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ORIGINAL PAPER

Synthesis, CMC Determination, Antimicrobial Activity and Nucleic Acid Binding of A Surfactant Copper(II) Complex Containing Phenanthroline and Alanine Schiff-Base

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Abstract A new water-soluble surfactant copper(II) complex [Cu(sal-ala)(phen)(DA)] (sal-ala = salicylalanine, phen = 1,10-phenanthroline, DA = dodecylamine), has been synthesized and characterized by physico-chemical and spectroscopic methods. The critical micelle concentration (CMC) values of this surfactant–copper(II) complex in aqueous solution were obtained from conductance measurements. Specific conductivity data (at 303, 308, 313. 318 and 323 K) served for the evaluation of the temperature-dependent CMC and the thermodynamics of micellization (ΔG^0m, ΔH^0m and ΔS^0m). The interaction of this complex with nucleic acids (DNA and RNA) has been explored by using electronic absorption spectral titration, competitive binding experiment, cyclic voltammetry, circular dichroism (CD) spectra, and viscosity measurements. Electronic absorption studies have revealed that the complex can bind to nucleic acids by the intercalative binding mode which has been verified by viscosity measurements. The DNA binding constants have also been calculated ($K_b=1.2\times10^5$ M^{-1} for DNA and $K_b=1.6\times10^5$ M^{-1} for RNA). Competitive binding study with ethidium bromide (EB) showed that the complex exhibits the ability to displace the DNA-bound-EB indicating that the complex binds to DNA in strong competition with EB for the intercalative binding site. The presence of hydrophobic ligands, alanine Schiff-base, phenanthroline and long aliphatic chain amine in the complex were responsible for this strong intercalative binding. The surfactant–copper (II) complex was screened for its antibacterial and antifungal activities against various microorganisms. The results were compared with the standard drugs, amikacin(antibacterial) and ketokonazole(antifungal).

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Introduction

Schiff base ligands have significant importance in chemistry; especially in the development of Schiff base complexes, because Schiff base ligands are potentially capable of forming stable complexes with metal ions [1]. Schiff base macro cycles have been of remarkable versatility in macrocyclic and supramolecular chemistry and therefore they have played an important role in the development of coordination chemistry as they readily form stable complexes with most of the transition metals. The research field dealing with transition metal complexes with macrocyclic Schiff base has expanded enormously and embraced wide and diversified subjects comprising vast areas of organometallic compounds [2]. In the area of bioinorganic chemistry the interest in the Schiff base complexes lies in that they provide synthetic models for the metal-containing sites in metalloproteins/enzymes and also contributed enormously to the development of medicinal chemistry, radio immunotherapy, cancer diagnosis and treatment of tumor [3]. Schiff bases are the compounds containing azimethine group (−HC=N−) which play an essential role in the field of coordination chemistry [4]. Interest in the binding of metal complexes to nucleic acid has been motivated not only by a desire to understand the basics of these interaction modes but also by the development of metal complexes into anti-inflammatory and anticancer agents. Hence, much attention has been targeted on the design of metal-based complexes, which can bind to DNA [5]. It is currently admitted that RNAs play a key role in many biological processes involving living cells and are considered as the essential

regulators of many steps of gene expression [6]. 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 function could be powerful tools for understanding and controlling gene expression [7]. Binding of metal complexes with 1,10-phenanthroline or modified phenanthroline ligands to DNA has attracted much attention [8]. But only a few reports have investigated the interactions between transition metal complexes and RNA [9]. In recent years, the interaction of cationic metal complexes containing multidentate aromatic ligands, as a new agent studying anti-cancer characteristics with DNA have gained a growing importance [10]. Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified. Because of its biological relevance, a large number of copper(II) complexes have been synthesized and explored for their biological activities [11]. Among these copper complexes, attention has been mainly focused on the copper(II) complexes of 1,10-phenanthroline ligand due to their high nucleolytic efficiency and antimicrobial activities etc. In the past decades, the interactions between nucleic acid and cationic surfactants have attracted immense interest in the context of separation and purification of DNA [12]. Surfactant-metal complexes can be defined as surfactant molecules that contain a metal complex ion as part of the head group. These systems represent a new class of materials in which the polar headgroup of the surfactant molecule contains a metal complex portion as an integral structural component. Haifz et al. has reported [13] on the synthesis, isolation and characterization of surfactant transition metal complexes. Though there are numerous reports to study surfactant solution without isolation, a few reports on the synthesis, isolation and characterization of surfactant transition metal complexes available.

We have been interested in the synthesis and micelle forming properties of copper(II) and cobalt(III) complexes containing lipophilic ligands [14–16]. In spite of the greatest effort and success in the study of surfactant metal complexes, such complexes still attract much attention due to the relative simplicity of their synthesis and their interesting properties. In this paper, we report the synthesis, critical micelle concentration (CMC) determination and DNA/RNA binding properties of a surfactant–copper(II) complex containing 1,10-phenanthoroline and alanine Schiff-base as ligands.

Experimental

Materials

Calf thymus DNA (CT-DNA), Ethidium bromide (EB), yeast tRNA, and L-alanine were purchased from Sigma-Aldrich, and were used as such. Copper(II) chloride monohydrate, 1,10-phenanthroline, and salicylaldehyde were purchased from Merck, India. All the experiments involving the interaction of the surfactant-copper(II) 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

The carbon, hydrogen, nitrogen and oxygen contents of the samples were determined at SAIF, Cochin University, Cochin, Kerala. Absorption spectra were recorded on a Shimadzu UV-Visible spectrophotometer using cuvettes of 1 cm path length, and emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. Infra-red spectra were recorded on FT-IR JASCO 460 PLUS spectrophotometer with samples prepared as KBr pellets. EPR spectra were recorded on JEOL-FA200 EPR spectrometer. 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.

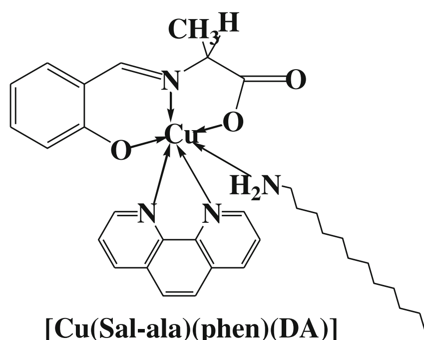
Synthesis of the Surfactant-Copper(II) Complex

The precursor metal complex [Cu(Sal-ala)(phen)(H_2O)] was prepared according to the literature procedure as follows [17]. A mixture of the ligands, 1,10-phenanthroline (5 mmol; 0.99 g in 20 mL methanol) and salicylalanine (5 mmol; 0.97 g in 20 mL methanol was homogenized and added dropwise to a solution of $CuCl_2.H_2O$ (5 mmol; 0.86 g in 20 mL water) while stirring at 50 °C. The bright green precipitate, [Cu(Sal-ala)(phen)(H_2O)] obtained was filtered off, washed with ethanol and dried in a desiccator.

The complex [Cu(Sal-ala)(phen)(H_2O)] (3.0 g) was dissolved in 20 cm^3 water. To this solution slightly more than the calculated amount of dodecyl amine in 3 cm^3 ethanol was added dropwise over a period of 30 min. The green solution gradually became red and the mixture was set aside at 45 °C for 4 days until no further change was observed. Slowly the surfactant copper(II) complex, [Cu(sal-ala)(phen)(DA)] was separated out as a pasty solid mass and was filtered off, washed with small amounts of alcohol and acetone, and then dried over air. The semidried solid was further dried in a drying pistol over fused calcium chloride and stored in vacuum desiccator. The amino acid Schiff-base surfactant copper(II) complex [Cu(sal-ala)(phen)(DA)] is shown in Scheme 1.

Critical Micelle Concentration (CMC) Value

CMC values of the surfactant copper(II) complex at different temperatures were obtained by through conductometric method. The conductivity cell (dip type with a cell constant of 1.0)



Scheme 1 Structure of [Cu(sal-ala)(phen)(DA)]

was calibrated with KCl solutions in the appropriate concentration range. The cell constant was calculated using molar conductivity data for KCl published by Shedluosky et al. [18]. Various concentrations of the complexes were prepared in the 10^{-5}–10^{-1} mol dm^{-3} range in aqueous solution. The conductivities were measured at 303, 308, 313, 318 and 323 K. The temperature of the thermostat was maintained constant within ±0.01 K.

Nucleic Acid Binding Experiments

The nucleic acid binding experiments were performed at 25.0 ±0.2 °C. The DNA concentration per nucleotide was determined by spectrophotometrically using the known molar extinction coefficient value of 6600 M^{-1} cm^{-1} and 9250 M^{-1} cm^{-1} for DNA and RNA respectively at 260 nm [19]. Milli-Q water was used to prepare tris buffer. The absorption titration was performed by using a fixed complex concentration to which increasing amount of the DNA/tRNA stock solution was added. Surfactant copper(II) complex-DNA solutions were allowed to incubate for 20 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.

Emission studies were done using ethidium bromide as the emitting agent. Fluorescence quenching experiments were done by using DNA/tRNA pretreated with ethidium bromide (EB) and kept for 30 min for incubation. The surfactant copper(II) complex was then added as a second molecule to this EB-DNA mixture and the effect on emission intensities were measured. Samples were excited at 450 nm and emission was observed between 500 and 700 nm.

The cyclic voltammetry (CV) experiments were performed in a single compartment cell with a three-electrode configuration glassy carbon working electrode, platinum wire auxiliary electrode and saturated calomel as reference electrode on a EG&G PAR 273 potentiostat equipped with a personal computer. The supporting electrolyte was tris buffer at pH 7.1. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements; during measurements a stream of nitrogen gas was passed over the solution.

Viscometric experiments were carried out using an Ubbelodhe viscometer of 2 mL capacity thermostated in a water bath maintained at 25 °C. The flow rates of the buffer (10 mM), DNA (100 μM) and DNA in the presence of Cu(II) complex at various concentrations (10–100 μM) were measured with a manually operated timer at least three times to agree within 0.2 s. The relative viscosity was calculated according to the relation, $\eta = t - t_0/t_0$ [20]. 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. A plot of $(\eta/\eta_0)^{1/3}$ versus 1/R, {R=[DNA]/[complex]} was constructed from viscosity measurements.

Circular dichroism spectra (220–320) of CT-DNA were recorded at 25 °C. It was scanned for each sample in buffer CT-DNA used was 2×10^{-5} M and surfactant-Cu(II) complex was added to a ratio of [Cu]/[DNA]=2:1. The CD spectra studies for each sample were repeated at least three times.

Antimicrobial Tests

The antimicrobial tests were carried out in the Bose clinical laboratory Madurai, India. Six species of micro-organisms namely Chromo bacterium, Serratia, Staphylococus aureus, Bacillus megaterium, Aspergillus and candida albicans (yeast) were used for this study.

Diffusion Tests

In vitro antibacterial and antifungal activities of the ligands and the resulting complexes were evaluated by the well-diffusion method. Muller-Hinton broth was employed as microbial growth medium. The micro-organisms were cultured for 18 h in an incubator. Some colonies of these were dissolved in a 0.9 % NaCl sterile solution and homogenized to obtain turbidity comparable to that of 0.5 on the McFarland scale. This suspension was then diluted to 1/100th to obtain the inoculums used which correspond to 1.5×10^6 CFU/Ml.

Molten nutrient agar was poured (to a height of 8 mm) into sterile 15 cm diameter petri dishes and alloed to solidify. The nutrient agar was trhen inoculated with the mmicro-organisms by inundation and the dishes were allowed to dry for 10 min at ambient temperature in an incubator. Holes of 6 mm diameter were then punched carefully using a sterile cork borer and these were filled with 80 uL of the compounds (DMSO and aqueous solutions 3 mg/mL). the plates were then kept at 4 °C for 30 min to allow for pre-diffusion and then transferred to an incubator maintained at 37 °C. The width of the growth inhibition zone around the well was measured after 24 and 48 h of incubation. Three replicas were made for each sample and mean values of the growth inhibition zone were calculated.

Results and Discussion

Characterization

The surfactant–copper(II) complex synthesized in the present study was characterized by UV–vis, IR, and EPR techniques. The purity of the complex was checked by, C, H, N, O analysis, and the results were found to be in good agreement with the calculated values. (Found: C, 66.6 %; H, 7.5 %; N, 8.6 %; O, 7.4 % $C_{36}H_{48}CuN_4O_3$ calcd: C, 66.1 %; H, 7.1 %; N, 8.9 %; O, 7.7 %) The electronic absorption spectra are often very helpful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements were used to assign the stereochemistries of the metal ions in the complexes based on the positions and number of d-d transition peaks. The electronic absorption spectra of the ligand and its complexes were recorded at room temperature. The intense absorption bands in the range 222 nm have been assigned to π-π* transition of the coordinated phenanthroline ligand while those at 272 nm assigned to the n-π* transitions of the salicylidene chromopore respectively [21]. The electronic spectrum of the Cu(II) complex exhibited only one broad d-d transition at 11904 cm^{-1} assigned to the ^2E_g-$^2T_{2g}$ transition, which is in conformity with the octahedral configuration around the copper ion. Though three transitions are expected in this case, they are very close in energy and often appear in the form of one broad band envelope [22].

The strong characteristic bands of 1,10-phenanthroline at 832 cm^{-1} (out of plane CH center ring [23] shift to lower frequencies upon complexation with copper). This shift can be explained by the fact that the nitrogen atoms of 1,10-phenanthroline ligands donate a pair of electrons each to the central cobalt metal forming a coordinate covalent bond [24]. The absence of bands in the region 2080–2140 cm^{-1} associated with the N-H bonds of the amino acids also confirms the presence of the Schiff-base in the resulting complex. The bands at 929–970 cm^{-1} have been assigned to Cu-O and those at 432–547 cm^{-1} to Cu-N [25]. The asymmetric stretch, γs (COO) is evidenced by the medium broad beaks observed in the range 1345–1426 cm^{-1}. This give rise to a Δγ[γas(COO)- γs(COO)] of 147–257 cm^{-1} characteristic of monodentate coordination of the carboxylate group [21]. The band around 2915 cm^{-1} and 2883 cm^{-1} can be assigned to C–H asymmetric and symmetric stretching vibration of aliphatic CH_2 of dodecylamine.

EPR studies of paramagnetic transition metal(II) complexes yield information about the distribution of the unpaired electrons and the nature of the bonding between the metal ion and its ligands. The solid state EPR spectra of our surfactant-copper(II) complex was recorded in X-band frequencies at room temperature as well as in frozen solution (77 K) (Fig. 1). The complex exhibits well defined single isotropic features near g=2.10 at room temperature (Inset plot of Fig. 1). Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species. At 77 K we observed two peaks. This is because the copper complex units have been mounted on a surfactant chain resulting in some spin–spin coupling between the copper complex units. The results exhibit g-tensor parameters with g-tensor parameters with g‖>g⊥>2.0023. This indicates that the copper site has a dx^2-y^2 ground state [26] characteristic of square planar or octahedral geometry in copper(II) complexes. The value of exchange interaction term G, estimated from the following expression is 0.96.

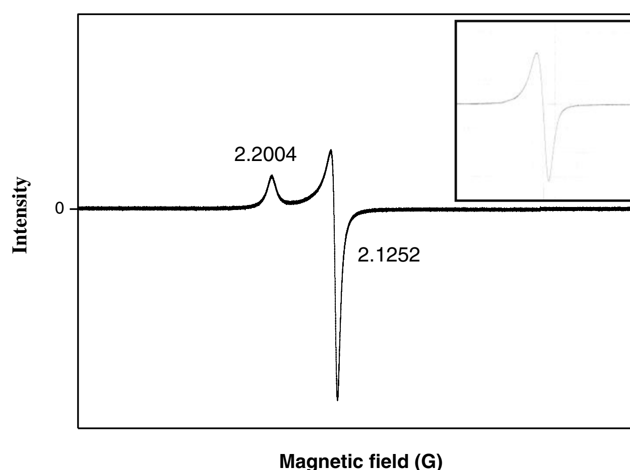


Fig. 1 EPR spectrum of [Cu(sal-ala)(phen)(DA)] at LNT (liquid nitrogen temperature)

$$G = g_{\|}-2.0023/g_{\perp}-2.0023$$

G>4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If G<4.0, significant exchange coupling is present and misalignment is appreciable. The observed value for the exchange interaction term G suggests that the complex has distorted octahedral geometry and the Schiff base ligand forming Cu(II) complex is considered as a strong field ligand.

Determination of Critical Micelle Concentration (CMC)

The CMC values of the surfactant copper(II) complex were computed from the slope of plots of conductivity versus complex concentration. The complex concentration at which the micellization starts was evident from the change in the slope of the plot and that particular concentration was taken at the CMC under the experimental conditions. 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 Fig. 2. Least-squares analysis was employed, and the correlation coefficients were greater than 99 % in all the cases.

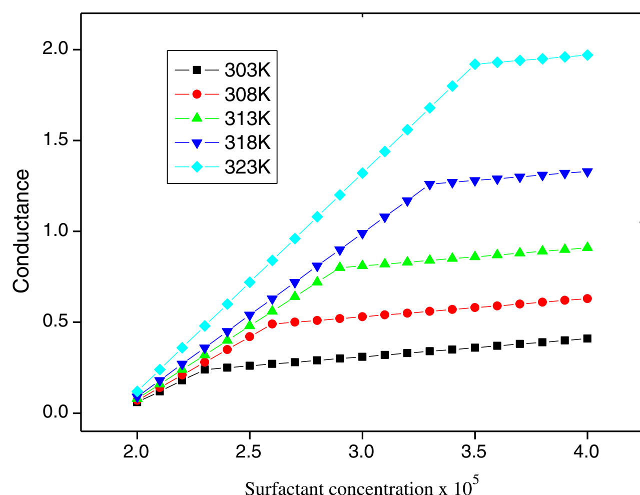


Fig. 2 Electrical conductivity vs. copper(II) complex concentration for aqueous [Cu(sal-la)(phen)(DA)] solutions

The conductivity measurements at five different temperatures (303, 308, 313, 318 and 323 K) were repeated three times and the accuracy of CMC values (Table 1) was found to be within ±2 %. The CMC value thus obtained for our complex is 2.37×10^{-5} mol dm^{-3} at 30 °C. It was observed normally that the CMCs increase with increase in the temperature for a given system. This is due to a decrease in hydration in the hydrophilic group and disruption of the water surrounding the hydrophobic group.

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. Gonzalez-Perez et al. has reported the change in the CMC with temperature has been analyzed in terms of the phase separation model of for micelle formation [27]. In accordance to these models, the standard free energy of micelle formation per mole of monomer,

$\Delta G^0{}_m$, is given by

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

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 [28, 29]), respectively. The enthalpy of micelle formation can be obtained by applying the Gibbs–Helmholtz equation to Eq. (1)

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

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

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

The thermodynamic parameters of micellization for surfactant copper(II) complex of the present study are compiled in Table 1. Since the changes of CMC with temperature are small, the values of $\Delta H^0{}_m$ and $\Delta S^0{}_m$ must be rather inaccurate and should be considered as only approximate. The negative values of enthalpy ($\Delta H^0{}_m$) of micellization indicate the exothermic nature of the micellization process.

The positive values of $\Delta S^0{}_m$ clearly indicate that the micellization of the studied surfactant copper(II) complex in aqueous solution is governed mainly by hydrophobic interactions between the surfactant complex molecules, resulting in the breakdown of the structured water surrounding the hydrophobic groups. Similar to our previous reports [30], the CMC values for the surfactant complex in the present study are very low compared to that of the simple organic surfactant, dodecylammonium chloride (CMC$=1.5\times10^{-2}$ mol dm^{-3}). Thus, it is suggested that our surfactant copper(II) complex molecules have more capacity to associate themselves into micellar aggregates when compared with common organic surfactants.

Nucleic Acid Binding Studies

Absorption Titration

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques [31]. Any complex binding with DNA through intercalation

Table 1 CMC values of [Cu(sal-ala)(phen)(DA)] in aqueous solution

Temperature	CMC×10^5	-$\Delta G^0{}_{mic}$ (kJ mol^{-1})	-$\Delta H^0{}_{mic}$(kJ mol^{-1})	T$\Delta S^0{}_{mic}$ (kJ mol^{-1})
298 K	2.37±0.3	40.07±0.1	10.03±0.4	30.04±0.2
303 K	2.66±0.3	43.35±0.1	11.09±0.4	32.26±0.2
308 K	2.91±0.1	44.87±0.4	11.71±0.1	33.16±0.3
313 K	3.31±0.2	46.37±0.3	12.35±0.3	34.02±0.1
318 K	3.55±0.4	47.07±0.1	12.81±0.2	34.26±0.4

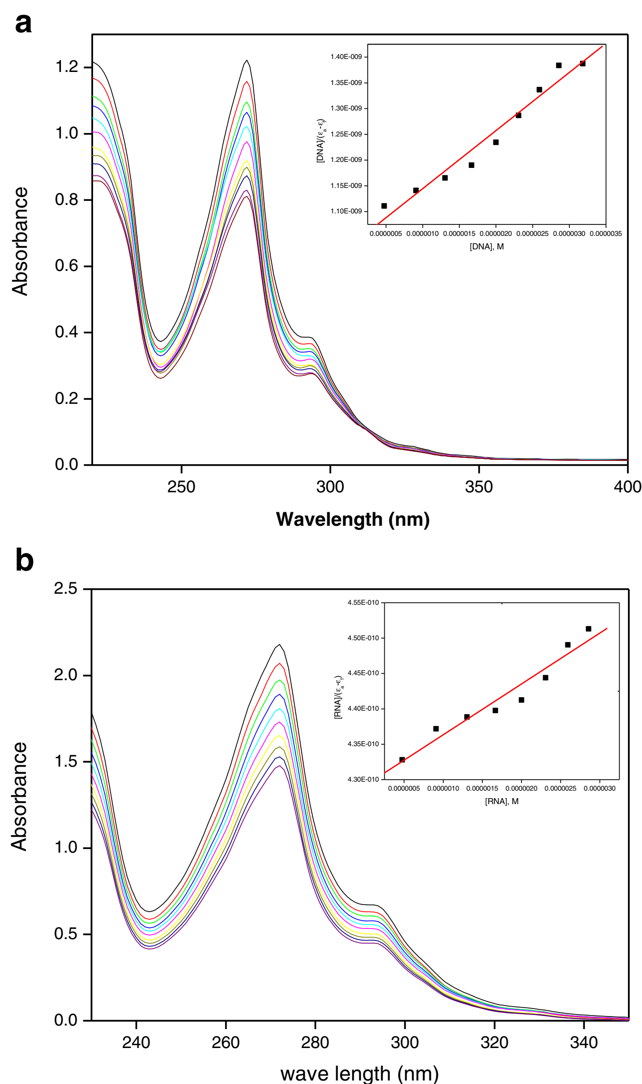


Fig. 3 Absorption spectra of [Cu(sal-ala)(phen)(DA): in the absence (*dotted line*) and in the presence (*solid lines*) of increasing amounts of CT DNA(A) and yeast tRNA(B). {Inset: Plot of [NA]/(εa-εf) vs. [NA]}. [complex]=5×10^{-4} M (**a**) and 5×10^{-5} M (**b**); [DNA]=0–4.8×10^{-7} M and [RNA]=0–1.5×10^{-6} M

usually results in hypochromism and bathochromism due to the intercalative mode. Thus, in order to provide evidence for the possibility of binding of complex to NAs, spectroscopic titration of the solutions of the surfactant-copper(II) complex with nucleic acids has been performed. The UV spectra have been recorded for a constant NA concentration in different [compound/NA] mixing ratios (r) UV spectra of nucleic acid in the presence and absence of our surfactant-copper(II) complex are shown in Fig. 3a and b. As seen from Fig. 3a and b with the increase of the concentration of CT-DNA or yeast tRNA, the absorption spectrum of our surfactant-copper(II) complex shows both hypochromism (32.29 % and 55.8 % for DNA and tRNA) respectively and slight red shift. Hypochromism was suggested to be due to a strong interaction between the electronic states of the intercalating salicylidene chromophore and that of the DNA bases [32]. Since the strength of this electronic interaction is expected to decrease as the distance of separation between the chromophore and the DNA bases, [33] the observed large hypochromism strongly suggests a close proximity of the salicylidene chromophore to the DNA bases. For example, intercalation of the salicylidene chromophore into the helix and strong overlap of the π-π* states of the salicylidene with the electronic states of the DNA bases are consistent with the observed spectral changes. Also copper(II) complex containing long aliphatic chain enhances hydrophobic interaction by strongly intercalating the base pairs of DNA. The observed spectroscopic changes are thus, consistent with intercalation of complex into the DNA base stacks. In order to further elucidate quantitatively the affinity of the surfactant-copper(II) complex to CT-DNA and yeast tRNA, the intrinsic binding constant, K_b has been determined using the equation [33],

$$[NA]/(\varepsilon_a-\varepsilon_f) = [NA]/(\varepsilon_0-\varepsilon_f) + 1/K_b(\varepsilon_0-\varepsilon_f)$$

where, [NA] is the concentration of DNA or RNA expressed in base pairs; ε_a, ε_f and ε_0 are the apparent, free and fully bound copper(II) complex extinction coefficients. A plot of $[NA]/(\varepsilon_a-\varepsilon_f)$ versus [NA] gives K_b as the ratio of the slope to intercept.

The K_b values of the surfactant-copper(II) complex with CT-DNA and yeast tRNA are given in Table 2. The binding constant K_b for the complex has been determined from the plot of $[NA]/(\varepsilon_a-\varepsilon_f)$ versus [NA] and was found to be 1.2×10^5 M^{-1} for DNA and 1.6×10^5 M^{-1} for RNA. This binding constant of the surfactant complex is higher than that of a similar type of complex containing bipyridine instead of phenanthroline [34]. Because phenanthroline ligand can provides more aromaticity extending from the metal center to overlap with the DNA base pairs by intercalation. Compared with those of the surfactant cobalt(III)/copper(II) complexes (2.2×10^4–3.4×10^4 M^{-1}), the binding constants (K_b) of the present system with nucleic acids is higher [35]. Also the K_b of our surfactant copper(II) complex is very much higher than that for the ordinary

Table 2 The binding constant (K_b) and Stern-Volmer constant (K_{SV}) of [Cu(sal-ala)(phen)(DA)], with DNA and RNA

Surfactant complex	K_b (M^{-1})		H%		K_{sv}	
[Cu(sal-ala)(phen)(DA)]	DNA	RNA	DNA	RNA	DNA	RNA
	1.2×10^5	1.6×10^5	22.29	25.80	1.24	1.27

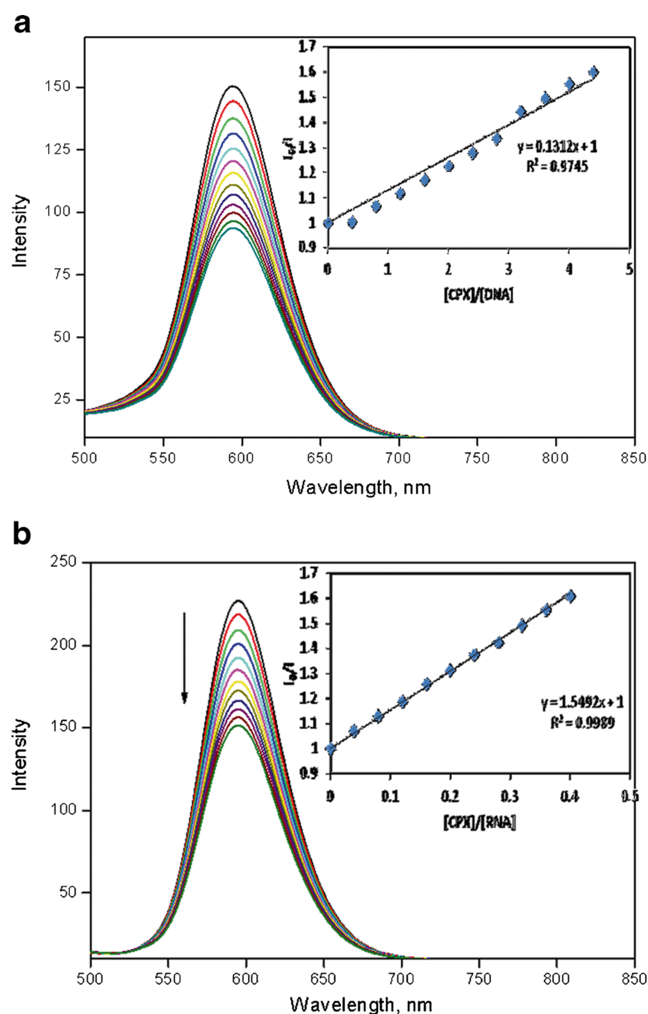


Fig. 4 Emission spectra of EB bound to CT DNA: **a** in the absence of the surfactant copper(II) complex (*dotted line*) and in the presence of the complex. {Inset: Plot of [complex]/[DNA] vs. I/Io}. [DNA]=1×10^{-4} M; [complex]=1.5×10^{-4} M; **b** Emission spectra of EB bound to RNA: in the absence of the complex (*dotted line*) and in the presence of the complex. {Inset: Plot of [complex]/[RNA] vs. I/Io}. [RNA]=1×10^{-4} M; [complex]=4×10^{-5} M

copper(II) complexes, like [Cu(salgly)(bpy)] $4H_2O$ [32], [Cu(salgly)(phen)]$4H_2O$ [33].

Fluorescence Spectroscopic Studies

Ethidium bromide (EB) emits intense fluoresence light in the presence of DNA, due to its strong intercalation between the adjacent DNA base pairs. It was previously reported that the enhanced fluoresence can be quenched by the addition of a second molecule [36]. The quenching extent of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA.

Our surfactant-copper(II) complex shows no fluorescence at room temperature in solution or in the presence of DNA/RNA, and their binding to DNA/RNA cannot be directly predicted through the emission spectra. Hence competitive binding studies have been performed using ethidium bromide (EB) to confirm the nature and the extent of binding of each complex with DNA/RNA. The emission spectra of EB bound to DNA/RNA in the absence and presence of the surfactant-copper(II) complex of the present study have been recorded for EB=1×10^{-6} M, [NA]=1×10^{-5} M with increasing amounts of copper(II) complex. The addition of the complex to NA, pretreated with EB, causes an appreciable reduction in emission intensity, ca. 50 % smaller than that observed in the absence of the complex. The emission band around 592 nm of the NA-EB system decreased in intensity upon the addition complex (Fig. 4a and b). This quenching of NA-EB fluorescence by our surfactant-copper(II) complex suggests that our complex displaces EB from the NA-EB complex and they interact with DNA/RNA by intercalative mode. This binding was analyzed through the canonical Stern-Volmer equation, [37].

$I_0/I=1+K\,r$, where I_0 and I are the fluorescence intensities in the absence and the presence of complex, respectively. K is a linear-Stern-Volmer quenching constant dependent on the ratio of r_{bE} (the ratio of the bound concentration of ethidium bromide to the concentration of NA). r is the ratio of the total concentration of complex to that of NA. From the linear plots of I_0/I versus r, the K values were obtained. The K values thus obtained for our surfactant-copper(II) complex are shown in the Table 2. The quenching plots illustrates that the quenching of EB bound to DNA by the complex is in good agreement with the linear Stern–Volmer equation, which proves that the surfactant copper(II) complex can bind to DNA. It also can be known that the replacement of EB bound to NA by the copper(II) complex results in a decrease of the EB bound to NA fluorescence intensity. The data suggest that the interaction of nucleic acid with the present complex is stronger than that of bipyridine complex (unpublished results), which is consistent with the above absorption spectral results.

Cyclic Voltammetry

Cyclic voltammetry techniques have been employed to study the interaction of the redox active surfactant-copper(II) complexes with DNA in order to further testing the DNA-binding

Table 3 Electrochemical parameters for the interaction of DNA with [Cu(sal-ala)(phen)(DA)]

Surfactant complex	E_{pc} (mV)	E_{pa} (mV)	Δ_{Ep} (mV)	$E_{1/2\ (mV)}$	i_{pa}/i_{pc}
[Cu(sal-ala)(phen)(DA)]	−692.5	33.49	725.99	−312.76	0.40
[Cu(sal-ala)(phen)(DA)] + DNA	−550.5	47.49	597.99	−402.76	0.59

Table 4 Electrochemical parameters for the interaction of RNA with [Cu(sal-ala)(phen)(DA)]

Surfactant complex	E_{pc} (mV)	E_{pa} (mV)	Δ_{Ep} (mV)	$E_{1/2\ (mV)}$	i_{pa}/i_{pc}
[Cu(sal-ala)(phen)(DA)]	−778.5	15.49	853.49	−313.76	0.30
[Cu(sal-ala)(phen)(DA)] + RNA	−606.5	47.49	653.99	−255.76	0.60

modes assessed from the spectral and viscosity studies [38]. The cyclic voltammogram (CV) of complex in phosphate buffer exhibits one redox couple in the potential range, +1000 to −1000 mV. The observed redox potentials in presence and absence of NA observed in the voltammetric studies of surfactant complex is given in Tables 3 and 4 and is assigned to metal centred redox reactions [39]. It is known that the electrochemical potential of a copper complex will shift positively when it intercalates into nucleic acid, and if it is bound to nucleic acid by electrostatic interaction, the potential would shift in a negative direction [34].

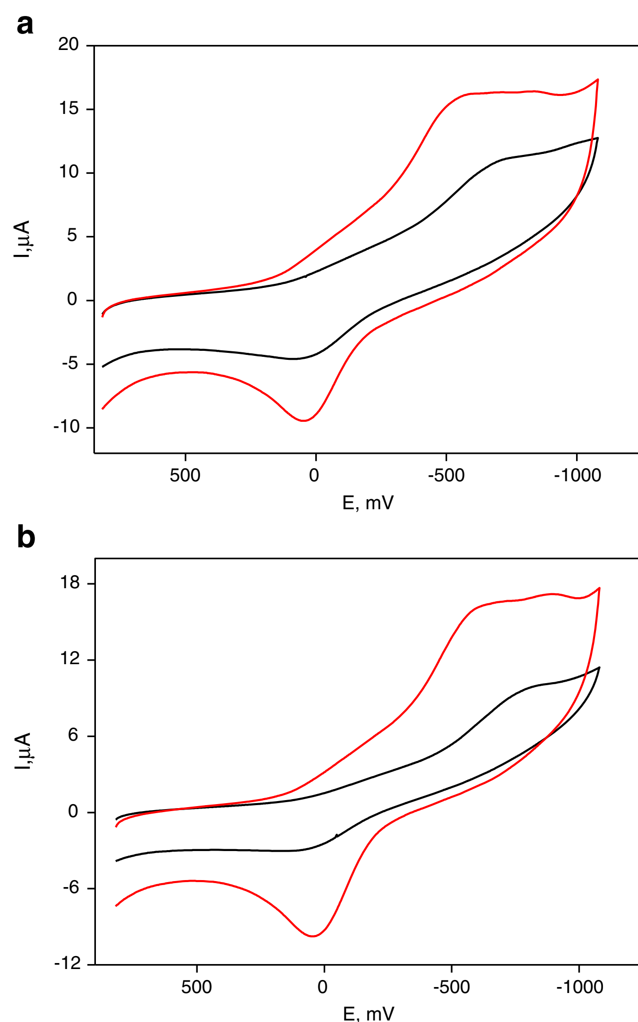


Fig. 5 **a** CV spectra of [Cu(sal-ala)(phen)(DA)] in the absence (*dotted line*) and in the presence (*solid line*) of ct DNA. [complex]=1×10^{-3} M; [DNA]=0–2.68×10^{-5} M; **b** In the absence (*dotted line*) and in the presence (*solid line*) of RNA. [complex]=1×10^{-3} M; [RNA]=0–2.68×10^{-5} M

The typical cyclic voltammograms (CV) of surfactant - copper(II) complex in the absence and presence of nucleic acids in Tris–HCl buffer solutions are shown in Fig. 5a and b. The cyclic voltammogram of [Cu(sal-ala)(phen)(DA)] in the absence of nucleic acids features reduction of Cu(II) to Cu(I) form at a cathodic peak potential, Epc of −692.5 mV versus SCE. Reoxidation of Cu(I) occurs, upon scan reversal, at 33.49 mV. The ratio of the oxidation peak current to reduction peak current (I_{pa}/I_{pc})=0.40, indicates that the redox process was quasireversible. No new peaks appeared after the addition of nucleic acid to the surfactant-copper(II) complex. During the course of the addition of nucleic acid (CT DNA/yeast tRNA) 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 indicating the intercalation of the copper(II) complex into the nucleic acid structure [34].

Circular Dichroism Studies

Circular dichroism spectral techniques may give us useful information on how the conformation of the CT DNA chain is influenced by the bound complex. The CD spectrum of CT DNA consists of a positive band at 274 nm that can be due to base stacking and a negative band at 243 nm that can be due to helicity and it is also characteristic of DNA in a right-handed B form [40]. The changes in CD signals of ct DNA observed on interaction with drugs may often be assigned to the corresponding changes in CT DNA structure. Thus simple groove

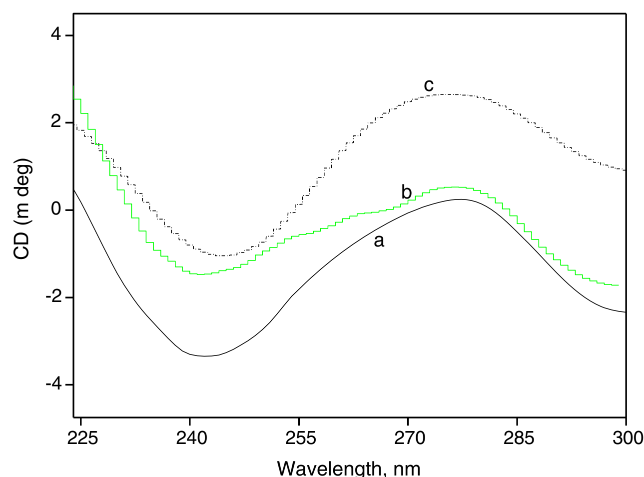


Fig. 6 Circular dichroism spectra in the absence (*dotted line*) and in the presence of increasing amount of surfactant copper(II) complex b and c [Cu(sal-ala)(phen)(DA)]. [complex]=1–5×10^{-5} M; [DNA]=1×10^{-4} M

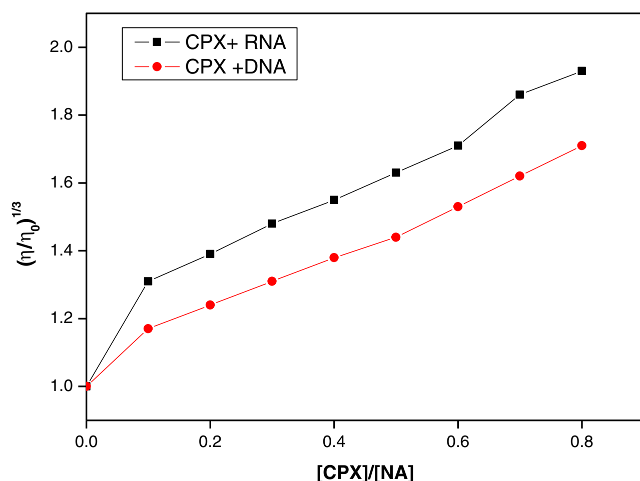


Fig. 7 Effects of increasing amounts of complex [Cu(sal-ala)(phen)(DA)] in presence of nucleic acid (DNA and RNA) on the relative viscosities of calf thymus DNA at 29.0 (±0.1)°C

binding and electrostatic interaction of small molecules show less or no perturbation on the base-stacking and helicity bands, whereas intercalation enhances the intensities of both the bands stabilizing the right-handed B conformation of CT DNA as observed for the classical intercalator methylene blue [41]. The CD spectrum of CT DNA was monitored in the presence of surfactant-copper(II) complex and the changes were observed. It exhibits a positive band at 279 nm (Fig. 6.) due to base stacking and a negative band at 246 nm due to helicity of B DNA [42]. The surfactant copper(II) complex makes increase in the intensity of positive and negative bands but there were not evident shifts in the band positions. A similar observation was also made for DNA binding of $[Ru(NH_3)_4(pip)]^{2+}$, $[Ru(NH_3)_4(hpip)]^{2+}$ and $[Ru(NH_3)_4(dip)]^{2+}$ which were known intercalators, [43]. So the surfactant copper(II) complex of the present study binds to DNA through intercalation.

Viscosity Studies

Optical or photophysical probes generally provide necessary, but not sufficient clues to support an intercala tive binding model. Under appropriate conditions intercalation causes a significant increase in the viscosity of DNA solutions due to the separation of base pairs at intercalation sites and hence increases the overall DNA contour length. By contrast, ligands that bind exclusively in the DNA grooves, under the same conditions, typically cause less pronounced changes (positive or negative) or no changes in DNA solution viscosity. Hydrodynamic measurements that sensitive to length change are regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data [39]. A classical intercalation model results in lengthening the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA/RNA viscosity. However, a partial and/or non-classical intercalation of ligand may bend (or kink) nucleic acid helix, resulting in the decrease of its effective length and, concomitantly, its viscosity. To further clarify the nature of the interaction between the title complex and DNA, viscosity measurements were carried out by keeping [NA] and varying the concentration of the complexes. The changes in relative viscosity of nucleic acid in the presence of the title complex are shown in Fig. 7. We can see that upon increasing the amounts of the title complex, the relative viscosity of DNA increases steadily. The results suggest that the title complex intercalates between the base pairs of DNA, in agreement with the other experimental results.

Antimicrobial Activity

The surfactant copper(II) complex were screened for activity against selected pathogenic bacterial and fungal species using the disc diffusion method. As expected, the surfactant complex has higher activity against the same microorganisms (48 h) under identical conditions (Table 5). This may be due to higher hydrophobic character of the complex which can damage the bacterial/fungal cellular membrane/wall. This increase in activity on chelation might be due to the partial sharing of the positive charge of the metal in chelated complex with the ligands donor atoms so that there is electron delocalization over the whole chelate ring. This may increase

Table 5 Antimicrobial activities of the [Cu(sal-ala)(phen)(DA)]

S.No	Test microorganisms	Diameter of zone inhibition (24 h)	Diameter of zone inhibition (48 h)	Standard disk Amikacin/ ketokonazole (Gram positive/ Gram negative)
		(mm)		
1	Chromo bacterium	13±0.58	18±0.58	18±0.58
2	Serratia	14±1.53	17±2.31	18±0.58
3	Staph. Aureus	14±0.58	19±0.58	22±0.58
4	Bacillus	19±0.58	22±0.58	19±0.58
5	Albicans	20±0.54	22±0.58	17±0.58
6	Aspergillus	25±0.54	29±0.58	30±0.58

liphophilic character of the metal chelate and thus enabling it to permeate the lipoid layers of the bacterial membrane [44].

Conclusion

A new surfactant copper(II) complex containing phenanthroline and alanine Schiff-base ligand has been synthesized and characterized. As mentioned in our previous reports [31], the critical micelle concentration value of this surfactant-copper(II) complex is also very low compared to that of the simple organic surfactant, dodecylammonium chloride (CMC=1.5×10^{-2} mol dm^{-3}). Thus it is concluded that this surfactant-copper(II) complex has more capacity to associate themselves, forming aggregates, compared to those of ordinary synthetic organic surfactants. The interaction of this complex with nucleic acids has been studied with electronic spectroscopy revealing their ability to bind to nucleic acid. Competitive binding study with EB has also been performed by fluorescence spectroscopy. All these experiments show that the interaction between the NA-EB complex and the surfactant copper(II) complex via the intercalative mode. Viscosity measurements of NA solutions in presence of our complex have confirmed that this intercalation as the most possible binding mode to NA. Cyclic voltammetric and circular dichroism studies have also established the intercalating binding nature of our surfactant copper(II) complex to nucleic acids. This intercalation is due to the presence of hydrophobic ligands alanine Schiff-base, phenanthroline and long aliphatic chain in the complex. The surfactant-copper(II) complex has good antimicrobial activity against Gram positive and Gram negative bacteria and fungi. Thus our results show that the surfactant-copper(II) complex can be a candidates for DNA binding reagents, as well as laying the foundation for the rational design of new useful DNA probes. It can also be considered for antifungal and antibiotic drugs [45, 46].

References

1. Souza P, Garcia-Vazquez JA, Masaguer JR (1985) Trans Met Chem 10:410–412
2. Sunaga SAS, Taniguchi T, Miyazaki H, Nabeshima T (2007) Inorg Chem 46:2959–2961
3. Quiroga AG, Ranninger CN (2004) Coord Chem Rev 248:119–133
4. Elerman Y, Kabak M, Elmali A (2002) Z Naturforsch B 57:651–656
5. Bhattacharya S, Mandal SS (1997) Biochim Biophys Acta 1323:29–44
6. Hermann T (2005) Curr Opin Struct Biol 15:355–366
7. Thomas JR, Hergenrother PJ (2008) Chem Rev 108:1171–1224
8. Ni Y, Li D, Kokot S (2006) Anal Biochem 352:31–242
9. Chow CS, Barton JK (1992) Biochemistry 31:5423–5429
10. Kashanian S, Gholivand MB, Ahmadi F et al (2007) Spectrochim Acta A 67:472–478
11. Uma V, Kanthimathi M, Weyhermuller T, Nair BUJ (2005) Inorg Biochem 99:2299–2307
12. Trewavas A (1967) Anal Biochem 21:324–329
13. Haifz AA (2005) J Surfactant Deterg 8:359–363
14. Kumaraguru N, Santhakumar K, Arunachalam S, Arumugam MN (2007) Int J Chem Kinet 39:22–28
15. Arumugam MN, Santhakumar K, Kumaraguru N, Arunachalam S (2003) Asian J Chem 15:191–196
16. Santhakumar K, Kumaraguru N, Arumugam MN, Arunachalam S (2006) Polyhedron 25:1507–1513
17. Ndifon TP, Moise OA, Julius NN, Mbom DY, Awawou GP, Lynda DN (2010) Res J Chem Environ 14:50–54
18. Shedlovsky T (1932) J Am Chem Soc 54:411–1428
19. Reichmann MF, Rice SA, Thomas CA, Doty P (1954) J Am Chem Soc 76:3047–3053
20. Dunn TM (1960) The visible and ultraviolet spectra of complex compounds in modern coordination chemistry. Interscience, New York
21. Garcia-Raso A (1999) Polyhedron 18:871–878
22. Tadros TF (2005) Applied surfactants, 1st edn. Wiley-VCH, Weinheim
23. Morris ML, Busch DH (1960) J Am Chem Soc 82:1521–1524
24. Strukl JS, Walter JL (1971) Spectrochim Acta A 27:223–238
25. Schilt AA, Taylor RC (1959) J Inorg Nuc Chem 9:211–221
26. Speie G, Csihony J, Whalen AM, Pie CG (1996) Inorg Chem 35: 3519–3524
27. Gonzalez-Perez A, Del Castillo JL, Czapkiewicz J, Rodriguez JR (2004) Colloids Surf A Physicochem Eng Asp 232:183–189
28. Zana RJ (1980) Colloid Interf Sci 78:330–337
29. Nusselder JJH, Engberts JBFN (1992) J Colloid Interf Sci 148:353–361
30. Kumaraguru N, Arunachalam S, Arumugam MN, Santhakumar K (2006) Trans Met Chem 31:250–255
31. Warring MJ (1965) J Mol Biol 13:269–282
32. Cantor C, Schimmel PR (1980) Biophy Chem Freeman WH, San Francisco, 2:398
33. Pyle AM, Rehmann JP, Meshoyrer R, Kumar CV, Turro NJ, Barton JK (1989) J Am Chem Soc 111:3051–3058
34. Carter MT, Rodriguez M, Bard AJ (1989) J Am Chem Soc 111: 8901–8911
35. Tamil Selvi P, Palaniandavar M (2002) Inorg Chim Acta 337:420–428
36. Zhao G, Lin H, Zhu S, Sun H, Chen Y (1998) J Inorg Biochem 70: 219–226
37. Lakowicz JR, Webber G (1973) Biochemistry 12:4161–4170
38. Shen Q, Liu J, Chao H, Xue G, Ji L (2001) J Inorg Biochem 83:49–55
39. Satyanaryana S, Daborusak JC, Chaires JB (1992) Biochemistry 31: 9319–9324
40. Uma Maheswari P, Palaniandavar M (2004) J Inorg Biochem 98: 219–230
41. Lincoln P, Tuite E, Norden B (1997) J Am Chem Soc 119:1454–1455
42. Ivanov VI, Minchenkova LE, Shchelkina AK, Poletaev AI (1973) Biopolymers 12:89–110
43. Satyanaryana S, Daborusak JC, Chaires JB (1993) Biochemistry 32: 2573–2584
44. Garcia-Raso A, Fiol JJ, Zafra AL, Mata I, Espinosa E, Molins E (2000) Polyhedron 19:673–680
45. Nyholm RS, Tobe ML (1956) J Chem Soc 1707–1718
46. Buckingham DA, Jones D (1965) Inorg Chem 4:1387–1392