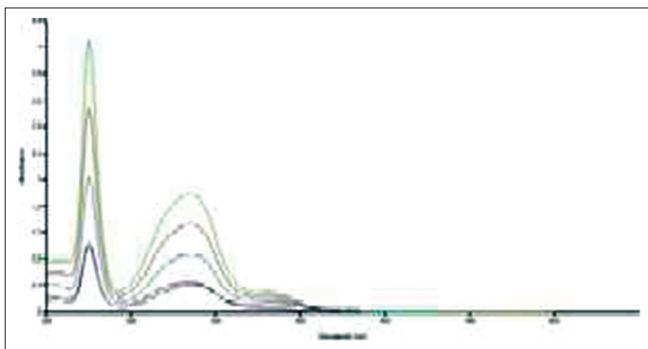


Fig. 4: Absorption spectra of Co(II) complex in the absence and presence of the CT-DNA concentration (0-100µM)

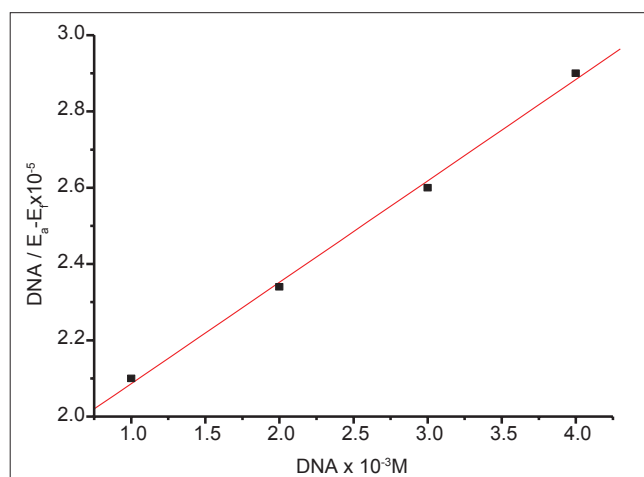


Fig. 5: Plot of $[DNA]/(\epsilon_b - \epsilon_f)$ versus $[DNA]$ for the complex with DNA

complex to determine the extent of binding between the molecule and DNA. EB is an indicator for fluorescence quenching [37]. The quenching extent of fluorescence EB bound to DNA is used to determine the DNA binding strength of the metal complex. The fluorescence quenching curves of EB bound to DNA in absence and presence of the complex was monitored. The addition of the metal complex to EB bound to DNA has shown a reasonable reduction in emission intensity indicating that the complex is bound to DNA at the sites occupied by EB (Fig. 6). The quenching plots indicate that the quenching of EB bound to DNA by the metal complex is in good agreement with the linear Stern-Volmer equation. In the plot of I_0/I versus $(\text{complex})/(\text{DNA})$, K is given by the ratio of the slope to the intercept (Fig. 7).

Viscosity measurements

Viscosity measurements are used to explore the binding modes of complex with DNA. Optical photophysical probes provide necessarily, but not sufficient, clues to support a binding model. To clarify further the interactions between the complex and DNA, viscosity measurements were carried out. Viscosity measurements that are sensitive to length change are regarded as the least uncertain and the most critical tests of a binding model in a solution in the absence of crystallographic structural data (Satyanarayana et al., 1992). A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to hold the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial intercalation ligand could bend the DNA helix, reduce its effective length and, in tandem, its viscosity (Satyanarayana et al., 1993). In Fig. 8, the relative viscosity increased with the addition of metal complex, showing that the cobalt complex bind with DNA in the classical intercalation mode.

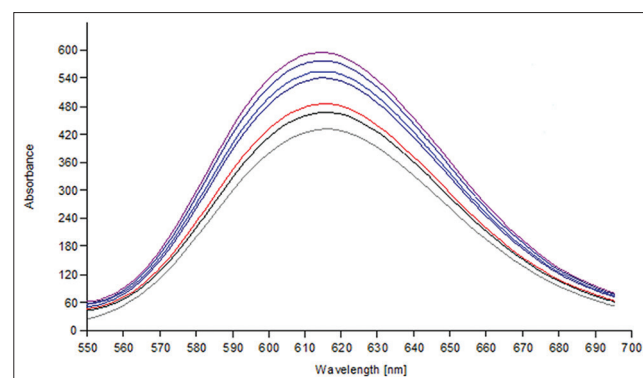


Fig. 6: Emission spectrum of EB bound to DNA in the absence and presence of Co (II) complex

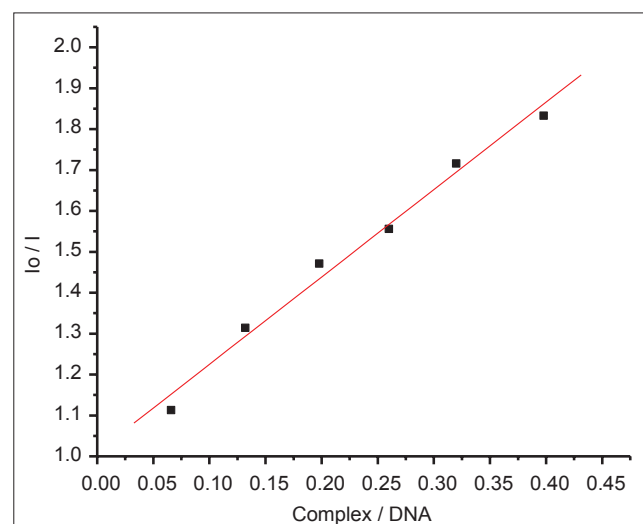


Fig. 7: Plot of emission intensity I_0/I versus $(\text{complex})/[\text{DNA}]$

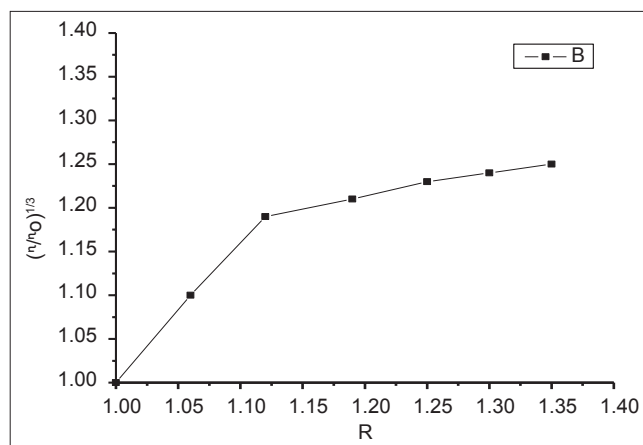


Fig. 8: Effect of increasing amounts of Co(II) complex on the relative viscosity of CT-DNA

CONCLUSION

In this paper, coordination chemistry of a Mannich base ligand obtained from the reaction of succinimide, methoxy benzaldehyde and thiourea is described. Mn (II), Co (II), Ni (II) and Cu (II) complexes have been synthesized using the above Mannich base ligand and characterized on the basis of analytical, magnetic and spectral data. The Mannich base coordinates through its thiourea nitrogen and oxygen of succinimide to the metal ion and acts as a neutral bidentate ligand. All the complexes exhibit octahedral geometry. The ligand and its metal complexes have shown significant antibacterial activity. The Co (II) metal complex showed efficient DNA binding ability and the binding constant value is consistent with other typical intercalators.

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