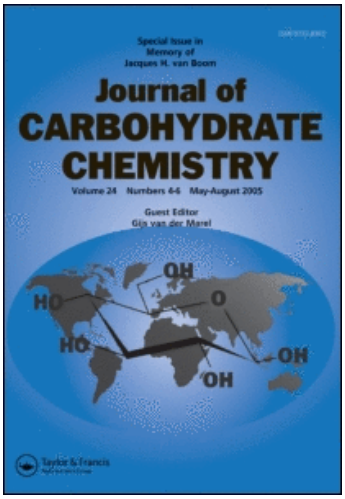

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Synthesis, Characterization, Solution Stability Studies, Electrochemistry, and DNA-Binding Behavior of Cu(II) Complexes of D-Gluconic Acid

Sartaj Tabassum[a]; Suvigya Mathur[a]
[a] Department of Chemistry, Aligarh Muslim University, Aligarh, India

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Synthesis, Characterization, Solution Stability Studies, Electrochemistry, and DNA-Binding Behavior of Cu(II) Complexes of D-Gluconic Acid

Sartaj Tabassum and Suvigya Mathur

Department of Chemistry, Aligarh Muslim University, Aligarh, India

The chemistry of saccharides has emerged as a new subarea of pharmaceuticals. Condensation reactions of D-gluconic acid with $[M(en)_2]Cl_2/[M(ea)_2]Cl_2$ where M = Cu, Ni, en = ethylenediamine, and ea = ethanolamine were carried out and a new series of chiral complexes have been isolated and characterized. Molar conductance measurements show that the complexes are ionic, and the spectral data are indicative of octahedral geometry of the complexes $[Cu(D\text{-}GlcCO_2H\ en^*)_2\ (H_2O)_2] \cdot Cl_2$ **(1b)**, $[Cu(D\text{-}GlcCO_2H\ ea^*)\ (H_2O)_2] \cdot Cl_2$ **(3b)** and $[Ni(D\text{-}GlcCO_2H\ ea^*)\ (H_2O)_2] \cdot Cl_2$ **(4)**, and the square planar geometry of complex $[Ni(D\text{-}GlcCO_2H\ en^*)_2] \cdot Cl_2$ **(2b)**. Polarimetric data along with CD spectra establish the chiral nature of complexes. Solution stabilities of these complexes were evaluated by cyclic voltammetric techniques as a function of pH. Electrochemical behavior of the complexes was studied in aqueous solution and showed an irreversible Cu^{II}/Cu^{I} couple. Kinetic studies of complex **1b** and **3b** with calf thymus DNA have been investigated spectrophotometrically under pseudo-first order conditions, and k_{obs} values have been evaluated. Circular dichroism, cyclic voltammetry determinations, and viscosity measurements have also been carried out to authenticate the binding of DNA with metal complexes. Complexes **1b** and **3b** bind to DNA by covalent bond formation.

Keywords Transition metal–D-gluconic acid complexes, DNA-binding behavior, Cyclic voltammetry, Solution stability, Viscosity measurements

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Address correspondence to Prof. Sartaj Tabassum, Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India. E-mail: tsartaj62@yahoo.com

INTRODUCTION

The metal binding properties of carbohydrates are of potential importance in the field of bioinorganic chemistry, owing to the fact that both carbohydrates and metal ions coexist in biologic systems.[1] Sugar-metal ion interactions are of fundamental importance in many biochemical processes such as the transport and storage of metal ions,[2] stabilization of membrane structures,[3] binding of glycoproteins to cell surfaces,[4] toxic metal metabolism,[5] binding of proteins to sugars,[6] and function and regulation of metalloenzymes, and among other processes. Furthermore, carbohydrates are established as potential ligands, not only because of their biocompatible nature, but also due to the presence of their multihydroxy functionality and well-defined stereochemistry, which is conducive for metal coordination.[7] Besides this, carbohydrates and their derivatives such as gluconic acid, which contain both $-OH$ and $-COOH$ functional groups, are used extensively as sequestering agents.[8] They can form chelates with toxic metal ions, thus making them less available to plants, showing their pertinent application to agricultural science.[9]

Insight into the binding modes of metals to carbohydrates may lead to the discovery of new chiral auxiliaries[10] for improved enantioselectivity in organic synthesis[11] and in the design of new metal-carbohydrate-based pharmaceuticals.[12] Chirality is one of the key structural factors of many biologic activities; thus, the homochiral nature of carbohydrates can be well exploited to synthesize potent anticancer drugs. Previous studies on basic aminoglycoside antibiotics reveal that these bind DNA at the negatively charged phosphate groups by electrostatic interaction[13] and are capable of modifying nucleic acids only in the presence of metal ions and oxygen.[14,15] Thus, interest arose to investigate whether a metal complex of a carbohydrate ligand could affect a DNA helix and thereby demonstrate potential antitumor activity. Although there is a paucity of reports on saccharide-metal complex DNA interactions, DNA cleavage activity of some of the saccharide complexes containing Fe(III) and Cr(III) metal ions has been investigated.[7,9] Furthermore, copper complexes are known to have a broad spectrum of biologic interactions[16] and have been extensively utilized as effective cleaving agents[17] or useful DNA probes.[18] However, the biointeraction studies of Cu(II)-saccharide complexes are still unexplored. Despite the significant importance of carbohydrates, their use in inorganic chemistry is limited because of the difficulty in isolating discrete compounds,[19] their extremely hygroscopic nature, their complicated stereochemistry,[8] and, moreover, their poor crystallinity.[20]

Herein, we describe the synthesis, characterization, solution stability studies, electrochemistry, and DNA binding properties of optically active complexes of D-gluconic acid derived from its reaction with bis(ethylenediamine/ethanolamine) Ni^{II}/Cu^{II} chloride.

EXPERIMENTAL

Materials

All reagents were of the best commercial grade and were used as received. Ethanolamine, ethylenediamine, $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, and tris buffer (GR) were procured from E. Merck. D-gluconic acid were obtained from s.d. Fine-Chem Ltd. Calf thymus DNA was purchased from Sigma Chemical Company. All the experiments involving interaction of the complexes with DNA were conducted in tris HCl buffer of pH 7.5. The CTDNA was dissolved in 0.01 M tris-HCl buffer and was dialyzed against the same buffer overnight. Solution of DNA gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} $\sim$1.9, indicating that the DNA was sufficiently free of protein. The extinction coefficient ε_{260} was taken as 6600 M^{-1} cm^{-1}.[21] Stock solutions were stored at 4°C. Experiments involving the interaction of complexes with CTDNA were carried out with varying concentrations of CTDNA $4-6.4 \times 10^{-5}$ M. The following abbreviations are used: en = 1,2, diaminoethane, ea = ethanolamine, en* = deprotonated/condensed 1,2, diaminoethane, ea* = deprotonated/condensed ethanolamine, D-GlcCO_2H = condensation product of D-gluconic acid, LNT = liquid nitrogen temperature, and TCNE = tetracyanoethylene.

Instrumentation

Final, purified products were characterized by several routine analytical, spectroscopic, and electrochemical techniques. The compounds were highly hygroscopic, and hence special precautions were necessary while handling. Carbon, hydrogen, and nitrogen contents were analyzed on a Carlo Erba Analyzer Model 1106. Molar conductances were measured at room temperature on a Digisun Electronic Conductivity Bridge. Fourier-transform IR (FTIR) spectra were recorded on an Interspec 2020 FTIR spectrometer as KBr pellets. The UV/vis absorption studies were carried on a Systronics119 spectrophotometer (ESP-300), and kinetics experiments were performed on an (USB 2000) Ocean Optics spectrometer. The solid-state EPR spectra were acquired on a Varian ESR-112 spectrometer. Circular dichroism spectra were recorded on a Jasco J-710 CD spectropolarimeter. The NMR spectra were obtained on a Bruker DRX-300 spectrometer. Specific rotations of the complexes were obtained on a Rodulf Autopole polarimeter at 20°C at the sodium D line in a 1-dm tube containing the complex dissolved in water. Cyclic voltammetric studies were performed on a CH Instrument Electrochemical analyzer using a three-electrode configuration composed of a Pt wire as the auxiliary electrode, a platinum micro-cylinder as the working electrode, and Ag/AgCl as the reference electrode. Electrochemical measurements were

made under nitrogen atmosphere. All electrochemical data were collected at 298 K and are uncorrected for junction potentials. Viscosity measurements were carried out using Ostwald's viscometer at $29 \pm 0.01^{\circ}C$. Flow time was measured with a digital stopwatch. Each sample was measured three times, and an average flow time was calculated. Data are presented as (η/η_0) versus binding ratio ([Cu]/[DNA]), where η is a viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution ($t > 100$ s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

Synthesis of bis(ethylenediamine) Cu(II)/Ni(II) Complexes (1a and 2a) and Synthesis of a bis(ethanolamine) Cu(II) Complex (3a)

All the complexes have been synthesized by the procedure reported earlier.[22]

Synthesis of $(Cu(D\text{-}GlcCO_2H\ en^*)_2\ (H_2O)_2) \cdot Cl_2$ (1b)

To a methanolic solution of **1a** (0.253 g, 1 mmol) was added D-gluconic acid (0.318 mL, 2 mmol). Immediately, a deep blue product precipitated was collected, washed with hexane, and dried in vacuo. Yield 0.37 g (65%): $[\alpha]_D^{25} + 29$ (c 0.2, H_2O): UV/Vis [λ_{max}; nm (ε; L cm^{-1} mol^{-1})]: 232 (1647), 295 (1740), 650 (83). Anal. calcd. for $C_{16}H_{36}Cl_2CuN_4O_{12}$: C, 31.52%; H, 5.20%; N, 9.19%, Found: C, 31.49%; H, 5.20%; N, 9.17%. IR (KBr disc, cm^{-1}): 1600 ν (C═N), 3175 ν (C–N), 1360 δ (N–H), 1460 [δ (OCH, CH_2, CCH)], 1085 [ν (CO, CC)], 760, 804 cm^{-1} [δ (CCH,CH) ν (CC · CO)], 535 ν (M–N).

Synthesis of $(Ni(D\text{-}GlcCO_2H\ en^*)_2) \cdot Cl_2$ (2b)

This complex was obtained with a procedure analogous to that for **1b** using **2a** in dry MeOH (0.744 g, 3 mmol). Yield 0.71 g (67%): $[\alpha]_D^{25} + 13$ (c 0.2, H_2O): UV/Vis [λ_{max}; nm (ε; L cm^{-1} mol^{-1})]: 232 (1647), 293 (1700), 580 (91). Anal. calcd. for $C_{16}H_{32}Cl_2N_4NiO_{10}$: C, 33.80%; H, 5.63%; N, 9.85%. Found: C, 33.79%; H, 5.62%; N, 9.83%. IR (KBr disc, cm^{-1}): 1602 ν (C═N), 3168 ν (C–N), 1358 δ (N–H), 1456 [δ (OCH, CH_2, CCH)], 1080 [ν (CO, CC)], 754, 800 cm^{-1} [δ (CCH,CH) ν (CC · CO)], 560 ν (M–N). ^1H NMR (D_2O, ppm): 6.5 (N–H), 3.2–4.8 (skeleton proton of D-gluconic acid). ^{13}C NMR (D_2O, ppm): 177 (C_1 = N), 75, 74, 73, 72 65 (skeleton carbon atoms of D-gluconic acid from C_2 to C_6), 41.6, 39.2 (CH_2–CH_2).

Synthesis of ($Cu(D\text{-}GlcCO_2H\ ea^*)\ (H_2O)_2) \cdot Cl_2$ (3b)

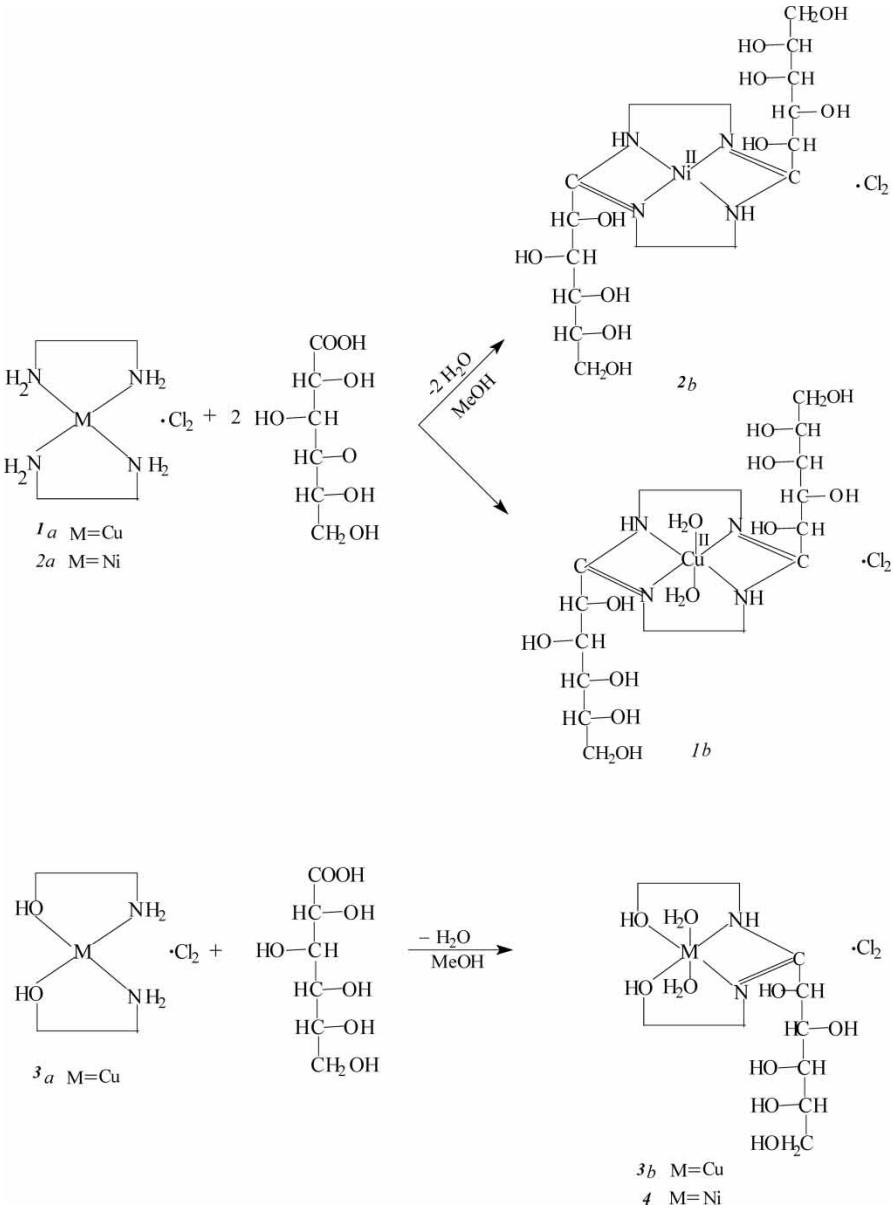
Complex **3a** (0.51 g, 2 mmol) was dissolved in MeOH (50 mL). To this solution was added D-gluconic acid (0.31 mL, 0.2 mmol) dropwise with constant stirring. A deep green amorphous product was obtained that was separated, washed with hexane, and dried in vacuo. Yield 0.55 g (68%): mp 210°C. $[\alpha]_D^{25}$ −59 (*c* 0.2, H_2O): UV/Vis [λ_{max}; nm (ε; L $cm^{-1}\,mol^{-1}$)]: 232 (1644), 300 (1750), 712 (75). Anal. calcd. for $C_{10}H_{26}Cl_2CuN_2O_9$: C, 26.20%; H, 5.76%; N, 6.26%. Found: C, 26.26%; H, 5.73%; N, 6.29%. IR (KBr disc, cm^{-1}): 1608 ν (C = N), 3170 ν (C–N), 1363 δ (N–H), 1462 [δ (OCH, CH_2, CCH)], 1082 [ν (CO, CC)], 758, 801 cm^{-1} [δ (CCH,CH) ν (CC · CO)], 570 ν (M–N), 547ν (M–O).

Synthesis of ($Ni(D\text{-}GlcCO_2H\ ea^*)\ (H_2O)_2) \cdot Cl_2$ (4)

A mixture of ethanolamine (0.12 mL, 2 mmol) and $NiCl_2 \cdot 6H_2O$ (0.237 g, 1 mmol) dissolved in 50 mL of MeOH gave a bluish green solution. The color of the solution turned to light green when D-gluconic acid (0.159 mL, 1 mmol) was added. The light green amorphous product precipitated within 5 min by setting the solution aside. The precipitate was isolated, washed with hexane, and dried in vacuum. Yield 0.387 g (75%): $[\alpha]_D^{25}$ −40 (*c* 0.2, acidic H_2O): UV/Vis [λ_{max}; nm (ε; L $cm^{-1}\,mol^{-1}$)]: 293 (1702), 390 (823), 690 (89). Anal. calcd. for $C_{10}H_{26}Cl_2N_2NiO_9$: C, 26.90%; H, 4.93%; N, 6.27%. Found: C, 27.91%; H, 5.00%; N, 6.24%. IR (KBr disc, cm^{-1}): 1610 ν (C═N), 3172 ν (C–N), 1361 δ (N–H), 1457 [δ (OCH, CH_2, CCH)], 1082 [ν (CO, CC)], 758, 801 cm^{-1} [δ (CCH,CH) ν (CC · CO)], 600 ν (M–N), 540 ν (M–O).

RESULTS AND DISCUSSION

The corresponding chiral Schiff-base complexes were synthesized via the condensation reaction of compounds **1a**, **2a**, and **3a** with D-gluconic acid as depicted in Sch. 1. Complexes **1b**, **2b**, **3b**, and **4** were found to be soluble only in water and were insoluble in common organic solvents. However, complex **4** is soluble in slight acidic aqueous solution. All the complexes exhibited aqueous molar conductivity, which is in good agreement with the proposed complex formulas and also correlate their 1 : 2 electrolyte behavior. Complexes **1b** and **2b** are hygroscopic, while complexes **3b** and **4** are not moisture sensitive and are acquired in good yields. All the complexes have been characterized extensively both in solid and in solution states. Furthermore, all the aqueous solution spectral analyses were carried out using freshly prepared solutions. Crystallization of the complexes was not easy, probably because of the structural flexibility of the metal-sugar complexes. And in the absence of crystal structure, a definite conclusion regarding the three-dimensional structure could not be obtained. Regardless of this fact, all the complexes were exhaustively characterized by IR, UV/vis, CD, and EPR ^1H, ^{13}C, and 2D COSY



Scheme 1

NMR spectroscopy. The optical rotation $[\alpha]_D^{25} + 29$ (*c* 0.2, H_2O), + 13 (*c* 0.2, H_2O), −59 (*c* 0.2, H_2O) and −40 (*c* 0.2, acidic H_2O) for complexes **1b**, **2b**, **3b**, and **4**, respectively, indicate that complexes are optically active. Kinetic experiments were performed using copper complexes (**2b** and **3b**), and analogous nickel complexes were synthesized only for NMR studies.

FTIR Studies

The FTIR spectra of all the sugar complexes were recorded as KBr pellets and exhibited merged and broadened bands with the loss of fine structures. Complexes **1b**, **2b**, **3b**, and **4** showed the presence of sharp ν (C=N)[23] stretching vibrations at 1600, 1602, 1608 and 1610 cm^{-1}, respectively, supporting the formation of the imino center. In addition, the bands at ~3180 $cm^{-1}$and 1351–1379 cm^{1} are ascribed to ν (N–H) and (C–N) groups, respectively. Free gluconic acid shows a ν (O–H) band in the region of 3500–3200 cm^{-1}.[24] However, the complexes exhibited a broad envelope around ~3400 cm^{-1}, which is further complicated due to overlapping of ν (O–H) of water molecules present as moisture in these hygroscopic compounds. The FTIR spectra of complexes **1b**, **2b**, **3b**, and **4** showed the occurrence of broad bands for all the vibrational modes, which rendered the individual assignments difficult; however, the ranges of different frequencies could be identified. The skeletal vibrations centered around 1465 cm^{-1} [δ (OCH, CH_2, CCH)], 1080 cm^{-1} [ν (CO, CC)], 765 and 810 cm^{-1} [δ (CCH,CH) ν (CC · CO)] depict the presence of D-gluconic acid in the metal complexes. All the metal complexes exhibited broadening of band around 530–605 cm^{-1}, which makes the assignment of ν (M–O) and ν (M–N) very difficult.

Solution Absorption Studies

Absorption spectra of freshly prepared aqueous solution of the complexes **1b**, **2b**, **3b**, and **4** were recorded in the region of 200–800 nm, as shown in Fig. 1. The bands observed in the range 232 nm to 253 nm are ascribed to possible ligand transitions influenced by the presence of metal ions. The electronic spectra of complex **2b** show ligand → metal charge transfer bands in the region 280 to 306 nm and a prominent broad band in the region 561–599 nm attributed to an $^1A_{1g} \rightarrow {}^1A_{2g}$ transition indicative of square planar geometry of the complex.[25] However, the solution spectra of complex **4** exhibits three principal bands around 293 nm, 390 nm, and 690 nm assigned to the three spin-allowed $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions, respectively, for an octahedral Ni^{II} complex.[26] Crystal field theory provides a simple interpretation of the spectra of Ni^{II} in the fields of O_h symmetry.[27] The ratio ν_2/ν_1 of the frequencies of the first and second band maxima lie in most octahedral Ni ^{II} complexes between 1.5 and 1.7. The value (1.7) obtained for complex **4** falls within this range, which supports the octahedral geometry around the Ni^{II} ion in complex **4**. In the UV/Vis spectra of complexes **1b** and **3b** recorded in water, bands are observed at ~650 nm and ~712 nm corresponding to *d-d* bands, followed by a high-intensity band at 295 nm and 300 nm, respectively. The *d-d* bands are assigned to the $^2E_g \rightarrow {}^2T_{2g}$ transition,[28] and the high-intensity bands are

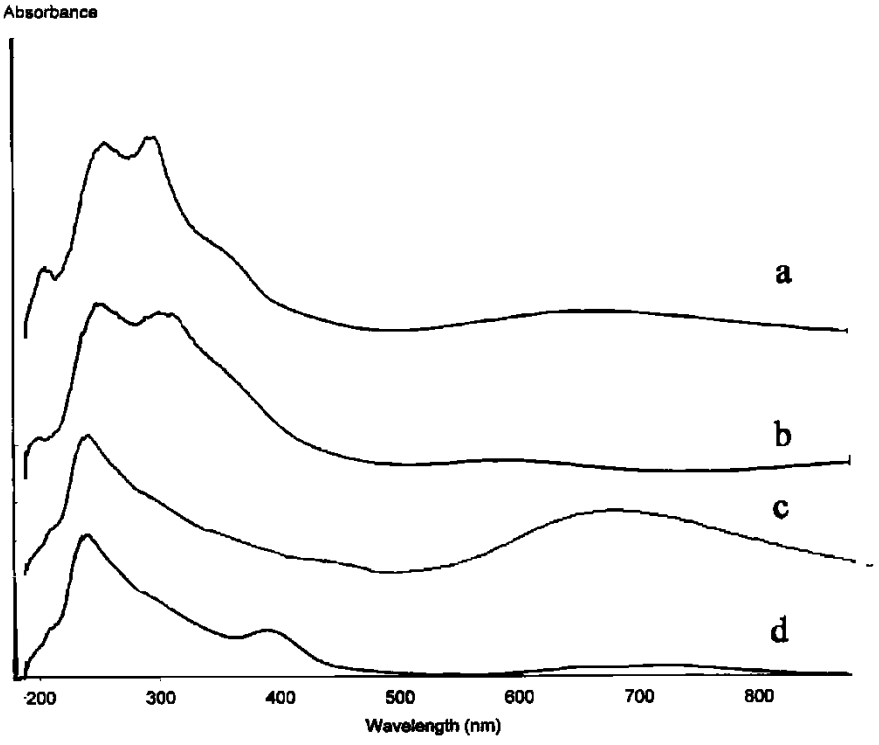


Figure 1: Absorption spectra of (a) complex **1b**, (b) complex **2b**, (c) complex **3b**, and (d) complex **4** in water.

attributed to ligand → metal charge transfer transitions, indicating the presence of a typical octahedral geometry around the Cu(II) ion.

EPR Studies

The X-band EPR spectra of complexes **1b** and **3b** have been recorded at liquid nitrogen temperature (LNT) in the solid state using TCNE ($g = 2.00277$) as a field marker. A typical EPR spectrum of complex **1b** is depicted in Fig. 2. The spectra are isotropic and exhibited a band at $g \approx 2$, which is characteristic for an octahedral copper complex.[29] Similar spectra having 'g' isotropic values were reported by C.P. Rao and co-workers[7,30] for metal-saccharide complexes composed of different transition metal ions.

NMR Studies

The ^1H NMR spectrum of complex **2b** in D_2O is not very informative due to the presence of broad signals. These broad signals with a bunch of fine structures in the region of 3.2–4.8 ppm are the result of a strongly coupled system of the hydroxyl protons of D-gluconic acid, which makes the

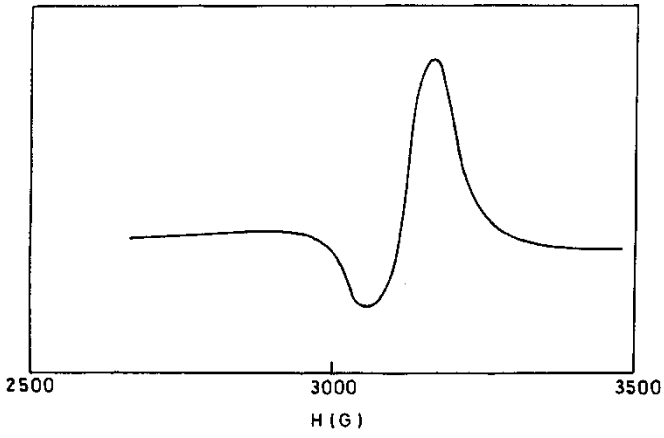


Figure 2: X-Band EPR spectrum at 77K of solid sample of complex **3b**.

assignment of individual resonances very difficult. Free gluconic acid shows hydroxyl protons in the region of 3.2–4.8 ppm. The retention of these signals in ^{1}H NMR spectrum of complex **2b** indicate that hydroxyl groups are not involved in the complex formation through deprotonation. However, the absence of a broad carboxylic group proton resonances of free gluconic acid at 8.5 ppm[31] indicate the formation of a metal-gluconic acid complex. Furthermore, the signal at 6.5 ppm in the 2D COSY ^{1}H NMR spectrum of complex **2b** (Fig. 3) is assigned to the N–H proton,[32] which confirms the condensation of gluconic acid with appropriate complex **2a**.

The ^{13}C NMR spectrum of complex **2b** (Fig. 4) is highly informative in comparison to ^{1}H NMR spectrum. The ^{13}C NMR spectrum of complex exhibits two signals at 41.6 and 39.2 ppm at the higher field ascribed to two carbon atoms of ethylenediamine, while six signals at the lower field were attributed to six carbon atoms of gluconic acid. The peak at 177 ppm is ascribed to $C_1{=}N$, indicating the formation of a Schiff base type complex. Furthermore, peaks of skeletal carbon atoms from C_{-2} to C_{-6} appeared at 75, 74, 73, 72, and 65 ppm, respectively, in the ^{13}C NMR spectrum of the complex **2b**, which is evident of the presence of D-gluconic acid in the complex.[33] Thus, the structure of complex **2b** is in good agreement with the observed ^{1}H and ^{13}C NMR data. However, complex **4** acquires octahedral geometry, as the Ni^{II} ion has two unpaired electrons. Due to the paramagnetic nature of this complex, the ^{1}H and ^{13}C NMR signals are featureless.[34]

Electrochemical Studies

Cyclic voltammetric studies were carried out in 0.4 M aqueous KNO_3 as supporting electrolyte with 10^{-4} M concentration of complexes (using Pt disc

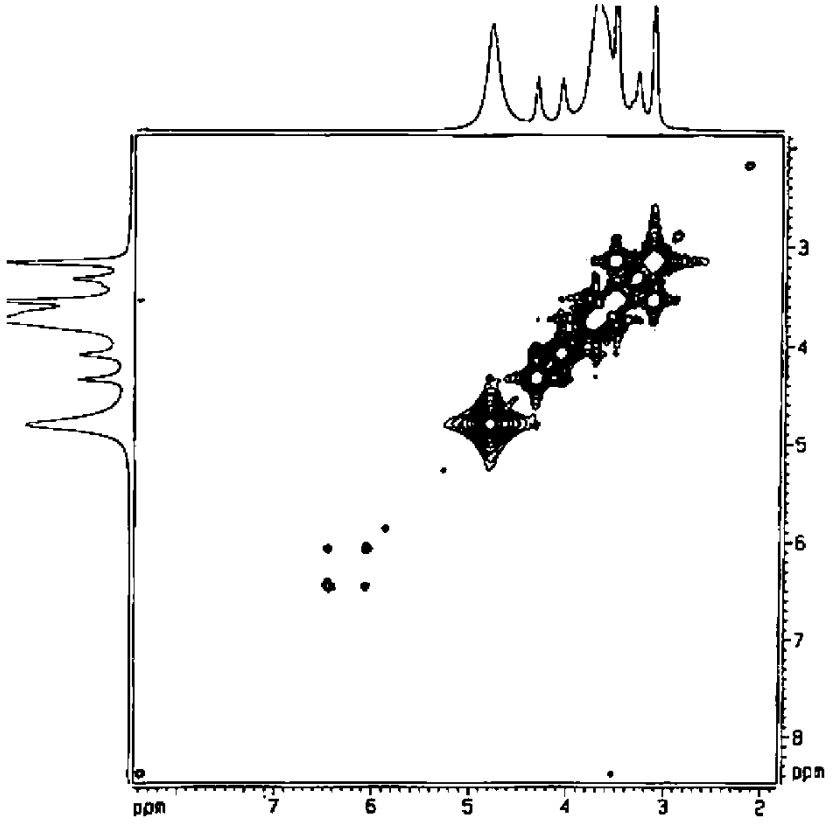


Figure 3: ^{1}H-^{1}H COSY spectrum of complex **2b** in D_2O solution.

as working electrode, Pt wire as counter electrode, and Ag/AgCl as reference electrode) in the potential range 0.1 to −0.1V. The complexes **1b** and **3b** exhibited an irreversible cathodic reduction peak for $Cu^{II} \rightarrow Cu^{I}$ at a scan rate of $0.1\,V\,s^{-1}$.

Interaction of Complexes with CTDNA

Electrochemical methods prove to be a complementary tool over previously used methods of investigation such as UV/Vis spectroscopy to study the interaction of complexes with DNA.[35] Strong cathodic reduction peaks are observed at −0.40 V and −0.63 V for complexes **1b** and **3b** with i_{pc} values of 6.41 A and 2.54 A, respectively. At different scan rates, the voltammograms do not show any major changes. On addition of CTDNA, the reduction potentials (E_{pc}) are found to be shifted to the more negative side (−0.674 V) for complex **3b**, while to the less negative side (−0.377 V) for complex **1b**. These shifts in E_{pc} values suggest that both Cu(II) and Cu(I) forms of the complexes

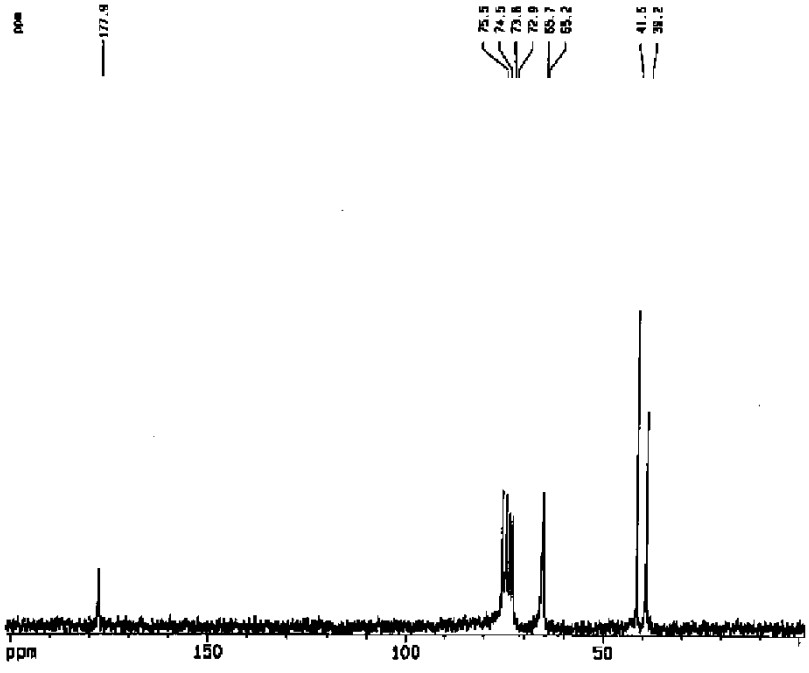


Figure 4: ^{13}C NMR spectrum of complex **2b** in D_2O solution.

1b and **3b** bind to DNA, but with different affinity. The voltammogram profiles of free complexes (**1b** and **3b**) and DNA-bound complexes are depicted in Fig. 5a and 5b. In addition to the change in E_{pc} values, the cathodic peak currents (i_{pc}) increases to 8.19 and 3.62 after the addition of CTDNA for complex **1b** and **3b**, respectively, indicating that the adsorption of the Cu(I) product tends to be suppressed in the presence of DNA.[36] The shifts in E_{pc} and i_{pc} values suggest that complex **1b** and **3b** bind strongly to CTDNA.

Solution Stability Studies

A cyclic voltammetric study was performed with complex **1b** and **3b** at various pH values (4–12) in aqueous solution. Both complexes show a single irreversible cathodic reduction $Cu^{II} \rightarrow Cu^{I}$ peak at all pH values. The E_{pc} values of both the complexes are very sensitive to changes in pH. In the pH range of 4–12, the voltammetric behavior of the complexes (**1b** and **3b**) was found to be reproducible, and the data for these complexes are listed in Table 1. The reduction potentials shift to less negative values with increase in pH in aqueous solution. This suggests that H_2O molecules are directly involved in the coordination sphere of the complexes. Fig. 6 showed the voltammograms of complex **1b** at various pH values. In both the complexes there is a

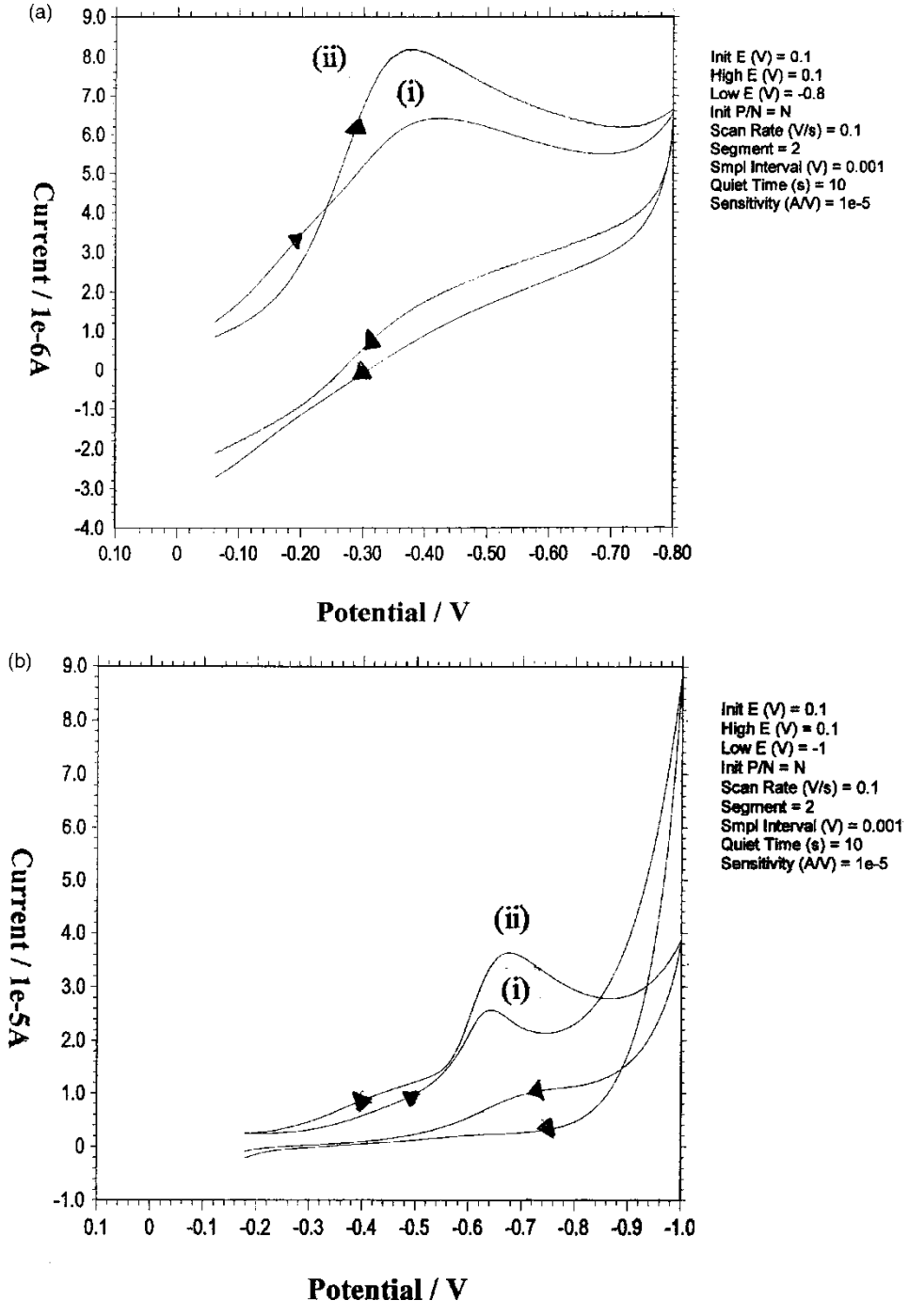


Figure 5: Cyclic voltammograms at the scan rate of 0.1 V s^{-1} (a) (i) complex **1b** alone and (ii) complex **1b** in presence of CTDNA and (b) (i) complex **3b** alone and (ii) complex **3b** in presence of CTDNA in aqueous solution.

Table 1: Cyclic voltammetic Data for Complexes **1b** and **3b** at various pH values.

	$-E_{pc}/V$	
pH	**1b**	**3b**
4.0	0.41	0.38
6.0	0.39	0.37
8.0	0.44	0.39
10.0	0.43	0.41
12.0	0.42	0.42

linear relationship between the cathodic potential E_{pc} and the various pH values as depicted in Fig. 7, indicating that complexes do not undergo any structural rearrangement.[37] The slopes of the lines (in mV/unit of pH) for complexes **1b** and **3b** are 10 and 11, respectively, which further authenticate the sensitivity of the overall structure of complexes towards the pH of environment.[9] Nonprecipitation and hydrolytic stability of the complexes at extreme pH conditions suggest the robust nature of these complexes in aqueous solution over a wide range of pH.[38]

Absorption Spectral Features of DNA Binding

The DNA-binding properties of complexes **1b** and **3b** were established by monitoring the changes in absorbance at 260 nm (λ_{max} of CTDNA) under pseudo-first order conditions, that is, [DNA] $\gg$ [complex] (Fig. 8a and b). Different sets of kinetic measurements were carried out at fixed concentration of complex **1b** and **3b** (0.4×10^{-5} M) with varying concentrations of CTDNA ($4-6.4 \times 10^{-5}$ M). The spectral changes were monitored as a function of time. During the interaction of the complex with CTDNA, the absorption band of calf thymus DNA is affected, exhibiting hyperchromism. This resulting hyperchromism upon interaction with DNA implies the binding of the complexes with DNA. Similar hyperchromism results were cited in the literature for a Cu(II) complex with a ligand bearing –NH– and –OH groups.[39] Changes in absorption spectral behavior are possibly due to the substitution of the H_2O molecule by the DNA base nitrogen atom and then coordination of these nitrogen bases to the cupric ion in the complex.[40]

The observed pseudo-first order reaction rate constants (k_{obs}) were calculated from the slopes of the straight lines obtained by plotting logarithmic peak growth vs. time for bands obtained upon interaction of complexes with CTDNA. The slopes of these straight lines are different for complex **1b** and complex **3b**, which may be ascribed to the structural difference in the two

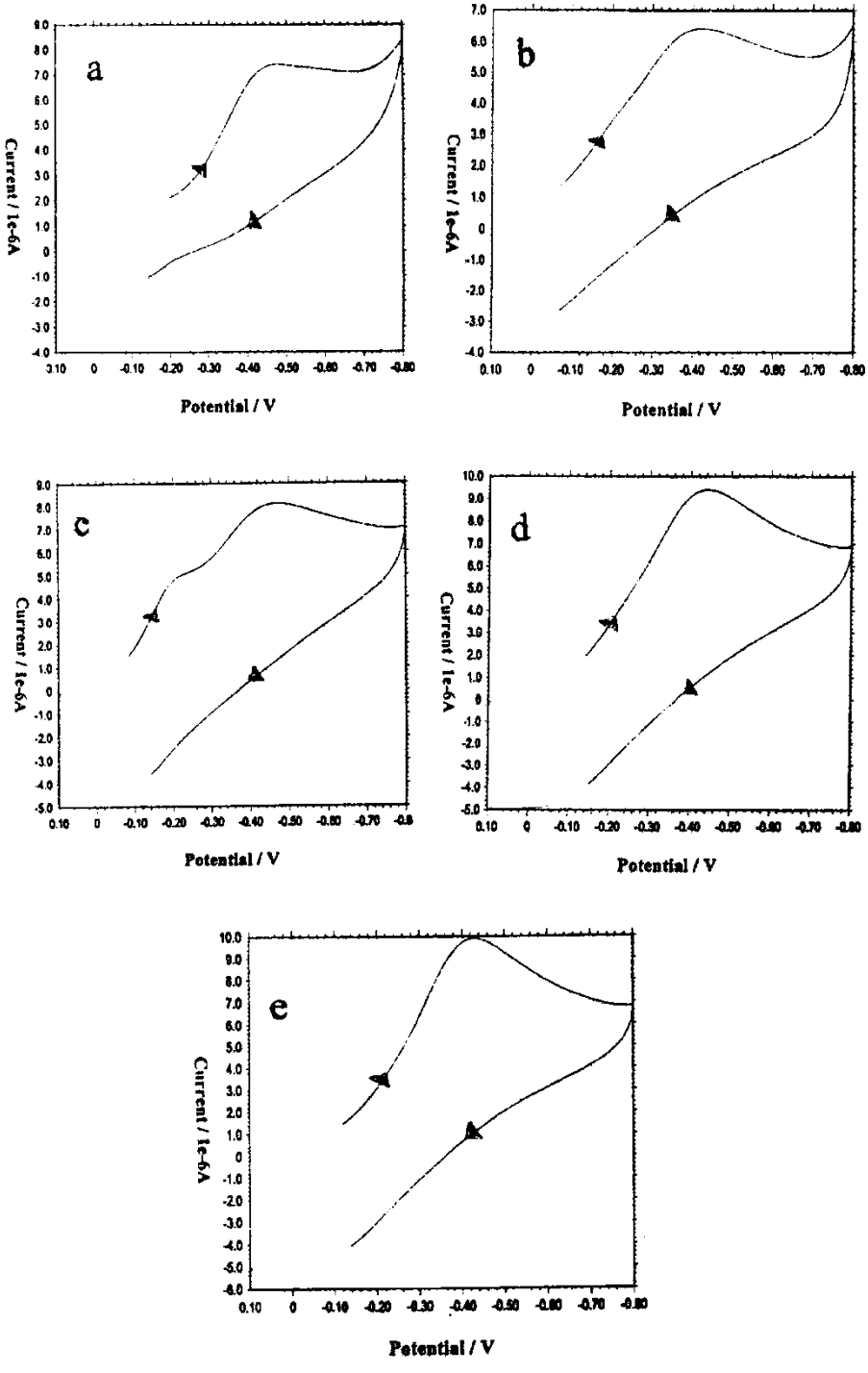


Figure 6: Cyclic voltammograms of complex **1b** at various pH (a) 4, (b) 6, (c) 8, (d) 10, and (e) 12.

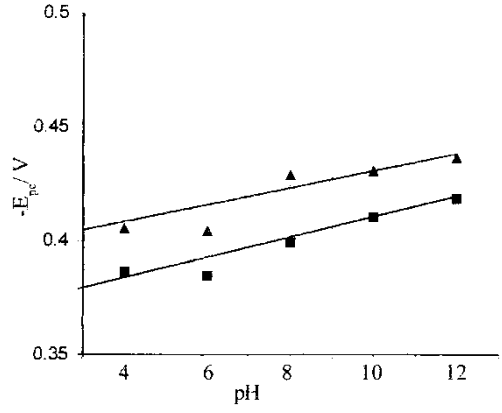


Figure 7: Plot of the $Cu^{II} \rightarrow Cu^{I}$ reduction potential (E_{pc}) as a function of pH for complex **1b** and complex **3b** at Pt microcylinder as the working electrode, Ag-AgCl as the reference electrode, at a scan speed of 0.1V s^{-1}. Complex **1b** (▲) Complex **3b** (■).

complexes, that is, complex **1b** bearing more –NH and –OH groups in comparison to complex **3b** is more reactive toward DNA. Hence, these are used to compare the relative binding of the two complexes with DNA. The following rate law has been derived[41]

$$k_{obs} = k_1 k_2 [\text{DNA}]/(k_{-1} + k_2) \quad (1)$$

where k_1 k_2 and k_{-1} are respective rate constants. The subscript describes the direction in which the rate constant is acting. The rate-determining step for DNA-complex formation is k_2. The rate law (1) holds good only if the plot of k_{obs} versus [DNA] is linear and the suggested mechanism derived from the kinetic data for binding of complexes **1b** and **3b** with CTDNA is correct, as shown in Sch. 2. Furthermore, the plots of k_{obs} values versus different CTDNA concentrations are found to exhibit excellent linear behavior, (Fig. 9), which provides supportive evidence for the proposed mechanism.

Circular Dichroism Studies

The stereochemical contribution to the CD spectra probably arises due to the presence of the asymmetric groups in the ligands. The CD spectra of complexes **1b** and **3b** were recorded in aqueous solution in the range of 200–400 nm (Fig. 10a and b). Complex **1b** exhibits a positive rise at 230 nm and a negative Cotton effect at 226 nm. The strong negative Cotton effect in complex **3b** indicates a large dissymmetry around the Cu^{II} ion. This is due to the inversion of the absolute configuration around the metal center and is

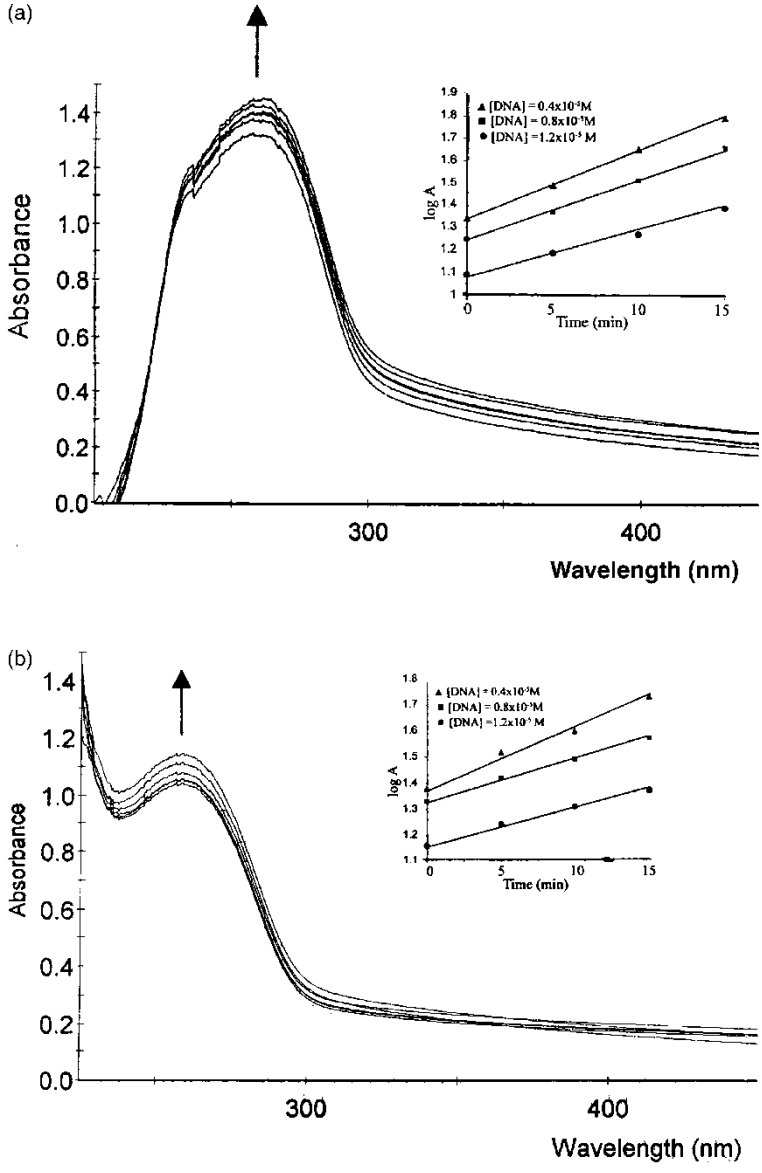
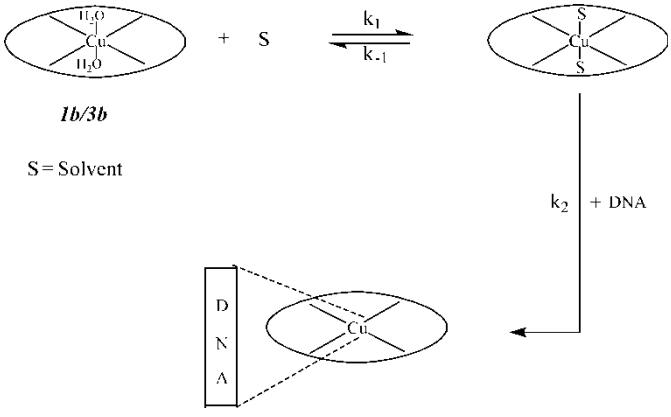


Figure 8: UV absorption spectra of CTDNA (a) in presence of complex **2b** and (b) in presence of **3b**. Arrows show the absorbance changes upon increasing DNA concentration. Inset: plots of kinetic measurements of different CTDNA concentration with respect to time ▲, ■, ● experimental data points, full lines linear fitting of the data.

attributed to the differences observed in the orientation of hydroxyl groups of D-gluconic acid.[42] These findings are also consistent with the polarimetric data. Thus, the CD responses of complex **1b** and **3b** indicate that Cu(II) was ligated by an optically active D-gluconic acid moiety.



Scheme 2

Circular dichroism (CD) was an effective method to examine the structural modification of DNA caused by an interaction with complex **1b** and **3b**. The UV circular dichroic spectrum of CTDNA exhibits a positive band at 282 nm due to base stacking and a negative band at 242 nm (Fig. 11a) due to helicity of DNA.[43] It is observed that addition of complexes **1b** and **3b** causes significant and distinct spectral perturbation in the CD spectrum of CTDNA. The induced CD spectrum of CTDNA in the presence of complex **1b** and **3b** exhibit an increase in positive ellipticity, while the intensity of negative ellipticity band decreases (Fig. 11b and c), with the latter being affected slightly more than the other. These results suggest that both the complexes **1b** and **3b** interact with CTDNA and the binding of these complexes with CTDNA induces certain conformational changes within the DNA molecule.[44]

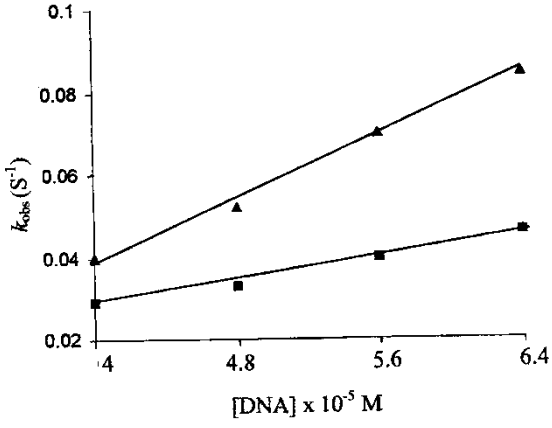


Figure 9: Plots of k_{obs} versus concentration of CTDNA. Complex **1b** (▲) and complex **3b** (■).

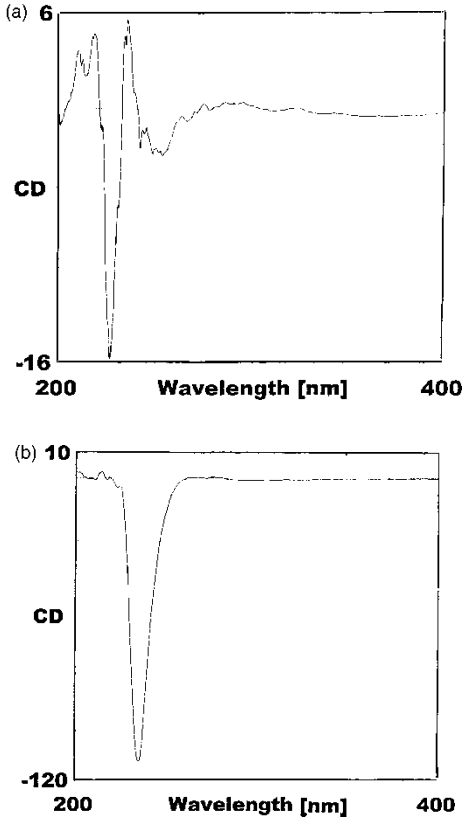


Figure 10: Circular dichorism spectra of (a) complex **1b** and (b) complex **3b** in water.

Viscosity Studies

Spectroscopic data obtained for the binding nature of complex **1b** and **3b** with DNA are not sufficient to support the binding mode of the complexes. In order to further authenticate the interaction of complexes with DNA and to clarify the mode of binding of complexes, hydrodynamic measurements such as viscosity studies were performed. These measurements are sensitive to length change and are considered to be as least ambiguous and most critical tests of binding in solution.[45] A classical intercalation model is expected to increase the length of the DNA helix, as base pairs are pushed apart to accommodate bound ligand. On the other hand, partial or nonclassical intercalators reduce the effective DNA length as they produce kinks in the DNA helix, which results in a decrease in viscosity.[46] The relative specific viscosity of DNA decreases with increment in the concentration of complexes **1b** and **3b** as shown in Fig. 12. The experimental results suggest that complexes **1b** and **3b** bind covalently to DNA to effect this change in viscosity.

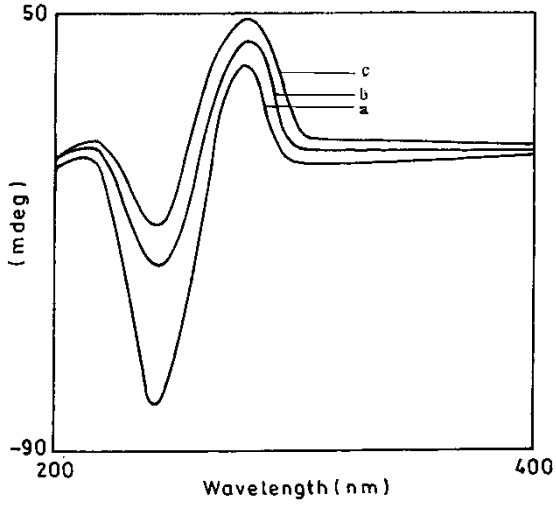


Figure 11: Circular dichroism spectra of (a) calf thymus DNA alone, (b) CTDNA in presence of complex **1b**, and (c) CTDNA in presence of complex **3b**. (DNA) = 3×10^{-4}M.

CONCLUSIONS

The present work offers a new attempt toward the synthesis of transition metal-saccharide complexes and their interaction with calf thymus DNA. Although extensive work is going on in this field, metal-carbohydrate DNA interaction is largely unexplored. As the crystal structures are scarce for transition metal-carbohydrate complexes, herein exhaustive analytical and spectroscopic investigations are carried out to ascertain the structure of the complexes. The UV/vis and EPR spectroscopy indicate the octahedral

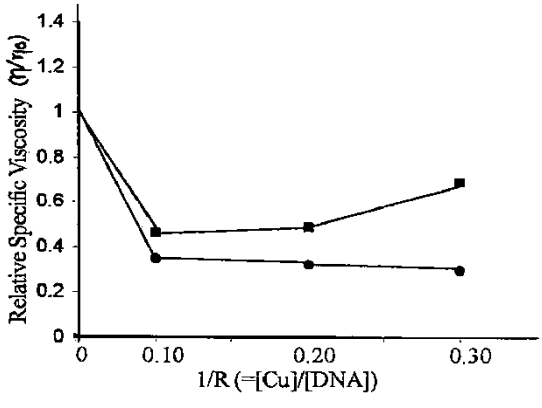


Figure 12: Effects of increasing amounts of complex **1b** (■) and complex **3b** (●) on the specific relative viscosity of CTDNA at 29 ± 0.01°C.

geometry around the metal ions in complexes **1b**, **3b**, and **4** and square planar geometry of complex **2b**. Solution stability studies of complexes **1b** and **3b** exhibit a linear relationship between pH and cathodic peak potentials suggestive of hydrolytic stability and the robust nature of these complexes in aqueous solution over a wide range of pH. Moreover, considering the biocompatibility of saccharides and bioessentiality of the copper ion, interest arose to study DNA-binding behavior of these complexes. Spectroscopic and electrochemical studies together with viscosity measurements support that complexes **1b** and **3b** bind covalently to calf thymus DNA. Thus, natural occurrence, biocompatibility, low toxicity, high solubility, and DNA interaction studies support that these complexes may prove to be useful as DNA inhibitors.

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