CHAPTER IV

DNA Cleavage activity of Cobalt(II) and Copper(II) complexes of a *β*-cyclodextrin based Azo functionalized Schiff base*

4.1. Introduction

Of late, there has been an increasing fascination for the development of transition metal complexes of Schiff bases with diverse dentisities and structures, because such complexes may interact and cleave DNA and thus they may find potential applications in the field of pharmaceuticals for cancer therapy[1-2] and in molecular biology, *etc*.[3-4] Actually Schiff bases represent a class of compounds with a wide spectrum of catalytic and biological activities, *viz*., antimicrobial, antifungal, antiviral including insecticidal, antitumor and cytotoxic activities, etc.[5-11] The biological activities associated with the Schiff bases are due to the presence of azomethine linkage in their structure.[12] However, the stability of Schiff bases is often poor, so introduction of an azo group into their structure may often improve stabilities of Schiff bases as well as their complexes through a highly delocalized conjugated system.[13] Azo functionalized Schiff base ligands and their metal complexes may show interesting biological activities.[14-17] For most anticancer and antiviral therapies, DNA is the primary target molecule.[18] Over the past decades the interactions between the nucleic acid and transition metal complexes have been studied extensively.[19,20] In general, the tumor cells can be destroyed by blocking the replication of the altered DNA. Transition metal Schiff base complexes often bind to DNA through the noncovalent ways like the groove, the intercalation and the electrostatic bindings.[21] That's why physiologically important transition metal complexes have been synthesized and investigated for their biological activities over the years.[22,23] For example, Cisplatin and its second generation compounds have been the most favorite metallodrugs for cancer treatment, but their uses have been limited by resistance, toxicity and other side effects.[24,25] Therefore, search for better alternative anti-carcinogenic metallodrug is always fascinating and essential. In this regard copper-based drugs are very attractive, because copper being an essential metal its complex exhibit less toxicity than those with nonessential ones. The interaction studies of many copper(II) complexes with DNA reveal that modification of the ligand's

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structure often lead to subtle or substantial changes in the binding mode, location, affinity and different cleavage mechanisms.[26-28] Similarly, cobalt complexes have also achieved importance for their applicability in the various biological fields *e.g.*, some Co(II) complexes such as hexamine cobalt was reported to induce DNA condensation.[29] But Schiff base metal complexes are mostly insoluble in aqueous solution and aqueous solubility is a prerequisite for availability in bio-fluids (bio-availability). It is reported that many antibiotics containing high aqueous soluble sugar or carbohydrate moieties readily interacts with DNA[30] and so, incorporation of β-cyclodextrin moiety, therefore, in the ligand structures of the metal complexes should enhance their aqueous solubility thereby making the complexes fairly acceptable through bio-fluids. Anyway, relatively few water-soluble azo-functionalized Schiff bases and their metal complexes have been reported and used as DNA cleaving agents.[9] Defiantly, incorporation of both the azo and azomethine groups as well as *β*-CD moiety in the ligand's structure and subsequently their presence in the metal complexes will augment the individual group properties in a single structure and enhance their bio-availability as well as their bio-activities. Therefore, in view of the above facts, herein this work synthesis, physic-chemical characterization, DNA cleavage activities of the Cu(II) and Co(II) complexes of a new β-cyclodextrin based azo functionalized schiff base ligand have been reported.

4.2. Experimental section

4.2.1. Materials and methods

o-phenylenediamine, 2- hydroxy benzaldehyde were obtained from Sigma Aldrich, Germany (with purity > 99%) and were used without further purification. In all the experiments double di-ionized distilled water was used. Solvents such as acetone, dimethylformamide (DMF), dimethylaminopyridine (DMAP) and ethanol were procured from S. D. Fine chemicals, India while cupper acetate was procured from Thomas Baker, India and cobalt acetate was procured from Sigma Aldrich, Germany. Elemental microanalyses (C, H and N) were performed with a Euro VECTOR EA 3000 analyzer. IR spectra were recorded on Perkin-Elmer Spectrum FT-IR spectrometer (RX-1) in the range 4000-400 cm^{-1} at ambient temperature using KBr pellets and UV-Visible spectra were recorded on a Jasco V-530 double beam spectrophotometer (using a quartz cell with path length of 1 cm) duly equipped with thermostated bath (maintained at 25±0.1 °C) using water and DMSO as solvent references. ^{1}H NMR spectra were recorded on a Bruker Advance-II 400 MHz

spectrometer by using D_2O and DMSO-d_6 as solvents at ambient temperature and chemical shifts (δ) were quoted in ppm with respect to TMS. Metal-contents of the complexes were ascertained by AAS (Varian, SpectraAA 50B) by using standard metal-solution from Sigma-Aldrich, Germany. Specific conductances of the aqueous solutions of the synthesized complexes were recorded (at room temperature) using a Systronics (India) conductivity meter (TDS-308). Fluorescence emission experiments were performed by using Photon Technologies International (Quantamaster-40, USA) spectrophotometer at ambient temperature and the thermal denaturation studies of DNA was conducted with the same UV-Vis spectrophotometer as already mentioned. Ostwald viscometer was used for viscosity studies at ambient temperature under atmospheric pressure. Magnetic susceptibilities were measured with a Sherwood Scientific Ltd magnetic susceptibility balance (Magway MSB Mk1) at ambient temperature. The mass spectra of the ligand and the complexes were recorded with Waters ZQ-4000 instrument. The detailed descriptions of the various analytical and spectroscopic methods and instruments have been discussed in chapter II.

4.2.2. Synthesis

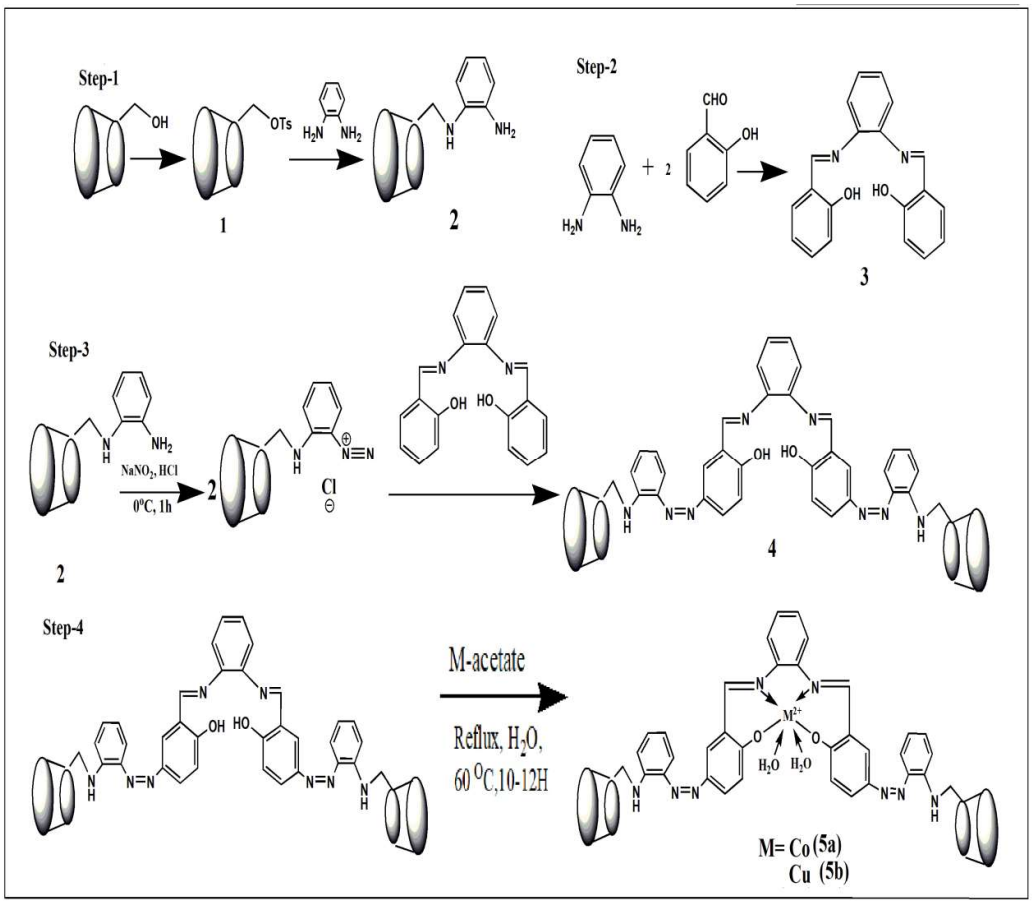
The synthetic path of the amino modified β-cyclodextrin supported azo Schiff base ligand and its Co(II) and Cu(II) complexes are shown in Scheme 4.1.

4.2.2.1. Synthesis of mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin (*β*-CDOTs) (1)

Mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin (*β*-CDOTs) (1) was prepared by following the method described in Chapter III (3.2.2.1).[31]

4.2.2.2. Synthesis of mono-6-deoxy-6-(1,2-diamino)-*β*-cyclodextrin (2)

o-phenylenediamine (140.18 mg, 0.775 mmol) was dissolved in dry DMF (2.0 mL) and warmed to 50 °C, followed by the addition of DMAP (dimethylaminopyridine) (97.0 mg, 0,795 mmol) and KI (32.2 mg, 0.194 mmol). After 5 minutes powdered β -CDOTs (**1**) (500 mg, 0.388 mmol) was added to the solution. The reaction mixture was stirred for 36 h at 50 °C and cooled to room temperature. Subsequent removal of the volatile materials in vacuo then yielded a chocolate brown mass. The crude product was dissolved in water and reprecipiteted by adding acetone to the aqueous solution. The light brown coloured precipitate was collected by filtration and dried in vacuum desiccators over anhydrous $CaCl_2$.



Scheme 4.1. Syntheses of compounds 1, 2, 3, 4, 5a and 5b.

Color: Light Brown; Yield (68%); IR cm^{-1}, KBr: 1641, 1565, 1409, 1251 cm^{-1}; UV: 219 nm, 274 nm; ^1H NMR (400 MHz, D_2O, 25 °C): δ = 7.85 (m, 1H, Ph), 7.68 (m, 1H, Ph), 7.49 (m, 1H, Ph), 7.25 (m, 1H, Ph), 5.91–5.85 (m, 14H, OH2 and OH3 CD), 4.99 (s, 1H, NH), 4.89-4.80 (m, 7H, H1 CD), 4.66 (s, 2H, NH_2), 4.52–4.50 (m, 6H, OH6 CD), 4.38 (m, 2H, H6' CD), 4.17 (m, 1H, H5' CD), 3.83–3.72 (m, 25H, H3, H5 and H6 CD), 3.60–3.51 (m, H2, H4 overlap with water) ppm; Anal. Calcd for $C_{48}H_{76}N_2O_{34}$: C, 47.05; H, 6.25; N, 2.28; O, 44.40. Found: C, 46.88; H, 6.08; N, 2.06; O, 43.97. m/z (ESI): calculated 1225.08, found 1226.09 $[M+H]^+$.

4.2.2.3. Synthesis of Schiff base (3)

To a warm ethanolic solution of *o*-phenylenediamine (0.1 mol), ethanolic solution of 2- hydroxy benzaldehyde (0.2 mol) was added dropwise with continuous stirring and the reaction mixture was refluxed for 2 h. When cooled to room temperature, the reaction mixture yielded a yellow colored solid product. It was

separated by filtration, washed with ethanol and diethyl ether and then dried in a vacuum desiccator.

Color: Yellow; Yield (88%); IR cm^{-1}, KBr: 3451, 1635, 1484, 1373, 1280; ^1H NMR (400 MHz, DMSO-D_6, 25 °C): δ = 12.96 (s, 2H, Phenolic -OH), 8.94 (s, 1H, CH=N), 7.48 – 6.96 (m, aromatic protons) ppm; UV: 273 nm, 345 nm; Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.09; N, 8.85; O, 10.11. Found: C, 75.18; H, 4.89; N, 8.39; O, 9.94. m/z (ESI): calculated 316.35, found 317.51 $[M+H]^+$.

4.2.2.4. Synthesis of amino modified *β*-cyclodextrin based azo Schiff base ligand (4)

A solution of the compound **2** (0.7358 mmol, 900.00 mg) was prepared by dissolving it in a dilute HCl solution (0.143mL of conc. HCl (1.671 mmol) plus 10 mL of distilled water) and immediately cooled to 0 ℃. 0.6 mL of aqueous $NaNO_2$ (0.7358 mmol, 50.76 mg) solution was added to it dropwise keeping the temperature between 0-5 ℃. The diazonium chloride formed was coupled with the Schiff base precursor, (**3**, 0.3679 mmol, 116 mg) dissolved in 10 mL of 10% aqueous NaOH. The mixture was stirred for 1 h at 0 ℃ and then acidified with conc. HCl to obtain a light brown coloured matrix/mass. From which a light reddish brown product was obtained on removal of the volatile materials in vacuo. The crude product was dissolved in water and reprecipiteted out with acetone. The precipitate was collected by filtration and the solid product was kept in a vacuum desiccator for drying properly.

Color: Light red ; Yield (66 %); IR cm^{-1}, KBr: 1666, 1458, 1383, 1232; ^1H NMR (400 MHz, D_2O, 25 °C): δ = 10.26 (s, 2H, Phenolic -OH), 8.09 (s, 1H, CH=N), 7.66 – 6.94 (m, aromatic protons), 5.87–5.81 (m, 28H, OH2 and OH3 CD), 5.25 (s, 1H, NH), 4.91–4.83 (m, 14H, H1 CD), 5.14–5.07 (m, 12H, OH6 CD), 4.31 (m, 4H, H6’ CD), 4.19 (m, 2H, H5’ CD), 3.88–3.71 (m, 30H, H3, H5 and H6 CD), 3.61–3.50 (m, H2, H4 overlap with water) ppm; UV: 281 nm, 349 nm, 456 nm; Anal. Calcd for $C_{116}H_{164}N_8O_{70}$: C, 49.92; H, 5.92; N, 4.01; O, 40.13. Found: C, 49.38; H, 5.39; N, 3.87; O, 40.01. m/z (ESI): calculated 2790.51, found 2791.63 $[M+H]^+$.

4.2.2.5. General procedure for synthesis of metal complexes of the amino modified β-cyclodextrin based azo Schiff base ligand (5a & 5b)

An aqueous solution of the metal acetates [0.0719 mmol of each acetate: 14.35 mg, $Cu(acac)_2.H_2O$ and 17.90 mg, $Co(acac)_2$] was added drop-wise to a well stirred aqueous solution of the ligand (**4**) (0.0719 mmol, 200 mg) at room temperature. The

solution mixture was stirred and refluxed for 10-12 h at 60 °C. The solution was then concentrated in a rotary evaporator to a volume of ~15 mL, followed by the addition of acetone (~100 mL). On standing overnight at room temperature the solution yielded a precipitate. The precipitate was filtered and washed with acetone, next it was dried under vacuum. All the prepared compounds are found to be air stable and fairly soluble in water.

Physical and spectral data for **5a** and **5b are as follows:**

4.2.2.5.1. Co(II) complex(5a)

Color: Pink; Yield (72%); IR cm^{-1}, KBr: 1630, 1453, 1343, 1210, 839, 770, 531, 424; UV: 283 nm, 362 nm, 453nm, 541nm; Anal. Calcd for $C_{116}H_{166}N_8O_{72}$ Co: C, 48.31; H, 5.80; N, 3.88; O, 39.94; Co, 2.04. Found: C, 48.02; H, 5.31; N, 3.47; O, 39.63; Co, 1.90. m/z (ESI): calculated 2883.45, found 2883.23 $[M]^+$.

4.2.2.5.2. Cu(II) complex(5b)

Color: Light green; Yield (70%); IR cm^{-1}, KBr: 1635, 1452, 1342, 1205, 863, 798, 510, 445; UV: 286 nm, 369 nm, 451 nm, 523 nm; Anal. Calcd for $C_{116}H_{166}N_8O_{72}$ Cu: C, 48.24; H, 5.79; N, 3.87; O, 39.88; Cu, 2.20. Found: C, 48.06; H, 5.21; N, 3.45; O, 39.16; Zn, 2.01. m/z (ESI): calculated 2888.07, found 2889.18 $[M+H]^+$.

4.2.3. DNA interaction study

The detailed description of the various analytical and spectroscopic methods and instruments for DNA interaction study has been discussed in chapter II (sections 2.3 and 2.4).

4.3. Results and discussion

Mono-6-deoxy-6-(1,2-diamino)-*β*-cyclodextrin (2) was prepared from mono-6-deoxy-6-(*p*-tosylsulfonyl)-*β*-cyclodextrin [*β*-CDOTs, 1] in presence of DMAP (acts as a base as well as a catalyst) and KI (acts as protecting agent for one $-NH_2$ group of 1,2-diamine). After synthesis of the amino modified *β*-cyclodextrin, it was tagged through the azo linkage with a schiff base (3) prepared earlier through the condensation of salicaldehyde and *o*-phenylenediamine. This tagging results into the ligand (4). Ligand (4) on reaction with cobalt acetate and copper acetate in aqueous solutions yielded the corresponding metal complexes (5a and 5b) (Scheme 4.1). The structures of the complexes were confirmed by various analytical and spectroscopic analyses like elemental analysis (CHN), FTIR, ^1H NMR, UV-visible and ESI-MS spectroscopies. The elemental analysis data of the ligand (4) and its Co(II) and Cu(II) complexes are showed in Table 4.1.

Table 4.1. Elemental analysis of ligand (4) and its complexes (5a and 5b).

Compounds	Colour	Elemental Analyses Found (calculated) %				
		C	H	N	O	Metal
Ligand (**4**)	Light Red	49.38	5.39	3.87	40.01	
		(49.92)	(5.92)	(4.01)	(40.13)	
Co(II) complex (**5a**)	Pink	48.02	5.31	3.47	39.63	1.90
		(48.31)	(5.80)	(3.88)	(39.94)	(2.04)
Cu(II) complex (**5b**)	Light green	48.06	5.21	3.45	39.16	2.01
		(48.24)	(5.79)	(3.87)	(39.88)	(2.20)

4.3.1. FTIR spectra

The functional groups of the compounds 1, 2, 3, 4, 5a and 5b were assigned by infrared spectra. The FTIR spectra of compounds 1 and 2 were shown in (Figure 4.1 and Figure 4.2).

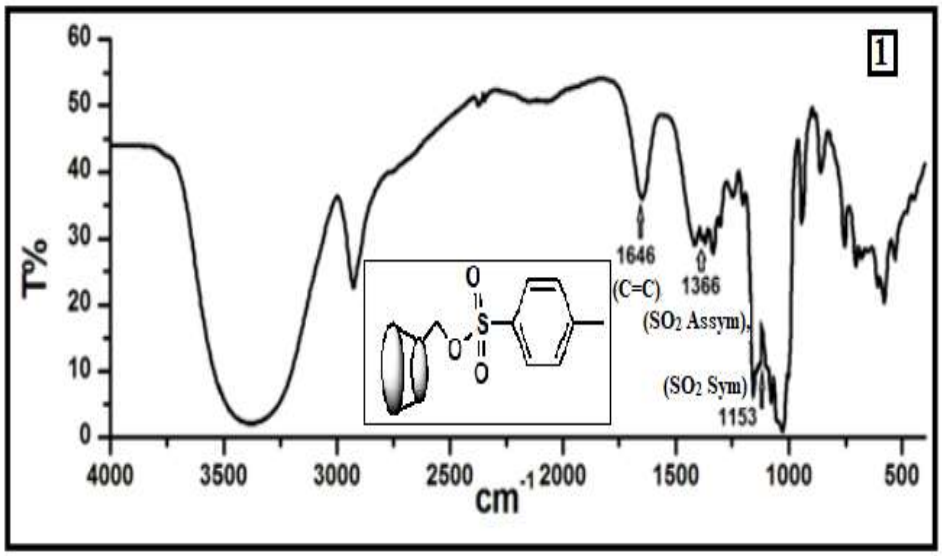


Fig 4.1. FTIR spectrum of Mono-6-deoxy-6-(p-tosylsulfonyl)-β-cyclodextrin (β-CDOTs) (1).

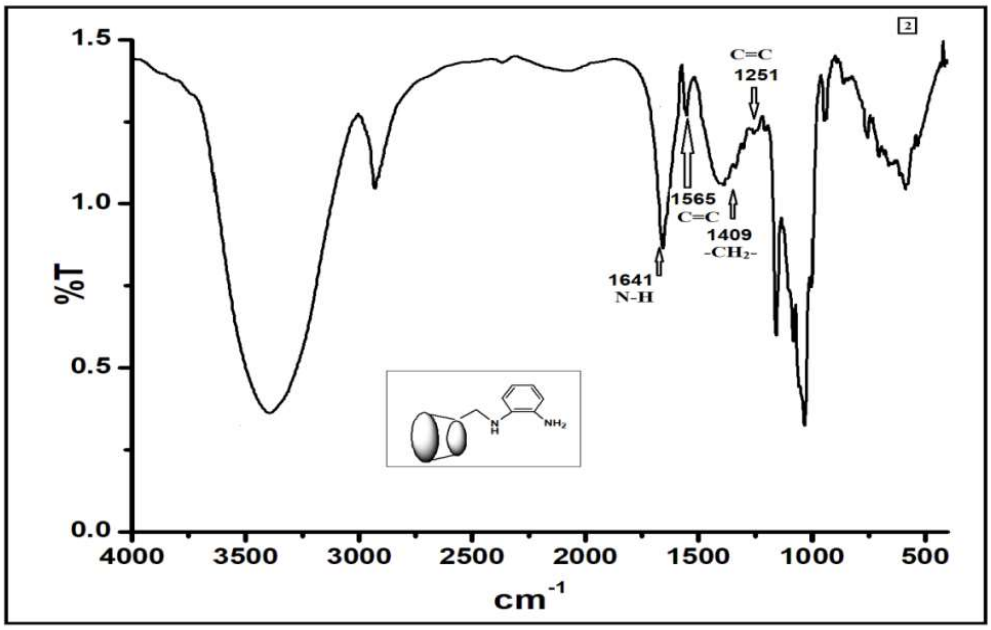


Fig 4.2. FTIR spectrum of mono-6-deoxy-6-(1,2-diamino)-β-cyclodextrin (2).

The characteristic absorption peaks at 1646 (C=C), 1366 (SO_2 assym), 1153 (SO_2 sym) cm^{-1} in the IR spectrum of 6-OTs-ß-CD (1)[32] [due to sulfonic acid group] have disappeared in the IR spectrum of the compound (2). On the contrary, four characteristic absorption peaks at 1641, 1565, 1409 and 1251 cm^{-1} appeared in the spectrum of the compound (2). The absorption peak at1641 cm^{-1} was assigned to N-H stretching vibration of the NH/NH_2 groups of *o*-phynelinediammine. The absorption peak at 1409 cm^{-1} was assigned to $-CH_2-$ stretching vibrations. The peaks at 1565 and 1251 cm^{-1} were assigened to C=C stretching vibrations for the phenyl moiety of the compound (2). These results confirmed that the p-toluenesulfonyl group had been substituted by *o*-phenylenediamine group. In IR spectra of the compound (3) (Figure. 4.3), the band at 1635 cm^{-1} is due to the azomethine ν(C=N) suggesting the formation of the Schiff base. The absorption peaks at 3451 cm^{-1}, 1373 cm^{-1} and 1280 cm^{-1} were assigned to phenolic –OH group and ν(C-O) stretching vibrations, thus these peaks suggest the presence of phenolic -OH group in compound (3). The FTIR spectrum of compound 4, 5a and 5b were shown in (Figure 4.4 - Figure 4.6). The important infra red bands of the synthesized ligand and the complexes are listed in Table 4.2. For the compound (4) the band at 1666 cm^{-1} was due to azomethine stretching vibrations. This band is shifted towards lower frequencies (1630-1635cm^{-1}) in the complexes (5a

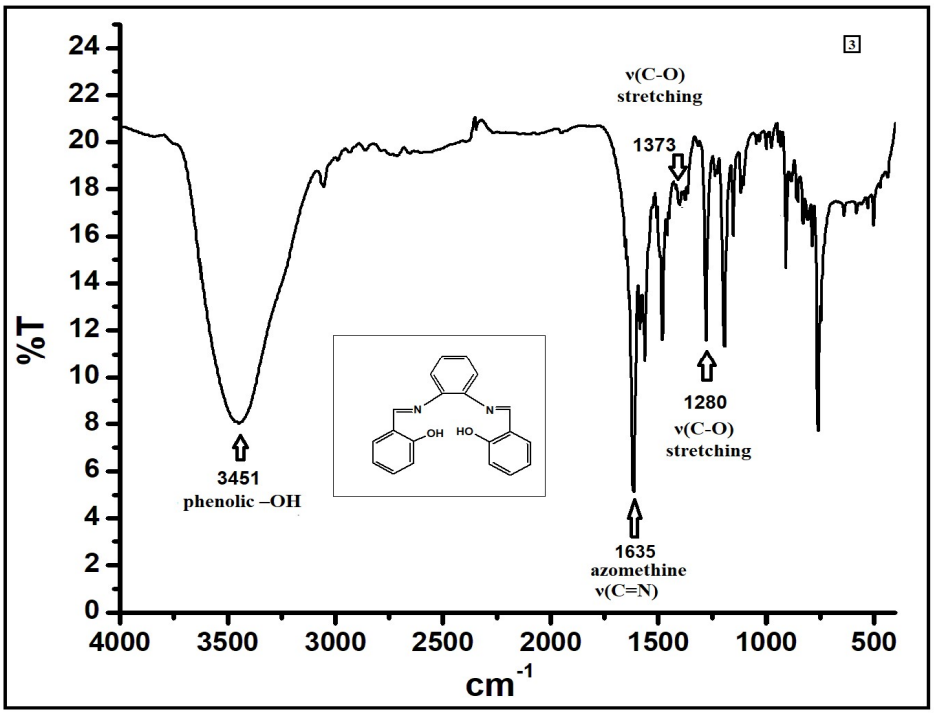


Fig 4.3. FTIR spectrum of the Schiff base (3).

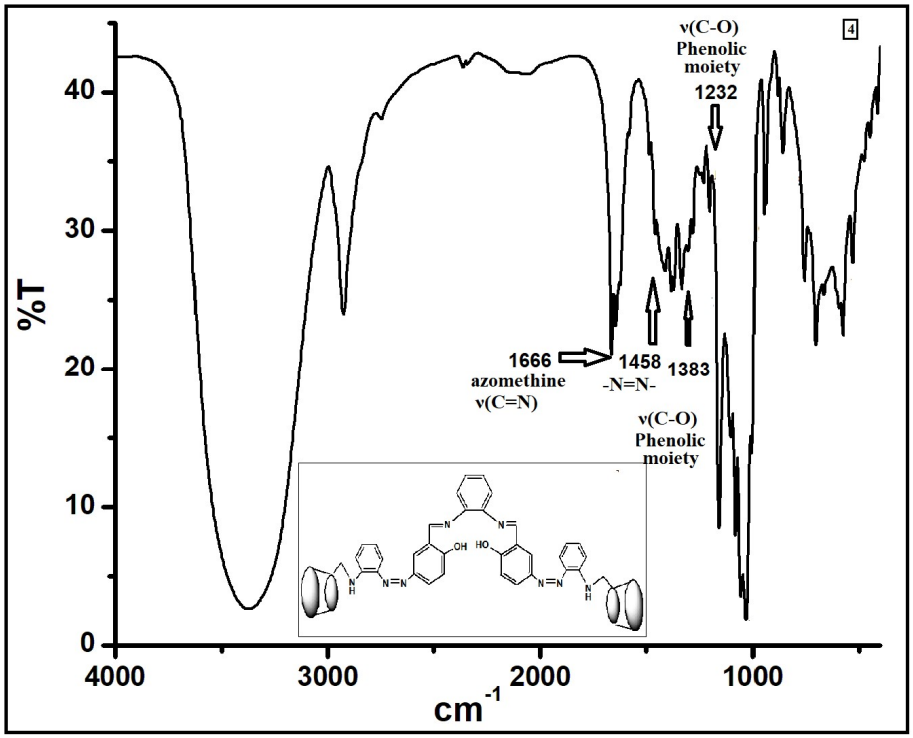


Fig 4.4. FTIR spectrum of the amino modified β-cyclodextrin supported azo Schiff base ligand (4).

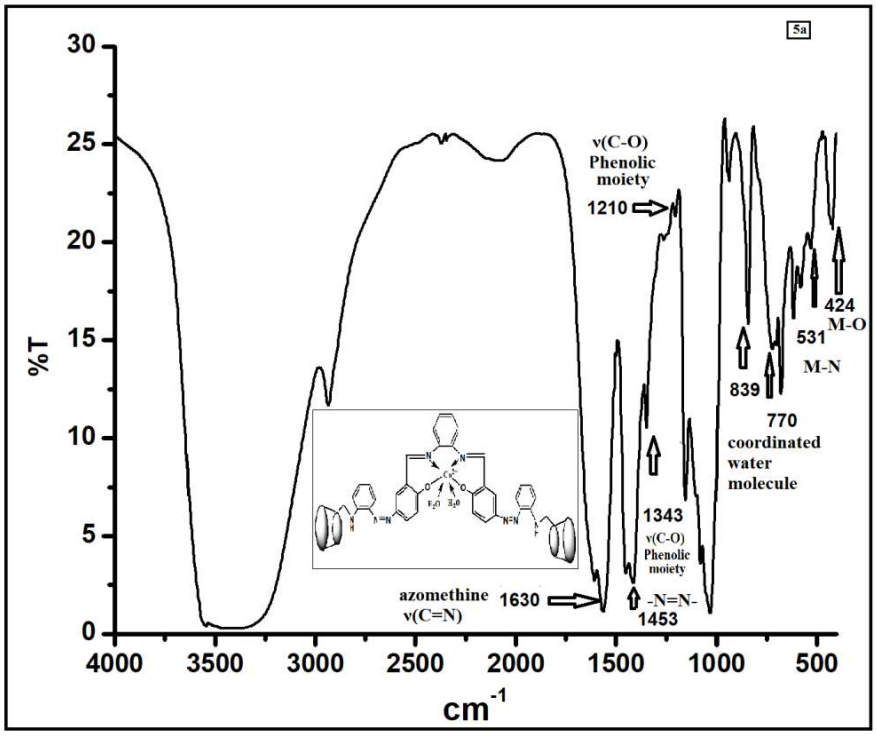


Fig 4.5. FTIR spectrum of Co(II) complexes of the amino modified β-cyclodextrin supported azo Schiff base ligand (5a).

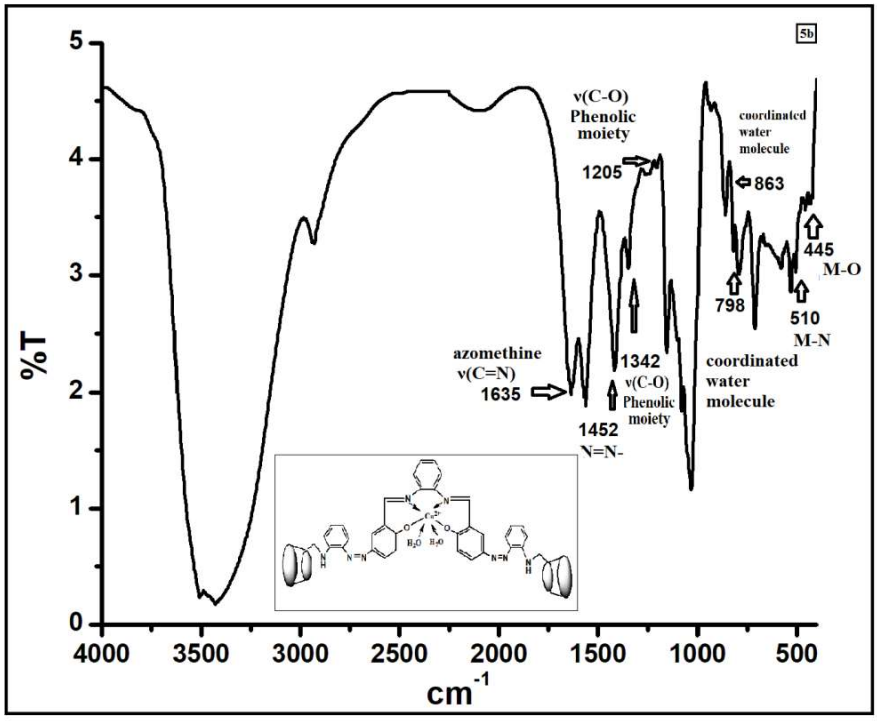


Fig 4.6. FTIR spectrum of Cu(II) complex of the amino modified β-cyclodextrin supported azo Schiff base ligand (5b).

and 5b) and this fact suggests the involvement of azomethine nitrogen atom (-C=N-) in complexation. The band for the diazo group in β-cyclodextrine based azo-linked Schiff base ligand (4) appeared at 1458 cm^{-1}. In the IR spectra of the both complexes (5a and 5b), the frequency of this group did not show any appreciable shift, because of the non-participation of diazo N-atoms in the complex formation. In the IR spectra of both the complexes, the bands that appeared at 839-863 cm^{-1} and 770-798 cm^{-1} were assigned for the rocking and wagging vibrations of water ligand, respectively.[31] The involvement of deprotonated phenolic moiety in complexes have been confirmed by the shift of ν(C-O) stretching bands [observed at 1383, 1232 cm^{-1}] in the free ligands (4) to a lower frequency at 1343, 1210 cm^{-1} and 1342, 1205 cm^{-1} in complexes (5a and 5b), respectively.[33] The shift of ν(C-O) bands to lower frequencies suggests the weakening of C-O bond and formation of stronger M-O bond. The bands observed at 424 and 445 cm^{-1} were attributed to ν(M-phenolic O) and the band observed at 531cm^{-1} and 510 cm^{-1} were attributed to ν(M-N) vibrations for the complexes (5a and 5b), respectively.[34]

Table 4.2. IR spectral data of the ligand (4) and the complexes (5a and 5b) in cm^{-1}.

Compounds	ν(-C=N-)	ν(-N=N-)	ν(C-O)	ν(H_2O)	ν(M-N)	ν(M- O)
Ligand (**4**)	1666	1458	1383, 1232			
Co (II) complex (**5a**)	1630	1453	1343, 1210	839, 770	531	424
Cu (II) complex (**5b**)	1635	1452	1342, 1205	863, 798	510	445

4.3.2. NMR spectra

^{1}H NMR spectra of the mono-tosyleted β-cyclodextrin (1), β-cyclodextrin based amine (2), Schiff base (3) and β-cyclodextrin based azo linked Schiff base (4) are recorded in D2O and DMSO-d_6 (shown in Figure 4.7- Figure 4.10).

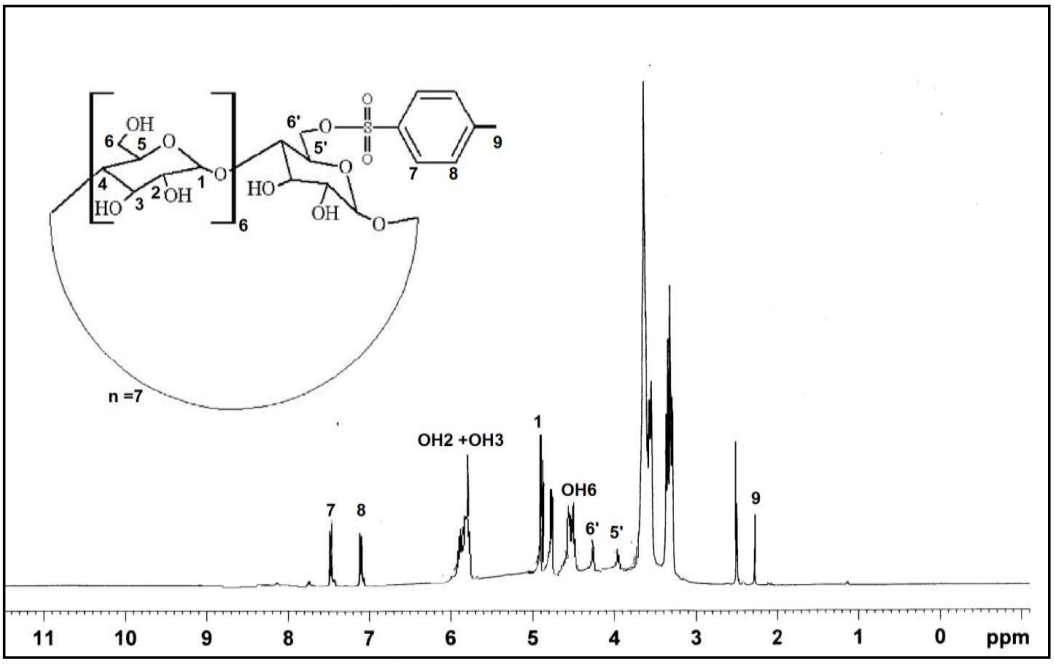


Fig 4.7. ^{1}H NMR of Mono-6-deoxy-6-(p-tosylsulfonyl)-β-cyclodextrin (β-CDOTs) (1).

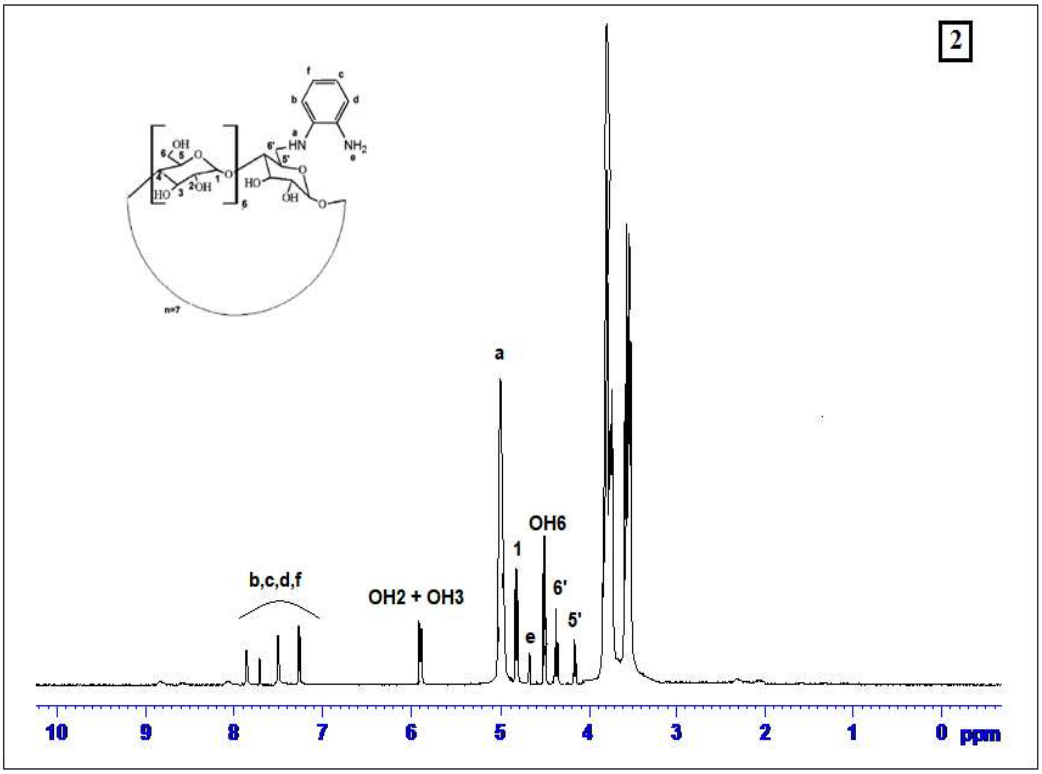


Fig 4.8. ^{1}H NMR of Mono-6-deoxy-6-(1,2-diamino)-β-cyclodextrin (2).

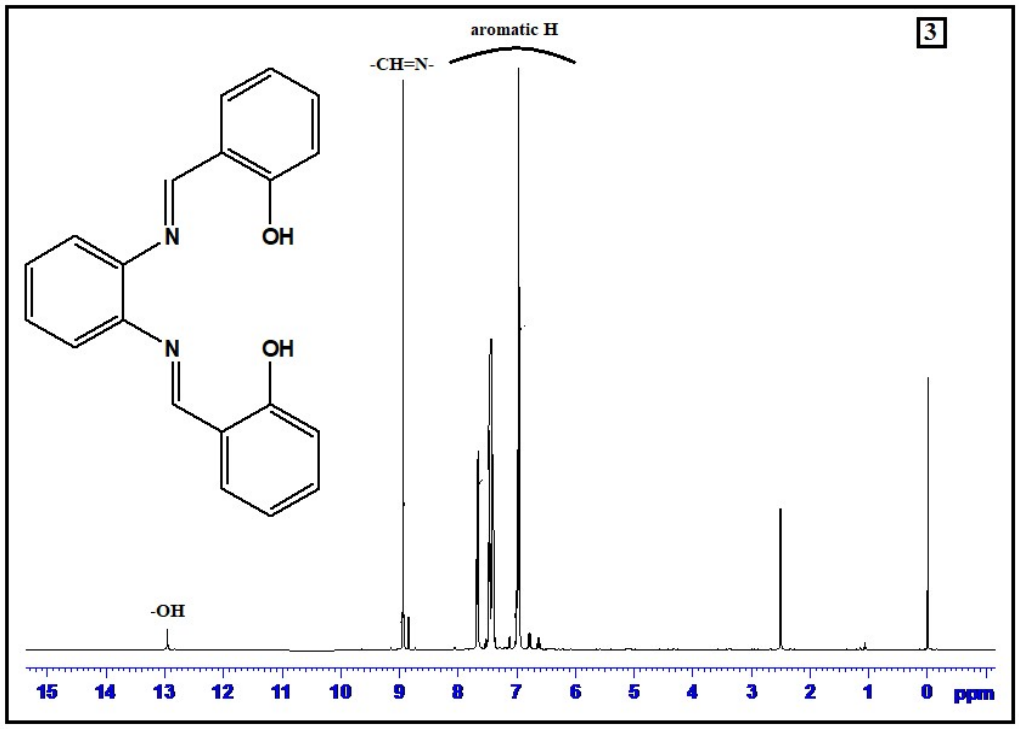


Fig 4.9. ^{1}H NMR of the Schiff base (3).

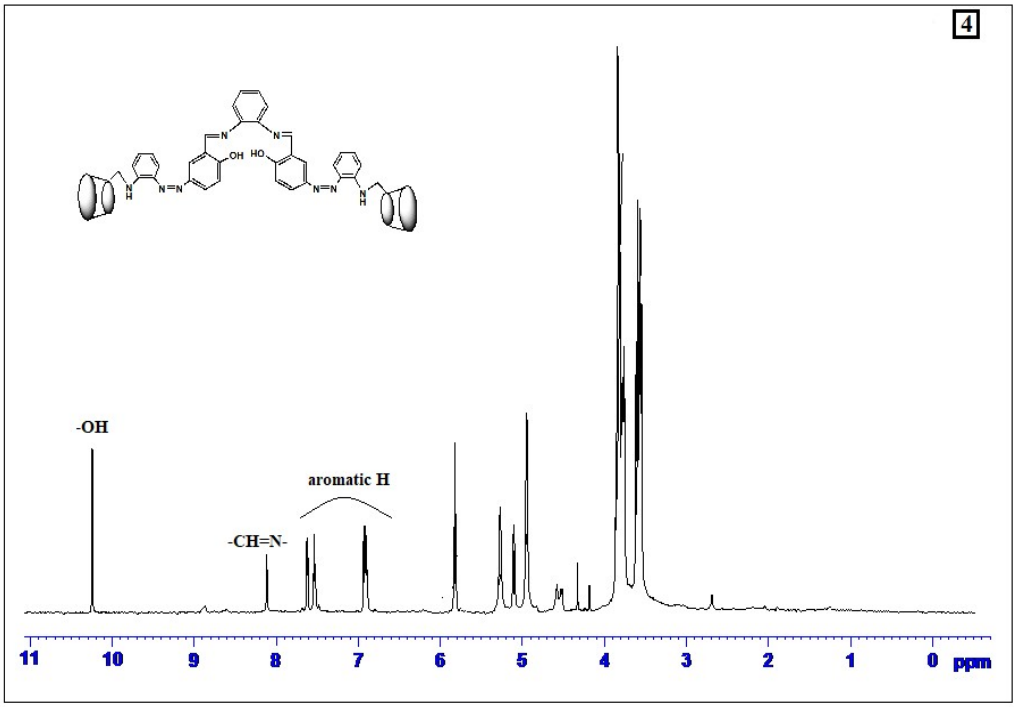


Fig 4.10.^{1}H NMR of the amino modified β-cyclodextrin supported azo Schiff base ligand(4).

^{1}H NMR spectra of the mono-tosyleted β-cyclodextrin (1), *β*-cyclodextrin based amine (2), Schiff base (3) and β-cyclodextrin based azo linked Schiff base (4) are recorded in D_2O and DMSO-d_6 (shown in Figure. 4.7- 4.10). The ^{1}H NMR spectra of the metal complexes could not be obtained, as they are paramagnetic in nature. ^{1}H NMR spectrum of the compound (2) shows that the peak at δ ≈ 4.99 ppm for –NH group. Signals of the aromatic protons appeared as multiplets in the characteristic range of δ ≈7.8–7.2 ppm and –NH_2 group appeared as a singlet in 4.66 ppm.[35] The signals of the H6 protons with an amino group (H6'a. H6'b) appear at approximate 0.11 ppm lower field than those of H6 protons of other rings of *β*-cyclodextrin suggesting the formation of mono-6-deoxy-6-(toluene-3,4-diamino)-*β*-cyclodextrin (2). After the reaction of ortho-phenylenediamine and salicyldehyde, the resulting Schiff base (3) shows the following signals: phenyl protons as multiplets at ≈ 6.9 to 7.4 ppm, the peak at δ ≈ 12.9 ppm for the phenolic protons present in the salicylaldehyde moieties and the peak at δ ≈ 8.9 ppm for the azomethine proton (–CH=N–), its downfield shift was due to the effect of the *o*-hydroxyl group of the aromatic ring. After the coupling reaction between the compound (2) and (3), the phenolic proton present in the salicylaldehyde moieties experience some upfield sfhift (δ ≈ 10.2 ppm) and the corresponding azomethine proton (–CH=N–) peak appears at δ ≈ 8.09 ppm for the compound (4) due to the conjugation with azo (-N=N) groups.

4.3.3. UV-visible spectra and Magnetic Moment

The UV-VIS spectra of the β-cyclodextrin based amine (2), Schiff base (3), *β*-cyclodextrin based azo linked Schiff base (4) and the complexes (5a and 5b) were recorded in water and DMSO (shown in Figures 4.11- 4.15). The modified amine (2) shows two bands at 219 and 274 nm due to the $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions. The electronic spectrum of the Schiff base (3) exhibits the absorption bands at 273 nm due to the $\pi\rightarrow\pi^*$ transition.[36] In addition to these peaks it showed a band at 373 nm attributable to $n\rightarrow\pi^*$ transition.[37,38] All the peaks of the ligand and the complexes are summarized in Table 4.3. In the spectrum of the ligand (4), three peaks appear at 281 nm, 349 nm and 456 nm and these peaks were attributed to $\pi\rightarrow\pi^*$ transition of the aromatic rings, to $n\rightarrow\pi^*$ transition of azomethine moiety and to $n\rightarrow\pi^*$ transition of azo nitrogen (–N=N–), respectively.[39,40] In both the metal complexes (5a and 5b), the first band of the ligand (4) was not significantly affected by coordination with metal ions and second band of the ligand (4) showed bathochromic shifts from 349 nm to

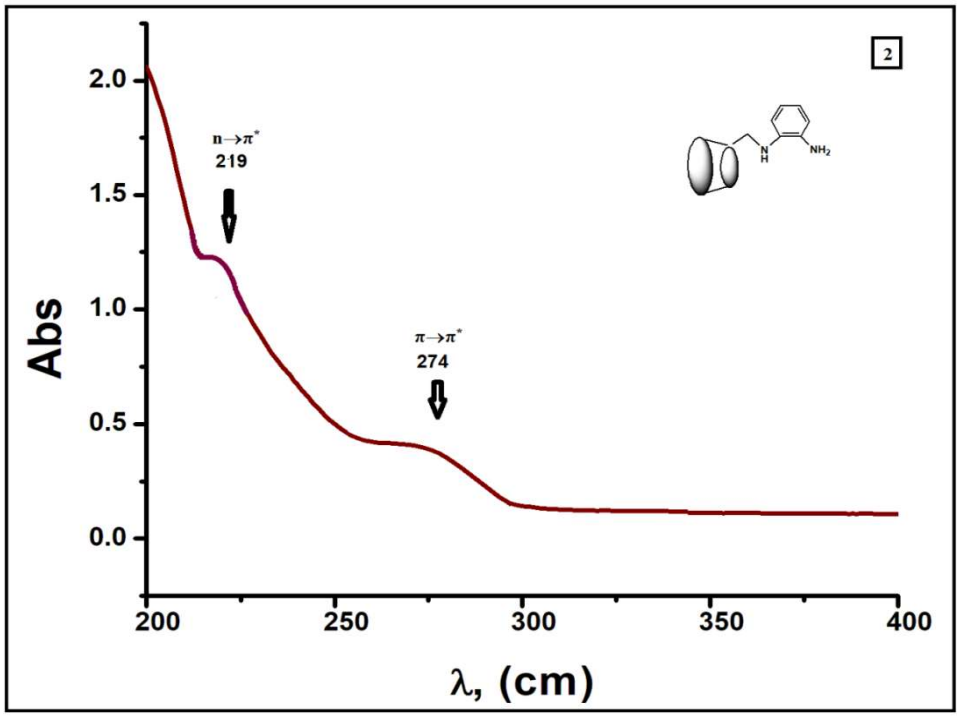


Fig 4.11. Absorption spectrum of Mono-6-deoxy-6-(1,2-diamino)-β-cyclodextrin (2).

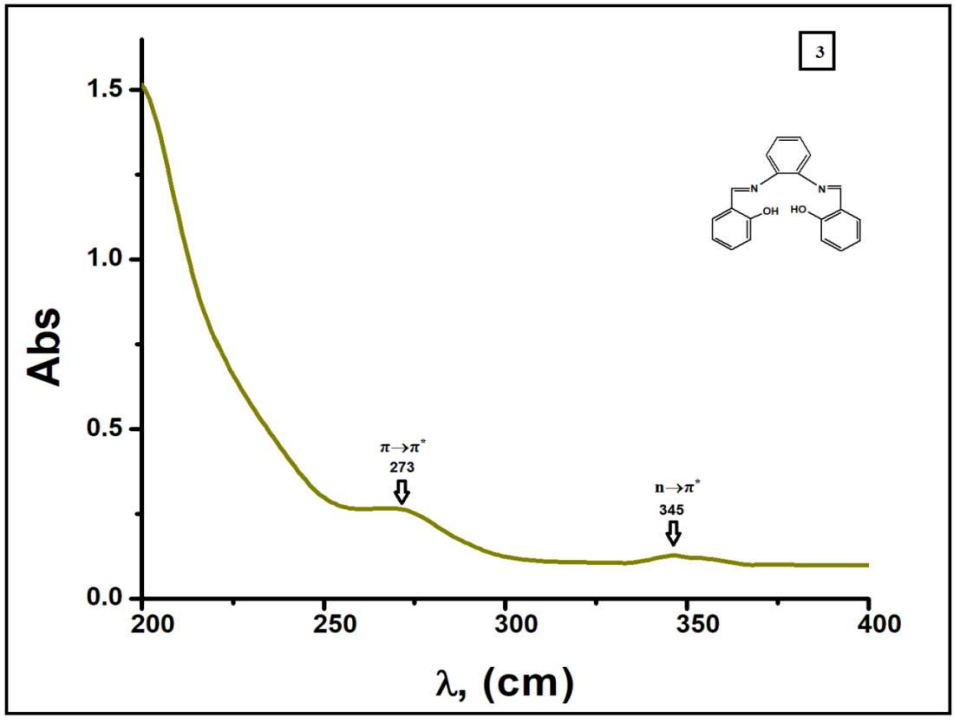


Fig 4.12. Absorption spectrum of the Schiff base (3).

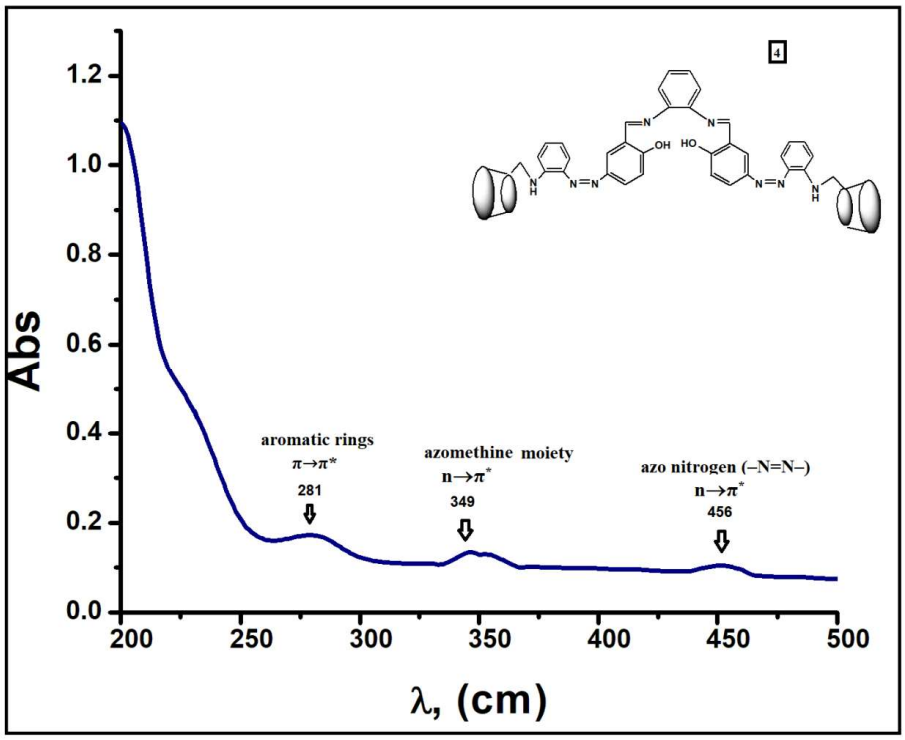


Fig 4.13. Absorption spectrum of the amino modified β-cyclodextrin supported azo Schiff base ligand(4).

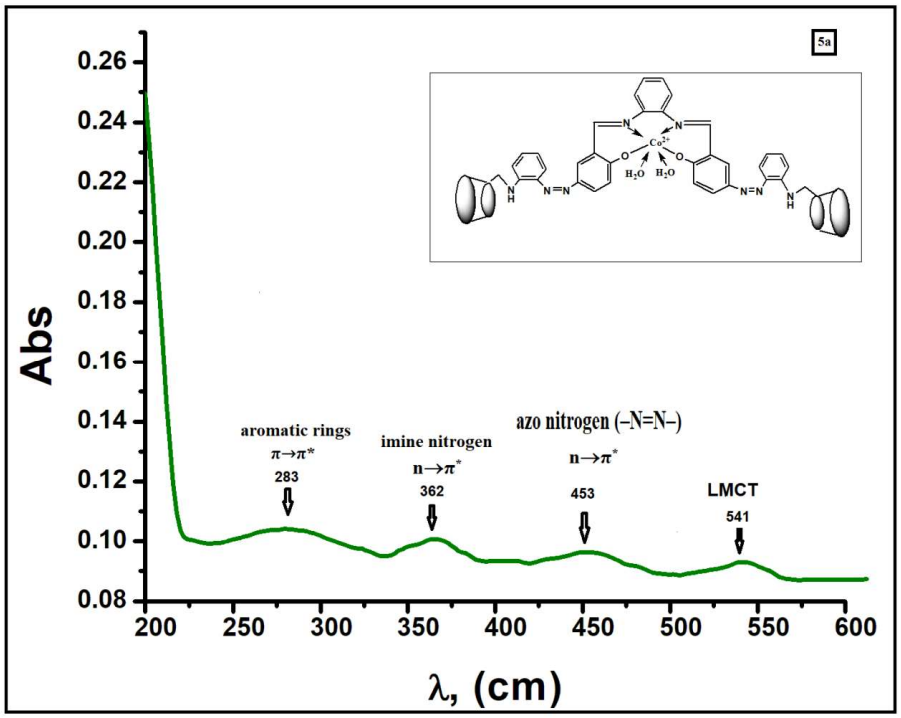


Fig 4.14. Absorption spectrum of Co(II) complexes of the amino modified β-cyclodextrin supported azo Schiff base ligand (5a).

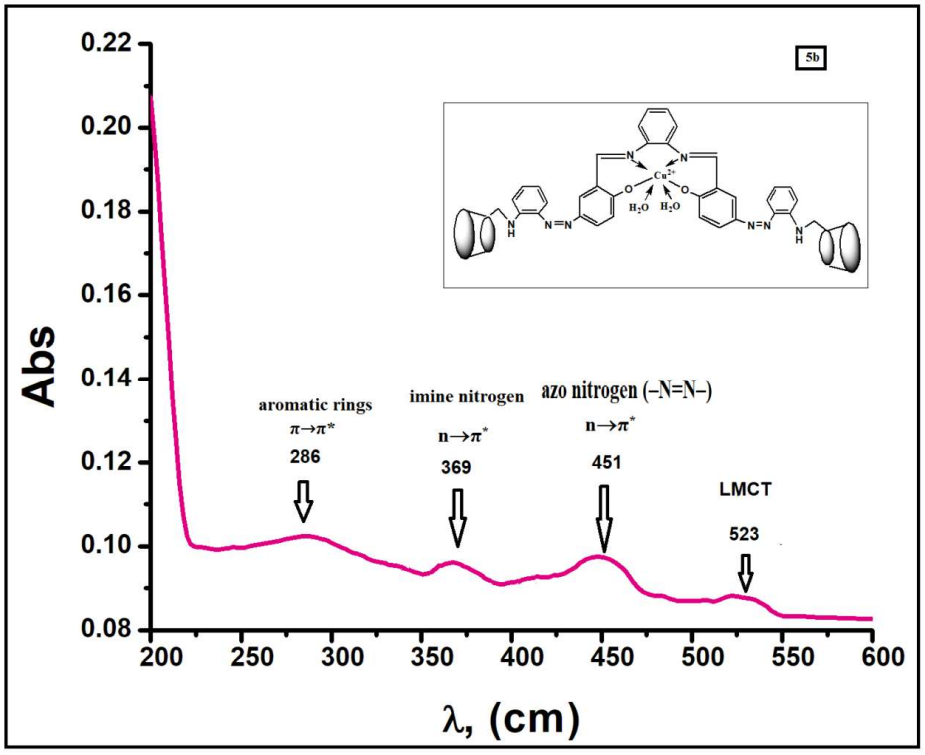


Fig 4.15. Absorption spectrum of Cu(II) complexes of the amino modified β-cyclodextrin supported azo Schiff base ligand (5b).

362 nm and 369 nm, respectively and thereby suggesting the coordination of the metal ions [Co(II) and Cu(II)] with imine nitrogen atom of β-cyclodextrin based azo linked Schiff base ligand (**4**).The –N=N– bands of the free ligands (456 nm) did not shift significantly in the corresponding metal complexes, suggesting that the diazo group did not participate in the complex formation. The peaks appearing at 541 nm for 5a and at 523 nm for 5b can be ascribed to ligand to metal charge transfer (LMCT) transitions. Therefore, these results confirmed that the β-cyclodextrin based azo linked Schiff base ligand coordinates to the metal ions through the oxygen atom of salicyledhyde and imine nitrogen.

Table 4.3. UV-visible spectra data of the ligand (4) and its complexes (5a and 5b).

Compounds	π→π* (aromatic rings)	n→π* (azomethine moiety)	n→π* (azo nitrogen –N=N–)	LMCT
Ligand (**4**)	281 nm	349 nm	456 nm	--
Co(II) complex (**5a**)	283 nm	362 nm	453 nm	541 nm
Cu(II) complex (**5b**)	286 nm	369 nm	451 nm	523 nm

The magnetic moment values (μ_{eff}) [1.67 B.M. for cobalt(II) complex (5a) and 1.52 B.M. for copper(II) complex (5b)] at room temperature suggested that both the Co(II) and Cu(II) complexes (5a and 5b) were paramagnetic in nature and thusindicating low-spin distorted octahedral structure for the Co(II) complex (5a) and distorted octahedral structure for the Cu(II) complex (5b).

4.3.4. ESI-MS

The ESI-MS spectra of the compounds 2, 3, 4 and the complexes (5a and 5b) were recorded given in figure (Figures 4.16 - 4.20). The compound 2 and 3 show m/z peak at 1226.09 and 317.51 corresponds to $[M + H]^+$. Also for the ligand m/z peak appeared at 2791.63 is assigned $[M + H]^+$ mass fragments. In both Co(II) and Cu(II) complexes (5a and 5b) the new peak appeared at 2883.23 (for $[M]^+$) and 2889.18 (for $[M + H]^+$), respectively, thus their peaks can be attributed to the molecular weights of respective complexes. Therefore these results were in good agreement with the respective structures already suggested by the elemental and other spectral analyses.

4.3.5. Molar Conductance

The synthesized Co(II) and Co(II) complexes (5a and 5b) were dissolved in water and their molar conductance (concentration range 10^{-3} mol dm^{-3}) were determined at room temperature. The molar conductances of 13.12 Ω^{-1} mol^{-1} cm^2 and 12.93 Ω^{-1} mol^{-1} cm^2, respectively for the Co(II) and Cu(II) complexes, indicate that the metal complexes are non-electrolytic in nature.[41]

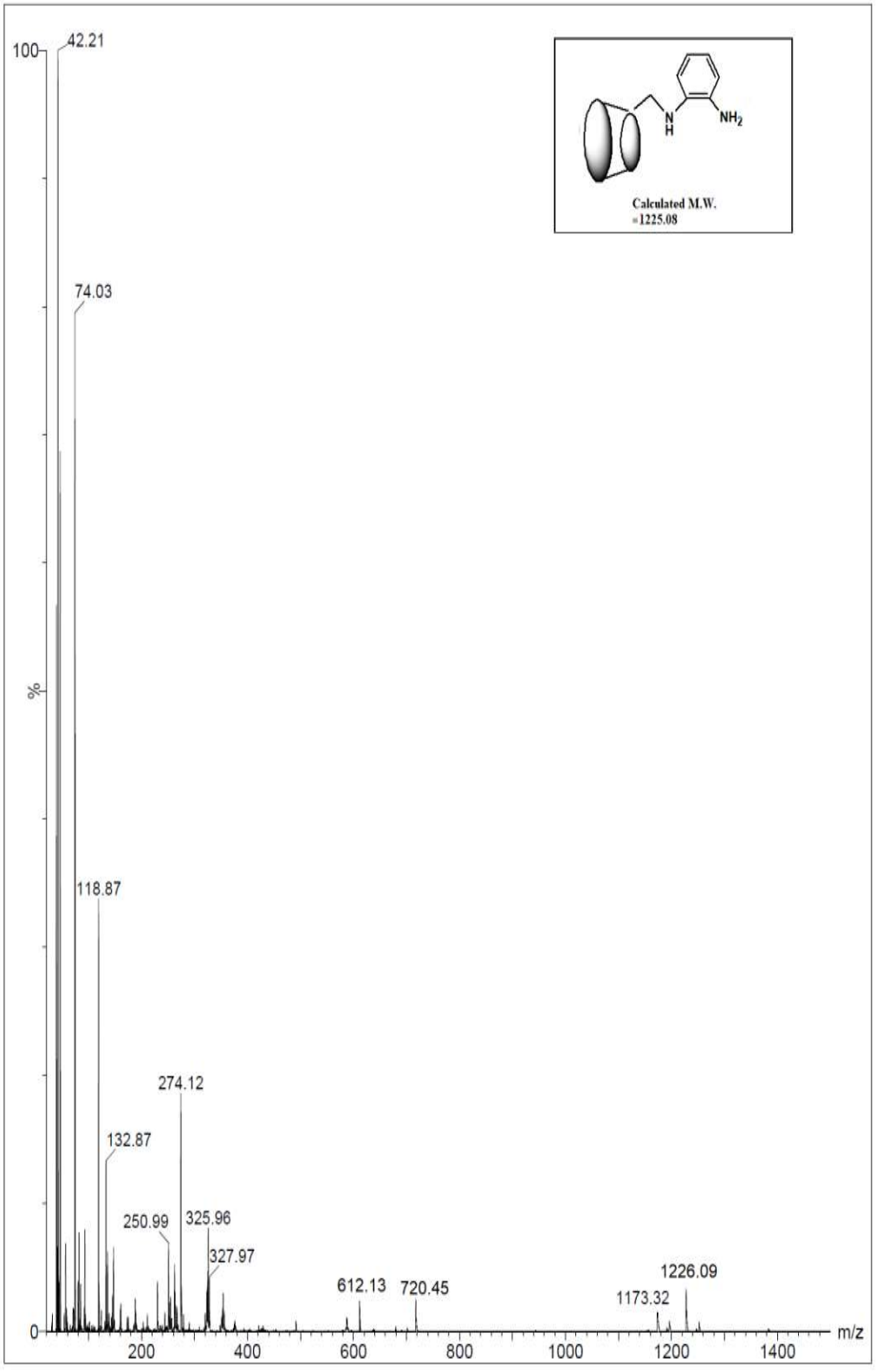


Fig 4.16. ESI-MS spectrum of mono-6-deoxy-6-(1,2-diamino)-*β*-cyclodextrin (2).

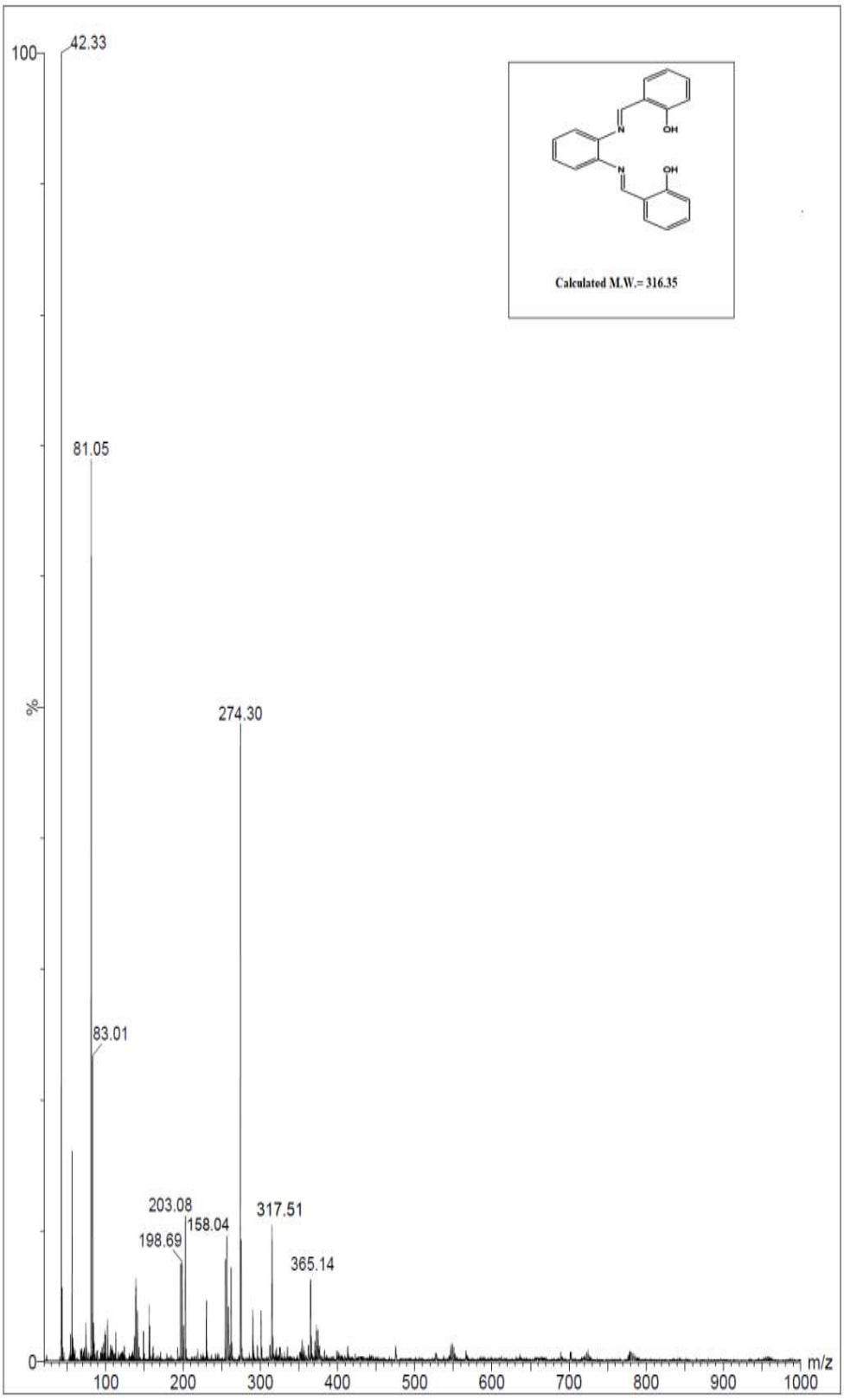


Fig 4.17. ESI-MS spectrum of the Schiff base (3).

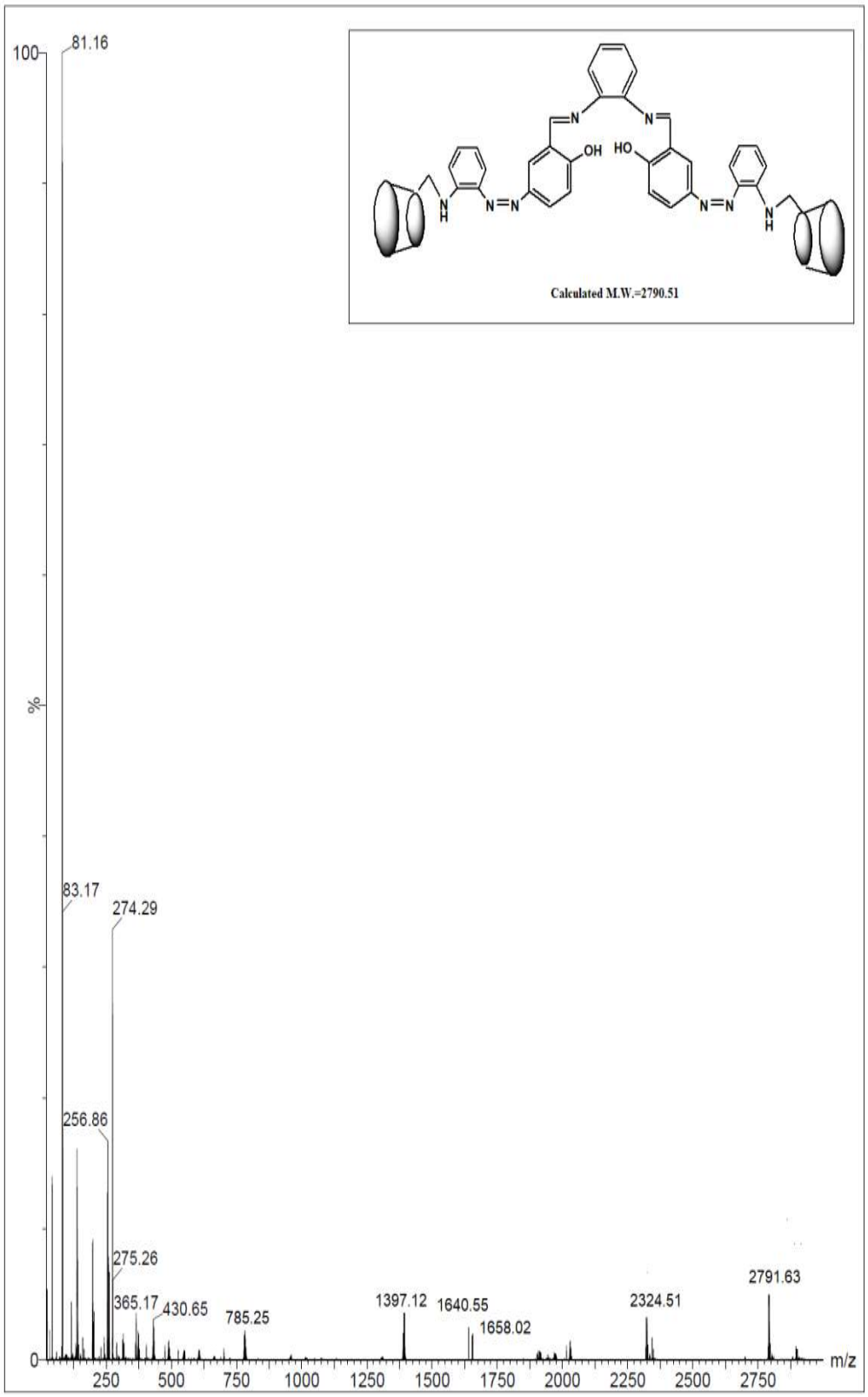


Fig 4.18. ESI-MS spectrum of the amino modified *β*-cyclodextrin supported azo Schiff base ligand(4).

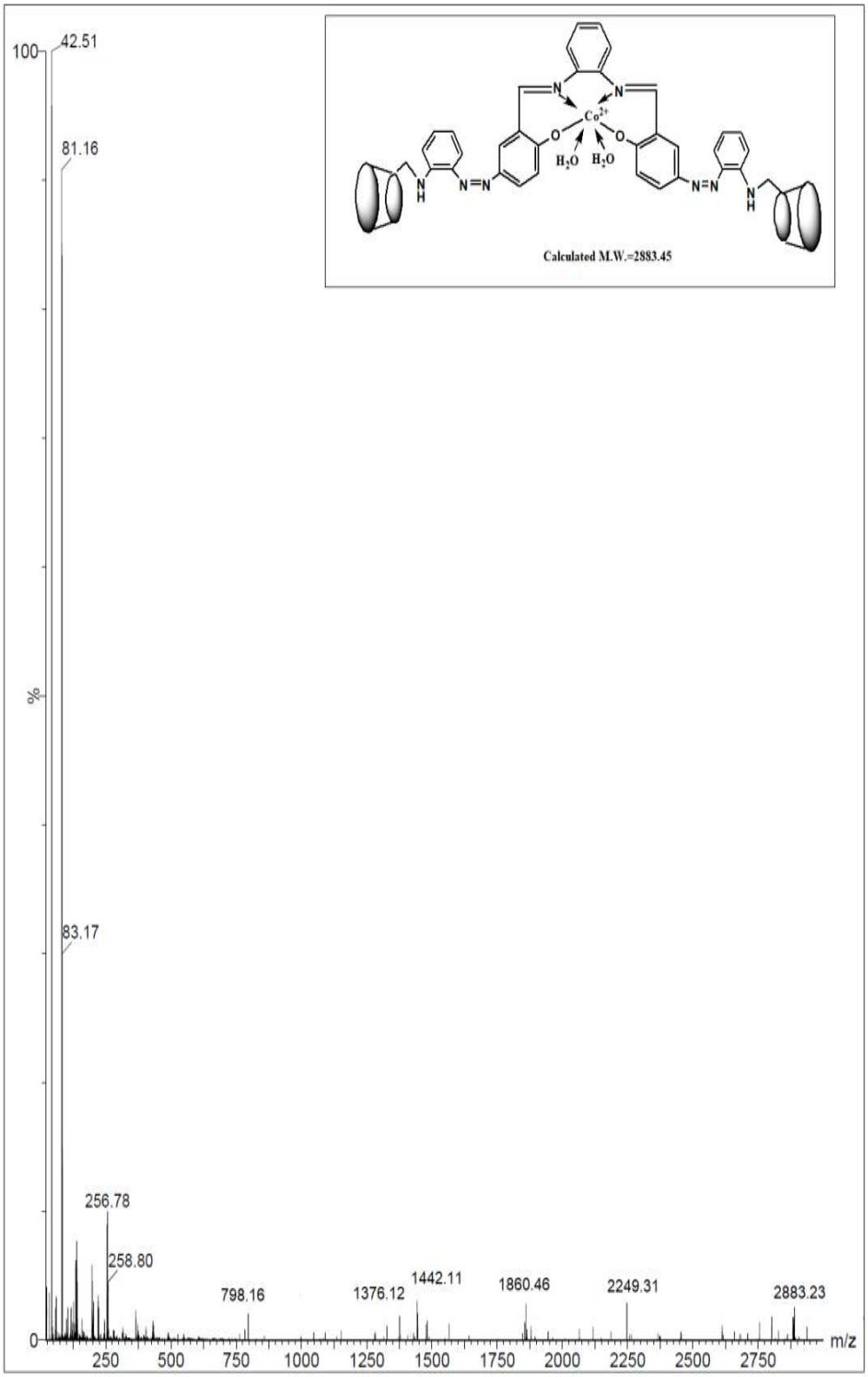


Fig 4.19. ESI-MS spectrum of Co(II) complex the amino modified β-cyclodextrin supported azo Schiff base ligand (5a).

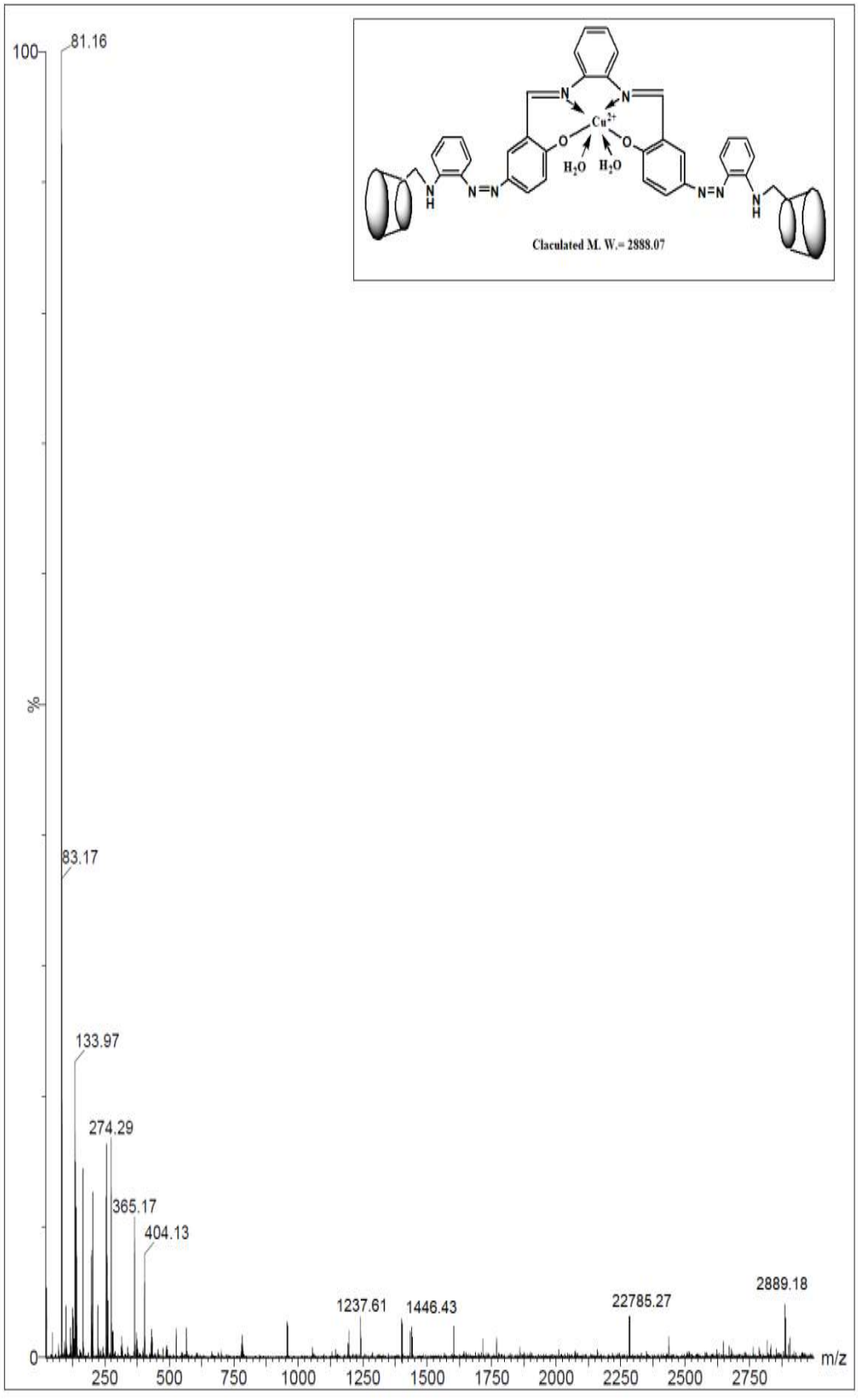


Fig 4.20. ESI-MS spectrum of Cu(II) complex of the amino modified *β*-cyclodextrin supported azo Schiff base ligand (5b).

4.3.6. DNA binding study

4.3.6.1. Electronic absorption titration

To study the binding mode of the metal complexes with DNA, electronic absorption spectroscopy serves as a most common tool. When a metal complex binds to DNA through intercalative mode (because of strong stacking interaction commence between an aromatic chromophore and DNA base pairs) results into hypochromism shift and bathochromism.[42] Absorption spectra of complexes (5a and 5b) in presence of DNA are shown in Figure 4.21 and Figure 4.22, respectively.

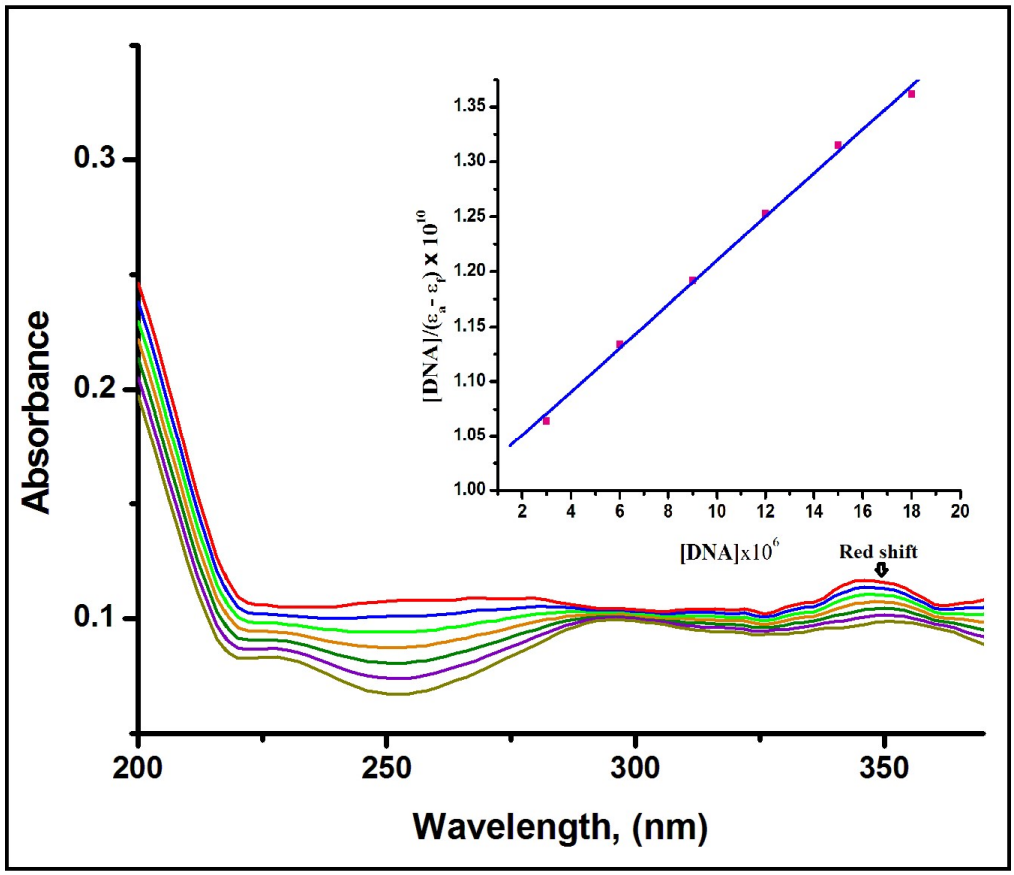


Fig 4.21. Absorption spectra of Co(II) complex (red line) in absence and presence of increasing amount of CT DNA (0 -15 μM), Inset: plot for binding constant (K_b).

The characteristic peak of the complex (**5a**) and the complex (**5b**) (around~360 nm) exhibited bathochromic shift (~4 nm for complex **5a** and ~3 nm for complex **5b** in n-π* band) and spectra show hypochromic shifts with increasing concentration of DNA in complex solutions indicate that they bind to CT-DNA by intercalation mode.[43] The intrinsic binding constants (K_b) of two complexes with DNA were determined using Wolfe-Shimer equation and the corresponding value for the complexes (5a and 5b)

were obtained as (1.97±0.06) x 10^4 and (1.15±0.06) x10^4 M^{-1}, respectively. Interestingly, the binding strength of the complex 5a is greater than the complex 5b.

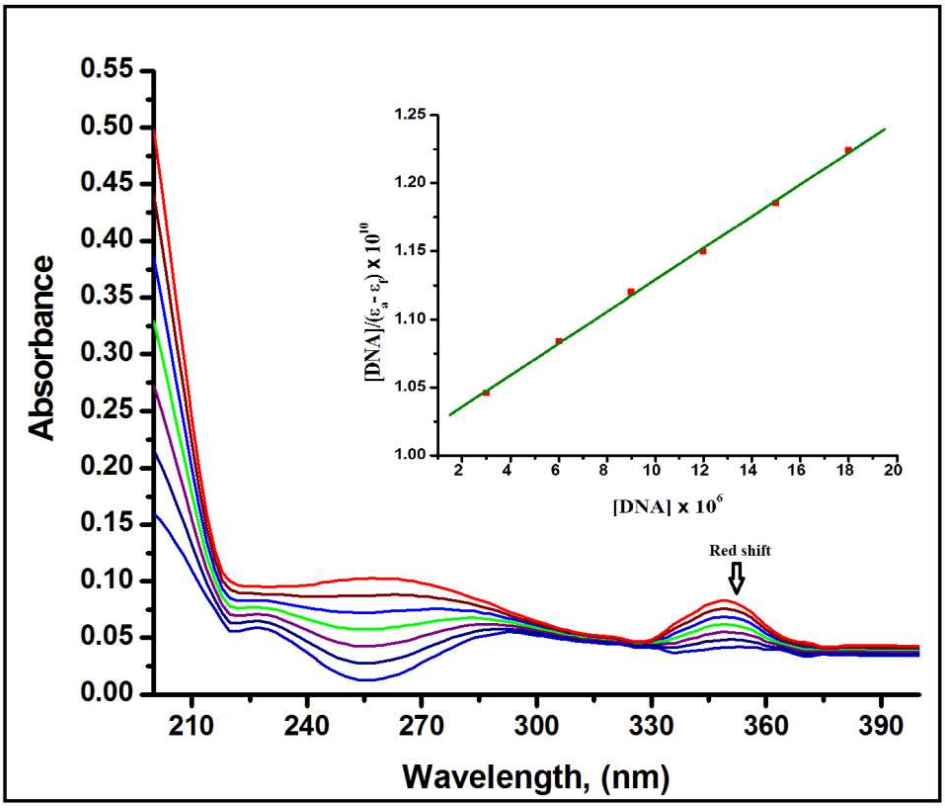


Fig 4.22. Absorption spectra of Cu(II) complex (red line) in absence and presence of increasing amount of CT DNA (0 -15 μM), Inset: plot for binding constant (K_b).

4.3.6.2. Ethidium Bromide (EB) competitive study with fluorescence emission spectroscopy

The probe ethidium bromide (EB) shows intense fluorescence at about 580-600 nm in the presence of CT-DNA, because of tenable intercalation between the adjacent DNA base pair. The fluorescence emission band of EB-DNA conjugate can be quenched significantly by the addition of a second molecule (intercalate to DNA equally or stronger than EB)[44] due to the displacement of the bound EB. The synthesized complexes (5a and 5b) do not exhibit any emission band in solution or in the presence of CT- DNA or EB when excited at 540 nm. So the changes occur in the emission spectra of EB-DNA solution on addition of the complexes are vital to test the EB displacement ability of the synthesized complexes. Fluorescence emission band intensities at 599 nm were observed to be quenched on increasing complex concentrations and thus revealed that complexes depose DNA- bound EB and itself

bind to DNA (Figure 4.23 and Figure 4.24). There figures show that the fluorescence quenching curve of EB-bound CT-DNA by complexes is in good agreement with the classical Stern –Volmer equation. The K_{sv} values of the Co(II) (5a) complex and the Cu(II) (5b) complex are $3.28x10^4$ and $4.17x10^4$, respectively, indicating the binding affinities of the complexes with CT-DNA are in the order of 5a > 5b. There results significantly suggest that the complexes competes with EB as far as intercalative mode of binding with DNA is concerned and they definitely bind to CT-DNA through intercalation mode.[43]

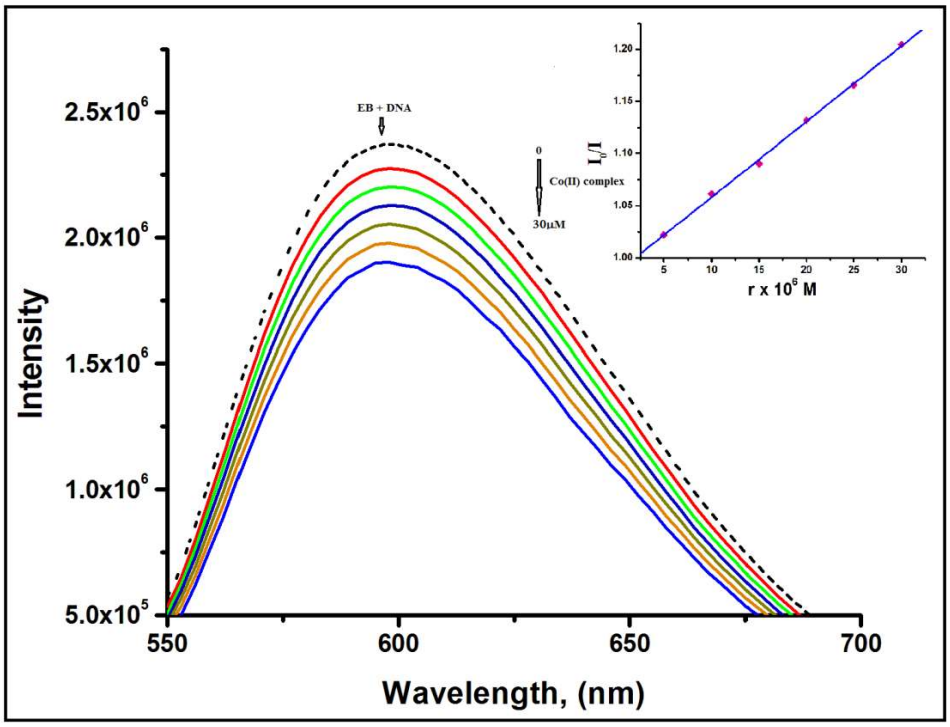


Fig 4.23. Emission spectra of EB bound to the DNA in absence and presence of increasing amount of Co(II) complex (0–30 μM), Inset: plot for quenching constant (Ksv).

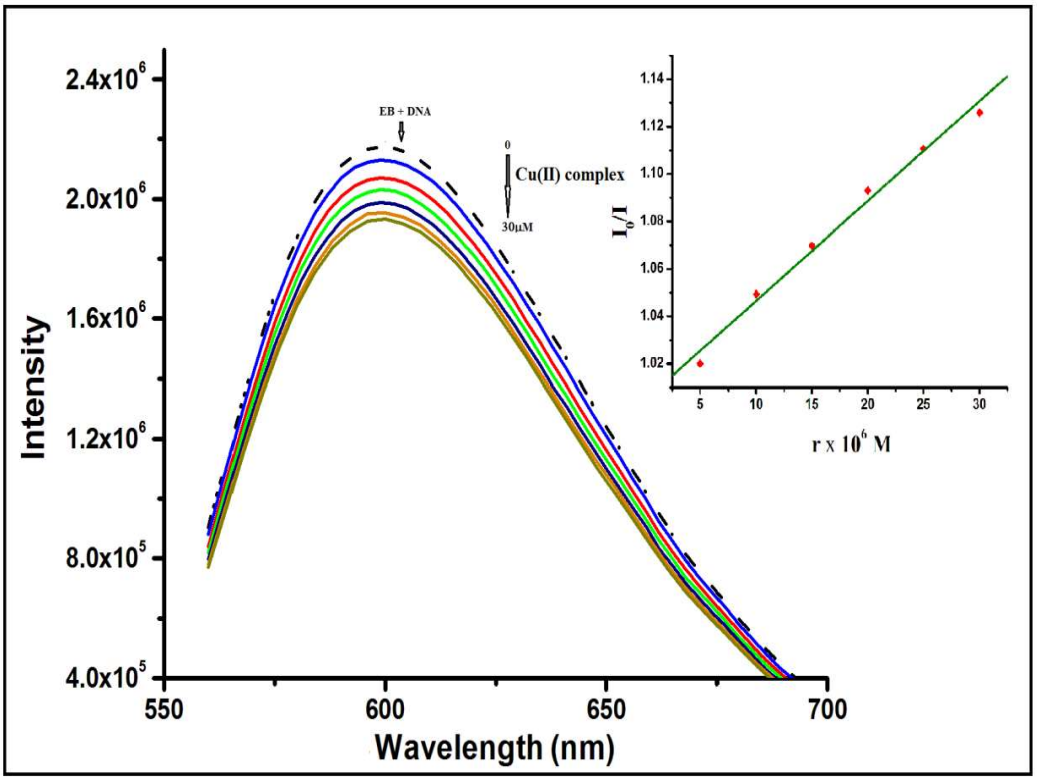


Fig 4.24. Emission spectra of EB bound to the DNA in absence and presence of increasing amount of Cu(II) complex (0–30 μM), Inset: plot for quenching constant (Ksv).

4.3.6.3. Thermal denaturation study

For establishing the degree of intercalation, the DNA melting experiment is a useful technique.[45] The melting temperature (T_m) of DNA generally augment with the intercalation of the complexes into DNA base pairs. [43] Thermal denaturization profile of CT-DNA solution both in absence and in presence of the metal complexes (5a and 5b) were showed in Figure 4.25 and Figure 4.26. The melting temperature (T_m) for CT-DNA solution was 78 $^{\circ}$C. But in presence of the Co (II) and Cu(II) complexes, T_m value increases dramatically to 84.0^{0}C and 81.0^{0}C respectively and the corresponding augments in presence of Co (II) (5a) and Cu (II) (5b) complexes ΔT_m= 6.0^{o}C and 3.0^{o}C respectively indicates intercalative binding of the complexes.[46] However the binding affinities of the complexes with CT-DNA are in the order of 5a > 5b.

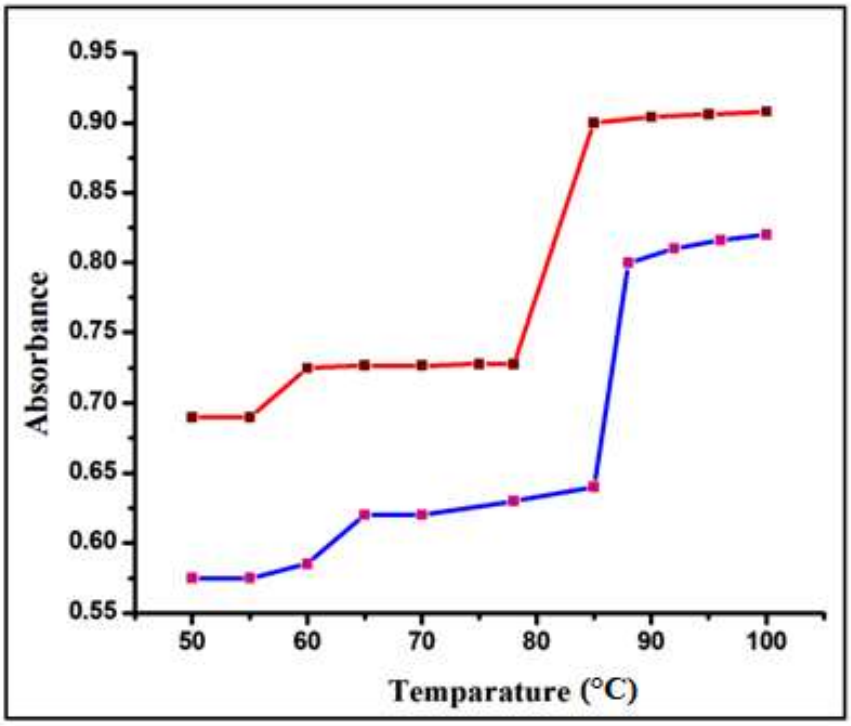


Fig 4.25. Plot of absorbance *vs.* temperature (°C) for the melting of CT DNA alone (red line) and CT DNA + Co(II) complex (blue line).

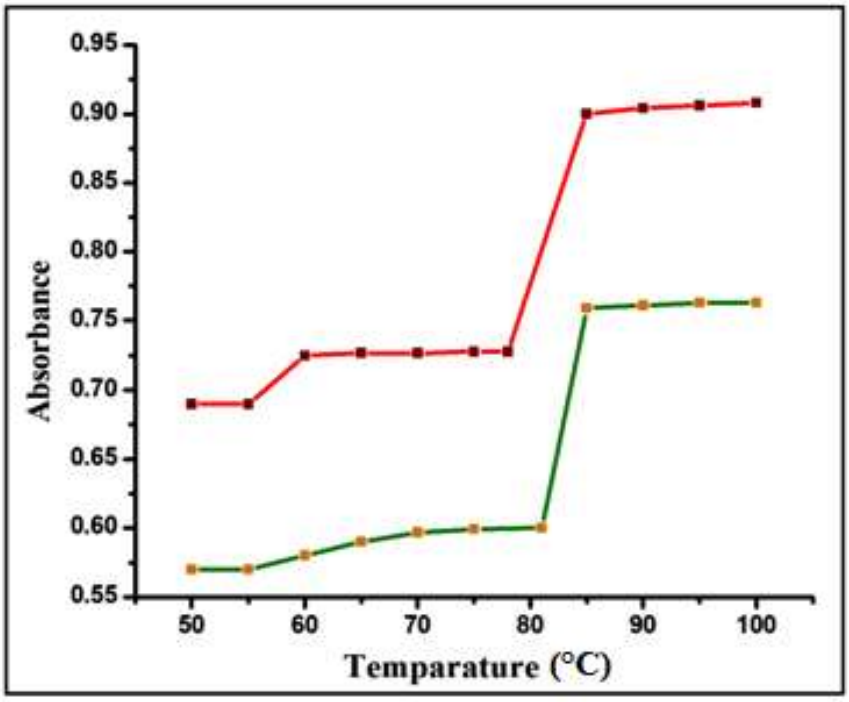


Fig 4.26. Plot of absorbance *vs.* temperature (°C) for the melting of CT DNA alone (red line) and CT DNA + Cu(II) complex (olive line).

4.3.6.4. Viscosity measurement

A classical intercalative mode of binding causes significant increase in viscosity of the DNA solution due to enhanced separation of the DNA base pairs at intercalation sites to accommodate host compounds and it results an increase in overall length of DNA causing an enhancement of the viscosity of the DNA solution.[46] But in contrast, complexes that bind with DNA via non-classic intercalations (like external groove-binding or electrostatic interaction) under the same conditions, results in a kink or bends in the DNA helix. This kink or bends in DNA helix causes slight shortening of the effective length of DNA helix with less pronounced or no change in viscosity. The relative viscosity of DNA increases in presence of Ethidium bromide (a well known DNA intercalator) due to lengthening of DNA helix.

Upon increasing the concentration of each complex (5a and 5b) in the CT-DNA solution individually, the relative viscosity of DNA solution increase steadily almost similar to the nature as in case of Ethidium Bromide (shown in Figure 4.27). In the case of the ligand (4**)** solution such viscosity changes are inconsistent. Therefore enhancement in viscosity in case of the complex solutions suggests that the synthesized complexes bind to DNA via intercalation mode.

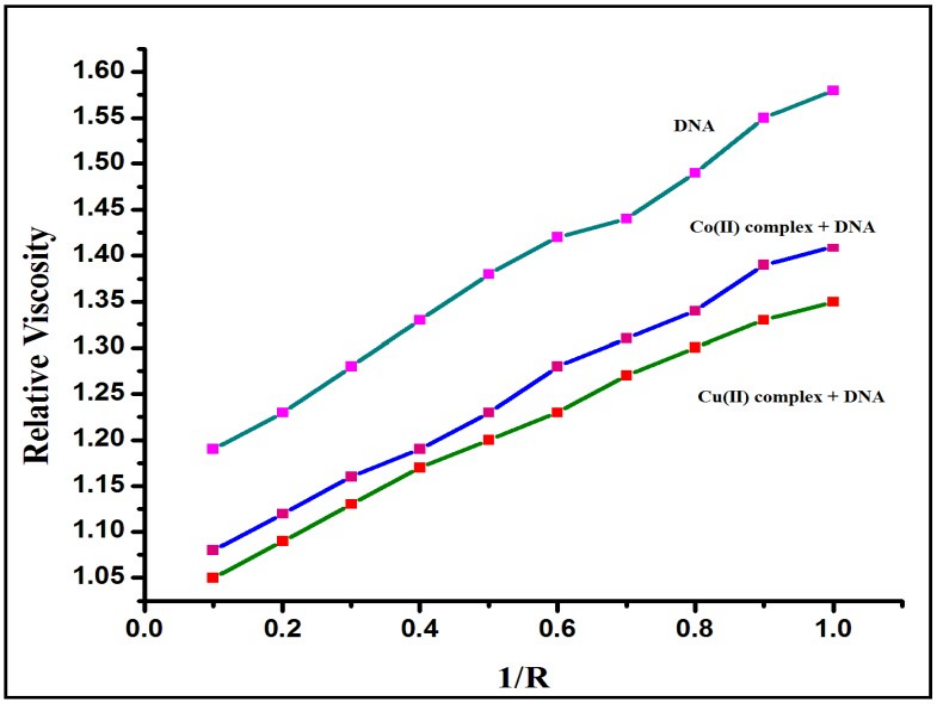


Fig 4.27. Effect of increasing amounts of (a) EB (b) Co(II) complex (c) Cu(II) complex on the relative viscosity of CT-DNA.

4.3.7. DNA cleavage study

The electrophoresis study clearly showed that both the Co(II) and Cu(II) complexes have acted on DNA as there was noticeable difference in bands of the complexes with respect to the band of the control DNA (Figure 4.28). The synthesized complexes are highly potent to transform the supercoiled form (SC) of DNA (Form I) into nicked circular (NC) form (Form II). The experiment is regulated by hydroxyl radical ($HO^{\bullet}$) originated from oxidant H_2O_2. This hydroxyl radical attacks DNA and results into strand scission. The surfacing of smears in the gel photograph supports radical cleavage.[47] This mechanism for oxidative cleavage was earlier suggested by Sigman.[48] As the synthesized complexes were established to cleave DNA quite significantly, so it can be inferred that these complexes will most probably inhibit the growth of pathogens.[49] However, the Co(II) complex shows more smears than the Cu(II) complex.

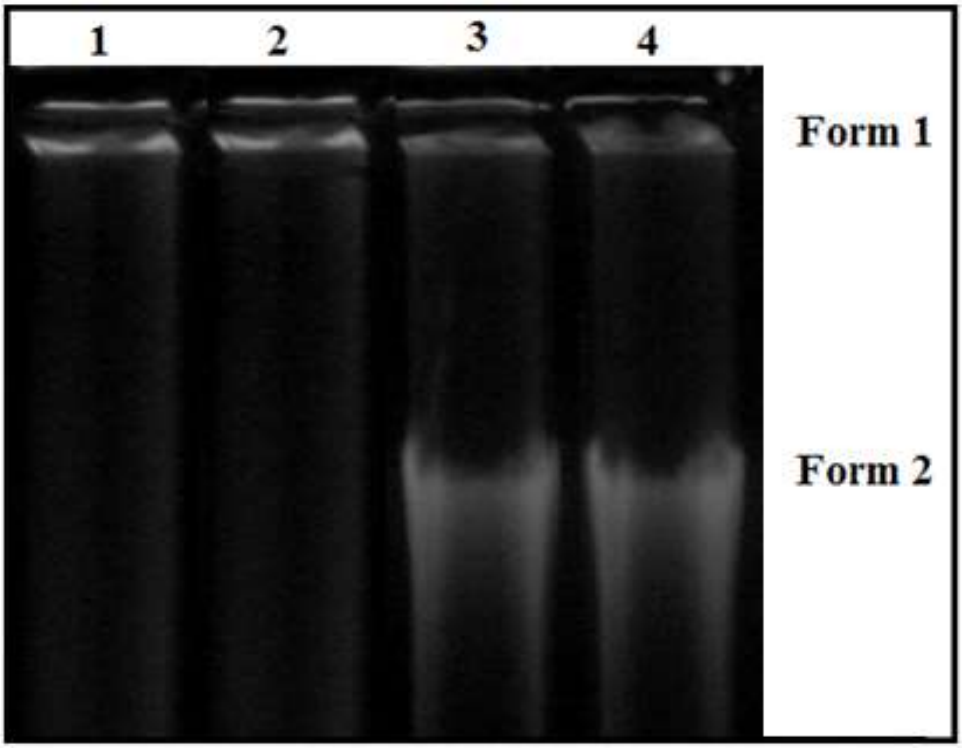


Fig 4.28. Changes in the agarose gel electrophoretic pattern of pBR322 plasmid DNA induced by H_2O_2 for Co(II) and Cu(II) complex. Lane (1): DNA control, Lane (2): DNA + H_2O_2, Lane (3): DNA+ Co(II) complex + H_2O_2, (4): DNA+ Cu(II) complex + H_2O_2.

4.4. Conclusion

In summary, both the synthesized Co(II) and Cu(II) complexes were paramagnetic in nature. The Co(II) complex possesses low-spin distorted octahedral structure and the Cu(II) complex acquires distorted octahedral structure. Both the synthesized complexes are fairly soluble in aqueous phase and highly potent to cleave DNA. It was found that the both complexes bind with CT-DNA through intercalative mode and the Co(II) complex showed better cleavage activity than the Cu(II) complex. Thus the present study revealed that the incorporation of β-CD, azomethine and diazogroup in the same ligand system not only increased the bio-availability of the complexes (through increased aqueous solubility) but also make them good DNA interaction.

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