J Fluoresc (2014) 24:1701–1714
DOI 10.1007/s10895-014-1457-1

ORIGINAL PAPER

Synthesis, Micellization Behaviour, DNA/RNA Binding and Biological Studies of a Surfactant Cobalt(III) Complex With Dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine

Karuppiah Nagaraj · Krishnan Senthil Murugan · Pilavadi Thangamuniyandi · Subramanian Sakthinathan

Received: 9 August 2014 / Accepted: 8 September 2014 / Published online: 27 September 2014

Abstract A new surfactant cobalt(III) complex, *cis*-[-$Co(dpqc)_2(DA)_2](ClO_4)_3$, where dpqc = dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine and DA = dodecylamine, has been synthesized and characterized by elemental analysis, UV-Visible, IR and NMR spectra. The critical micelle concentration (CMC) value of this surfactant cobalt(III) complex in aqueous solution was obtained from conductance measurements. The conductivity data (at 303, 308, 313, 318 and 323 K) were used for the evaluation of the temperature-dependent CMC and the thermodynamics of micellization (ΔG^0_m, ΔH^0_m and ΔS^0_m). Absorption, fluorescence, cyclic voltammetry, circular dichroism and viscosity experiments have been carried out to study the interaction of the surfactant cobalt(III) complex with DNA and RNA. The results suggest that the complex can bind to nucleic acids by intercalation via both the dpqc ligand and the long aliphatic chain of the complex into the base pairs of DNA/RNA. In vitro cytotoxicity experiments show that the surfactant cobalt(III) complex exhibits cytotoxic activity against the HepG2 (human hepatocellular liver carcinoma) tumor cell lines and found to be active.

Keywords Surfactant cobalt(III) complex · Critical micelle concentration · DNA · RNA · Anticancer · Intercalation · Hydrophobicity

Introduction

During the past decade, there has been tremendous interest in studies pertaining to the interaction of various transition metal complexes with nucleic acids [1–3]. Deoxyribonucleic acid plays an important role in the life process, because it bears heritage information and instructs the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells. DNA is a particularly good target for metal complexes as it offers a wide variety of potential metal binding sites [4, 5]. Such sites include the electron rich DNA bases or phosphate groups that are available for direct covalent or coordination to the metal center. There are non-covalent binding modes as well, such as hydrogen bonding and electrostatic binding to grooved regions of the DNA and intercalation of planar aromatic ligands into the stacked base pairs. Due to unusual binding properties coordination compounds are suitable candidates as DNA secondary structure probes and antitumor drugs [6]. RNA is a versatile molecule that plays essential roles in many biological processes, and consequently, it is an attractive target for potential therapeutics. The structure diversity present in RNA molecule has led to specific drug recognition sites. It is currently admitted that RNA plays a key role in many biological processes involving living cells and is considered as the essential regulator of many steps of gene expression [7]. Furthermore, many viruses such as HIV, HCV, influenza and flaviviruses are encoded by a unique RNA molecule that serves as the main genetic material. Therefore, metal complexes that bind to RNA and disturb RNA functions could be powerful tools for understanding and controlling gene expression [8]. Many useful

Electronic supplementary material The online version of this article (doi:10.1007/s10895-014-1457-1) contains supplementary material, which is available to authorized users.

K. Nagaraj (✉) · S. Sakthinathan
School of Chemistry, Bharathidasan University, Tiruchirapalli 620024, Tamilnadu, India
e-mail: kanagarajnagaraj@gmail.com

K. Senthil Murugan
Post Graduate and Research Development of Chemistry, Vivekananda College, Tiruvedakam West, Madurai 625234, Tamilnadu, India

P. Thangamuniyandi
Department of Polymer Science, University of Madras, Guindy Campus, Chennai 600 025, Tamilnadu, India

applications of these complexes require that the complex binds to nucleic acids through an intercalative mode. However, the majority of such studies have been focused on complexes of ruthenium, and to a far lesser extent, on other metal complexes. Much attention has been paid to the complexes containing symmetric aromatic ligands such as 1, 10-phenanthroline and its derivatives. Some of these complexes exhibit interesting properties upon binding to DNA [9–11]. Aromatic ring stacking between nucleobases is considered to be a major driving force for these bindings, which depends on the size and electron density of the interacting aromatic rings, as well as on the combined effect of hydrophobic and hydrophilic interactions.

Studies of complex formation between DNA and a cationic amphiphilic molecule have implications for gene therapy, a conceptually new approach for the treatment of human disease [7], which is rapidly progressing from basic research to clinical applications [12]. In gene-therapy, DNA-cationic surfactant complexes are very useful. Due to the polyanionic nature of DNA, its diffusion through the eukaryotic cell is difficult. To remove such difficulties, various synthetic gene transfer vectors, e.g. cationic lipids and related surfactants, have been recently developed [13–15]. Properties such as structure, thermodynamics, and morphology of cationic lipid-DNA complexes have been widely investigated by a variety of methods [16–20]. These studies shows that variations in length, degree of unsaturation, flexibility, and chemical structure of the lipid chain and the nature of the counter ion can have large effects on the interaction process and transfection efficiency. A change or replacement of the surfactant with another amphiphile having different molecular structural features may bring back high transfection efficiency [21–26]. The elucidation of the optimal transfection recipe for any given cell line therefore appears to require a prior knowledge of the surfactant. This in turn necessitates clear understanding of the nature of interaction between various surfactants and DNA. Several surfactants also find utility in the purification of DNA through precipitation [27–29]. Recently, cationic surfactants are shown to induce coil to globular transition of DNA and this has been demonstrated by using fluorescence microscopy [30]. Modeling of electrostatic, hydrophobic, and hydration forces in such interactions has also been widely studied [31].

A variety of mixed ligand cobalt(III) complexes having phenanthroline ligand and its derivatives serve as bioinorganic model compounds [32]. They exhibit antiviral activities by their interaction with nucleic acid templates and inhibit proviral DNA synthesis. Ji et al. have synthesized cis-$[Co(phen)_2(pdtb)]^{3+}$ (phen=1,10-phenanthroline and pdtb=3-(pyridine-2-yl)-5,6-diphenyl-as-triazine) and its binding properties to calf thymus DNA indicate that the size and shape of the intercalated ligand pdtb had a marked effect on the binding affinity of the complex to DNA [33–36]. Although some experimental investigation on RNA-metal complex interactions have been carried out during the past decade as described above, many questions still need to be answered for a better understanding of the mechanisms and the biological implications of the interactions. One of the questions is that how the structures of nucleic acid affect the binding behaviours of metal complexes. It is well known that yeast tRNA is different from calf thymus DNA (CT DNA) not only in composition of bases, but also in structure. CT DNA is a B form configuration, while yeast tRNA has A form configuration with a L-shaped structure. Therefore, a comparative study of the interactions of metal complexes with yeast tRNA and CT DNA will be really important for understanding the mechanism of the interactions and the biological impact of metal complexes.

Cobalt is recognized as an essential metal element widely distributed in the biological systems such as cells and body, and the interaction of cobalt(III) complexes of diimine [37, 38] and polypyridyl ligands [39, 40] with DNA has attracted much attention [41–43]. Hence, investigation of cobalt(III) complexes containing substituted phenanthroline ligand is of importance in the search for more effective drugs. Recently, we have reported synthesis, structure, DNA binding properties and cytotoxic activity of a series of surfactant cobalt(III) complexes with bipyridine, and phenanthroline ligands, and found that the complexes have good DNA binding properties and antitumour activities [44–46]. As continuation of our work, herein, we present synthesis, structure, CMC determination, antitumor activities, and DNA/RNA interaction of a surfactant cobalt(III) complex with modified phenanthroline (dpqc) ligands. The results should be valuable in understanding the interaction mode of the complex with DNA. Also, the cytotoxicity of this complex has been evaluated by MTT assay method (MTT=(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and some staining experiments.

Experimental

Materials and Methods

Calf Thymus (CT) DNA, yeast (t-RNA) and ethidium bromide were purchased from Sigma-Aldrich, Germany, and used as received. The nucleic acid binding experiments were performed at 25.0±0.2 °C. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ~1.8–1.9:1, indicating that the DNA was sufficiently free from protein [47]. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using the known molar extinction coefficient value of 6,600 M^{-1} cm^{-1} and 9,250 M^{-1} cm^{-1} respectively for DNA and RNA at 260 nm [48]. Stock solutions were stored at 4^0 C and used within 4 days. All the experiments involving the interaction of the surfactant cobalt(III) complex with nucleic acids were carried out with twice distilled water in buffer containing 5 mM Tris–HCl/50 mm NaCl at pH 7.0.

Physical Measurements

CHN analysis of the sample was carried out at CECRI (Central Electro Chemical Research Institute), Karaikudi, India. Absorption spectra were recorded on a Shimadzu UV-3101PC spectrophotometer using cuvettes of 1 cm path length, and emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. Cyclic voltammetry measurements were made on Princeton EG and G-PARC model potentiostat. Circular dichroism spectra were recorded on a JASCO J-716 spectropolarimeter equipped with a peltier temperature control device. Thermal denaturation studies were recorded on a Shimadzu UV-3101PC spectrophotometer using cuvettes of 1 cm path length with temperature controller of Peltier system. Conductivity studies were done in aqueous solutions of the complex with an Elico conductivity bridge type CM 82 and a dip type cell with a constant of 1.0.

Syntheses of Modified Phenanthroline Ligand

The modified phenanthroline ligand, dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine (dpqc) was synthesized from the starting material 1,10-phenanthroline-5,6-dione according to the reported procedures [49–55].

1,10-phenanthroline-5,6-dione

Four grams of 1,10-phenanthroline and 4 g of KBr were added to ice cold mixture containing conc.H_2SO_4 (40 ml) and conc. HNO_3 (20 ml). The mixture was heated to reflux for 3 h and the hot solution was poured over 500 ml of ice cold solution and neutralized with NaOH until neutral to slightly acidic pH. From this solution the titled compound was extracted with chloroform and then the chloroform solution was evaporated in a rotary evaporator at 40 °C until a yellow solid was separated out. This solid was recrystallized from methanol.

Dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine (dpqc)

A mixture of 1,10-phenanthroline-5,6-dione (4.8 mmol) and 2-diaminocyclohexane (6.3 mmol) in ethanol (120 ml) was refluxed for 2 h. The resulting yellow solution was reduced in volume by rotary evaporation at 50 °C until a pale yellow solid was separated out. This solid was filtered off and recrystallized from methanol to give pale yellow needles of the titled complex.

Syntheses of Complex

Cis-[Co(dpqc)$_2$Cl$_2$]Cl

To a refluxed methanolic solution of the ligand, dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine (10 mM), anhydrous cobalt chloride (5 mM) in methanol (10 ml) was added while hot. The solution then turned from yellow to dark brown. Chlorine gas (generated by mixing potassium permanganate and concentrated hydrochloric acid) was passed through the solution. A dark brown precipitate separated out and it was then dissolved in 10^{-3} M hydrochloric acid (50 ml) by warming at 60^0 C for 30 min. To this, a solution of 4 M hydrochloric acid (20 ml) was added and the mixture was kept as such for overnight. The resulting crystalline complex *cis*-[Co(dpqc)$_2$Cl$_2$]Cl was filtered off, washed with ethanol and dried under vacuum over $CaCl_2$.

cis-[Co(dpqc)$_2$(DA)$_2$](ClO$_4$)$_3$

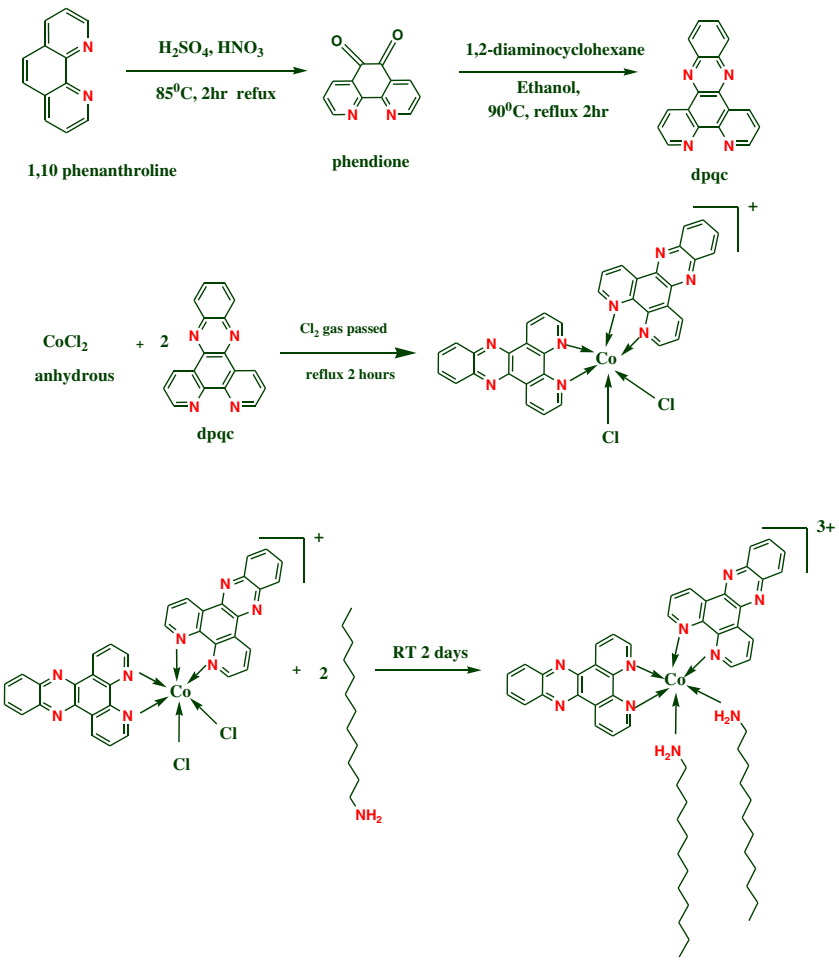
The surfactant cobalt(III) complex (Scheme 1) synthesized as follows. To a solution of three grams of *cis*-[Co(dpqc)$_2$Cl$_2$]Cl, a slightly more than the calculated amount of dodecylamine in ethanol was added drop by drop over a period of 30 min. The mixture was set aside at room temperature for 2 days. Afterwards a saturated solution of sodium perchlorate in perchloric acid was added to it. Slowly a pasty solid mass separated out and it was filtered, washed with small amounts of alcohol followed by acetone, and then dried over air. The semi-dried solid was further dried in a drying pistol over fused calcium chloride and then stored in a vacuum desiccator.

Elemental Analysis

The purity of the surfactant complex was checked by the analysis of carbon, hydrogen, nitrogen and cobalt present in them. C, H, and N analysis were carried out at CECRI (Central Electro Chemical Research Institute), Karaikudi, India. The cobalt content of the surfactant cobalt(III) complex was estimated by the following Kitson's method [56]. A known weight of the complex was reduced with tin and concentrated hydrochloric acid. The reduced aqueous cobalt(II) ion was made up to 10 ml in a volumetric flask using 0.1 M perchloric acid. Two millilitres of this solution and 1 ml of 50 % ammonium thiocyanate solution were pipetted out into a 10 ml volumetric flask and made up to the mark with acetone. The absorbance of this solution was measured at 625 nm against a blank reagent. From the absorbance, the concentration of cobalt was calculated.

Determination of Critical Micelle Concentration (CMC) Values

The critical micelle concentration value of the surfactant cobalt(III) complex was determined by conductivity method. Appropriate concentration range for surfactant cobalt(III) complex was chosen in aqueous solution. The conductivity cell was calibrated with KCl solutions in the appropriate



Scheme 1 Formation of cis-Bis(dodecylamine) bis(dipyrido[n-a:2′,4′-c] phenazine)cobalt(III) ion

concentration range and the conductivities were measured at 303, 308, 313, 318 and 323 K. The temperature of the thermostat was maintained constant to within±0.01 °C. The conductance was measured after thorough mixing and temperature equilibrating at each dilution. The establishment of equilibrium was checked by taking a series of readings after 15-min intervals until no significant change in the conductivity was observed.

Nucleic Acid (NA) Binding Experiments

A constant concentration of surfactant cobalt(III) complex was maintained for UV-Visible absorption measurements to which increments of the nucleic acid stock solutions were added. The solutions were allowed to incubate for 10 min before the absorption spectra were recorded. Equal solution of nucleic acid was added to both complex solution and reference solution to eliminate the absorbance of nucleic acid itself.

For fluorescence quenching experiments, nucleic acids were pretreated with ethidium bromide (EB) for 30 min. Solutions of surfactant cobalt(III) complex was added to this mixture and their effect on the emission intensity was measured. The samples were excited at 450 nm and emission was observed between 500 and 700 nm.

For the cyclic voltammetry experiments, the electrode surfaces were freshly polished with alumina powder and then sonicated in ethanol and distilled water for 1 min prior to each experiment and the electrode was rinsed with doubly distilled water thoroughly between each polishing step. Finally, each electrode was cleaned thoroughly in an ultrasonic cleaner again with doubly distilled water. These experiments were performed at 25.0±0.2 °C in a single compartment cell with a three-electrode configuration, glassy carbon working electrode, platinum wire auxiliary electrode and saturated calomel as reference electrode using tris buffer. Before experiments, all solutions were deaerated with dry nitrogen gas for 10 min to remove dissolved oxygen and kept under nitrogen atmosphere throughout the experiments.

Viscosity experiments were carried out on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 30±0.1 °C. DNA samples of approximately

0.5 mM were prepared by sonication in order to minimize complexities arising from DNA flexibility. Flow time was measured with a digital stopwatch, and each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the concentration of the surfactant cobalt(III) complex, where η is the viscosity of DNA solution in the presence of the complex, and η_0 is the viscosity of DNA solution alone. Viscosity values were calculated from the observed flow time of DNA solution containing solutions ($t>100$ s) corrected for the flow time of the buffer alone (t_0): $\eta=t - t_0/t_0$ [57].

Circular dichroism experiments were recorded from 220–320 nm at 25 °C using a quartz cell of 1 cm path length. Each CD spectrum was collected after averaging over at least 4 accumulations using a scan speed of 100 nm min^{-1} and 1 s response time. The region between 220 nm and 320 nm was scanned for each sample. The spectra were recorded both in the absence and in the presence of surfactant cobalt(III) complex.

Biological Studies

Cytotoxicity Assay

The cytotoxicity of the surfactant cobalt(III) complex was measured in the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay as described earlier [58]. The complex was first dissolved quantitatively in dimethyl sulfoxide (DMSO, Sigma, USA) to make the stock solution. Briefly, cells were seeded at a density of 5×10^4 HepG-2 liver cancer cells/well into 96-well plates. After 24 h, the cells were treated with surfactant cobalt (III) complex at various concentrations (10, 30, 60, 90 μg/ml) and incubated for 24 and 48 h as indicated. At the end of the incubation, 10 μl of 3-(4–5 dimethylthiozol-2-yl) 2–5 diphenyl-tetrazolium bromide (MTT) (5 mg/ml) per well was added and incubated in dark at 37 °C for 4 h. The formazan crystals formed after 4 h were solubilized in 100 μl of DMSO after aspirating the medium. The absorbance was monitored at 570 nm (measurement) and 630 nm using a 96 well plate reader (Bio-Rad, Hercules, CA, USA). The IC_{50} value was defined as the concentration of compound that produced a 50 % reduction of cell viability.

Evaluation of Apoptosis (Acridine Orange and Ethidium Bromide Staining)

Acridine orange and ethidium bromide staining was performed as described by Spector et al. [59] Twenty-five microliters of cell suspension of sample (both attached, released by trypsinization, and floating), containing 5×10^5 cells, was treated with AO and EB solution (one part of 100 mg/mL AO and one part of 100 mg/mL EO in PBS) and examined under a fluorescent microscope (Carl Zeiss, Germany) using an UV filter (450–490 nm). Three hundred cells per sample were counted in tetraplicates for each dose point. Cells were scored as viable, apoptotic or necrotic as judged by the staining, nuclear morphology and membrane integrity, and percentages of apoptotic and necrotic cells were then calculated. Morphological changes were also observed and photographed.

Dye Preparation and Drug Preparation

The amount of 200 μL of dye mixture (100 μL/mg AO and 100 μL/mg EB in distilled water) was mixed with 2 mL cell suspension (30,000 cells/mL) in 6-well plate. The suspension was immediately examined and viewed under Olympus inverted fluorescence microscope (Ti-Eclipse) at 200× and 400×magnification. We observed untreated cells as controls and cells treated with testing material IC_{50} concentrations for 24 h of exposure.

Drug Treatments

HepG2 were seeded in a 24-well plate (50,000 cells per well). After 24 h of cells incubation, the medium was replaced with 100 μL medium containing IC_{50} dose of testing material. Untreated cells served as the control. After 24 h, aspirate the media and treat with prepared dye and observe under the fluorescent microscope.

Hoechst 33342 Staining

This procedure is very sensitive to cell concentration and pH of the media. Cells should be approximately $1–2\times10^6$ ml, in buffered media, pH 7.2. It is also helpful to include 2 % fetal calf serum to maintain the cells.

Drug was added and incubated for 24 and 48 h. Homogenously aspirated and spent media was removed and 1 ml of saline was added and centrifuged at 1,500 rpm for 10 mins. The cells were stained with 0.5 mL of Hoechst 33342 solution (3.5 μg/mL in PBS) and incubated for 30 min at 37 °C incubator. After 30 min the Hoechst 33342 solution was discarded and the cells observed at 490–520 nm of fluorescent microscope. Time is a critical factor due to the transport of the dye. Typically, 30 min is a minimum, but it is important to remember that the signal may begin to degrade after ~120 min. It is recommended that the staining kinetics be empirically defined. Analyze apoptosis under fluorescent microscope after incubation. Washing is not recommended.

Results and Discussion

Spectroscopic Characterization

The surfactant cobalt(III) complex synthesized in the present study was characterized by UV–Vis, IR, and NMR techniques. The purity of the complex was checked by Co, C, N, H analysis, and the results were found to be in good agreement with the calculated values. Element, % found (% calculated): Co 4.48(4.39), C 55.64(55.34), N 10.64(10.78) and H 6.58(6.47). The electronic absorption spectra often provide quick and reliable information about the ligand arrangement in transition metal ion complexes [60, 61]. The UV-Visible absorption spectrum of this complex shows two distinctive bands at 332 and 348 nm due to ligand-based π-π* bands. Infrared spectroscopy is used to distinguish the mode of coordination of the ligands with the central metal ion. The IR bands, δ (C–H) 830, 731 cm^{-1}, observed for dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine are redshifted to 811 and 731 cm^{-1} in the surfactant cobalt(III)–dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine complex. These shifts can be explained on the basis of the fact that the nitrogen atoms of the ligands dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine donate pair of electrons to the central cobalt metal, forming a coordinate covalent bond [62]. Besides, it is also confirmed by the shift of γ (C=N) of dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine from about 1,460 cm^{-1} in the free ligand to 1,413 cm^{-1} after coordination [63]. For the surfactant cobalt(III) complex, the bands exhibit around 2,925 cm^{-1} and 2,853 cm^{-1} can be assigned to C-H asymmetric and symmetric stretching vibrations of aliphatic-CH_2 group of dodecylamine. Also the perchlorate bands appeared around 1,100, 925, 620, and 450 cm^{-1} belong to an ionic species; this means that the counter-ion of the complex is not involved in the cobalt-ligand coordination [64].The methylene protons of the long chain moiety (dodecylamine) gave rise to a multiplet at 1.2–1.9 ppm, whereas the terminal methyl group of the hydrocarbon chain substituent gave a triplet around 0.9-0.7 ppm. The aromatic protons of dipyrido[3,2-a:2′,4′-c](6,7,8,9-tetrahydro)phenazine ligands at 8.4-9.7 ppm .

Critical Micelle Concentration Values (CMC)

The specific conductivity of the solutions of surfactant cobalt(III) complex increases with the complex concentration and temperature. When plots are made of specific conductivity versus concentration of the complex (SI Fig. 1), the slope is reduced after a particular value of the concentration. This particular value of concentration at which slope of the plot changes shows micellization and this concentration is chosen as CMC. The CMC values were determined at five different temperatures (303, 308, 313, 318 and 323 K). At all temperatures a break in the conductance versus concentration in the plots, characteristic of micelle formation, was observed. The CMC values were determined by fitting the data points above and below the break to two equations of the form y= mx+c and solving the two equations simultaneously to obtain the point of intersection. Least-squares analysis was employed, and the correlation coefficients were greater than 0.98 in all the cases. The conductivity measurements were repeated three times and the accuracy of CMC values (Table 1) was found to be within ±2 % error. It was observed that the CMC values increased with increase in the temperature for a given system. An increase in temperature will disrupt the water surrounding the hydrophobic group leading to retardation of micellization. So CMC value increased with temperature.

The CMC values for the surfactant cobalt(III) complex in the present study are very low compared to that of the simple organic surfactants [5, 65] like cetyltrimethylammonium bromide (CMC 9.2×10^{-4} mol dm^{-3}) and sodiumdodecylsulphate (CMC 8.0×10^{-3} mol dm^{-3}). Thus it is suggested that these metal surfactant complex has more capacity to associate themselves forming aggregates (more than hundred times) compared to those of ordinary synthetic organic surfactants. That is introduction of a metal complex to the hydrophilic part of the amphiphile has remarkably enhanced the ability of aggregation of the surfactant.

Thermodynamics of Micellization

The study of dependence of CMC with temperature is often undertaken to obtain information on various thermodynamic parameters of micelle formation. The change in the CMC with temperature is generally analyzed in terms of the phase separation or the equilibrium model for micelle formation [66–68]. According to these models, the standard Gibbs free energy of micelle formation per mole of monomer, ΔG^0_m, is given by

$$\Delta G^0_m = RT(2-\alpha_{ave})\ln CMC, \quad (1)$$

where R, T and α_{ave} are gas constant, absolute temperature and average degree of micellar ionization (the micelle ionization degree at the CMC is equal to the ratio between the slopes of the nearly linear specific conductance versus [complex] plots above and below the CMC) [34], respectively.

The enthalpy of micelle formation can be obtained by applying the Gibbs–Helmholtz equation to Eq. (1)

$$\Delta H^0_m = -RT^2(2-\alpha_{ave})d\ \ln CMC/dT. \quad (2)$$

From the Gibbs free energy and the enthalpy of micelle formation, the entropy of micelle formation can be determined

Table 1 CMC values of $[Co(dpqc)_2(DA)_2](ClO_4)_3$ in aqueous solution

Temperature	CMC×10^7	$-\Delta G^0_{mic}$ (kJ mol^{-1})	$-\Delta H^0_{mic}$(kJ mol^{-1})	$T\Delta S^0_{mic}$ (kJ mol^{-1})
303 K	0.71	61.24	20.50	40.73
308 K	0.87	62.21	21.28	40.93
313 K	0.95	62.17	21.72	40.44
318 K	1.05	63.45	22.70	40.74
323 K	1.25	64.12	23.47	40.64

as,

$$\Delta S^0_m = \left(\Delta H^0_m - \Delta G^0_m\right)/T \quad (3)$$

The thermodynamic parameters thus obtained are shown in Table 1. As seen from the table the Gibbs free energy of micellization is found to be negative for the surfactant complex indicates that the micellization process of all the systems is spontaneous within the temperature range studied. Further ΔS^0_m of micellization is positive for the complex. This positive values of ΔS^0_m for the surfactant cobalt(III) complex indicate that the micellization of the surfactant complex in aqueous solution is governed mainly by hydrophobic interaction between the surfactant complex cations, resulting in the breakdown of the structured water surrounding these cationic head groups.

Nucleic Acid Binding Studies

Uv-Visible Absorption Studies

Metal complexes can bind to nucleic acids in different binding modes on the basis of their structure and charge and type of ligands. Titration with UV-visible spectroscopy is an effective method to examine the binding mode of nucleic acids with the metal complex since the observed changes of the spectra may give evidence of the existing interaction mode [69, 70]. In general, hypochromism along with red shift will be observed in the case of the intercalative binding mode [71, 72]. Figure 1 and SI Fig. 1 show the UV-visible spectra of the surfactant cobalt(III) complex, titrated with CT DNA and yeast tRNA. As seen from Fig. 1 and SI Fig. 2 with increase of the concentration of CT-DNA or yeast tRNA, the absorption spectrum of the surfactant cobalt(III) complex shows strong hypochromism and red shift. These observations for the surfactant cobalt(III) complex are confirmed by the following observations. When the surfactant cobalt(III) complex intercalates the base pairs of CT-DNA, the π*-orbital of the intercalated substituted phenanthroline in the surfactant cobalt(III) complex can couple with the π-orbital of the base pairs, thus decreasing the π–π*transition energy and resulting in bathochromism. Furthermore, the coupling π*-orbital is partially filled by electrons, thereby decreasing the transition probabilities and concomitantly resulting in hypochromism. This observed strong hypochromism along with red shift may be due to large strong hydrophobic interaction between the modified phenanthroline along with the long aliphatic chain amine ligands of the surfactant cobalt(III) complex and base pairs of nucleic acids leading to strong intercalative binding mode [72–74].

In order to elucidate quantitatively the affinity of the surfactant cobalt(III) complex to CT-DNA and yeast tRNA, the intrinsic binding constant, K_b has been determined using the equation [75],

$$[\mathbf{NA}]/(\varepsilon_\mathbf{a}-\varepsilon_\mathbf{f}) = [\mathbf{NA}]/(\varepsilon_\mathbf{b}-\varepsilon_\mathbf{f}) + \mathbf{1}/\mathbf{K_b}(\varepsilon_\mathbf{b}-\varepsilon_\mathbf{f})$$

where, [NA] is the concentration of DNA or RNA expressed in base pairs; ε_a, ε_f and ε_b are the extinction coefficients of apparent, free and fully bound cobalt(III) complex. A plot of $[NA]/(\varepsilon_a-\varepsilon_f)$ versus [NA] gives K_b as the ratio of the slope to intercept.

The characteristics of UV and visible spectrum, as well as the binding constants obtained (Table 2) demonstrate that the complex binds with tRNA more strongly than with CT DNA. A possible explanation for this observation may be due to the A-form configuration and the L-shaped tertiary structure of yeast tRNA, in which the major groove, is wide and shallow, thus its base pairs are well exposed and can be attacked by complex easily. In addition, the surfactant cobalt(III) complex may bind to the bugle region of yeast tRNA.

Further the binding constant of surfactant cobalt(III) complex with nucleic acids at below their CMC values is lower than that of at above their CMC values. At above cmc values the surfactant complex present as both monomers as well as micelles. In the micellar form due to aggregation, the probability for interaction of each complex molecule with nucleic acids will be more.

The binding constants (K_b) of the surfactant cobalt(III) complex is very much higher than that for the ordinary metal complex containing the same modified phenanthroline ligand, $[Co(dpqc)_2Cl_2]Cl$ (K_b, 3.97×10^4 M^{-1}) [76]. This indicates that the long aliphatic chain amine ligand present in the surfactant cobalt(III) complex plays a definite role in the intercalative binding between the surfactant cobalt(III)

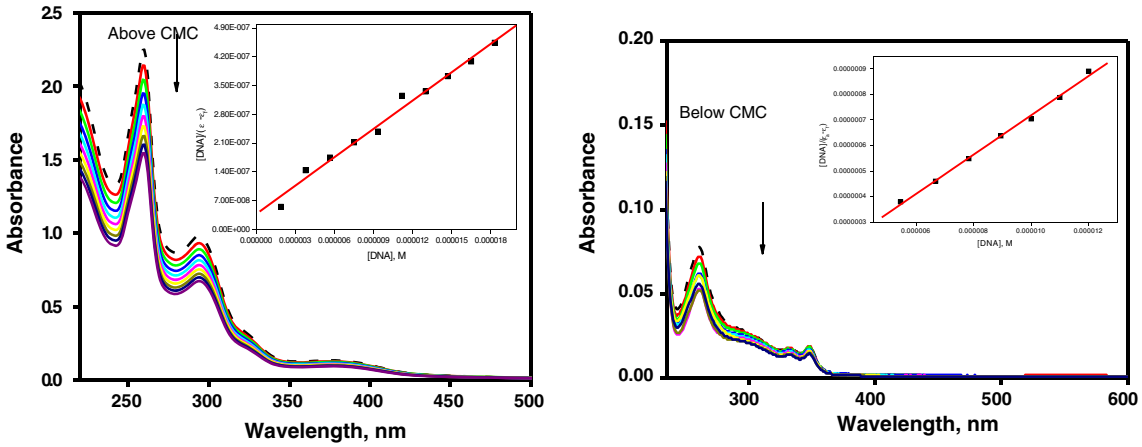


Fig. 1 Absorption spectra of complex in the absence (*dotted lines*) and in the presence of increasing amounts of DNA (*solid lines*), [Complex]= 5.0×10^{-7} M (Below CMC), 5.0×10^{-4} M (Above CMC), [DNA]=0–1.0×10^{-5} M, *Arrow* shows the absorbance changes upon increasing DNA concentrations. Inset: plot of [DNA]/ ($\varepsilon_a - \varepsilon_f$) versus [DNA]

complex and nucleic acids through their hydrophobic effect. Such strategy should be valuable in understanding the DNA-binding behaviors of this class of complexes as well as laying a foundation for the design of powerful agents for probing and targeting nucleic acids, providing insight into the field of DNA interactions.

Thermal Studies and Thermodynamic Parameters of the Binding

The DNA melting experiments are useful in confirming the intercalative binding, because the intercalation of the complex into DNA base pairs causes stabilization of base stacking and therefore raises the melting temperature of the double-stranded DNA[77]. It is well-accepted that when the temperature of the DNA solution increases, the double-stranded DNA gradually dissociates to single strands, generating a hyperchromic effect in the absorption spectra of the DNA bases. So the transition temperature for the conversion of of double strands to single strand can be determined by monitoring the absorbance of the DNA bases as a function of temperature [78, 79]. Literature reports [49, 50], indicate that the intercalation of metal complexes with CT DNA generally results in a considerable increase of melting temperature (T_m). The melting curves of CT-DNA in the absence and presence of surfactant cobalt(III) complex are presented in Fig. 2. Melting temperature (T_m) of CT DNA in buffer was determined as 79.0 ± 0.2^0 C under the experimental conditions. The DNA intrinsic binding constant of the surfactant cobalt(III) complex at T_m was calculated by using McGhee's equation (Eq. (4)) [80, 81], where T^0_m is the melting temperature of CT-DNA alone, T_m is the melting temperature in the presence of the cobalt(III) complex, ΔH_m is melting enthalpy of DNA (per base pair), R is the gas constant, K is the DNA-binding constant at T_m, L is the free complex concentration (approximated to the total complex concentration) at T_m, and n is the size of the binding site.

$$1/T^0{}_m - 1/T_m = (R/\Delta H_m)[\ln(1 + KL)]^{1/n} \quad (4)$$

By appropriately substituting the value of ΔH_m (6.9 kcal mol^{-1}) and n (2.0) from the literature [53–55] for CT DNA used in these studies under identical solution conditions, the K values were determined for these surfactant cobalt(III) complex and are shown in Table 3. As seen from this table K values followed the same trends as observed through uv-visible absorption studies.

Only few thermodynamic parameters such as free energy, enthalpy and entropy changes upon binding of metal

Table 2 The intrinsic binding constants (K_b) and hypochromism (%) of the surfactant cobalt(III) complexes; [Complex]=5.0×10^{-7} M (below CMC), [Complex]=5.0×10^{-4} M (Above CMC), [DNA]=0–1.8×10^{-5} M

Surfactant Cobalt(III) Complex	K_b (M^{-1})				Hypochromism (%)			
	DNA		RNA		DNA		RNA	
	Below CMC	Above CMC	Below CMC	Above CMC	Below CMC	Above CMC	Below CMC	Above CMC
Complex	3.6×10^6	4.8×10^6	5.1×10^6	5.7×10^8	34.91	38.71	44.82	41.40

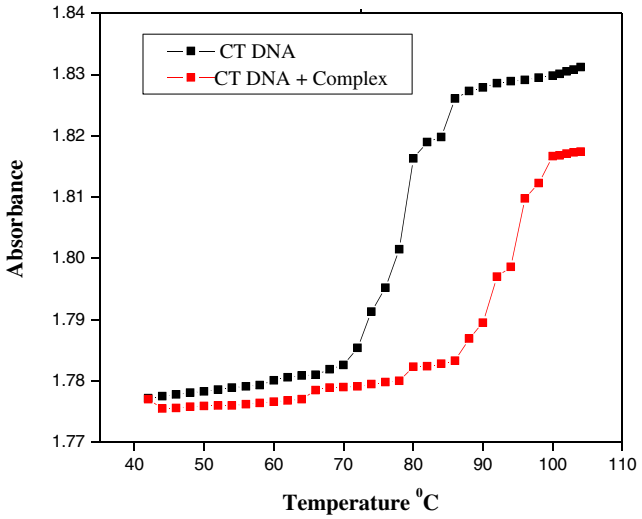


Fig. 2 DNA melting curves at 260 nm in the absence and presence of complex at 8 μM; [DNA]=80 μM

complexes to CT DNA have been measured. In fact, the thermodynamic parameters of DNA-complex formation are essential for a thorough understanding of driving forces of the binding of metal complexes to DNA [82]. The change of standard enthalpy was determined according to the van't Hoff's equation (Eq. (5)). The changes of standard free energy and standard entropy of the binding of all these complexes to CT DNA were determined according to Eqs. (6) and (7), where K_1 and K_2 are the DNA-binding constants of the complex at the temperatures T_1 and T_2, respectively. ΔG^0, ΔH^0, and ΔS^0 are the changes of standard free energy, standard enthalpy, and standard entropy of the binding of the complex to CT DNA, respectively.

$$\ln(K_1/K_2) = \Delta H^0/R(T_1-T_2/T_1T_2) \quad (5)$$

$$\Delta G^0 = -RT\ln K \quad (6)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (7)$$

The value of ΔH^0 obtained is −41.45 kJ mol^{-1} for complex by substituting $K_1=5.70\times10^5$ M^{-1} (T_1=298 K) and $K_2=3.97\times10^5$ M^{-1} (T_2=357 K) into Eq. (5). By substituting $K_1=5.70\times10^5$ M^{-1} (T_1=298 K) and $\Delta H^0=-41.45$ kJ mol^{-1} into Eqs. (6) and (7), $\Delta G^0{}_{298\ K}=-37.40$ kJ mol^{-1} and $\Delta S^0=-13.58$ J mol^{-1} K^{-1} at 25^0 C were arrived.

It is clearly observed from the above experimental results that the complex formation between surfactant cobalt(III) complex and CT DNA for this case was spontaneous with negative ΔG^0 values. Generally from the thermodynamic data it is possible to find out the mode of interaction between small molecules and DNA [83]. It is well known that if both ΔH^0 and ΔS^0 are shown positive it will indicate that hydrophobic forces play a dominant role in the binding. If ΔH^0 and ΔS^0 are shown negative values then one can assume that van der Waals and hydrogen bonding interactions are the predominant forces of the binding. If ΔH^0 is shown as positive but ΔS^0 is negative it is possible to assume that the interaction forces are electrostatic. Making use of these information the type of binding force between the surfactant cobalt(III) complex in the present study and CT DNA was found out. In the present case, both ΔH^0 and ΔS^0 are shown negative indicating that intercalative interactions are probably the main forces in the binding of the investigated complex to CT DNA.

Competitive Binding Studies

To further verify the interaction mode between the surfactant cobalt(III) complex and CT DNA, EB fluorescence displacement assay was used. The intrinsic fluorescence intensity of DNA is very low and that of EB in Tris–HCl buffer is also not high due to quenching by solvent. However, on addition of DNA, the fluorescence intensity of EB is enhanced by intercalative binding to nucleic acid. A competitive binding of the metal complex to nucleic acid can result in the reduction of the emission intensity due to the decreasing of the binding sites of nucleic acid available for EB [84] EB (=3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide) is a phenanthridine fluorescence dye and is a typical indicator of intercalation, forming soluble complexes with nucleic acids and emitting intense fluorescence in the presence of CT DNA due to the intercalation of the planar phenanthridinium ring between adjacent base pairs on the double helix [85]. Bhattacharya and Mandal [86] have reported that the addition of cationic surfactants to EB–DNA complex can result in quenching of the fluorescence due to displacement of EB by the surfactants. Recently, Zhao et al. [87] found that the fluorescence quenching of EB–DNA by the Gemini surfactant may be due to the replacement of the DNA intercalator, i.e., EB.

When surfactant cobalt(III) complex solutions are added to nucleic acid pretreated with EB, the nucleic acid-induced emission intensity of EB decreases (Fig. 3 and SI Fig. 3). Addition of surfactant cobalt(III) complex has quenched the EB emission by replacing EB bound to nucleic acid. The binding was analyzed through Stern-Volmer equation, $I_0/I=1+K_{sv}[Q]$, where I_0 and I are the fluorescence intensities in the absence and presence of the complex, respectively, K_{sv} is the Stern-Volmer constant and Q is the concentration of surfactant cobalt(III) complex [75]. A plot of I_o/I vs. [Q] was drawn and shows linear plots. The K_{sv} values were obtained (Table 3) from slope. The observed quenching of DNA-EB fluorescence by surfactant cobalt(III) complex suggests that they

Table 3 The Melting Temprature (ΔT_m) and Stern-Volmer constant (K_{SV}) of surfactant cobalt(III) complex with CT DNA. [EB]=2×10^{-5} M, [DNA]=1×10^{-4} M, [Complex]=0–1.43×10^{-6}

Surfactant Cobalt(III) Complex	$K_{sv}\times10^3$ M^{-1}		ΔT_m (±0.2 °C) Binding Constant (M^{-1})	
	DNA	RNA	DNA	DNA
Complex	8.81	9.89	15	1.55×10^6

displace EB from the DNA-EB complex and they can interact with nucleic acid by the intercalative mode [88].

Cyclic Voltammetry Studies

The binding nature of surfactant cobalt(III) complex with nucleic acids was further confirmed by cyclic voltammetry experiments. Based on the shift of the formal potentials in the cyclic voltammograms of metal complexes the relative binding affinities and binding modes of the metal complexes with DNA can be determined [89]. The typical cyclic voltammograms (CV) of surfactant cobalt(III) complex in the absence and presence of nucleic acids are shown in Fig. 4 and SI Fig. 4 and the peak potentials are provided in the SI Table 2 and 3 . When nucleic acid is added to a solution of the surfactant cobalt(III) complex both the anodic and cathodic peak current heights of the complex is decreased in the same manner of increasing additions of nucleic acids, (Fig. 4 and SI Fig. 3). Also during nucleic acids addition the anodic peak potential (E_{pa}), cathodic peak potential (E_{pc}), and $E_{1/2}$ (calculated as the average of E_{pc} and E_{pa}) all showed positive shifts. These positive shifts are considered as evidences for intercalation of the complex into the nucleic acids. Had the molecule bound electrostatically to the negatively charged deoxyribose-phosphate backbone of DNA, negative peak potential shifts should have been detected. Therefore, the positive shift in the CV peak potentials of complex is indicative of intercalative binding mode of the complex with nucleic acids [90]. Thus, the electrochemical results are in agreement with the spectral studies, reinforcing the conclusion that the surfactant cobalt(III) complex binds to CT DNA in an intercalation mode.

Viscosity Studies

In order to prove conclusively the intercalative nature of the interaction between the surfactant cobalt(III) complex and nucleic acids viscosity measurements were carried out on DNA/RNA solution both in presence and absence of surfactant cobalt(III) complex. Spectroscopic data are necessary, but not sufficient to support for intercalative binding mode. Hydrodynamic measurements which are sensitive to length increases (i.e. viscosity, sedimentation, etc.) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structure data [91]. A classical intercalation mode can cause a significant increase in viscosity of DNA/RNA solution due to increase in separation of base pairs of nucleic acids at intercalation sites and hence an increase in overall nucleic acid contours length. By contrast, a partial and/or non-classical intercalation of a complex to the DNA helix will reduce its effective length and in turn, the solution viscosity [92]. The effect of the surfactant cobalt(III) complex on the viscosity of nucleic acid solution is given in SI Fig. 5. As illustrated in this figure, with increasing

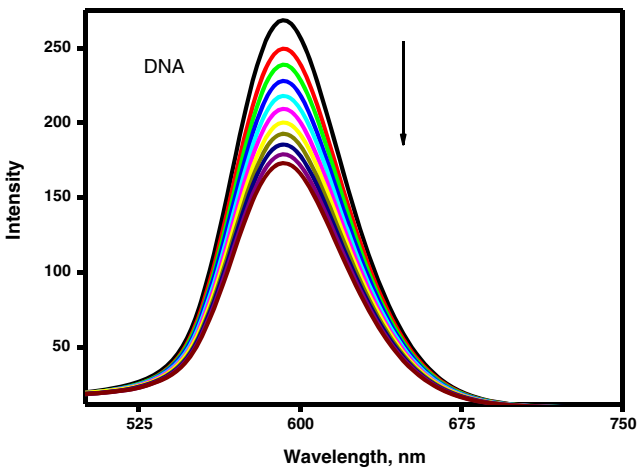


Fig. 3 Emission spectra of EB bound to DNA: in the absence and in the presence of the surfactant cobalt(III) complex. [DNA]=1×10^{-4} M; [complex]=5×10^{-4} M

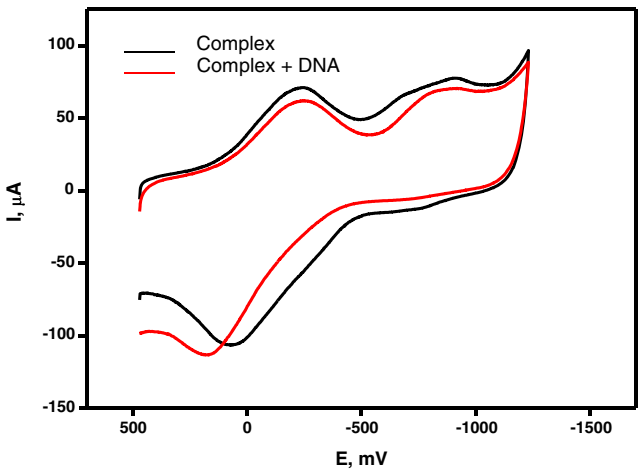


Fig. 4 CV spectra of Surfactant cobalt(III) complex in the absence (*black line*) and in the presence (*red line*) of DNA; [complex]=1×10^{-3} M; [DNA]=0–2.68×10^{-5} M

concentration of surfactant complex the relative viscosity of nucleic acids increases steadily, proving that surfactant cobalt(III) complex binds to nucleic acids by intercalation. Thus, the results of viscosity studies further validate those obtained from electronic absorption titration, fluorescence titration, and electrochemical titration.

The most direct evidence for the actual binding of the DNA with surfactant cobalt(III) complex might come from X-ray crystallographic studies. Unfortunately, all our efforts to grow crystals of the system for the surfactant cobalt(III) complex intercalating into CT DNA suitable for X-ray structure determination have been unsuccessful. However, based on the above discussion of the electronic absorption titration, thermal denaturation, EB fluorescence displacement experiment, electrochemical titration, and viscometry measurement, one can conclude that the intercalation is the most probable binding mode between the surfactant cobalt(III) complex and CT DNA.

Circular Dichroism Studies

Circular dichroism studies were conducted in order to determine the extent of change in conformation of DNA upon binding of complexes. The B form conformation of DNA shows two conservative CD bands in the UV region, a positive band at 278 nm due to base stacking and a negative band at 246 nm due to polynucleotide helicity as seen in Fig. 5 and SI Fig. 6. The CD spectrum of nucleic acid was monitored in the presence of increasing amounts of surfactant cobalt(III) complex. The positive band showed increase in molar ellipticity with a red shift of the band maxima when the concentration of each complex was progressively increased. This increase in intensity with a red shift in positive bands suggests that the surfactant cobalt(III) complex bind to nucleic acids via intercalation. In general, the intercalating molecules show increase in CD band intensity and red shift. If the complex binds via non-intercalating mode such as groove or electrostatic binding, they won't show any shift or small changes in CD band.

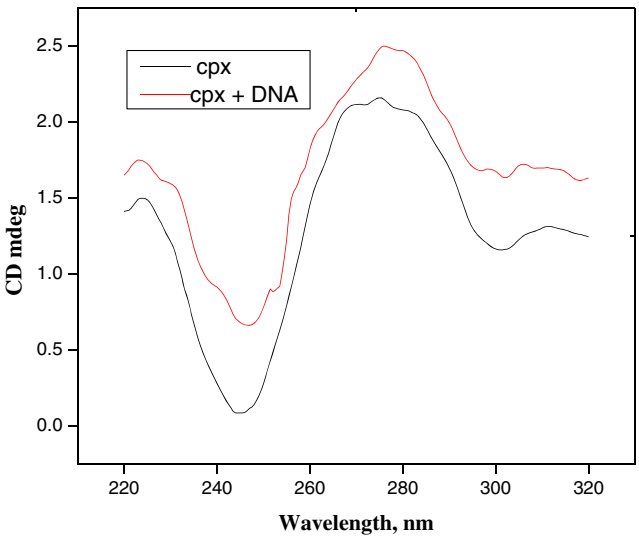


Fig. 5 Circular dichroism spectra of CT DNA in the absence (*black line*) and in the presence of surfactant cobalt(III) complexes (*red line*). [Complex]=1×10^{-5} M; [CT DNA]=1×10^{-5} M

Cytotoxity Studies

MTT Assay

We examined the cytotoxicity of the effects of the surfactant cobalt(III) complex on cultured HepG2 liver cancer cells by exposing cells for 24 and 48 h to the medium containing the complex at 10-90 μg/mL concentration. In vitro antitumor activity of the complex was determined according to the percentage of nonviable cells (%NVC) which was calculated by the following equation:

$$\text{NVC\%} = [\text{number of NVC}/\text{total number of cells}] \times 100$$

The results of these experiments are shown in SI Table 3. As shown in table increasing the concentration of surfactant cobalt(III) complex was accompanied by progressive decrease in the VC %. This is due to the fact that by increasing the concentration of cationic surfactant complex the adsorption of ions on cell membranes increases, leading to increase in penetration and antitumor activity.

The inhibition of cell viability percent showed that the surfactant cobalt(III) complex is the most active one (24 h) at a concentration of 90 μg/ml, the VC % reaching up to 7.2 for surfactant complex. This means that the drug at this concentration causes the death of most of the tumor cells. For 24 h treatment period, higher concentrations of the complex were required to kill the cells whereas for 48 h treatment the cell killing occurred at lower concentrations. The results of the cytotoxic activity on human tumor cell lines was determined according to the dose values of drug exposure required to reduce survival in the cell lines to 50 % (IC_{50}). The IC_{50} value of the complex was slightly higher for the 24 h treatment groups, i.e., in the range of 7.2–90 μg/mL, whereas for the 48 h treatment groups the IC_{50} value fell in the range of 5.4–90 μg/mL. It should be noted that the action of the complex as antitumor agents is found to be dependent on the type of tumor cell line tested but, as shown from the results, surfactant cobalt(III) complex show excellent cytotoxic activity against tumor cell lines and, at very low concentrations, reduces the survival to 50 %. This is due to the fact that cobalt complex have a capacity to reduce the energy status in tumors as well as to enhance tumor hypoxia, which also influences their antitumor activities. It may be also concluded that the level of cellular damage

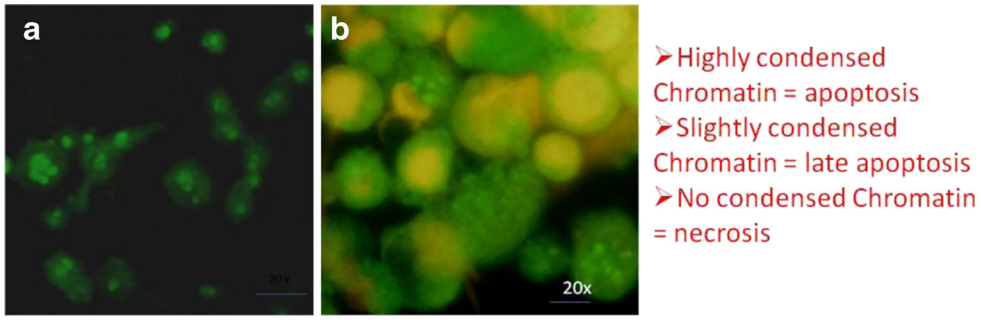


Fig. 6 Photomicrographs of control and AO and EB stained HepG2 liver cancer cells incubated for 24 h with surfactant cobalt(III) complex. A. Untreated control cells. B. surfactant cobalt(III) complex treated control cells.; Viable (light green), early apoptotic (bright green fluorescing), late apoptosis (red to orange fluorescing) and necrosis (red fluorescing) cells were observed. Magnification at 20×

inflicted by these complex depends on the nature of their axial ligands. It is known that phenanthroline-containing metal complexes have a wide range of biological activities such as antitumor, antifungal, apoptosis [93, 94] and interaction with DNA inhibiting replication, transcription and other nuclear functions and arresting cancer cell proliferation so as to arrest tumor growth. In general, the high selectivity of action by redox-active cobalt complex upon tumors is due to their specific reactivity [95]. From this results, surfactant cobalt(III) complex seems to offer promise due to the high electron affinity of the metal (which increases its ability to bind DNA) and the ready reducibility of the compounds [96].

Fluorescence Microscopic Analysis of apoptotic cell death (AO and EB staining)

AO/EB staining adopting fluorescence microscopy also revealed apoptosis from the perspective of fluorescence. After HepG2 liver cancer cells were exposed to the concentrations of surfactant cobalt(III) complex for 24 h. In this study, we used acridine orange/ethidium bromide (AO/EB) double staining assay [97]. Acridine orange is taken up by both viable and nonviable cells and emits green fluorescence if interrelated into double stranded nucleic acid (DNA) or red fluorescence if bound to single stranded nucleic acid (RNA). Ethidium bromide is taken up only by nonviable cells and emits red fluorescence by intercalation into DNA. We distinguished four types of cells according to the fluorescence emission and the morphological aspect of chromatin condensation in the stained nuclei: (1) viable cells showing light green fluorescing nuclei with highly organized structure; (2) early apoptotic cells having bright green fluorescing nuclei with chromatin condensation and nuclear fragments; (3) late apoptotic cells having orange to red fluorescing nuclei with condensed or fragmented chromatin; and (4) necrotic cells having red fluorescing without chromatin fragmentation. Viable cells have uniform bright green nuclei with organized structure. Apoptotic cells have orange to red nuclei with condensed or fragmented chromatin. Necrotic cells have a uniformly orange to red nuclei with condensed structure (Fig. 6). Our results indicate that the surfactant cobalt(III) complex induced apoptosis at the concentrations evaluated, in agreement with the cytotoxic results. The results suggest that the complex treatment caused more cells to take to death in HepG2 liver cancer cells.

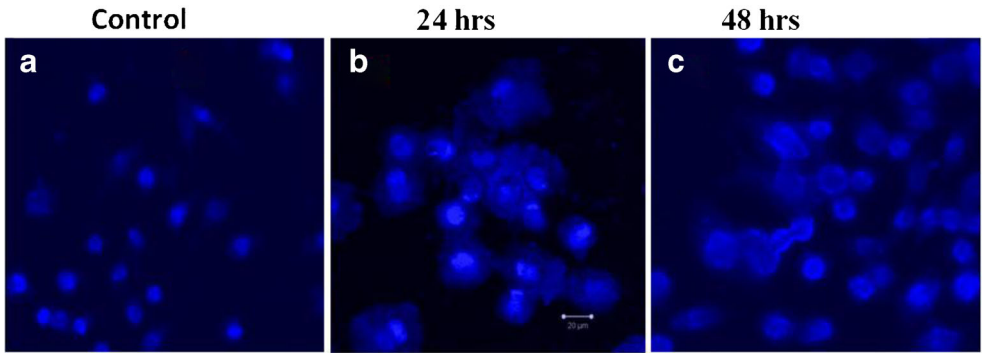


Fig. 7 Surfactant cobalt(III) complex induces apoptosis in HepG2 liver cancer cells. Representative fluorescent micrographs of HepG2 liver cancer cells stained with Hoechst 33258 fluorescent dye after the compound exposure for 24 and 48 h. A Untreated control cells; B, C surfactant cobalt(III) complex treated control cells. B-24 h; C-48 h. Magnifications at 20×

Apoptosis Detection Hoechst 33342 DNA Staining

It is possible to perform apoptosis detection assay with Hoechst 33342 (Sigma B-2262), but the increase in fluorescence seen in the apoptotic cells may be less dramatic. Hoechst dyes can also be obtained from Molecular Probes. H3342 is a "vital" DNA stain that binds preferentially to A-T base-pairs. The cells require no permeabilization for labeling, but do require physiologic conditions since the dye internalization is an active transport process. This condition typically varies among cell types (Stander et al. 2009). The procedure for Staining and analysis of cells using Hoechst 33342 (H342): To investigate if HepG2 liver cancer cells were triggered to undergo apoptosis due to the exposure of surfactant cobalt(III) complex, morphological changes of apoptosis was performed in the treated cells by Hoechst 33342 staining. Apoptosis is one of the major pathways that lead to the process of cell death [98]. After the cells were treated with IC_{50} concentrations of all the surfactant cobalt(III) complex (10–90 μg/mL) for 24 and 48 h the cells were observed for cytological changes adopting Hoechst 33342 staining. The observations revealed that the complex brought about cytological changes such as chromatin fragmentation, binucleation, cytoplasmic vacuolation, nuclear swelling, cytoplasmic blabbing and late apoptosis indication of dot-like chromatin and condensation (Fig. 7) whereas untreated cells did not show such changes. Data collected from the manual counting of cells with normal and abnormal nuclear features. Both apoptotic and necrotic cells increased in dose-dependent manner. These data clearly indicated that higher doses of all the surfactant cobalt(III) complex resulted in remarkable chromatin condensation and nuclear fragmentation in HepG2 liver cancer cells.

Conclusions

The synthesis and characterization of a new surfactant cobalt(III) complex, *cis*-$[Co(dpqc)_2(DA)_2](ClO_4)_3$ has been reported. As in our previous reports [44, 73], the critical micelle concentration value of this surfactant cobalt(III) complex is 0.7×10^7 M^{-1} and it has more capacity to associate forming aggregates, than ordinary synthetic organic surfactants. The nucleic acid binding property of the complex was investigated by electronic absorption, fluorescence, cyclic voltammetry and viscosity measurements. The binding towards nucleic acids reveal that the surfactant cobalt(III) complex interacts with nucleic acid by intercalation. In vitro cytotoxic activities suggest that the surfactant cobalt(III) complex is active against human liver carcinoma cancer cell line. Comparing the nucleic acid binding properties and in vitro anticancer activities with the previous surfactant cobalt(III) complexes reported by us, it is obvious that the modified phenanthroline ligands in the more hydrophobic systems influence the structure and DNA-binding ability. Activities of the surfactant cobalt(III) complex to cancer cell lines are in accord with their nucleic acid-binding abilities, suggesting that the cytotoxic activities of this surfactant cobalt(III) complex may be associated with or originate from their ability to intercalate the base pairs of DNA. The present investigation shows that the modified phenanthroline ligand containing surfactant systems can create interesting differences in the configuration and electron density distribution, which result in differences in DNA and RNA-binding behaviors and cytotoxic activities. This strategy should be valuable in understanding the DNA and RNA binding properties of the surfactant cobalt(III) complex as well as laying a foundation for the design of powerful agents for probing and targeting nucleic acids.

Acknowledgments We are grateful to the UGC- COSIST and DST-FIST programmes of the Department of Chemistry, Bharathidasan University, and UGC-RFSMS fellowship to one of the authors, K. Nagaraj, by Bharathidasan University. Financial assistance from the CSIR (Grant No. 01(2461)/11/EMR-II), DST (Grant No. SR/S1/IC-13/2009) and UGC (Grant No. 41-223/2012(SR) sanctioned to S. Arunachalam are also gratefully acknowledged.

References

1. Hegg EL, Burstyn JN (1998) Coord Chem Rev 173:133–165
2. Komiyama M, Sumaoka J (1998) Curr Opin Chem Biol 2:751–757
3. Norden B, Lincoln P, Akerman B, Tuite E (1996) In: Sigel A, Sigel H (eds) Marcel Dekker, New York. 33:177–252
4. Chan S, Wong WT (1995) Coord Chem Rev 138:219–296
5. Prativel GJ, Bernadou B, Meunier S (1998) Adv Inorg Chem 45:251
6. Liang F, Wang P, Zhou X, Li T, Li ZY, Lin HK, Gao DZ, Zheng CY, Wu CT (2004) Bioorg Med Chem Lett 14:1901–1904
7. Gallego J, Varani G (2001) Acc Chem Res 34:836–843
8. Chow CS, Barton JK (1992) Biochemistry 31:5423–5429
9. Morgan RJ, Chatterjee S, Baker AD, Strekas TC (1991) Inorg Chem 30:2687–2693
10. Zou XH, Ye BH, Li H, Zhang QL, Chao H, Liu JG, Ji LN (2000) J Biol Inorg Chem 6:143–150
11. Chao H, Mei WJ, Huang QW, Ji LN (2002) J Inorg Biochem 92:165–170
12. Radler JO, Koltover I, Salditt TCR (1997) Science 275:810–814
13. Khun PS, Levin Y, Barbosa MC (1999) Phys A Stat Mech Appl 274: 8–18
14. Akao T, Fukumoto T, Ihara HA (1996) FEBS Lett 391:215–219
15. Reimer DL, Zhang YP, Kong S, Wheeler JJ, Graham RW, Bally MB (1995) Health Environ Res Online 34:12877
16. Dan N (1997) Biophys J 73:1842–1846
17. Barreleiro PCA, Olofsson G, Alexandridis P (2000) J Phys Chem B 104:7795–7802
18. Melnikov SM, Sergeyev VG, Yoshikawa K (1995) J Am Chem Soc 117:2401–2408
19. Melnikov SM, Sergeyev VG, Yoshikawa K (1995) J Am Chem Soc 117:9951–9956
20. Lasic DD, Strey H, Stuart MCA, Podgornik R, Pederik PM (1995) J Am Chem Soc 119:832–833

21. Matulis D, Rouzina I, Bloomfield VA (2002) J Am Chem Soc 124: 7331–7342
22. Silvius JR (1991) Biochim Biophys Acta (BBA) Biomembr 1070: 51–59
23. Prager MD, Baechtel FS, Gordon WC, Maullin S, Steinberg J, Sanderson A, Tom BH, Six HR (eds) (1980) Elsevier, New York, 39
24. Menger FM, Littau CAJ (1993) J Am Chem Soc 115:10083–10090
25. Menger FM, Littau CA (1997) J Am Chem Soc 113:1451–1452
26. Shukla D, Tyagi VK (2006) J Oleo Sci 55:381–390
27. Zana R (2002) Adv Colloid Inter Sci 97:205–253
28. Zana T (2002) J Colloid Inter Sci 248:203–220
29. Hamaguchi J, Geiduschek EP (1962) J Am Chem Soc 84:1329–1338
30. Geck P, Nasz I (1983) Anal Biochem 135:264–268
31. Bhairi SM, Mohan C Calbiochem Detergents A guide to the properties and uses of detergents in biological systems, *EMD* Biosciences San Diego CA
32. Dias RS, Innerlohinger J, Glatter O, Miguel MG, Lindman B (2005) J Phys Chem B 109:10458–10463
33. Meidan VM, Cohen JS, Amariglio N, Hirsch-Lerner D, Barenholz Y (2000) Biochim Biophys Acta Biomembr 1464:251–261
34. Srinivasan S, Annaraj J, Athappan P (2005) J Inorg Biochem 99:876–882
35. Li WX, Hui C, Hong L, Lan HX, Jun LY, Feng TL, Nian JL (2004) J Inorg Biochem 98:1143–1147
36. Senthil kumar R, Arunachalam S Biophy Chem 136:136–144
37. Senthilkumar R, Arunachalam S, Periasamy VS, Paul CP, Riyasdeen A, Akbarsha MA (2009) J Inorg Biochem 103:117–127
38. Kumaraguru N, Santhakumar K, Arunachalam S, Arumugham MN (2006) Polyhedron 25:3253–3260
39. Wang XL, Chao H, Li H, Hong XL, Liu YJ, Tan LF, Ji LN (2004) J Inorg Biochem 98:1143–1193
40. Shimakoshi H, Kaieda T, Matsuo T, Sato H, Hisaeda Y (2003) Tetrahedron Lett 44:5197–5199
41. Zhang QL, Liu JH, Ren XZ, Xu H, Huang Y, Liu JZ, Ji LN (2003) J Inorg Biochem 95:194–198
42. Vaidyanathan VG (2003) Nair BU J Inorg Biochem 94:121–126
43. Barton JK, Raphael A (1985) Proc Natl Acad Sci U S A 82:6460–6464
44. Nagaraj K, Arunachalam S (2013) New J Chem. doi:10.1039/c3nj00832k
45. Nagaraj K, Arunachalam S (2013) Z Phys Chem 227:1–19
46. Nagaraj K, Sakthinathan S, Arunachalam S (2013) J Floures. doi:10.1007/s10895-013-1332-5
47. Tamilselvi P, Palaniandavar M (2002) Inorg Chim Acta 337:420–428
48. Zhang QL, Liu JG, Xu H, Li H, Liu JZ, Zhou H, Qu LH, Ji LN (2001) Polyhedron 20:3049–3055
49. Indumathy R, Kanthimathi M, Weyhermuller T, Nair BU (2008) Polyhedron 27:3443–3450
50. Marmur J (1961) J Mol Biol 3:208–218
51. Reichmann MF, Rice SA, Thomas CA, Doty P (1993) J Am Chem Soc 76:3047–3053
52. Collins JG, Sleeman AD, Aldrich-Wright JR, Greguric I, Hambley TW (1998) Inorg Chem 37:3133–3141
53. Wu JZ, Ye BH, Wang L, Ji LN, Zhou JY, Li RH, Zhou ZY (1997) J Chem Soc Dalton Trans 1395–1401
54. Cai J, Yue Y, Rui D, Zhang Y, Liu S, Wu C (2011) Macromolecules 44:2050–2057
55. Ghosh S, Barve AC, Kumbhar AA, Kumbhar AS, Puranik VG, Datar PA, Sonawane UB, Joshi RR (2006) J Inorg Biochem 100:331–343
56. Vlcek AA (1967) Inorg Chem 6:1425–1427
57. Kitson RE (1950) Anal Chem 22:664–667
58. Zana R (1980) J Colloid Inter Sci 78:330–337
59. Izumrudov VA, Zhiryakova MV, Goulko AA (2002) Langmuir 18: 10348–10356
60. Kelly TM, Tossi AB, McConnell DJ, Strekas TC (1985) Nucleic Acids Res 13:6017–6029
61. Yasui Y, Shimura Y (1963) Bull Chem Jpn 36:1286–1293
62. Nyholm RS, Tobe ML (1956) J Chem Soc 1691–1707
63. Oulaghan B (1978) Inorg Chem 17:2197–2202
64. Fordyee WA, Sherdan PS, Zinato E, Riccieri P, Adamson AA (1977) Inorg Chem 16:1154–1159
65. Jaeger DA, Reddy VB, Arulsamy N, Bohle DS (1998) Langmuir 14: 2589–2592
66. Shanthakumar K, Kumaraguru N, Arunachalam S, Arumugam MN (2006) Polyhedron 25:1507–1513
67. Castelleno S, Gunther H, Ebersole S (1965) J Phys Chem 69:4166–4176
68. Mukerjee P (1962) J Phys Chem 66:1375–1376
69. Galan JJ, Perez AG, Rodriquez JR (2003) J Therm Anal Calorim 72: 465–470
70. Perez AG, Castillo JD, Czapkiewicz T, Rodriquez JR (2002) Colloid Polymr Sci 280:503–508
71. Pyle AM, Rehmann JP, Meshoyrer R, Kumar CV, Turro NJ, Barton JK (1989) J Am Chem Soc 111:3051–3058
72. Long EC, Barton JK (1990) Acc Chem Res 23:271–273
73. Nagaraj K, Arunachalam S (2013) Int J Biol Macro Mol 62:273–280
74. Song Y, Wu Q, Yang P, Luan N, Wang L, Liu Y (2006) J Inorg Biochem 100:1685–1691
75. Carter MT, Rodriguez M, Bard AJ (1989) J Am Chem Soc 111:8901
76. Nehru S, Arunachalam S, Renganathan A, Premkumar K (2013) J Biomol Stru Dyn
77. Tselepi-Kalouli E, Katsaros N (1989) J Inorg Biochem 37:271–282
78. Cory M, McKee DD, Kagan J, Henry DW, Miller JA (1985) J Am Chem Soc 107:2528–2536
79. Kumar CV, Asuncion EH J Am Chem Soc 115:8547–8553
80. Waring MJ (1965) J Mol Biol 13:269–282
81. Neyhart GA, Grover N, Smith SR, Kalsbeck WA, Fairley TA, Cory M, Thorp HH (1993) J Am Chem Soc 115:4423–4428
82. McGhee JD (1976) Biopolymers 15:1345–1375
83. Satyanarayana S, Dabrowiak JC, Chaires JB (1993) Biochemistry 32: 2573–2584
84. Chan HL, Liu HQ, Tzeng BC, You YS, Peng SM, Yang M, Che CM (2002) Inorg Chem 41:3161–3171
85. Gao F, Chao H, Zhou F, Xu LC, Zheng KC, Ji LN (2007) Helv Chim Acta 90:36–51
86. Bhattacharya S, Mandal SS (1998) Biochemistry 37:7764–7777
87. Lincoln P, Tuite E, Norden B (1997) J Am Chem Soc 119:1454–1455
88. Satyanarayana S, Dabroniak JC, Chaires JB (1992) Biochemistry 31: 9319–9324
89. Sangeetha Gowda KR, Bhoiya Naik HS, Vinay Kumar B, Sudhamani CN, Sudeep HV, Avikumar Naik TR, Krishnamurthy G (2013) Spectro Chim Acta A Mol Biomol Spectrosc 105:229–237
90. Zhao G, Lin H, Zhu S, Sun H, Chen Y (1998) J Inorg Biochem 70: 219–226
91. Lakowicz JR, Webber G (1973) Biochemistry 12:4161–4170
92. Barton JK, Danishefsky AT, Goldberg JMJ (1984) Am Chem Soc 106:2172–2176
93. Coyle B, Kinsella P, McCann M, Devereux M, OConnor R, Clynes M, Kavanagh K (2004) Induction of apoptosis in yeast and mammalian cells by exposure to 1,10-phenanthroline metal complexes. Toxicol Vitro Int J Published Assoc BIBRA 18: 63
94. Coyle B, McCann M, Kavanagh K, Devereux M, Geraghty M (2003) Biometals 16:321–329
95. Osinsky S, Levitin I, Bubnovskaya L, Sigan A, Ganusevich I (2004) Exp Oncol 26:140–144
96. Badawi AM, Mekawias M, Mohamed MZ, Khowdairy MM (2007) J Can Res Ther 3:198–206
97. Baskic D, Popovic S, Ristic P, Arsenijevic NN (2006) Cell Biol Int 30:924–932
98. Stander A, Marais S, Stivaktas V (2009) J Ethnopharmacol 124:45–60