Asian Journal of Chemistry; Vol. 31, No. 9 (2019), 1931-1942

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21990

Surfactant-Ruthenium(II) Complexes: Synthesis, Characterization, DNA Binding, Anticancer and Antimicrobial Activity

R. SUGANTHI DEVI[1] and N. KUMARAGURU[2,*]

[1]Department of Chemistry, Government Arts College, Tiruchirapalli-620022, India
[2]Department of Chemistry, Periyar E.V.R. College, Tiruchirapalli-620023, India

*Corresponding author: E-mail: nkguru@gmail.com

Received: 8 February 2019; *Accepted*: 12 April 2019; *Published online*: 31 July 2019; AJC-19479

The interaction of ligand bound ruthenium(II) complexes with DNA have grown fame because of their relevance in the development of new reagents for medicinal applications and the impact of dominating cisplatin. Surfactant-ruthenium(II) complexes $[Ru(DMP)_2(DA)Cl](ClO_4)$ (**1**) and $[Ru(DMP)_2(DA)_2](ClO_4)_2$ (**2**) with primary ligand as DMP (2,9-dimethyl[1,10]-phenanthroline) and secondary ligand as dodecyl amine (DA) were synthesized and characterized. The critical micelle concentration (CMC) of complexes in aqueous solution were obtained from conductivity measurements. The interaction of surfactant-ruthenium(II) complexes with CT-DNA has been explored by spectroscopic technique and viscosity dimensions. These complexes were tested for cytotoxic and antimicrobial activities with human cervical cancer cell line (HeLa) and pathogenic microorganisms. The results indicate that the complex **2** binds more strongly to DNA than complex **1** further affecting the viability of the cells significantly and also showed moderate antimicrobial activity.

Keywords: Surfactant-ruthenium(II) complexes, DNA binding, Cytotoxic activity, Antimicrobial activity.

INTRODUCTION

Ruthenium metalointercalators is a curious element in the science of drug development. The chemistry of ruthenium goes hindmost to the days where the metal evidenced to be a promising alternative for platinum which had a dominating role as an anticancer drug. The high neurotoxicity and nephrotoxicity of cisplatin, restricts the chances for attaining therapeutic aids from dose intensification which initiated the exploration for new options by transition metal complexes [1-3].

Ruthenium complexes draws attention because of their ability to bind with nucleic acids under various modes and pave a pathway for pharmacological study. Ruthenium itself is a good candidate for the design and synthesis of novel drug delivery systems as the three dimensional configuration of ruthenium facilitates ligand coordination and its functional attributes aimed at well-defined bio macromolecular targets.

The ability of ruthenium to interchange with nitrogen and oxygen donor molecules, octahedral geometry to interact with nucleic acids, the ease to access the oxidation states II and III in the biological fluids, the high transportation into abnormal tumor cells by transferrin mechanism, iron mimicking capability and the pattern of DNA binding supported the metal as highly potent anticancer alternative [4,5]. The efficiency of an anticancer drug mainly depends on the binding character, mode and specific selectivity of the DNA base pairs. Generally, a complex bind to DNA helix by three modes namely electrostatic interaction, groove binding and intercalation. Binding nature usually depends on the distinguishing property of the ligand bound ruthenium complex [6].

In the past decades the phenanthrolines have found a wide range of applications in the redox reactions, DNA assays, interfaces to nucleic acids and in tumor activity. Substituted phenanthrolines occupy a priority role as probes in drug delivery. The adaptable properties of these substituted phenanthrolines provide a comfortable environment in the binding to DNA. Furthermore, phenanthroline is stiff and its insertion within cyclic or acyclic structures can impart to the resulting ligand a high degree of pre-organization [7], resulting in discriminating complexing agents. Reported to literature only a few reports are available in the binding of DNA to bulky phenanthroline moieties as ancillary ligands.

Surfactants, are amphipathic molecules possessing two diverse portions one moiety is hydrophilic and second moiety being hydrophobic, are chief building wedges of several physical, chemical and biological systems. Among them, the cationic species show some surplus advantages over the other classes of surfactants. These substances, besides their surface activity, show antitumor, antimicrobial and antiparasitic properties as well [8]. For example, the double-chain cationic surfactant such as dimethyl dialkylammonium bromide show remarkable immune-adjuvant properties and cetylpyridinium chloride is single-chain cationic surfactant with well-known antibacterial properties [9-11]. Cationic surfactant-DNA interactions have been the focus of many studies over the past few decades because they are of interest both in fundamental science and in biotechnological applications [12-14].

The amphiphilic coordination complexes possessing surfactants with hydrophobic tail and metal complex in the hydrophilic head were designed and are commonly named as metalosurfactants. These metalosurfactants have been an area of keen research owing to the properties exhibited by both the central metal ion and the surfactant ligand. These complexes are a special type of surfactants, where a coordination complex contains a central metal ion which is surrounded by ligands coordinated to the metal.

Like any other well-known surfactants, these metallosurfactant complexes also form micelles at a specific concentration called the critical micelle concentration (CMC) in aqueous solution. By manipulating the aspects springing out of such a curious class of combinations can lead to fascinating applications. There are few reports [15-18] on the synthesis, characterization and biological application of surfactant transition metal complexes, in contrast to numerous reports of the formation and study of such surfactants in solution without isolation. Ruthenium complexes bearing 2,9-dimethyl[1,10]-phenanthroline (DMP) as primary ligand and dodecyl amine (DA) surfactants as secondary ligands have been studied for their DNA binding mode, cytotoxicity and antimicrobial activities.

In the present study, we account the synthesis, CMC determination, DNA binding properties, anticancer and antimicrobial activity of various surfactant–ruthenium(II) complexes using different physico-chemical methods.

EXPERIMENTAL

The reagents used for the analysis were of analytical grade (Aldrich and Merck). The calf thymus DNA (CT-DNA) and dodecyl amine was obtained from Sigma-Aldrich, Germany for binding study. All the spectroscopic titrations were carried out in buffer (50 mM NaCl-5 mM Tris-HCl, pH-7) at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance 1.8–1.9:1 at 260 and 280 nm, specifying that the DNA was adequately free of protein [19]. Milli-Q water was used in the preparation of all the required solutions for the analysis.

FT-IR spectra are considered as one of the key tools in the characterization of the samples, were documented on a FT-IR Shimazdu Japan spectrophotometer with samples prepared as KBr pellets. ^{1}H spectra were documented on a BRUKER Spectrometer using DMSO as solvent. The mass fragmentation spectra of the surfactant complexes have been recorded on LC-TOF/MS (Synapt, Waters, USA). The 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.

The absorption titration experiments were documented 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. The antimicrobial screening studies were carried out at Indian Bio-track Research Institute, Thanjavur, India. The cytotoxic screening was carried out at KMCH College of Pharmacy, Coimbatore. The human cervical cancer cell lines (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10 % fetal bovine serum (FBS).

Synthesis of $Ru(DMP)_2Cl_2$: The following adaptation of the preparation of this complex developed by Weaver [20] was applied to provide good yields of the complex. Commercially obtained $RuCl_3{\cdot}3H_2O$ (1.95 g, 7.46 mmol) along with 2,9-dimethyl[1,10]phenanthroline (DMP) and (2.34 g, 11.24 mmol) and LiCl (1.0 g, 23.59 mmol) were heated at reflux in reagent grade dimethylformamide (15 mL) for 9 h. The reaction mixture was continuously stirred magnetically during this reflux. Later the reaction mixture was air cooled to room temperature, 45 mL of reagent grade acetone was gradually added and the subsequent solution was cooled at 0 °C overnight. Then the solution was filtered and a red black solution with a dark brownish yellow product was obtained. The precipitate was washed four times with 30-mL portions of distilled water followed by three 30 mL portions of diethyl ether and finally it was dried up by suction. Yield: 1.4 g. Anal. Calc. for $C_{28}H_{24}N_4Cl_2Ru$: C, 57.15, H, 4.11, N, 9.52. Found: C, 57.98, H, 4.02, N, 9.21 TOF-MS: *m/z* = 589.168 $[M+1]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 8.59 (4H), 8.11 (4H), 7.87 (4H), 2.51 (6H). IR (KBr, ν_{max}, cm^{-1}): 2921, 2847 (C-H), 1615, 1591 (C-N), 1143, 1139 (N-H), 1369, 1357 (C=C).

Synthesis of $[Ru(DMP)_2(DA)Cl](ClO_4)$ (1): A mixture of $Ru(DMP)_2Cl_2$ (1.104 g, 1.87 mmol) and dodecylamine (3.18 g, 17.15 mmol) in ethylene glycol (25 mL) was added and gradually stirred for 5 to 10 min, during which the solution turned brown. After the solution was left undisturbed at room temperature for 48 h. A dark brown precipitate was obtained by addition of 4-fold excess of a saturated aqueous $NaClO_4$ solution. All the perchlorate salts are possibly explosive and consequently the reaction was controlled in small quantity with utmost care. The precipitate was repetitively washed with acetone and filtered. A light chocolate brownish precipitate was obtained and it was dried up to eliminate water particles. Yield: 0.1 g. Anal. Calc. for $C_{40}H_{51}N_5O_4Cl_2Ru$: C, 57.36, H, 6.12, N, 8.36. Found: C, 57.65, H, 6.17, N, 8.48. TOF-MS: *m/z* = 839.21 $[M+2]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 8.15(4H), 7.75 (4H), 7.28 (4H), 2.48 (12H), 1.71 (2H), 1.34 (2H), 1.25-1.29 (16H) 0.89 (3H), IR (KBr, ν_{max}, cm^{-1}): 3333 (N-H), 2955, 2919, 2850 (C-H), 1647, 1568 (C-N), 1113 (N-H), 2333 (-NH_2), 1489 (C=C), 816, 720 (C-H), 1090, 625 (ClO_4).

Synthesis of $[Ru(DMP)_2(DA)_2](ClO_4)_2$ (2): A mixture of $Ru(DMP)_2Cl_2$ (1.104 g, 1.87 mmol) and dodecylamine (6.36

g, 34.31 mmol) in ethylene glycol (35 mL) was added and slowly stirred for 5 to 10 min, during which the solution turned light brown. After the solution was left untouched at room temperature for 72 h. A dark blackish brown precipitate was obtained by addition of 4-fold excess of a saturated aqueous $NaClO_4$ solution. All the perchlorate salts are potentially explosive and therefore the reaction was controlled in small quantity with utmost care. The precipitate was repeatedly washed with acetone and filtered. A dark brown chocolate precipitate was obtained and it was dried to remove water particles. Yield: 0.17 g. Anal. Calc. for $C_{52}H_{78}N_6O_8Cl_2Ru$: C, 57.45, H, 7.24, N, 7.74. Found C, 57.41, H, 7.17, N, 7.21. TOF-MS: *m/z* =1088.37 $[M+2]^+$. ^1H NMR (500 MHz, DMSO-d_6): 8.17 (4H), 7.80 (4H), 7.32 (4H), 2.44 (12H), 1.74 (4H), 1.38 (4H), 1.25-1.29 (32H) 0.88 (6H), IR (KBr, ν_{max}, cm^{-1}): 2956, 2921, 2851 (C-H), 1651 (C-N), 1151 (N-H), 2348 (-NH_2), 1458, 1485, 1439 (C=C), 1081, 635 (ClO_4).

Determination of CMC: The concentration of the surfactant complexes as a function of specific conductance was determined conductometrically using a specific conductivity meter [21]. Initially the cell was calibrated with standard KCl solutions in appropriate concentration range. By using the molar conductivity data for KCl, the cell constant was noted [22]. The surfactant-ruthenium(II) complexes of various concentrations were prepared in the range 10^{-2} to 10^{-4} mol dm^{-3} in aqueous solution.

As temperature has an impact on micellization, four different temperatures *i.e.*, 25, 35, 45 and 55 °C were chosen to study the conductivity of the complex solutions. For each series of measurement the specific conductance was noted after complete mixing of the solutions. The complex solution was then titrated under controlled constant temperature for each specific addition. The equilibrium was tested by taking the readings at fixed time intervals of 10 min till no major change was observed.

DNA binding study: The absorption spectral titration experiments were executed by using a constant concentration of the surfactant-ruthenium(II) complex to which sequential increasing volumes of the DNA solution was added. The surfactant-ruthenium(II) complex-DNA solutions were put to incubation for 0.5 h before the absorption spectra were documented. The concentration of the DNA was determined using an extinction coefficient value of 6600 M^{-1} cm^{-1} at 260 nm [23].

The fluorescence quenching experiments were carried out by using the CT-DNA pretreated with a standard dye ethidium bromide (EB) and kept for 20-30 min under incubation. The surfactant-ruthenium(II) complex was then added consequently as a secondary molecule to this EB-DNA mixture and the subsequent effect on its corresponding emission intensities were well documented. The samples were excited at 500 nm and emission was set in the range of 500-700 nm. These experiments were carried out in 50 mM NaCl and 5 mM Tris-HCl buffer at pH 7.1 in aqueous media.

The viscosity measurements were carried out using an Ubbelodhe type viscometer sustained at a constant temperature of 25 °C. The CT-DNA sample solutions were prepared by sonication in order to diminish the convolutions arising from DNA flexibility. The flow time was measured using a digital stop clock and the time duration for each sample was measured at least for triplicates. The data obtained are presented as $(\eta/\eta_0)^{1/3}$ *versus* ratio of [Complex]/[DNA], where η is the relative viscosity of DNA in the presence of the complex and η_0 is the viscosity of DNA only [24].

Cytotoxicity study

Cell culture: The HeLa cervical cancer cells taken in single layer were isolated with trypsin-ethylenediamine tetraacetic acid (EDTA) to obtain single cell suspensions. The feasible cells were counted by tryphan blue exclusion using a hemocytometer and diluted with medium containing 5 % FBS to get a final concentration of 1×10^5 cells/mL. The cell suspension of 100 µL per well were broadcasted into 96 well plates at plating concentration of 10,000 cells per well and incubated for cell attachment at conditions of 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity. After a time duration of 24 h the cells were treated with successive concentrations of the test samples [25].

MTT assay: The surfactant-ruthenium(II) complexes **1** and **2** were primarily dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final test concentration with serum less medium. An additional of four serial dilutions were made to afford a total of five sample concentrations. The aliquots of 100 µL of these different sample dilutions were added to the appropriate wells already containing 100 µL of medium, resulting in the required final sample concentrations. Following the sample addition, the plates were incubated for 24 and 48 h at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity. The medium containing without samples served as control and triplicate was maintained for all concentrations. 3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a good yellow water soluble tetrazolium salt [25]. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the quantity of formazan produced is directly proportional to the number of viable cells [26].

After 24 and 48 h of incubation, 15 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well, wrapped in aluminium foil and incubated at 37 °C for 4 h. The medium with MTT was then discarded and the formed purple formazan crystals were solubilized in 100 µL of DMSO and then measured for the absorbance at 570 nm using micro plate reader. The data was collected for four replicates and used for calculation. The percentage cell inhibition was determined using the following formula:

$$\text{Cell inhibition (\%)} = 100 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Non-linear regression graph was then plotted between percentage of cell inhibition and concentration of the complex and IC_{50} was determined. The half maximal inhibitory concentration (IC_{50}) value was determined as the complex concentration that is required to reduce the absorbance to half that of the control.

Morphological changes apoptosis assay (AO/EB staining): The cells were grown in MEM (Eagles Minimum

Essential Medium) supplemented with 10 % fetal bovine serum (FBS) [27,28]. Then the cells were detached by the addition of equal volume of trypsin (0.25 %)/EDTA (0.02 %) and versene (0.1 %) followed by observation under a microscope to confirm complete dissociation of the cells. Then approximately cells ranging 1×10^5 in number were sown in the well plate and further incubated at 37 °C for 24 h. Finally, the surfactant-ruthenium(II) complexes as samples were added at different concentrations in duplicates. The cells without the sample served as the control. The plate was incubated for 24 h. After incubation, the medium was totally removed and rinsed with phosphate buffer saline (PBS). The AO-EtBr stain (1 µg/mL) was added in each well and the morphological changes were observed under inverted phase contrast microscope.

Morphological changes apoptosis by tryphan blue staining: The viability of the cells when treated with surfactant-ruthenium(II) complexes were assessed by the tryphan blue staining assay [29,30]. Approximately the detached cells ranging 1×10^5 in numerals were sown in the well plates and incubated at 37 °C for 24 h. This was followed by the addition of the surfactant-ruthenium(II) complexes as samples of various concentrations. The plates were further put to incubation for 24 h. After incubation, the medium was wholly removed and washed with Phosphate Buffer Saline (PBS). Tryphan blue stain (0.4 %) was then added in each well for 30 min and the images were observed under inverted phase contrast microscope.

Antimicrobial screening: The antibacterial activities of complexes were evaluated using disk diffusion method by Kirby–Bauer disc diffusion process [31]. This method is extremely effective for fast emergent microorganisms and the actions of the test complexes are noted by assessing the diameter of the zone of inhibition [28,32]. The surfactant-ruthenium(II) complexes were taken in extreme dry condition at room temperature and dissolved in 1 % DMSO. The bacterial microorganisms *i.e.*, the Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) were grown a medium of nutrient agar and incubated at 37 °C for 48 h followed by regular subculture to fresh medium and used as test bacteria. Subsequently, fresh cultural discs were inoculated with a loop full of bacterial culture and it was spread throughout the discs homogeneously using a sterile glass spreader. This was followed by the addition of 100 µL sample concentrations of 50 and 100 mM to each disc. The plates were incubated at 37 °C for 24-48 h. The discs where the solvents were added served as the control. Inhibitory activity was noted by measuring the diameter of the visible zone after the period of incubation.

RESULTS AND DISCUSSION

Spectroscopic characterization: The coordination of the primary and ancillary ligands to the central ruthenium atom is studied by IR vibrational spectroscopy [33]. The results of the spectral observation of phenanthroline metal complexes were studied by Strukl and Walter [34] and Schilt & Taylor [35]. They showed that fewer noticeable alterations in the spectrum have been stated to arise upon coordination irrespective of the metal.

The infrared spectral data of several bipyridyl and phenanthroline complexes were studied by Strukl & Walter and Schilt & Taylor [34,35] and observed that the spectral shape of the dipyridine and phenanthroline complexes as ligands were fairly analogous, considering both the ligands. The bands for free phenanthroline at (C–H) 856, 738 cm^{-1}, was shifted to 816 and 720 cm^{-1} in the surfactant-ruthenium(II) complexes as nitrogen atoms of phenanthroline ligands donate a pair of electrons each to the central ruthenium metal, resulting in the formation of a coordinate covalent bond [36]. Moreover, the shift of (C–N) of phenanthroline from about 1670 cm^{-1} in the free ligand to 1651 cm^{-1} and 1647 cm^{-1} after coordination gives a confirmation [37]. The bands for perchlorate at 1090 and 625 cm^{-1} belong to an ionic species and stressing that this counterion is not involved in the ruthenium–ligand coordination [38].

For the surfactant–ruthenium(II) complexes **1** and **2**, the bands around 2921, 2919 cm^{-1} and 2851, 2850 cm^{-1} can be assigned to the C–H asymmetric and symmetric stretching vibration of aliphatic CH_2 of dodecylamine [33].

The ^1H NMR spectrum of the surfactant–ruthenium(II) complexes were studied. The methylene protons of the long chain moiety (dodecylamine) gave rise to a multiplet at 1.2-1.8 ppm, whereas the terminal methyl group of the hydrocarbon chain substituent gave a triplet around 0.89 ppm. The aromatic protons of phenanthroline ligands of the two complexes appeared in the region 7-10 ppm and an intense peak at 2.51 ppm is assignable to methylene proton in phenanthroline ligand [39-41].

Critical micelle concentration values (CMC): The aggregation attributes of the surfactant-ruthenium (II) complexes **1** and **2** in aqueous solution across a specific range of temperatures remarkably showed a variation with the concentration of the complexes [42-44]. A saturation point is observed after a gradual increase in the concentration and further increase in concentration does not show a significant change in specific conductance. This saturation at which the slope diverges specifies the micellization charm and is noted as the critical micelle concentration (CMC) [45]. As temperature has an impact on micellization, the CMC values were tabulated at different temperatures *viz.*, 25, 35, 45 and 55 °C. Accordingly at these temperatures, concentration of the complexes *versus* specific conductance studies were performed. The saturation point of the concentration where an interruption in the conductance occurs shows the CMC and is a typical feature of micelle formation as illustrated in the plot for the complexes **1** and **2** (Fig. 1). By using the data points from the plot two equations in the form $y = mx + c$, above and below the break in the conductance was obtained. Solving the equations gives the point of intersection as the CMC. These measurements were repeated thrice and accuracy of CMC values was maintained (Table-1). The initiation of micellization tends to happen at higher concentrations as there is an increase in temperature for a specific system. This performance is mainly due to the presence of minimum free energy state in the aqueous solution. Moreover an increase in temperature favours an increase in entropy when there is distortion of the water structure by the hydrophobic part leading to the breakdown of the water molecule surrounding the hydrophobic groups. Also a decrease in the hydration

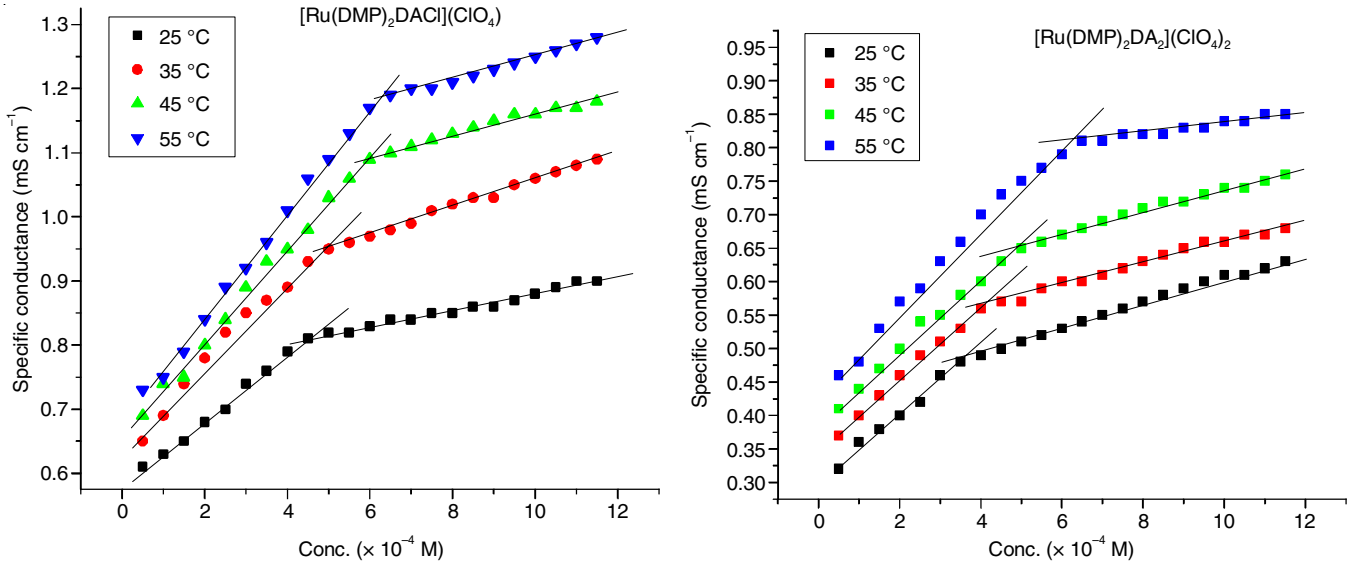

Fig. 1. Plots of surfactant-ruthenium(II) complex concentration *versus* specific conductance of complexes **1** and **2**

TABLE-1
CMC VALUES OF THE SURFACTANT–RUTHENIUM(II) COMPLEXES IN AQUEOUS SOLUTION

Surfactant-ruthenium(II) complexes	CMC Values (× 10^{-4} M) in °C			
	25	35	45	55
$[Ru(DMP)_2(DA)Cl](ClO_4)$	4.648	4.854	5.896	6.469
$[Ru(DMP)_2(DA)_2](ClO_4)_2$	3.455	4.208	4.861	6.261

of the hydrophilic group favours micellization. The effective level of the above two contrasting impressions determines CMC performance of the surfactant-ruthenium(II) complexes.

DNA Binding study

Electronic absorption spectral titration: The binding nature of any small molecule with the double helix polynucleotide comprises of three modes namely groove, electrostatic and intercalation, for which the UV absorption spectroscopy is widely applied.

The absorption spectra of the surfactant complexes $[Ru(DMP)_2(DA)Cl](ClO_4)$ (**1**) and $[Ru(DMP)_2(DA)_2](ClO_4)_2$ (**2**), in the absence and in the presence of CT-DNA, are shown in Fig. 2. The impact of increase in concentration of the CT-DNA were observed in the absorption bands ensuing the affinity of hyperchromism and a slender blue shift. The binding interactions of the complexes with DNA is provided with the evidence of hypochromicity of the absorption spectra with increasing concentrations of DNA.

Surfactant-ruthenium(II) complexes composed of methylene crowds of elongated chain aliphatic amine (dodecylamine), possess the prospect of interrelating to CT-DNA by manipulating π-π interactions, van der Waals forces or ionic forces [46]. Further the presence of hydrogen bonding between the –NH part of the complexes and the base sugars of the polynucleotide could lead to specific interactions [47-50]. The interface of surfactant ruthenium(II) complexes with CT-DNA could be finely tuned by the nature of the head group of the complexes.

As such the complexes synthesized contains substituted phenanthroline ligands, providing an aromatic moiety dispersing from the metal core giving a pathway for overlapping with the base pairs of DNA by intercalation. This is said to favour the hydrophobicity of the complexes enhancing better interaction. Nevertheless, in present study a hyperchromism

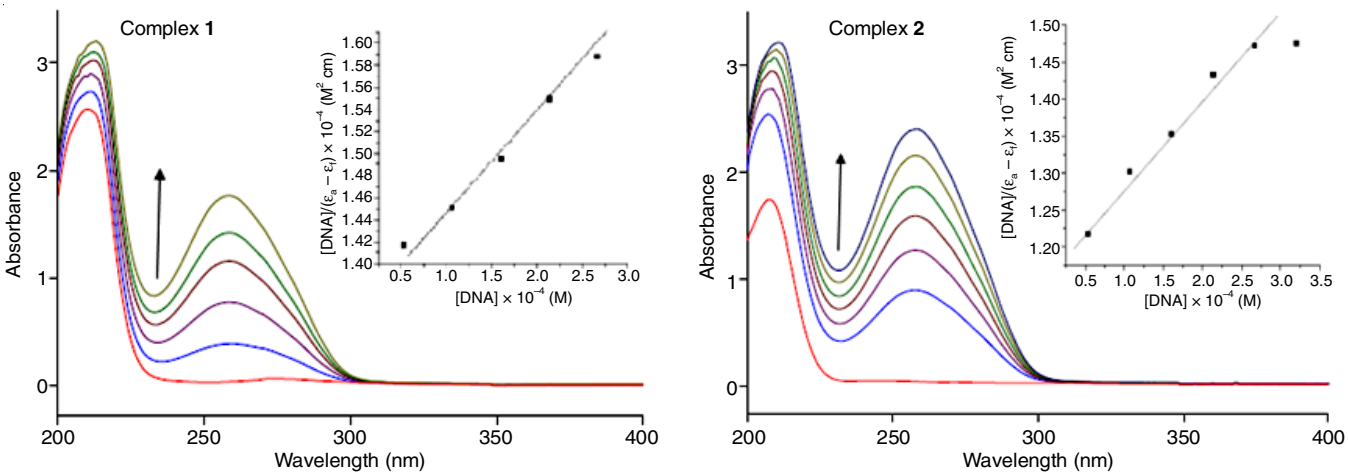

Fig. 2. Absorption spectra of complexes **1** and **2** in the absence (red line) and in the presence of increasing amounts of DNA, [Complex] = 10 μM, [DNA] = 0.5 -2.5 μM. Inset: Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA] experimental data points; solid line, linear fitting of the data

effect was observed in due of both the complexes which suggests that there is a tough hydrophobic association amid the methylene chain of the surfactant and the hydrophobic core of the nucleic acid. For further confirmation, the binding strengths of the complexes were compared and the intrinsic binding constant (K_b) was determined using the equation [51]:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad (1)$$

where [DNA] is the concentration of CT-DNA in base pairs, ε_a, ε_f and ε_b correspond to Abs_{obsd}/[Complex], the extinction coefficient of the free surfactant-ruthenium(II)complex and the extinction coefficient of the complex in the fully bound form, correspondingly and K_b is the intrinsic binding constant. The ratio of slope to intercept in the plot of [DNA]/ε_a – ε_f) *versus* [DNA] gives the value of K_b (inset Fig. 2). The intrinsic binding constants for the surfactant– ruthenium(II) complexes **1** and **2** are $6.0188 \times 10^4\ M^{-1}$ and $8.4919 \times 10^4\ M^{-1}$, respectively (Table-2) indicating that both complexes binds DNA with greater affinity. The K_b values are lower than the classical intercalator *i.e.* ethidium bromide ($7.16 \times 10^5\ M^{-1}$), which is the widely used reference in absorption titration [52]. Therefore, the above results indicate that the complexes may bind with DNA *via* intercalative mode. Also the effect of the primary ligands (phenanthroline moiety) in the binding of the complexes to DNA is enhanced. It is also evident that the binding constant of complex **2** is higher than complex **1**. The presence of surfactant as ancillary ligand greatly effects the binding. The change in the length of the surfactant dodecylamine from single chain to double chain consistently varies the binding to the nucleic acid due to the increase in the bulky hydrophobic group. The surfactant-ruthenium(II) complexes with single chain surfactant show less binding affinity in comparison to double chain surfactant ligand. Further the phenanthroline ring and its planarity which easily intercalates into the base pairs is thus enabled. Hence from the table the binding of surfactant-ruthenium(II) complexes follow the order *i.e.*, $[Ru(DMP)_2(DA)_2](ClO_4)_2$ > $[Ru(DMP)_2(DA)Cl](ClO_4)$.

Competitive binding between ethidium bromide (EB) and surfactant–ruthenium(II) complexes: Information regarding the interaction of the surfactant–ruthenium(II) complexes to the nucleic acid was narrowed down by means of the competitive binding experiment using ethidium bromide (EB) dye, the most sensitive fluorescent probes that can display maximum binding to DNA [53-55]. The intercalation of ethidium ion into the DNA helix establishes a vivid increase in fluorescence proficiency. A displacement of the dye is keenly observed on the addition of an alien molecule to DNA solution showing the extent of quenching.

Commonly, fragments with cationic species [56], multi polyamines and macro polypeptides, may displace the EB dye from DNA, proving quenching of EB fluorescence. According to Bhattacharya and Mandal the variation in the quenching of fluorescence in EB-DNA solution by the addition of cationic surfactant takes place confidently due to effective displacement by the surfactant head group [11]. As can be seen in Fig. 3. the sequential addition of the surfactant-ruthenium(II) complex to DNA bound with EB caused substantial drop in the emission intensity, displacing the EB fluorophore by the surfactant complex.

Applying the classical Stern–Volmer equation (eqn. 2) [57]:

$$I_0/I = 1 + K_{sv}[Sur\text{-}Ru] = 1 + K_q\tau_0[Sur\text{-}Ru] \quad (2)$$

where I_0 and I are the fluorescence intensities in the absence and presence of the surfactant-ruthenium(II) complex, respectively, K_{sv} is the Stern-Volmer constant and [Sur-Ru] is the concentration of surfactant-ruthenium(II) complexes. A plot of I_o/I

TABLE-2
BINDING CONSTANTS OF THE SURFACTANT–RUTHENIUM(II) COMPLEXES WITH CALF THYMUS DNA

Surfactant-ruthenium(II) complexes	$K_b \times 10^4\ M^{-1}$	$K_{SV} \times 10^4\ M^{-1}$	$K_{app} \times 10^6\ M^{-1}$	$K_q \times 10^{12}\ M^{-1}\ s^{-1}$
$[Ru(DMP)_2(DA)Cl](ClO_4)$	6.0188	2.930	3.170	1.332
$[Ru(DMP)_2(DA)_2](ClO_4)_2$	8.4919	3.410	3.871	1.550

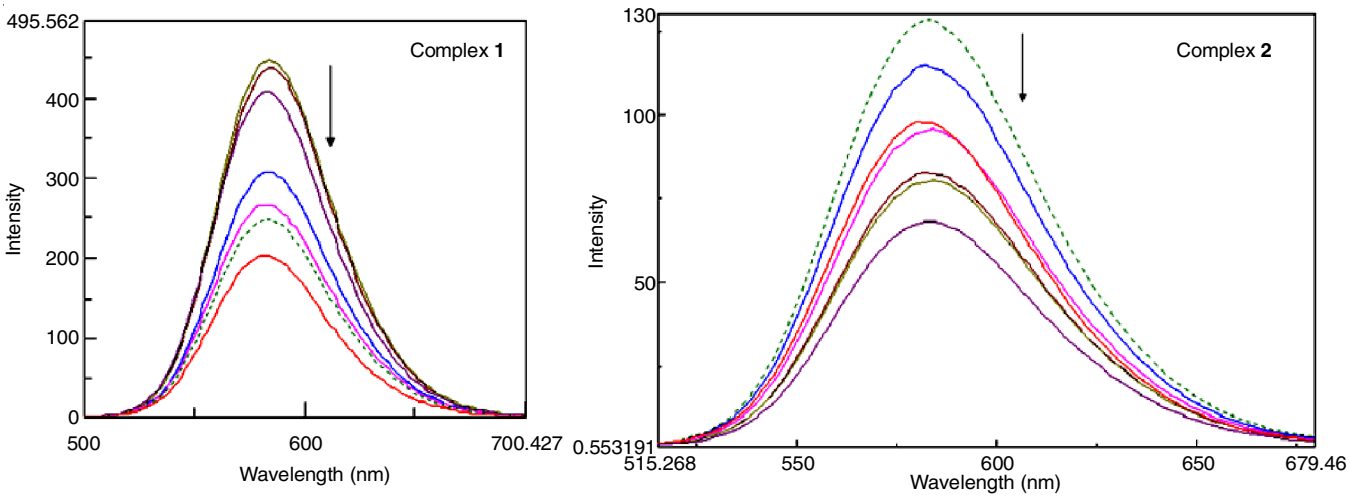

Fig. 3. Emission spectra of EB bound to DNA in the absence and presence of complex **1** and **2**, [EB] = 8×10^{-5} M, [DNA] = 8×10^{-5} M, [Complex] = 0 -24 μM. Arrow shows intensity changes upon increasing concentration of the complexes

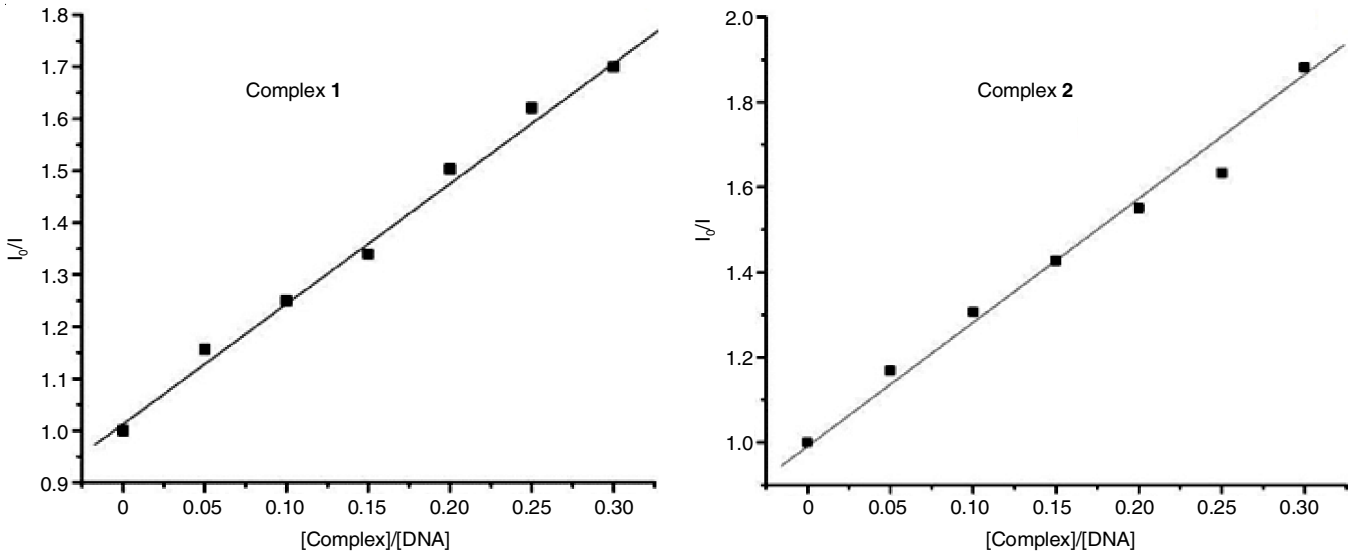

Fig. 4. Fluorescence quenching plot of EB bound to DNA by surfactant-ruthenium(II) complexes **1** and **2**

vs. [Q] was drawn and it shows good linearity (Fig. 4). The K_{sv} values were obtained from the slope and the linearity confirms the quenching behaviour. The K_{sv} is a measure of the effectiveness of the surfactant-ruthenium(II) complex as a quencher and the values for our surfactant-ruthenium(II) complexes, thus obtained, are shown in Table-2.

Moreover, in order to evaluate the strength of binding, the apparent binding constant (K_{app}) was expressed (eqn. 3)

$$K_{app} = K_{EB}\,[EB]/[Ru]_{50\,\%} \quad (3)$$

K_{EB} is the binding constant for ethidium bromide and the value is taken as $1.2 \times 10^6\ M^{-1}$ and $[Ru]_{50\,\%}$ is the value of the concentration of the surfactant-ruthenium(II) complex that causes 50 % drop of the EtBr-DNA initial fluorescence [58,59]. The K_{app} values are specified in Table-2 and found to be in the range of 10^6 which is analogous to the results from absorption spectral titration and implicates that ruthenium complexes exhibit good binding affinity. Further the strength of quenching followed in these interactions was determined by the bimolecular quenching constant K_q (eqn. 2) by utilizing the value of τ_0 as 22 ns which is the average lifetime of EtBr-DNA complex [58,60]. The values of K_q were found to be in the order of $10^8\ M^{-1}s^{-1}$ which is less than the limiting value of $10^{10}\,M^{-1}s^{-1}$ [61]. Hence it can be concluded that the complexes show good quenching affinity towards the nucleic acid binding.

The data puts forward that surfactant-ruthenium(II) complexes binds strongly with DNA, which is reliable with the spectroscopic results described above. Hence the order of binding is, $[Ru(DMP)_2(DA)_2](ClO_4)_2 > [Ru(DMP)_2(DA)Cl](ClO_4)$. This shows that the length of the surfactant chain has an impact on the emission study giving a change in the intercalation of the complexes with DNA. It also lays emphasis on the importance of hydrophobicity and its impact between surfactant ruthenium(II) complexes and DNA irrespective of the head group. The greater the chain length higher is the Stern Volmer constant, K_{app} and K_q values, which proves greater binding affinity.

Viscosity measurements: To investigate the specific binding mode, relative viscosities of the nucleic acid with gradual increase in concentrations of the surfactant-ruthenium(II) complexes were examined. Generally hydrodynamic approaches, which are delicate to increase the length of the nucleic acid helix, are regarded as the least indistinct and the most acute investigations of binding nature in solution [24,62].

The viscosity of the DNA is found to be strictly dependent on the changes in the length due to the interaction of the double helix with any small molecule. An intercalative metal complex under applicable conditions separates the base pairs causing a significant increase in the viscosity of DNA solutions and, thereby increasing the DNA helix length. Contrastingly ligands could also decrease the DNA viscosity when the binding exclusively occurs at the groove sites initiating a typical bend in the nucleic acid helix. The viscosity measurements of the surfactant-ruthenium(II) complexes **1** and **2** on the relative viscosity of DNA are displayed in Fig. 5. The results obviously shows the presence of an intercalative mode of interaction by the complexes with the base pairs of helix.

The binding of surfactant-ruthenium(II) complexes **1** and **2** to DNA showed a remarkable increase in the relative specific

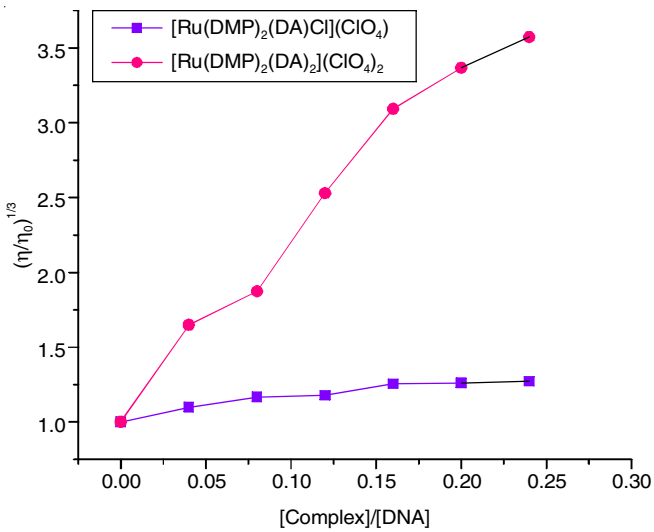

Fig. 5. Effects of increasing amount of complex **1** and **2** on the relative viscosities of the calf thymus DNA

viscosity of DNA while the complex **2** exerted a huge increase in DNA viscosity. The surfactant ruthenium(II) complex causes a split-up of the base pairs, to accommodate the complex into the helix, increasing its length and thereby its viscosity [63]. For the two surfactant-ruthenium(II) complexes, relative viscosity increases upon increasing the concentration ratio of complex to DNA. This confirms that both complexes bind to DNA through intercalative fashion as there is an increase in the graphical plot. Furthermore from the plot it is evident that the order of intercalation of DNA with the complexes follows, $[Ru(DMP)_2(DA)_2](ClO_4)_2 > [Ru(DMP)_2(DA)Cl](ClO_4)$.

The influence of the surfactant alkyl chain length and its hydrophobicity is also clearly visible in the extension of DNA binding. The surfactant with the double chain, binds more willingly to DNA leading to the separation of base pairs, thereby increasing the viscosity drastically when compared to surfactant with single chain [64].

Cytotoxicity studies

MTT assay: To deliver an indication for anticancer efficacy both the surfactant ruthenium(II) complexes were incubated with human cervical cancer cell lines (HeLa) with a concentration grade of each surfactant complex and evaluated the impact on cell viability through half inhibitory IC_{50} concentrations by MTT activity assay.

MTT assay decides the cytotoxicity of any drug based upon the activity of mitochondrial dehydrogenase enzyme present in the cells [65]. The cytotoxic nature of the surfactant-ruthenium(II) complexes were observed by exposing cells for 24 and 48 h to the medium containing the respective ruthenium complexes at 0.25-100 μM concentration. The maximum cytotoxic behaviour was observed at 100 μM concentration of the complexes (Fig. 6). The surfactant-ruthenium (II) complexes inhibited the growth of the cervical cancer cells significantly in a dose- and duration-dependent manner (Fig. 7).

The outcomes of the cytotoxic activity on human cervical cancer cell lines were carried out based on the quantitative values obtained on exposing the complex essential to decrease the survival of the cell lines to 50 % (IC_{50}). The half inhibitory IC_{50} concentration values for our surfactant-ruthenium (II) complexes (Table-3) clearly depicted that both the complexes showed high dynamic cytotoxic activity against the cervical cancer cell lines at very low concentrations. This resultant could be in due of the fact that surfactant-ruthenium(II) complexes possess the proficiency to minimize the energy level in anomalous tumor cells and which would also enhance the antitumor activity [66]. Further the presence of bulky ligands and their nature possess a high impact in the cellular mutilation inflicted by these surfactant-ruthenium(II) complexes. It is well documented that metal complexes possessing phenanthroline moieties being hetero aromatic enjoy biological activities such as antimalignancy, antimicrobial, apoptosis and interface with nucleic acids hindering cell proliferation so as to seize tumor progression [67-69]. The IC_{50} value for the surfactant-ruthenium(II) complex **2**, $[Ru(DMP)_2(DA)_2](ClO_4)_2$, was comparatively lower than for the complex **1**, $[Ru(DMP)_2(DA)Cl](ClO_4)$, also the values show

TABLE-3
IC_{50} VALUE OF THE SURFACTANT-RUTHENIUM(II) COMPLEXES FOR HeLa CANCER CELLS

Complexes	IC_{50} (μM)	
	24 h	48 h
$[Ru(DMP)_2(DA)Cl](ClO_4)$	58.95 ± 13.07	49.46 ± 3.8
$[Ru(DMP)_2(DA)_2](ClO_4)_2$	45.01 ± 5.01	38.30 ± 12.6

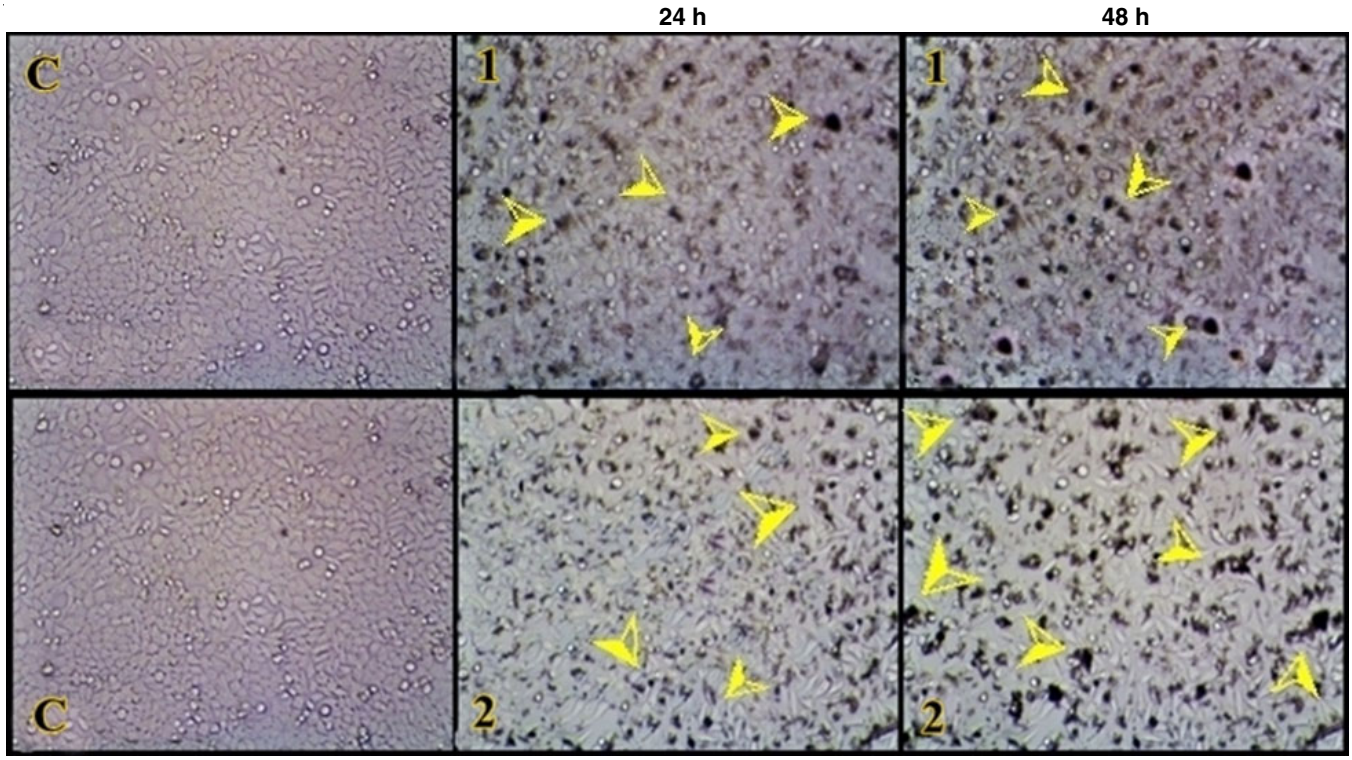

Fig. 6. Cytotoxic microscopic micrographs of HeLa cancer cells treated with complexes **1** and **2** in 24 and 48 h at 100 μM concentration. C: Control, 1: Complex **1**, 2: Complex **2**. Arrowheads points to abnormal cells

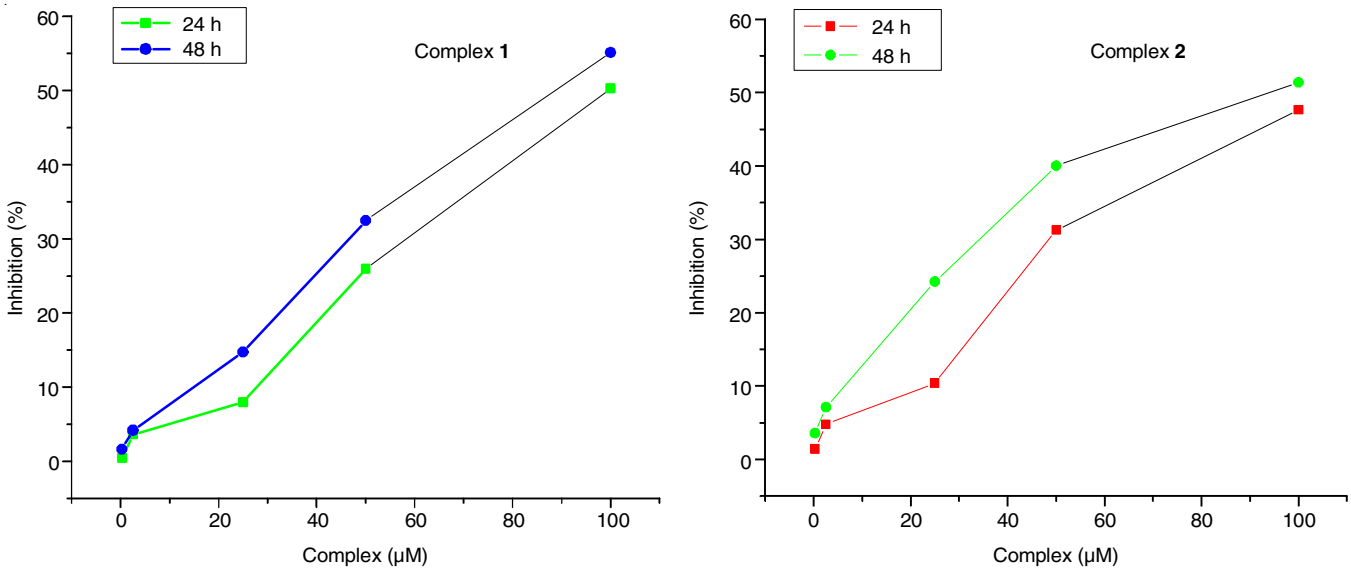

Fig. 7. Plot showing the percentage inhibition *vs.* variable concentrations of the complexes exposed to HeLa cancer cells for 24 and 48 h. The complexes effect the viability of the cells in a dose and duration dependent mode

a drastic variation with regard to duration (Fig. 8). The IC_{50} values were low when the cells were exposed to 48 h with comparison to 24 h where the value was high comparatively. This indicates that the complexes inhibited the growth of the cancer cells considerably in a dose and duration dependent manner.

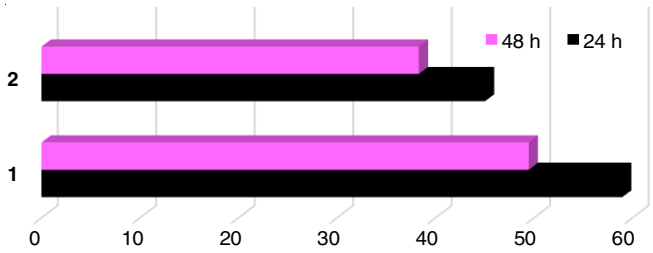

Fig. 8. Plot showing the comparison of IC_{50} values of the surfactant-ruthenium(II) complexes exposed to HeLa cancer cells for 24 and 48 h. The complexes effect the viability of the cells in a duration depen-dant manner

Morphological changes through apoptosis assay (AO/EB staining): Apoptosis and necrosis are the two main classes of cell demise accompanied by structural alterations and widespread DNA fragmentation [70]. This is a physiological process which is necessary to take away abnormal cells that are not required for cellular functions. This method of staining is very sensitive to DNA and shows a great change in nuclear morphology. The acridine orange dye has the capability to diffuse through the cell membrane and is accrued by viable and non-viable cells, whereas EB is accrued merely by non-viable cells. The apoptotic cells generally show changes like shrinking of the nucleus, condensation of the membrane cell wall, fragmentation and formation of irregular membrane clusters. The viable cells have been stained bright green in an orderly arranged normal homogenous green nuclei (Fig. 9C). The HeLa cells were then treated with 100 μM of the complexes **1** and **2** for 24 h. The observation revealed that the cells with apoptotic features underwent morphological changes further exhibiting characteristics such as cell blebbing and chromatin condensation in clusters (Fig. 9, 1 and 2).The observation of the cell images revealed that the apoptotic cells were in bright green clusters with irregular cell structure and necrotic cells possessed orange to reddish nuclei. The changes showed that the cells were found to induce apoptosis and necrosis at low concentration of both the complexes.

Morphological changes through tryphan blue staining: The tryphan blue staining is based on the principle that living

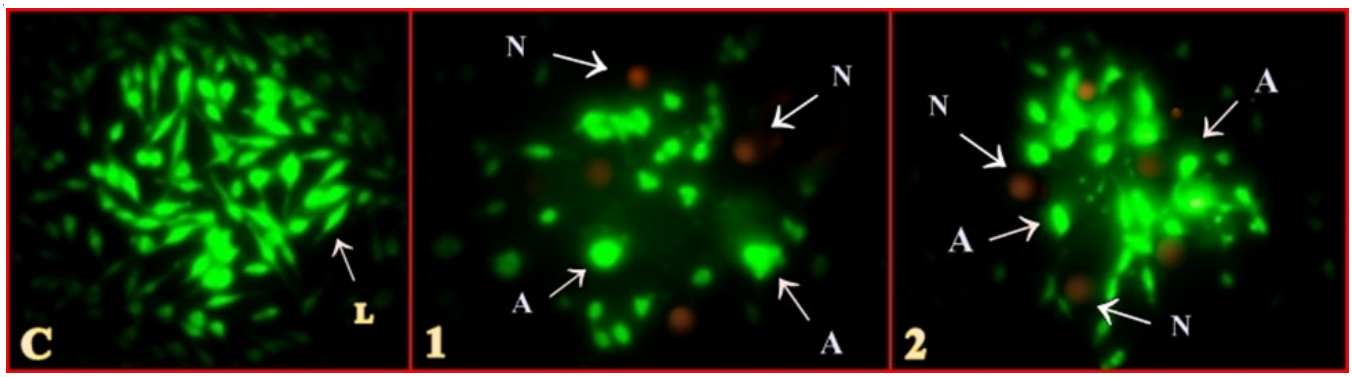

Fig. 9. AO/EB staining of HeLa cells for 24 h. C: Control, 1: Complex **1**, 2: Complex **2** treated with complexex. L: Livng cells, A: Apoptic cells and N: Necrosis cells respectively

cells possess intact cell membranes that intake dyes like tryphan blue, eosin or propidium. This staining method is employed to distinguish viable cells and non-viable cells by fluorescent microscopy. Further the cells with abnormal cytoplasm absorbs the dye and is seen as blue stained and these cells are non-viable. The living cells remain as normal without any morphological colour change. However, the surfactant-ruthenium(II) complexes when put forth for this staining with HeLa cell line absorbed tryphan blue selectively resulting in the loss of cell membrane and exhibited thick blue coloration. The morphological changes were observed at low concentrations of the complexes **1** and **2** (Fig. 10, 1 and 2).

Screening for antibacterial activity: Antimicrobial activity of surfactant–ruthenium(II) complexes **1** and **2** were screened *in vitro* against certain pathogenic bacterial species using the disk diffusion method and the results given in Table-4. The outcomes indicate good antibacterial activity of the complexes against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumonia*. This good activity may be observed due to selective proficient diffusion of the metal complexes through a protein rich porin cell membrane leading to a higher zone of inhibition. The microbial cell membrane being negatively charged facilitates the adsorption of the surfactant complexes by electrostatic interactions. Further the presence of enhanced hydrophobic interactions favour the penetration of surfactant alkyl tail to the interior of the bacterial cell wall mutating the microbial cell [71]. Moreover, as expected the bulky complex **2** exhibited better activity than complex **1** counter parts. The change in this may be due to the existence of greater hydrophobicity of the complex **2**, which can diffuse profusely through the bacterial cell wall. Furthermore, double chain surfactant ruthenium complex which is more hydrophobic shows better inhibitory activity than the single chain surfactant ruthenium complex. It may be concluded that present surfactant-ruthenium(II) complexes are in general capable of inhibiting the growth of bacteria to a good reach. Zoroddu *et al.* [72] have reported that phenanthroline ligand alone showed null significant activity against the Gram-negative and Gram-positive bacteria but the surfactant-ruthenium(II) complexes exhibited considerable activity against Gram-positive and Gram-negative bacteria.

Conclusion

A newfangled class of surfactant-ruthenium(II) complexes were synthesized and characterized by IR, NMR and mass spectrometry techniques. The critical micelle concentration values of surfactant-ruthenium(II) complexes in the present study were observed to be low compared to those of the simple organic surfactant, dodecyl ammonium chloride (CMC = 1.5 $\times 10^{-2}$ M). Thus, it is resolved that these metal surfactant complexes have more ability to associate among themselves, forming aggregates, than ordinary synthetic organic surfactants. The binding behaviour of these surfactant-ruthenium(II) complexes with calf thymus DNA was characterized by absorption titration study, displacement fluorescence quenching and viscosity measurements. The results indicate that both the complexes can effectively bind to CT-DNA *via* intercalation mode. This study specifies that the surfactant-ruthenium(II) complexes, containing double chain surfactant as secondary ligand, shows more strong affinity towards CT-DNA than the surfactant-ruthenium(II) complex containing single chain surfactant ligand. The surfactant-ruthenium(II) complexes are found to be cytotoxic to cervical cancer cells and the inhibitory concentration (IC_{50}) values were found to be less for double chain surfactant-ruthenium(II) complexes comparatively to

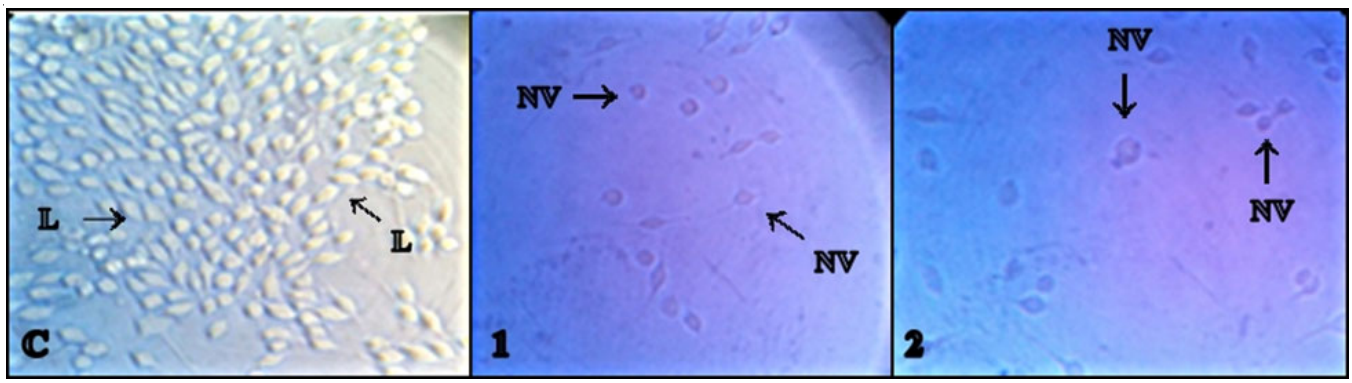

Fig. 10. Tryphan blue staining HeLa cells for 24 h. C: Control, 1: Complex **1**, 2: Complex **2** treated with complexes. L and NV: Living cells and non-viable cells respectively

TABLE-4
ANTIBACTERIAL ACTIVITY OF THE SURFACTANT-RUTHENIUM(II) COMPLEXES

Test organism	Zone of inhibition (mm)			
	$[Ru(DMP)_2(DA)Cl](ClO_4)$		$[Ru(DMP)_2(DA)_2](ClO_4)_2$	
	50 mM	100 mM	50 mM	100 mM
Escherichia coli	5	8	19	24
Klebsiella pneumonia	16	20	7	14
Staphylococcus aureus	8	11	16	18
Bacillus cereus	12	15	16	25

Standard: Ciprofloxacin; Solvent: DMSO (showed nil activity against microorganism tested)

single chain surfactant-ruthenium(II) complexes. The morphological assessment of the tumor cells were carried out by AO/EB and tryphan blue staining assays and the complexes were found to induce structural changes in the cells leading to apoptosis and necrosis. Therefore, these complexes might prove to be of application in target-based malignancy. Both the surfactant-ruthenium(II) complexes showed moderate antimicrobial activity against Gram-positive and Gram-negative bacteria.

ACKNOWLEDGEMENTS

The authors are thankful to Indian Bio-track Research Institute, Thanjavur, India for their assistance in antimicrobial studies and K.M.C.H. College of Pharmacy, Coimbatore, India for cytotoxicity studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. I. Garnuszek, J. Lici'anska, S. Skierski, M. Koronkiewicz, M. Mirowski, R. Wiercioch and A.P. Mazurek, *Nucl. Med. Biol.*, **29**, 169 (2002); https://doi.org/10.1016/S0969-8051(01)00294-3.
2. P.J. Loehrer Sr., S.D. Williams and L.H. Einhorn, *J. Natl. Cancer Inst.*, **80**, 1373 (1988); https://doi.org/10.1093/jnci/80.17.1373.
3. A. Keder, M.E. Cohen and A.I. Freeman, *Cancer Treat. Rep.*, **62**, 819 (1978).
4. E. Musgrove, C. Rugg, I. Taylor and D. Hedley, *J. Cell. Physiol.*, **118**, 6 (1984); https://doi.org/10.1002/jcp.1041180103.
5. A. Bergamo, C. Gaiddon, J.H.M. Schellens, J.H. Beijnen and G. Sava, *J. Inorg. Biochem.*, **106**, 90 (2012); https://doi.org/10.1016/j.jinorgbio.2011.09.030.
6. J. Liu, W. Zheng, S. Shi, C. Tan, J. Chen, K. Zheng and L. Ji, *J. Inorg. Biochem.*, **102**, 193 (2008); https://doi.org/10.1016/j.jinorgbio.2007.07.035.
7. A. Bencini and V. Lippolis, *Coord. Chem. Rev.*, **254**, 2096 (2010); https://doi.org/10.1016/j.ccr.2010.04.008.
8. M.M. Khowdairy, A.M. Badawi, M.A.S. Mohamed and M.Z. Mohamed, *J. Cancer Res. Ther.*, **3**, 198 (2007); https://doi.org/10.4103/0973-1482.38994.
9. M.D. Prager, F.S. Baechtel, W.C. Gordon, S. Maullin, J. Steinberg and A. Sanderson, eds.: B.H. Tom and H.R. Six, Liposomes and Immunobiology, Elsevier: New York 39 (1980).
10. C.L. Huyck, *Am. J. Pharm.*, **116**, 50 (1944).
11. S. Bhattacharya and S.S. Mandal, *Biochim. Biophys. Acta*, **1323**, 29 (1997); https://doi.org/10.1016/S0005-2736(96)00171-X.
12. D. McLoughlin and D. Langevin, *Colloids Surf. A Physicochem. Eng. Asp.*, **250**, 79 (2004); https://doi.org/10.1016/j.colsurfa.2004.04.096.
13. X. Zhao, Y. Shang, H. Liu and Y. Hu, *J. Colloid Interface Sci.*, **314**, 478 (2007); https://doi.org/10.1016/j.jcis.2007.04.059.
14. N. Jiang, P. Li, Y. Wang, J. Wang, H. Yan and R.K. Thomas, *J. Phys. Chem. B*, **108**, 153 (2004).
15. N. Aydogan and N.L. Abbott, *Langmuir*, **17**, 5703 (2001); https://doi.org/10.1021/la010178e.
16. H. Choi and M.P. Suh, *Inorg. Chem.*, **42**, 1151 (2003); https://doi.org/10.1021/ic025971p.
17. J. Bowers, K.E. Amos, D.W. Bruce and J.R.P. Webster, *Langmuir*, **21**, 1346 (2005); https://doi.org/10.1021/la0478705.
18. X. Jia-Qing, X. Bin, X. Bin, H. Wei and Z. Xian-Cheng, *J. Dispers. Sci. Technol.*, **27**, 481 (2006); https://doi.org/10.1080/01932690500374227.
19. J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961); https://doi.org/10.1016/S0022-2836(61)80047-8.
20. T.R. Weaver, Unpublished Results, The University of North Carolina, Chapel Hill, N.C., (1974).
21. N. Kumaraguru and K. Santhakumar, *J. Coord. Chem.*, **62**, 3500 (2009); https://doi.org/10.1080/00958970903118053.
22. J. Barthel, F. Feuerlein, R. Neueder and R. Wachter, *J. Solution Chem.*, **9**, 209 (1980); https://doi.org/10.1007/BF00648327.
23. M.F. Reichmann, S.A. Rice, C.A. Thomas and P. Doty, *J. Am. Chem. Soc.*, **76**, 3047 (1954); https://doi.org/10.1021/ja01640a067.
24. S. Satyanarayana, J.C. Dabrowiak and J.B. Chaires, *Biochemistry*, **31**, 9319 (1992); https://doi.org/10.1021/bi00154a001.
25. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4.
26. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo and M. Boyd, *J. Natl. Cancer Inst.*, **83**, 757 (1991); https://doi.org/10.1093/jnci/83.11.757.
27. D.L. Spector, R.D. Goldman and L.A. Leinwand, Cell: A Laboratory Manual, Cold Spring Harbor, New York, 34.1–34.9. (1998).
28. R. Kumar, S. Arunachalam, V. Periasamy, C. Preethy, A. Riyasdeen and M. Akbarsha, *J. Inorg. Biochem.*, **103**, 117 (2009); https://doi.org/10.1016/j.jinorgbio.2008.09.010.
29. R.G. Azrak, C.L. Frank, X. Ling, H.K. Slocum, F. Li, B.A. Foster and Y.M. Rustum, *Mol. Cancer Ther.*, **5**, 2540 (2006); https://doi.org/10.1158/1535-7163.MCT-05-0546.
30. S. Veeralakshmi, G. Sabapathi, S. Arunachalam, P. Venuvanalingam, S. Nehru, P. Kumar, C. Anusha and V. Ravikumar, *RSC Adv.*, **5**, 31746 (2015); https://doi.org/10.1039/C5RA02763B.
31. A.W. Bauer, N.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493.
32. M.S. Hossain, S. Easmin, M.S. Islam and M. Rashid, *J. Pharm. Pharmacol.*, **56**, 1519 (2004); https://doi.org/10.1211/0022357044913.
33. R.S. Kumar, S. Arunachalam, V.S. Periasamy, C.P. Preethy, A. Riyasdeen and M.A. Akbarsha, *Aust. J. Chem.*, **62**, 165 (2009); https://doi.org/10.1071/CH08281.
34. J.S. Strukl and J.L. Walter, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **27**, 223 (1971); https://doi.org/10.1016/0584-8539(71)80028-4.
35. A.A. Schilt and R.C. Taylor, *J. Inorg. Nucl. Chem.*, **9**, 211 (1959); https://doi.org/10.1016/0022-1902(59)80224-4.
36. L. Jin and P. Yang, *Polyhedron*, **16**, 3395 (1997); https://doi.org/10.1016/S0277-5387(97)00042-9.
37. T.I.A. Gerber, A. Abrahams, P. Mayer and E. Hosten, *J. Coord. Chem.*, **56**, 1397 (2003); https://doi.org/10.1080/00958970510001641691.
38. M.R. Rosenthal, *J. Chem. Educ.*, **50**, 331 (1973); https://doi.org/10.1021/ed050p331.
39. S. Ghosh, A.C. Barve, A.A. Kumbhar, A.S. Kumbhar, V.G. Puranik, P.A. Datar, U.B. Sonawane and R.R. Joshi, *J. Inorg. Biochem.*, **100**, 331 (2006); https://doi.org/10.1016/j.jinorgbio.2005.11.022.
40. J.D. Miller and R.H. Prince, *J. Chem. Soc. A*, 519 (1969); https://doi.org/10.1039/j19690000519.
41. S. Castellano, H. Gunther and S. Ebersole, *J. Phys. Chem.*, **69**, 4166 (1965); https://doi.org/10.1021/j100782a018.
42. J.M. Kelly, A.B. Tossi, D.J. McConnell and C. OhUigin, *Nucleic Acids Res.*, **13**, 6017 (1985); https://doi.org/10.1093/nar/13.17.6017.
43. J.K. Barton, A.T. Danishefsky and J.M. Goldberg, *J. Am. Chem. Soc.*, **106**, 2172 (1984); https://doi.org/10.1021/ja00319a043.
44. S.A. Tysoe, R.J. Morgan, A.D. Baker and T.C. Strekas, *J. Phys. Chem.*, **97**, 1707 (1993); https://doi.org/10.1021/j100110a038.
45. R.F. Pasternack, E.J. Gibbs and J.J. Villafranca, *Biochemistry*, **22**, 2406 (1983); https://doi.org/10.1021/bi00279a016.

46. K. Liu, L. Zheng, C. Ma, H.R. Gostl and A. Herrmann, *Chem. Soc. Rev.*, **46**, 5147 (2017); https://doi.org/10.1039/C7CS00165G.
47. J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang and L. Ji, *J. Inorg. Biochem.*, **91**, 269 (2002); https://doi.org/10.1016/S0162-0134(02)00441-5.
48. C. Liu, J.Y. Zhou, Q.X. Li, L.J. Wang, Z.R. Liao and H.B. Xu, *J. Inorg. Biochem.*, **75**, 233 (1999); https://doi.org/10.1016/S0162-0134(99)00037-9.
49. S. Zhang, Y. Zhu, C. Tu, H. Wei, Z. Yang, L. Lin, J. Ding, J. Zhang and Z. Guo, *J. Inorg. Biochem.*, **98**, 2099 (2004); https://doi.org/10.1016/j.jinorgbio.2004.09.014.
50. M.T. Carter, M. Rodriguez and A.J. Bard, *J. Am. Chem. Soc.*, **111**, 8901 (1989); https://doi.org/10.1021/ja00206a020.
51. A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro and J.K. Barton, *J. Am. Chem. Soc.*, **111**, 3051 (1989); https://doi.org/10.1021/ja00190a046.
52. A. Wolfe, G.H. Shimer Jr. and T. Meehan, *Biochemistry*, **26**, 6392 (1987); https://doi.org/10.1021/bi00394a013.
53. V.A. Izumrudov, M.V. Zhiryakova and A.A. Goulko, *Langmuir*, **18**, 10348 (2002); https://doi.org/10.1021/la020592u.
54. V.A. Izumrudov, M.V. Zhiryakova and S.E. Kudaibergenov, *Biopolymers*, **52**, 94 (1999); https://doi.org/10.1002/1097-0282(1999)52:2<94::AID-BIP3>3.0.CO;2-O.
55. B.C. Baguley and M. LeBret, *Biochemistry*, **23**, 937 (1984); https://doi.org/10.1021/bi00300a022.
56. T.B. Wyman, F. Nicol, O. Zelphati, P.V. Scaria, C. Plank and F.C. Szoka, *Biochemistry*, **36**, 3008 (1997); https://doi.org/10.1021/bi9618474.
57. J.R. Lakowicz and G. Weber, *Biochemistry*, **12**, 4161 (1973); https://doi.org/10.1021/bi00745a020.
58. F.A. Beckford, J. Thessing, M. Shaloski Jr., P.C. Mbarushimana, A. Brock, J. Didion, J. Woods, A. Gonzalez-Sarrías and N.P. Seeram, *J. Mol. Struct.*, **992**, 39 (2011); https://doi.org/10.1016/j.molstruc.2011.02.029.
59. J. Peberdy, J. Malina, S. Khalid, M.J. Hannon and A. Rodger, *J. Inorg. Biochem.*, **101**, 1937 (2007); https://doi.org/10.1016/j.jinorgbio.2007.07.005.
60. K.S. Ghosh, B.K. Sahoo, D. Jana and S. Dasgupta, *J. Inorg. Biochem.*, **102**, 1711 (2008); https://doi.org/10.1016/j.jinorgbio.2008.04.008.
61. J.R. Lacowicz, Principles of Fluorescence Spectroscopy, Springer: New York, edn 3 (2006).
62. S. Satyanarayana, J.C. Dabrowiak and J.B. Chaires, *Biochemistry*, **32**, 2573 (1993); https://doi.org/10.1021/bi00061a015.
63. B. Peng, H. Chao, B. Sun, H. Li, F. Gao and L.N. Ji, *J. Inorg. Biochem.*, **101**, 404 (2007); https://doi.org/10.1016/j.jinorgbio.2006.11.008.
64. R. Dias, S. Mel'nikov, B. Lindman and M.G. Miguel, *Langmuir*, **16**, 9577 (2000); https://doi.org/10.1021/la000640f.
65. P. Kumar, S. Senthamilselvi, M. Govindaraju and R. Sankar, *RSC Adv.*, **4**, 46157 (2014); https://doi.org/10.1039/C4RA06664B.
66. D.C. Ware, B.D. Palmer, W.R. Wilson and W.A. Denny, *J. Med. Chem.*, **36**, 1839 (1993); https://doi.org/10.1021/jm00065a006.
67. B. Coyle, P. Kinsella, M. McCann, M. Devereux, R. O'Connor, M. Clynes and K. Kavanagh, *Toxicol. In Vitro*, **18**, 63 (2004); https://doi.org/10.1016/j.tiv.2003.08.011.
68. M. McCann, M. Geraghty, M. Devereux, D. O'Shea, J. Mason and L. O'Sullivan, *Met. Based Drugs*, **7**, 185 (2000); https://doi.org/10.1155/MBD.2000.185.
69. B. Coyle, K. Kavanagh, M. McCann, M. Devereux and M. Geraghty, *Biometals*, **16**, 321 (2003); https://doi.org/10.1023/A:1020695923788.
70. J.A. Collins, C.A. Schandl, K.K. Young, J. Vesely and M.C. Willingham, *J. Histochem. Cytochem.*, **45**, 923 (1997); https://doi.org/10.1177/002215549704500702.
71. V.I. Martin, R.R. de la Haba, A. Ventosa, E. Congiu, J.J. Ortega-Calvo and M.L. Moya, *Colloids Surf. B Biointerfaces*, **114**, 247 (2014); https://doi.org/10.1016/j.colsurfb.2013.10.017.
72. M.A. Zoroddu, S. Zanetti, R. Pogni and R. Basosi, *J. Inorg. Biochem.*, **63**, 291 (1996); https://doi.org/10.1016/0162-0134(96)00015-3.