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Synthesis, micellar properties, DNA binding and antimicrobial studies of some surfactant–cobalt(III) complexes

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ABSTRACT

A new class of surfactant–cobalt(III) complex ions of the type, *cis*-$[Co(X)_2(C_{14}H_{29}NH_2)Cl]^{2+}$ (where X = ethylenediamine (en), or 2,2'-bipyridyl (bpy), or 1,10-phenanthroline (phen)) and *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl]^{2+}$ (trien = triethylenetetramine) were synthesized and characterized by IR, NMR, UV–visible electronic absorption spectra, elemental analysis and metal analysis. The critical micelle concentration (CMC) values of these surfactant–cobalt(III) complexes in aqueous solution were obtained from conductance measurements. Specific conductivity data (at 298, 308, 318 and 328 K) served for the evaluation of the temperature-dependent CMC and the thermodynamics of micellization (ΔG^0_m, ΔH^0_m and ΔS^0_m). Interactions between calf thymus DNA and the surfactant–cobalt(III) complexes in aqueous solution have been investigated by electronic absorption spectroscopy, emission spectroscopy and viscosity measurements. The electrostatic interactions, van der Waals interactions and/or partial intercalative binding have been observed in these systems. The surfactant–cobalt(III) complexes were screened for their antibacterial and antifungal activities against various microorganisms. The results were compared with the standard drugs, Ciprofloxacin and Fluconazole respectively.

1. Introduction

DNA plays a fundamental role in the storage and expression of genetic information in a cell. DNA is not only an important biological material with a unique double helical rodlike structure, but also an interesting anionic polyelectrolyte. Studies on the interaction of transition metal complexes with DNA have been pursued in recent years [1–4]. These complexes are stabilized in binding to DNA through a series of weak interactions, such as the π-stacking interactions associated with intercalation of a planar aromatic group between the base pairs, hydrogen-bonding and van der Waals interactions of functionalities bound along the groove of the DNA helix, and the electrostatic interaction of the cation with phosphate group of DNA. Studies directed toward the design of site- and conformation-specific reagents provide rationales for new drug design as well as a means of developing sensitive chemical probes of nucleic acid structure.

Surfactants, sometimes called suface-active agents, are among the most versatile chemicals available. They are amphiphilic molecules consisting of a hydrophilic head group and a hydrophobic (lipophilic) tail and are, thus, able to interact with both polar and non-polar compounds. Accordingly, surfactants are often classified as non-ionic or ionic (cationic, anionic or zwitterionic). Surfactants are major building blocks of many physical, chemical and biological systems. They have been introduced into several commercial products such as antiseptic agents in cosmetics and as germicides [5], and also have found a wide range of applications because of their unique solution properties such as detergency, solubilization and surface wetting capabilities, in diverse areas such as mining, petroleum and pharmaceutical industries [6]. Cationic surfactants offer some additional advantages over other classes of surfactants. These substances, besides their surface activity, do show antitumor properties [7]. Cationic surfactant–DNA interactions have been the subject of many studies over the past few decades because they are of interest both in fundamental science and in biotechnological applications [8–10]. Studies have shown that the binding of surfactant to DNA is cooperative based on the binding isotherm, and is similar to the interaction of surfactant with a synthetic polymer [11–13].

Surfactant–metal complexes are a special type of surfactants, where a coordination complex (containing a central ion with surrounded ligands coordinated to the metal) acts as the surfactant (Scheme 1). In these surfactants, the metal complex part containing the central metal ion with its primary coordination sphere acts as the head group and the hydrophobic part of one or more ligands acts as tail part. Like any other well-known surfactants, these metallosurfactant complexes also form micelles at a specific concentration called critical micelle concentration (CMC) in aqueous solution. There are but a few reports [14–17] on the synthesis, isolation and characterization of surfactant transition metal complexes, in contrast to numerous reports of the formation and study of such surfactants in solution without isolation. We have been interested in the synthesis and micelle forming properties of cobalt(III)/chromium(III) complexes

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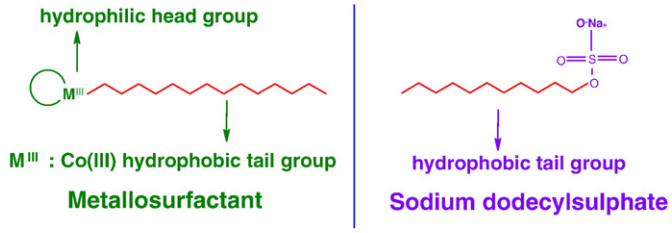

Scheme 1.

containing lipophilic ligands for a long time [18–21]. As in biology, such compounds may exhibit novel physical and chemical properties with interesting and useful associated applications.

A characteristic feature of transition metals is their ability to form complexes with a variety of neutral molecules such as bipyridine (bpy) and phenanthroline (phen). These are widely used as a classical *N,N* - bidentate ligand to prepare mixed-ligand complexes in coordination chemistry. Metal complexes of bipyridine and phenanthroline chelators are of great interest since they exhimit numerous biological properties such as antitumor, anticandida and antibacterial activity [22–24]. At the same time, metal complex bearing ethylenediamine have also been interest because in the classical antitumor agent *cis*-platinum, one of the ligands must be a N-donor and posses at least one hydrogen atom attached to the nitrogen [25].

In spite of the greatest effort and success in the study of metallosurfactants of cobalt(III) complexes, such complexes still attract much attention due to their interesting properties and the relative simplicity of their synthesis. To the best of our knowledge no previous studies are available to find the interaction of DNA with metallosurfactants. From this point of view the results presented here are of interest . In the present paper, we report the synthesis, CMC determination and DNA binding properties of various surfactant–cobalt(III) complexes using different physico-chemical methods. Also we have reported the antibacterial and antifungal activities of these surfactant–cobalt(III) complexes against certain human pathogenic microorganisms.

2. Experimental

2.1. Materials and methods

All the reagents were of analytical grade (Aldrich and Merck). Calf thymus DNA obtained from Sigma-Aldrich, Germany, was used as such. The spectroscopic titration was carried out in the buffer (50 mM NaCl-5 mM Tris–HCl, pH 7.1) at room temperature. 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 of protein [26]. Milli-Q water was used to prepare the solutions.

Absorption spectra were recorded on a UV–VIS–NIR Cary300 Spectrophotometer using cuvettes of 1 cm path length, and emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. FT-IR spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. ^{1}H & ^{13}C NMR spectra were recorded on a BRUKER 500 MHz Spectrometer using DMSO as solvent. Conductivity studies were done in aqueous solutions of the complexes with an Elico conductivity bridge type CM 82 and a dip-type cell with a cell constant of 1.0.

2.2. Synthesis of single chain metallosurfactants of cobalt(III) complexes

2.2.1. *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) and *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (2)

These surfactant–cobalt(III) complexes, were prepared by a similar method to those complexes reported in our previous literature [21].

2.2.2. *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (3)

To a solution of *cis*-$[Co(bpy)_2Cl_2]Cl_2$ [27] (0.53 g) dissolved in water (10 mL), tetradecylamine (0.25 g) in ethanol (2 mL) was added drop by drop over a period of 30 min. The dark violet colour solution gradually became brown during the reaction. The mixture was set aside at room temperature for 2 days until no further change was observed. Afterwards a saturated solution of sodium perchlorate in very dilute perchloric acid was added. Slowly a pasty solid mass separated out and it was filtered off, washed with small amounts of alcohol followed by acetone, and then it was dried over air. The semi-dried solid was further dried in a drying pistol over fused calcium chloride and stored in a vacuum desiccator.

The procedure for synthesizing *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (4) complex is similar to that described above, but in the place of bipyridyl complex, phenanthroline complex *cis*-$[Co(phen)_2Cl_2]Cl$ [27] was used.

Caution: Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of the material should be prepared and handled with great care.

2.3. Cobalt analysis

Cobalt content in the surfactant–cobalt(III) complexes were estimated by following Kitson's method [28]. A known weight of the complex was reduced with tin and concentrated hydrochloric acid. The reduced aqueous cobalt(II) ion was made upto 10 mL in volumetric flask using 0.1 M perchloric acid. 2 mL of this solution and 1 mL of 50% ammonium thiocyanate solution were pipetted out into a 10 mL volumetric flask and made upto the mark with acetone. The absorbance of this solution was measured at 625 nm against a reagent blank. From the absorbance, the concentration of the cobalt was calculated. The percentage of cobalt thus obtained for our surfanctant-cobalt(III) complexes, are shown in Table 1.

2.4. Determination of critical micelle concentration (CMC)

The CMC values of the complexes were determined conductometrically using a specific conductivity meter. The conductivity cell was calibrated with KCl solutions in the appropriate concentration range. The cell constant was calculated using molar conductivity data for KCl [29]. Various concentrations of surfactant–cobalt(III) complexes were prepared in the range 10^{-5}–10^{-1} mol dm^{-3} in aqueous solution. The

Table 1
Microanalysis and visible spectra of surfactant–cobalt(III) complexes

Surfactant–cobalt(III) complexes	λ_{max} (nm)	Yield (%)	(%) Found (Calc.)			
			Co	C	N	H
cis-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	513	62	9.57 (9.40)	34.72 (34.49)	11.32 (11.17)	7.38 (7.56)
cis-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	484	57	9.45 (9.03)	36.53 (36.79)	10.58 (10.73)	7.69 (7.56)
cis-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$	519	51	6.78 (6.75)	33.29 (33.02)	4.58 (4.81)	4.73 (4.50)
cis-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$	512	64	6.54 (6.40)	33.82 (33.90)	4.39 (4.56)	4.35 (4.26)

conductivity of these solutions was measured at 298, 308, 318 and 328 K. The temperature of the thermostat was maintained constant to within ±0.01 K. 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 occurred.

2.5. DNA-binding experiments

The DNA concentration per nucleotide was determined by absorption spectroscopy using the known molar extinction coefficient value of 6600 M^{-1} cm^{-1} at 260 nm [30]. Absorption titrations were performed by using a fixed surfactant–cobalt(III) complex concentration to which increments of the DNA stock solution were added. Surfactant–cobalt(III) complex-DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded.

For fluorescence experiments, DNA was pretreated with ethidium bromide (EB) for 30 min. The surfactant–cobalt(III) complexes were then 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. These experiments were carried out in 50 mM NaCl-5 mM Tris–HCl at pH 7.1 in aqueous media.

Viscosity experiments were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 29.0±0.1 °C. Calf thymus DNA samples of approximately 200 base pair of average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility [31]. Data were presented as $(\eta/\eta^0)^{1/3}$ versus binding ratio [32], where η is the viscosity of DNA in the presence of the complex, η_0 is the viscosity of DNA alone. The relative specific viscosity was calculated according to the relation $\eta=(t-t_0)/t_0$, where t_0 is the flow time for the buffer and t is the observed flow time for DNA in the presence and absence of the complex.

2.6. Microbial assay

The *in vitro* antimicrobial screening of the surfactant–cobalt(III) complexes were tested for their effect on certain human pathogenic bacteria and fungus by disc diffusion method [33]. The complexes were stored dry at room temperature and dissolved in DMSO (1%). Both the Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria were grown in nutrient agar medium and incubated at 37 °C for 48 h followed by frequent subculture to fresh medium and were used as test bacteria. The yeast *Candida albicans* grown into Sabouraud dextrose agar medium, incubated at 27 °C for 72 h followed by periodic subculturing to fresh medium and were used as test fungus. Then the petri dishes were inoculated with a loop full of bacterial or fungal culture and spread throughout the petri dishes uniformly with a sterile glass spreader. To each disc the test samples (10 μg/mL) and reference Ciprofloxacin (5 μg/disc for bacteria) or Fluconazole (10 μg/disc for fungus) was added with a sterile micropipette. The plates were then incubated at 35±2 °C for 24–48 h and 27±1 °C for bacteria and fungus, respectively. Plates with disc containing respective solvents served as control. Inhibition was recorded by measuring the diameter of the inhibitory zone after the period of incubation. All the experiments were repeated thrice and the average values are presented.

3. Results and discussion

3.1. Spectroscopic characterization

Infrared spectroscopy is used to distinguish the mode of coordination of the ligand with the central metal ion. Various workers have employed the NH_2 deformation mode (1700–1500 cm^{-1} region), the CH_2 rocking mode (950–850 cm^{-1} region) and Co–N stretching mode in the 600–500 cm^{-1} region to distinguish between *cis* and *trans* isomers [34,35]. The *cis*-isomers always show two peaks, whereas the *trans*-isomers usually have only one peak in the CH_2 rocking region. In the present study, the NH_2 deformation mode shows two bands in the region 1600–1530 cm^{-1} [36], two bands for the CH_2 or NH_2 twist mode in the region 1060–980 cm^{-1} [37], and two bands for the CH_2 rock mode in the region 900–850 cm^{-1}. Strukl and Walter [38], and Schilt and Taylor [39] studied the infrared spectra of several bipyridyl (bpy) and phenanthroline (phen) complexes. They observed that the spectral shape of the bpy and phen complexes were quite similar, taking into account the similarity of both ligands. The IR bands, δ (C-H) 853 cm^{-1}, 737 cm^{-1} observed for phenanthroline are redshifted to 844 and 714 cm^{-1} in the surfactant–cobalt(III)-phenanthroline complex, *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (4). This shift can be explained by the fact that the nitrogen atoms of phenanthroline ligands donate a pair of electrons each to the central cobalt metal forming a coordinate covalent bond [40]. Besides it is also confirmed by the shift of ν(C–N) of phenanthroline from about 1670 cm^{-1} in the free ligand to 1630 cm^{-1} after coordination [41]. The spectra of bipyridine complexes in general are less complicated than those of the phen complexes. Only three strong bands are present, one near 760 ascribed to out of plane bending of ring hydrogens, and one near 1450 cm^{-1} which is probably a ring frequency, and a ring frequency near 1600 cm^{-1}. For all the surfactant–cobalt(III) complexes, the bands exhibit around 2925 cm^{-1} and 2853 cm^{-1} can be assigned to C–H asymmetric and symmetric stretching vibration of aliphatic CH_2 of tetradecylamine. Perchlorate bands at *ca.* 1100 and 620 cm^{-1} belong to an ionic species; this means that this counter-ion is not involved in the cobalt-ligand coordination [42]. The IR spectroscopic data therefore clearly indicate a *cis* configuration for the surfactant–cobalt(III) complexes.

In the ^1H NMR spectrum of *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) complex, due to the lower symmetry, methylene protons of ethylenediamine show a more complex absorption around 2.1–2.9 ppm. The methylene protons of the long chain moiety (tetradecylamine) give rise to a multiplet usually at 1.2–1.8 ppm, whereas the terminal methyl group of the hydrocarbon chain substituent gives a triplet around 0.85 ppm). The ^1H NMR spectral data for the *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (2) complex, exhibit signals in the region 2.5–3.2 ppm attributable to the $-CH_2$ group of the triethylenetetramine chelate ring. In the ^1H NMR spectrum of *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (4) and *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (3) complexes, the aromatic protons appear in the region 7–10 ppm and assigned in a manner similar to the case of their respective paranet complexes [27,43]. The protons of long chain moiety (tetradecylamine) appear in the same region like surfactant–cobalt (III)-ethylenediamine and triethylenetetramine complexes.

The ^{13}C NMR spectrum of surfactant–cobalt(III) complexes exhibits only one signal around 35–40 ppm because of merging of tetradecylamine and chelating ligand signals. For long chain tetradecylamine, the aliphatic methylene carbons of the all the surfactant–cobalt (III) complexes appeared around 15–40 ppm and the terminal carbon atom appeared around 14 ppm. The ^{13}C NMR spectra of *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (4) and *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (3) complexes, exhibit signals in the aromatic region (130–160 ppm) corresponding to phenanthroline/bipyridine rings.

3.2. Critical micelle concentration values (CMC)

The specific conductivities of the surfactant complexes change with the total surfactant concentration and with temperature. The specific conductivity increases sharply in the pre-micellar region with the surfactant concentration, but is somewhat reduced at certain concentrations, which reflects the CMC. The CMC values were computed from the slope of [Complex] *versus* specific conductance data. The complex concentration at which the micellization starts was

Table 2
CMC values of some surfactant–cobalt(III) complexes in aqueous solution

Surfactant–cobalt(III) complexes	CMC ($mol\ dm^{-3}$)				ΔG^0_m ($kJ\ mol^{-1}$)	ΔH^0_m ($kJ\ mol^{-1}$)	$T\Delta S^0_m$ ($kJ\ mol^{-1}$)
	298 K	308 K	318 K	328 K			
cis-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	3.47×10^{-4}	3.95×10^{-4}	4.61×10^{-4}	4.89×10^{-4}	−34.18	−16.54	17.64
cis-$[Co(trien)\ (C_{14}H_{29}NH_2)Cl]\ (ClO_4)_2$	3.01×10^{-4}	3.57×10^{-4}	3.96×10^{-4}	4.41×10^{-4}	−35.76	−14.19	21.57
cis-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl]\ (ClO_4)_2$	6.95×10^{-5}	7.43×10^{-5}	8.01×10^{-5}	8.49×10^{-5}	−40.17	−9.04	31.13
cis-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	6.38×10^{-5}	6.45×10^{-5}	6.94×10^{-5}	7.43×10^{-5}	−41.95	−7.12	34.83

evident from the change in the slope of the plot and that particular concentration is the CMC under the experimental conditions. The CMC values were determined at four different temperatures (298, 308, 318 and 328 K). At all temperatures a break in the conductance *versus* concentration plots, characteristic of micelle formation, was observed. The CMC values were determined by fitting the data points above and below the break to two equation of the form $y=mx+c$ and solving the two equations simultaneously to obtain the point of intersection. Least-squares analysis was employed and correlation coefficients were greater than 0.98 in all the cases. The conductivity measurements at four different temperatures were repeated three times and the accuracy of CMC values (Table 2) was found to be within ±2% error. Fig. 1 illustrates the plot for the complexes, *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) and *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (3); similar plots (not shown) were obtained for the other surfactant–cobalt(III) complexes. It was observed that CMC values increases on increasing the temperature for a given system. This behavior may be related to two competitive effects. Firstly, a temperature increase causes a decrease in hydration in the hydrophilic group, which favors micellization. Secondly, a temperature increase also disrupts the water surrounding the hydrophobic group, and this retards micellization. The relative magnitude of these two opposing effects will determine CMC behavior.

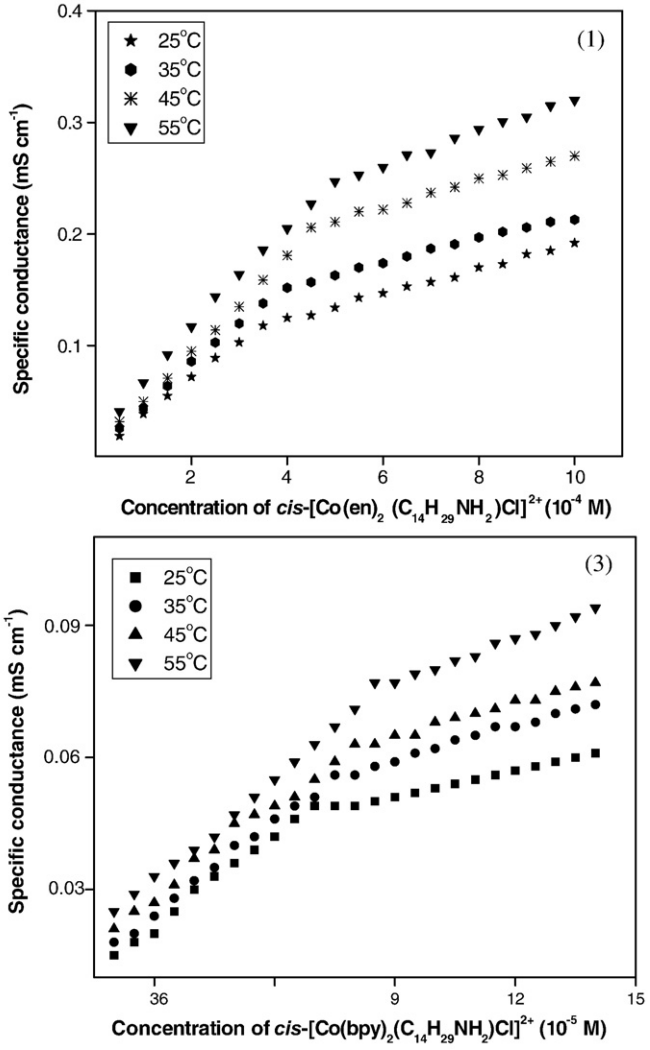

Fig. 1. Plots of concentration versus conductance of surfactant-cobalt(III) complexes 1 and 2 in aqueous solution.

3.3. Thermodynamics of micellization

The study of CMC versus temperature is often undertaken to obtain information on hydrophobic and head group interactions. This involves deriving 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 [44–46]. According to these models, the standard Gibbs free energy of micelle formation per mole of monomer, ΔG^0_m, is given by

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

where R, T and α_{ave} are gas constant, absolute temperature and average degree of micellar ionization (The micelle ionization degree at the CMC, was obtained as the ratio between the slopes of the nearly linear specific conductance *versus* [Complex] plots above and below the CMC [47]), respectively.

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

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

Once the Gibbs free energy and the enthalpy of micelle formation are obtained, obviously the entropy of micelle formation can be determined by

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

The thermodynamic parameters of micellization for the surfactant–cobalt(III) complexes are compiled in Table 2. The observed more negative Gibbs free energy of micellization indicates more favored micellization for the system under study. Moreover, since the changes of CMC with temperature are small, the value of ΔH^0_m and ΔS^0_m must be rather inaccurate and should be considered as only approximate. Nusselder and Engberts [48] have suggested that for negative ΔH^0_m values, London-dispersion forces play a major role in the micelle formation. Positive values of ΔS^0_m clearly indicate that the micellization of the studied surfactants in aqueous solution is governed mainly by hydrophobic interactions between the surfactant cations, resulting in the breakdown of the structured water surrounding the hydrophobic groups, and indicates that the cationic surfactant formation is an entropy driven process. As mentioned in our previous reports [20,21], the CMC values for surfactant–cobalt(III) complexes in the present study are also very low compared to that of the simple organic surfactants. Thus it is suggested that these metal surfactant

complexes have more capacity to associate themselves forming aggregates compared to those of ordinary synthetic organic surfactants. Moreover, introduction of a metal complex to the hydrophilic part of the amphiphile can remarkably enhance the ability of aggregation.

3.4. DNA binding studies

3.4.1. Electronic absorption spectral studies

Electronic absorption spectra are initially employed to study the binding of surfactant–cobalt(III) complexes and DNA. Intercalative binding of complex with DNA generally results in hypochromism and bathochromism, due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [49,50]. The extent of hypochromism generally indicates the intercalative binding strength. The absorption spectra of the complex **4**, *cis*-[Co(phen)$_2$($C_{14}H_{29}NH_2$)Cl](ClO_4)$_2$ (below CMC and above CMC), in the absence and in the presence of CT-DNA are shown in Fig. 2. With increasing concentration of calf thymus DNA, the absorption bands of the complex are affected, resulting in the obvious tendency of hyperchromism and slight blue shift. Similar hyperchromism has been observed for the Soret bands of certain porphyrins when interacted with DNA but has not yet been clearly explained [51].

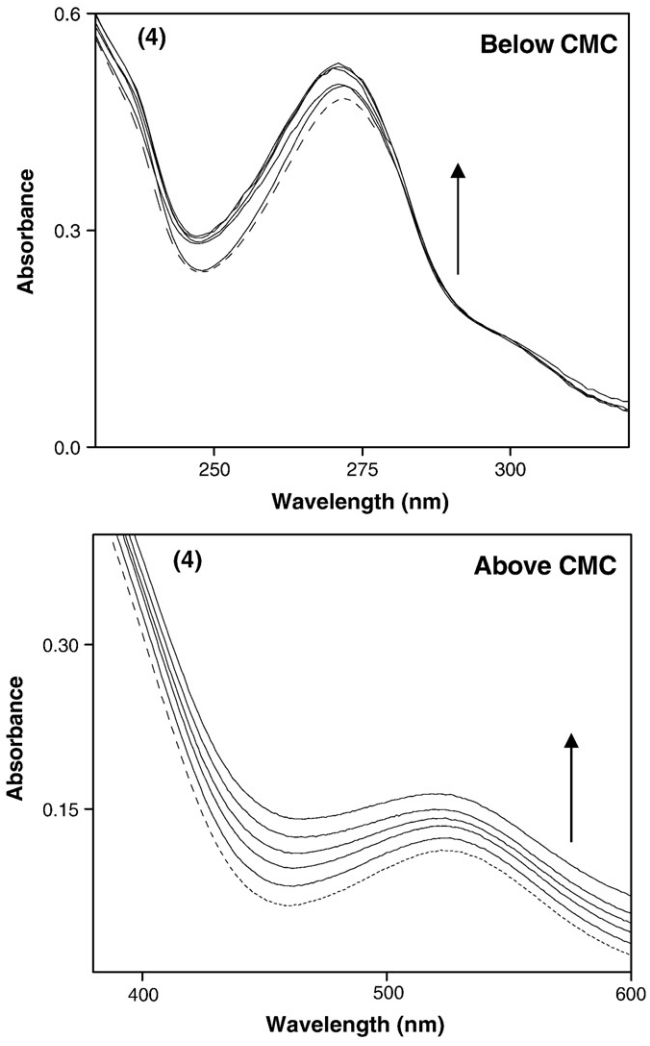

Fig. 2. Absorption spectra *cis*-[Co(phen)$_2$($C_{14}H_{29}NH_2$)Cl](ClO_4)$_2$ (4) in the absence (dotted lines) and in the presence of increasing amounts of DNA(solid lines), [Complex] = 10 μM (below CMC), 0.75 mM (above CMC), [DNA] = 2–9 μM. Arrow shows the absorbance changes upon increasing DNA concentrations.

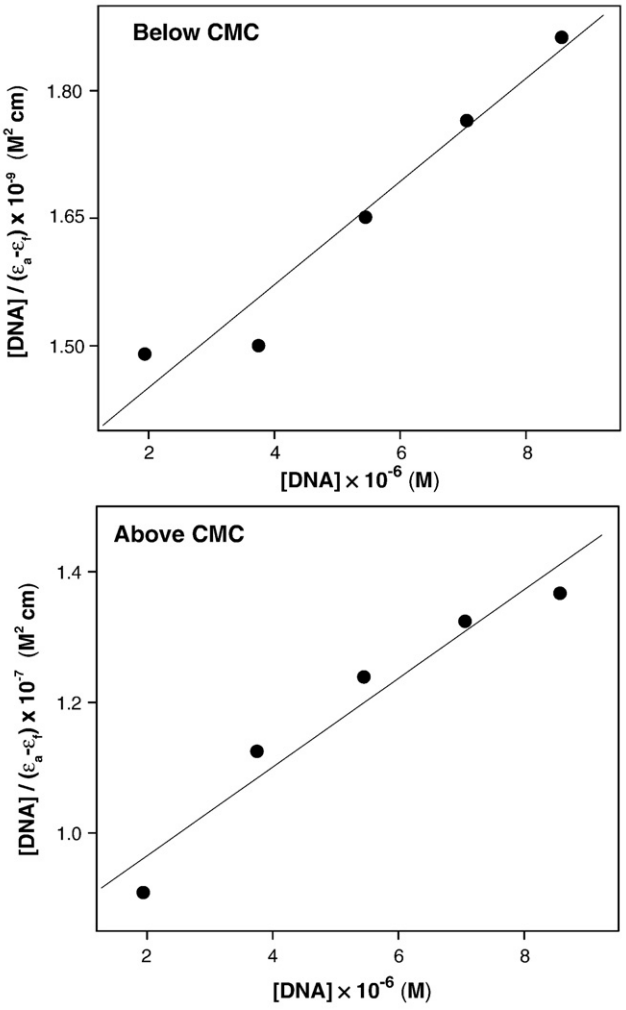

Fig. 3. Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA] for the absorption titration of DNA with *cis*-[Co(phen)$_2$($C_{14}H_{29}NH_2$)Cl](ClO_4)$_2$ (4); ●, experimental data points; solid line, linear fitting of the data.

The surfactant–cobalt(III) complexes can bind to the DNA in different binding modes on the basis of their structure, charge and type of ligands. The surfactant in the complex contains several methyl groups, binds to DNA by van der Waals interactions between the methylene groups and the thymine methyl group, and hydrophobic interactions between the methyl group in the complex and DNA interior [52–54]. The surfactant–cobalt(III) complexes, **1** and **2** do not contain any fused aromatic ring to facilitate intercalation, classical intercalative interaction would be impossible. In complex **3**, bipyridyl ligand which is expected to be non-planar and possesses a smaller π system than pheanthroline, a classical intercalation is precluded [55]. Since the surfactant–cobalt(III) complex **4**, contains phenanthroline ligand, which should provide an aromatic moiety extending from the metal center through which overlapping occurs with base pairs of DNA by intercalation. However, the hyperchromism effects observed in the present study suggest that there is a strong hydrophobic association between the hydrocarbon chain (of surfactant) and hydrophobic interior of DNA.

In order to compare the binding strengths of the complexes, the intrinsic binding constant, K_b, was determined by using the equation [56],

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_o - \varepsilon_f) + 1/K_b(\varepsilon_o - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_o correspond to A_{obsd}/[Co], the extinction coefficient of the free cobalt complex and the extinction coefficient of the complex in the fully

Table 3
The intrinsic binding constants (K_b) of surfactant–cobalt(III) complexes with calf thymus DNA

Surfactant–cobalt(III) complexes	K_b (M^{-1})	
	Below CMC	Above CMC
cis-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	–	2.59×10^4
cis-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	–	3.83×10^4
cis-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	2.86×10^4	5.95×10^4
cis-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$	4.54×10^4	8.19×10^4

bound form, respectively, and K_b is the intrinsic binding constant. The ratio of slope to intercept in the plot of [DNA]/($\varepsilon_a-\varepsilon_f$) versus [DNA] gives the value of K_b (Fig. 3). We have calculated the intrinsic binding constant value for our surfactant–cobalt(III) complexes at below CMC and above CMC. At below CMC as the concentration of complexes **1** and **2** are very low we are not able to measure absorbance for the complexes, *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) and *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (2). The intrinsic binding constants for the surfactant–cobalt(III) complexes are shown in Table 3. The table shows that the binding constant of complex **4** is higher than the other surfactant–cobalt(III) complexes **1**, **2** and **3**. Also the binding constants at below CMC values are lower than those of the corresponding constants at above the CMC values. Due to the presence of phenanthroline ligand and higher hydrophobicity, complex **4**, binds with DNA more strongly than the other complexes.

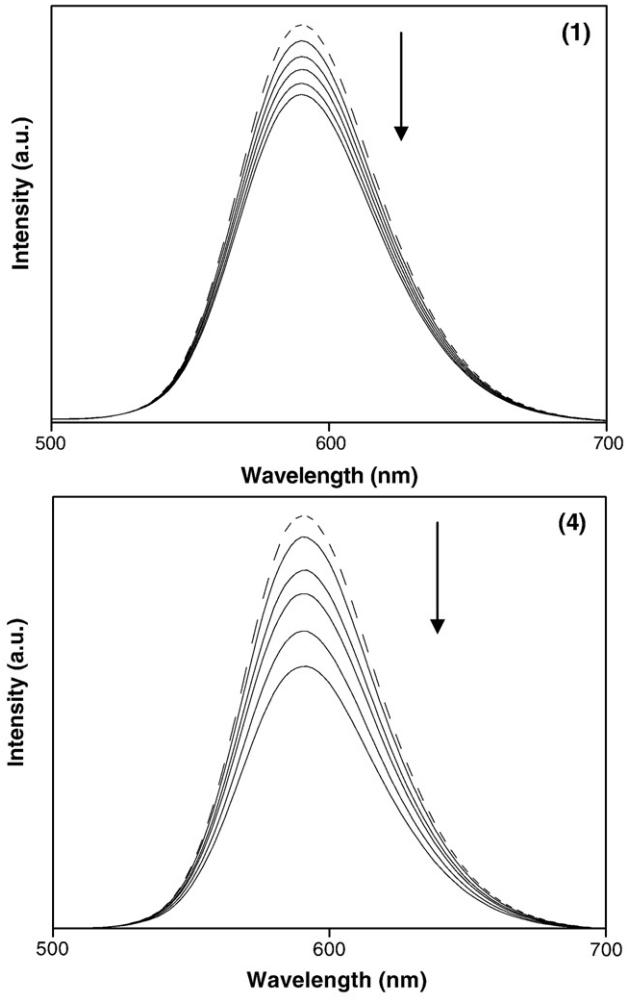

Fig. 4. Emission spectra of EB bound to DNA in the absence(- - -) and in the presence of *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) and *cis*-$[Co(phen)_2(C_{14}H_{29}NH_{20})Cl](ClO_4)_2$ (4), [EB]=4 μM, [DNA]=4 μM, [Complex]=0–20 μM. Arrow shows intensity changes upon increasing complex concentrations.

3.4.2. Competitive binding between ethidium bromide(EB) and surfactant–cobalt(III) complexes

The surfactant–cobalt(III) complexes, however, are non-fluorescent on excitation in the visible region. Hence, competitive binding studies using ethidium bromide (EB) bound to DNA were carrided out for surfactant–cobalt(III) complexes. Ethidium bromide (EB) is a well known cationic dye widely used as probe for native DNA [57]. The fluorescence intensity of EB is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double-stranded DNA. It was previously reported that the fluorescent light could be quenched by the addition of a second molecule [58,59]. Positively charged species [60], such as small cationic ions, polypeptides, and polyamines with linear or branched structures, may competitively displace the dye from DNA to solution, leading to the evident quenching of EB fluorescence. Bhattacharya and Mandal [61] have reported that the addition of cationic surfactant to EB-DNA complex can result in quenching of the fluorescence due to the displacement of EB by the surfactant. Recently, Zhao *et al.* [9] have observed that the fluorescence quenching of EB-DNA by the gemini surfactant may be due to the replacement of the DNA intercalator, *i.e.* EB.

The emission spectra of EB bound to DNA in the absence and the presence of the surfactant–cobalt(III) complex is given in Fig. 4. The addition of the complex to DNA pretreated with EB causes appreciable reduction in the emission intensity, indicating that the replacement of the EB fluorophore by the complex results in a decrease of the binding constant of the ethidium bromide to the DNA.

According to the classical Stern–Volmer equation [59]:

$$I_0/I = 1 + K_{sv}r$$

where I_0 and I are the fluorescence intensities in the absence and the presence of complex, respectively. K_{sv} is a linear Stern–Volmer constant and r is the ratio of the total concentration of complex to that of DNA. The fluorescence quenching curve of EB bound to DNA by the surfactant–cobalt(III) complexes are shown in Fig. 5. The quenching plots illustrates that the quenching of EB bound to DNA by the surfactant–cobalt(III) complexes are in good agreement with the linear Stern–Volmer equation, which also indicates that the complexes binds to DNA. In the plot of I_0/I *versus* [Complex]/[DNA], K_{sv} is given by the ratio of the slope to intercept (Fig. 5). The K_{sv} values for our surfactant–cobalt(III) complexes **1**, **2**, **3** and **4** are 0.036, 0.045, 0.053 and 0.077, respectively. These data suggest that the interaction of complex **4**, with DNA is strongest, followed by complexes **3**, **2** and then **1**, which is consistent with the above absorption spectral results.

3.4.3. Viscosity measurements

To further explore the binding of the surfactant–cobalt(III) complexes, viscosity measurements were carried out on calf thymus

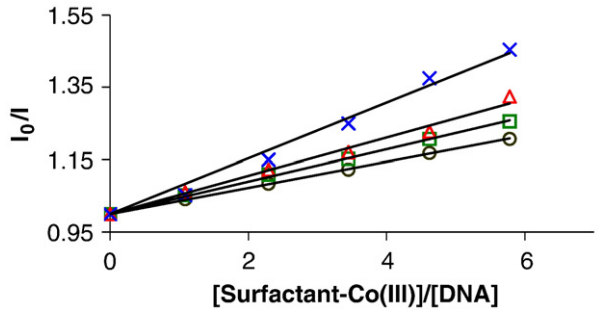

Fig. 5. Fluorescence quenching curves of EB bound to DNA by *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (○), *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (□), *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (Δ), and *cis*-$Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (×).

DNA by varying the concentration of the added complexes. Hydrodynamic method, such as determination of viscosity, which is exquisitely sensitive to the change of length of DNA, may be the most effective means studying the binding mode of complexes to DNA in the absence of X-ray crystallographic or NMR structural data [62]. Optical or photophysical probes generally provide necessary, but not sufficient clues to support an intercalative binding model. Intercalation of the complexes to DNA is known to cause a significant increase in the viscosity of a DNA solution due to the separation of the base pairs at the intercalation site and, hence an increase in the overall DNA molecular length. In contrast, groove-face or electrostatic interactions typically cause a bend (or kink) in DNA helix reducing its effective length and thereby its viscosity.

The effects of the surfactant–cobalt(III) complexes **1**, **2**, **3** and **4**, together with ethidium bromide (EB) on the viscosity of DNA were shown in Fig. 6. The intercalator ethidium bromide (EB) significantly increases the relative specific viscosity of DNA as expected for the lengthening of the DNA double helix resultant from well-characterized intercalation. In contrast, the binding of surfactant–cobalt(III) complexes **1**, **2** and **3**, to DNA greatly decreases the relative specific viscosity of DNA while for the complex **4**, it exerts slight increase on DNA viscosity. Based on the viscosity results, it was observed that the surfactant–cobalt(III) complexes **1**, **2** and **3**, could bind DNA by external contact (surface binding) or groove binding whereas the complex **4**, involves a partial and/or nonclassical intercalation mode. This is probably related to the molecular structure of the complex. In complex **4**, the phenanthroline (phen) ligand is somewhat sterically hindered from planarity and not completely intercalated with DNA. The complex would act as a "wedge" to pry apart once side of a base pair stack but not fully separate the stack as required by the classical intercalation model. So it is likely that the observed slight increase in relative viscosity of complex **4**, is not due to intercalative interaction but due to partial and/or nonclassical interaction.

3.5. Antibacterial and antifungal screening

The surfactant–cobalt(III) complexes (**1**–**4**) were screened *in vitro* for their microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. These complexes were found to exhibit considerable activity against Gram positive

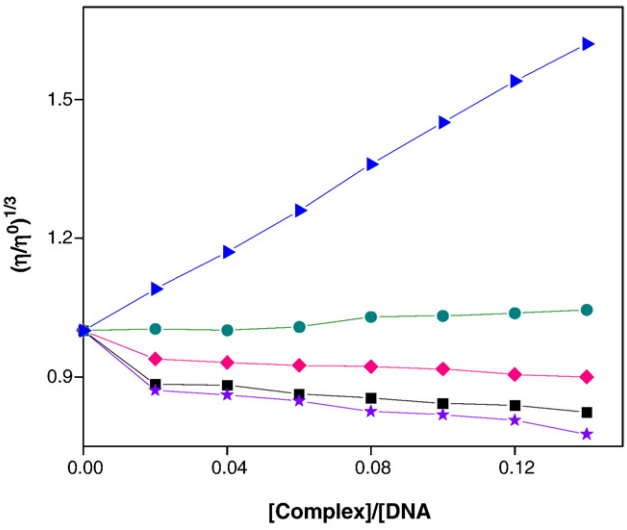

Fig. 6. Effects of increasing amounts of *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (■), *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (★), *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (◆) *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (●) and EB (▲) on the relative viscosities of calf thymus DNA at 29.0 (±0.1) °C.

Table 4
Antimicrobial activities of surfactant–cobalt(III) complexes, *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1), *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (2), *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (3) and *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2{\cdot}3H_2O$ (4)

Test organisms	Diameter of inhibition zone (mm)				
	Surfactant–cobalt(III) complexes				
	1	2	3	4	Standard
Staphylococcus aureus	16	18	8	15	32
Bacillus subtilis	13	16	12	11	34
Escherichia coli	13	15	8	12	32
Pseudomonas auregenosa	8	10	f	f	32
Candida albicans	13	20	8	11	25

Standard — Ciprofloxacin for bacteria and Fluconazole for fungus.
Solvent — DMSO (Showed nil effect against the microorganisms under test).
f — No inhibition zone is determined.

(*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and the pathogenic yeast *Candida albicans*. The test solutions were prepared in dimethyl sulphoxide (1%) and the results of the antimicrobial activities are summarized in Table 4.

The surfactant–cobalt(III) complexes showed significant microbial activity against Gram positive, Gram negative bacteria and fungus. In our biological experiments, using surfactant–cobalt(III) complexes, we have observed high antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) than Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The surfactant–cobalt(III) complexes are also very active against the yeast *Candida albicans*. The activity of these surfactant–cobalt(III) complexes may be due to an efficient diffusion of the metal complexes into the baterial/fungal cells and/or interaction with the bacterial/fungal cells [63]. The antimicrobial activity of these surfactant–cobalt(III) complexes were also compared with standard drugs Ciprofloxacin (for bacteria) and Fluconazole (for fungi). Out of the four surfactant–cobalt (III) complexes, the complex, **2** possessed very good activity against all the microorganisms. This may be due to higher hydrophobic character of the complex which can damage the bacterial/fungal cellular membrane/wall. It may be concluded that our surfactant–cobalt(III) complexes **1**–**4**, inhibit the growth of bacteria and fungi.

4. Conclusion

As mentioned in our previous reports [20,21], the critical micelle concentration values of surfactant–cobalt(III) complexes in the present study are also very low compared to that of the simple organic surfactant, dodecylammonium chloride ($CMC = 1.5 \times 10^{-2}$ mol dm^{-3}). Thus it is concluded that these metal surfactant complexes have more capacity to associate themselves, forming aggregates, compared to those of ordinary synthetic organic surfactants. The binding behavior of these surfactant–cobalt(III) complexes with calf thymus DNA were characterized by absorption titration, fluorescence quenching and viscosity measurements. The results show that the binding constants followed the order: *cis*-$[Co(phen)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (4) > *cis*-$[Co(bpy)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (3) > *cis*-$[Co(trien)(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (2) > *cis*-$[Co(en)_2(C_{14}H_{29}NH_2)Cl](ClO_4)_2$ (1) indicating that the surfactant–cobalt(III) complex containing phenanthroline ligand binds strongly than the other surfactant–cobalt(III) complexes. We conclude that electrostatic interaction, van der Waals interaction and/or partial intercalation binding modes have been observed in these systems. The surfactant–cobalt(III) complexes showed good antimicrobial activity against Gram positive and Gram negative bacteria and fungi. Thus our results show that the surfactant–cobalt(III) complexes can be a candidates for DNA binding reagents, as well as laying the foundation for the rational design of new useful DNA probes. They can also be considered for antifungal and antibiotic drugs.

Acknowledgements

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