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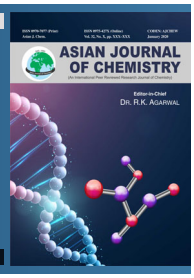

Interaction of CT-DNA with Ruthenium(II) Metallosurfactant Complexes: Synthesis, CMC Determination, Antitumour and Antimicrobial Activities

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To enhance the application of metalosurfactants in the field of drug delivery, it is essential to acquire the role of surfactants in terms of micellization, hydrophobicity, interaction with nucleic acids, reactive changes with abnormal cells and pathogenic organisms. A new class of two ruthenium(II) metallosurfactant complexes $[Ru(DMP)_2(CA)Cl](ClO_4)$ (**1**) and $[Ru(DMP)_2(CA)_2](ClO_4)_2$ (**2**), where DMP = 2,9-dimethyl[1,10]phenanthroline) and CA = cetyl amine were synthesized and characterized. The critical micelle concentration (CMC) and the thermodynamic parameters of micellization were determined and the variations suggest the expression of hydrophobic interaction in these complexes. The binding affinity of ruthenium(II) metallosurfactant complexes with CT-DNA has been investigated by spectroscopic and viscosity magnitudes. The outcomes expose that the complexes associate with CT-DNA through intercalation mode. Subsequently the complexes were taken for *in vitro* anticancer and antimicrobial inhibition study against human cervical cancer cell lines (HeLa) and pathogenic microorganisms and found that the complexes exhibited remarkable inhibitory action. The cytotoxic nature of the complexes towards, HeLa cells, was adopted by MTT assay and apoptosis were examined by AO/EB (acridine orange/ethidium bromide) and tryphanblue staining methods showing that complexes affected the viability of the cells significantly.

Keywords: Ruthenium(II), Metallosurfactant, Critical micelle concentration, DNA binding, Cytotoxicity, Antimicrobial.

INTRODUCTION

Impelled on by the accomplishment of platinum-based drugs in cancer chemotherapy, the research is still concentrating on novel beneficial metal based drugs that offer enhanced feasibility and higher efficacy [1-4]. Despite the tremendous achievement of cisplatin as an anticancer drug, it poses limitation due to its high nephrotoxicity, ototoxicity and myelosupression [5]. Attention has then been shifted to design non classical drugs capable to act in a mechanism unlike to cisplatin. In the search for such metallodrugs, ruthenium fascinated itself to the researchers and found its characteristics in synonym with the dominating platinum drugs. Ruthenium by itself possessed many in built properties such as biologically accessible oxidation states, low systemic toxicity, iron mimicking skill, selective transference of iron by transferrin mechanism, *etc*. to evidence its activity in inhibition of DNA replication, exhibiting mutagenic activity, bind to nuclear DNA, and as chemotherapeutic drugs in biomedical applications [6]. Generally, ruthenium complexes exhibit octahedral geometry, which is widely exploited to construct highly effective antitumor agents with high selectivity and minimized side effects compared to platinum drugs [7].

1,10-Phenanthroline (phen) is an archetypal chelating bidentate ligand for transition metal ions that has played a major role in the development of coordination chemistry and still continues to be of substantial interest as a versatile opening material for organic, inorganic and supramolecular chemistry [8-10]. Phenanthroline is a stiff planar, hydrophobic, electron-poor heteroaromatic system whose nitrogen atoms are charmingly positioned to perform cooperatively in cationic binding. These structural features determine its coordination ability toward metal ions [11].

Ruthenium(II) metallosurfactant complexes have been explored for usage as antitumour agents, topoisomerase inhibitors and antimicrobial tools. These ruthenium(II) metallo-

surfactant complexes with polypyridyl ligands possess a wide spread application and has the tendency to bind DNA *via* non-covalent interactions such as electrostatic interaction, groove binding and intercalation. Polypyridyl ligands can be easily modified providing DNA binding mode despite its octahedral configuration and three dimensional arrangements [12-14]. Indeed they enjoy photoluminescent, photo physical and electro-luminescent characteristics. These features enabled them in multidimensional applications from biotechnology to therapeutics. Ruthenium(II) metallosurfactant polypyridyl complexes diversifies the DNA binding modes of electrostatic interactions, groove binding, intercalation and covalent cross-linking [12-14]. Many ruthenium(II) metallosurfactant complexes exhibit interesting properties upon binding to nucleic acids.

Conventional surfactants consist of atleast one hydrophilic head with polar groups and hydrophobic tail constituted by carbon chains owing to which they possess wide applications in foaming and solubilization [15-17]. The interaction between cationic surfactants and nucleic acids has acknowledged a pronounced attention from the biomedical sciences. Cationic surfactants containing different head groups could be used as antimicrobial agents and as drug delivery targets [18-20]. They also possess the capacity to form complexes with a wide range of biomolecules and are utilized to formulate advanced functional nano target materials.The strong associative behaviour between DNA and cationic surfactant systems is well known in literature and by violating this behaviour through surfactant based metal complexes could pave way for extensive target based drug delivery systems.

Even though multi-methodologies were developed to design metal based drugs, the development of surfactant based metal complexes as metallodrugs is a confident approach to identify the effect of their hydrophobic tail part on the interaction with bio macromolecules such as nucleic acids and biopolymers [21]. In tune with this, double chain systems of surfactants exhibit better antitumor activity as their hydrophobic tail part makes them efficiently pierce into the nucleic acids. So, this kind of tuning of the hydrophobicity of metallodrugs will lead to optimization of DNA binding and its cytotoxicity behaviour. Metallosurfactants are tuned with a central metal ion surrounded by bulky ligands coordinated to the long chain surfactant. The better performance of metallosurfactant is associated to the flexibility delivered by tailored alterations in their molecular construction, namely in the length and nature of hydrophobic tails and the head groups. In these views, our intention is to create the structural foundation for the design of new surfactant metal complexes, which possess more potent DNA binding affinities. This property plays a vital role in biotechnological and pharmacological applications, particularly for the possibility of utilizing such systems for *in vitro* gene delivery and gene transfer [22]. Furthermore, the positive exploitation of surfactant metal drugs in antitumor studies stood as an area of abundant curiosity due to the characteristics exhibited by both the central metal and its surfactant ligand in a solitary intricate. The development of anticancer surfactant metal-based drugs was attempted by reacting dodecylamine with copper and cobalt cationic complexes showed the presence of anticancer property [23]. The hydrophobic nature of the complexes is well suitable for interference with the most fundamental aspect of cancer cells *i.e*. the rapid dividing ability, paving a road for antitumor exploration.

From the literature several reports have been reported for the interaction of DNA with conventional surfactants but boons on surfactant based metal complexes are limited. Thus, the present study focuses on some single and double chain ruthenium(II) metallosurfactant complexes and their interface with CT-DNA. The binding capability and nature of binding style of ruthenium(II) metallosurfactant complexes with CT-DNA was studied by UV-visible absorption, fluorescence emission quenching study and viscometry measurements. In addition, the cytotoxic nature and antimicrobial activities of the complexes were examined in order to clarify the mechanism of biological activity.

EXPERIMENTAL

The constituents and reagents used for the study were of analytical grade (Aldrich and Merck). The calf thymus DNA (CT-DNA) and cetylamine was procured from Sigma-Aldrich, Germany and used straight without further purification. The reagents essential for the absorption and emission examinations were carried out in buffer (50 mM NaCl, 5 mM Tris-HCl, pH 7) at room temperature. DNA solutions were standardized by quantifying the absorption by means of molar absorption coefficient, $\varepsilon_{260} = 6600\ L\ mol^{-1}\ cm^{-1}$ at 260 nm and the ratio of absorbance at 260 and 280 nm was 1.8-1.9:1, agreeing that the DNA was agreeably free of protein [24]. Ultrapure water attained by deionizing distilled water using a Milli-Q Reagent Grade water system was used for synthesis and to prepare solutions for all physical measurements.

The functional group of the metal complexes were identified by FT-IR Shimazdu Japan spectrophotometer with samples prepared as KBr pellets. ^1H spectra were documented on a BRUKER Spectrometer using DMSO as solvent. The conductivity measurement studies were carried out in aqueous solutions of synthesized complexes with an Elico-conductivity bridge type CM 82 and a dip-type cell with a cell constant of 1.0. The fragmentation mass spectrum of the complexes have been recorded on LC-TOF/MS, Synapt, Waters USA.

The absorption spectral studies were documented on a UV-VIS-NIR Cary300 Spectrophotometer by means of cuvettes of 1 cm path length, and fluorescence emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. The human cervical cancer cell lines (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune, India and grown in eagles minimum essential medium containing 10 % fetal bovine serum (FBS).

Synthesis of $Ru(DMP)_2Cl_2$: Commercial $RuC1_3{\cdot}3H_2O$ (1.95 g, 7.46 mmol), 2,9-dimethyl[1,10]phenanthroline (DMP) (2.34 g, 11.24 mmol) and LiCl (1.0 g, 23.59 mmol) were heated at reflux in reagent grade DMF (15 mL) for 8 h. The reaction blend was stirred magnetically throughout this period. After the reaction mixture was cooled to room temperature, 50 mL of reagent grade acetone was slowly added and the resultant solution was cooled at 0 ºC overnight. Filtering the solution, yielded a red black solution and a light brownish yellow microcrystalline product was recovered. The solid was washed three

times with 25 mL portions of water followed by 3 × 25 mL portions of diethyl ether and then it was dried by suction (yield 1.3 g). Anal. calcd (found). % for $C_{28}H_{24}N_4Cl_2Ru$: C, 57.15 (57.98), H, 4.11 (4.02), N, 9.52 (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 (CN), 1143, 1139 (N-H), 1369, 1357 (C=C).

Synthesis of $[Ru(DMP)_2(CA)Cl](ClO_4)$: A combination of $Ru(DMP)_2Cl_2$ (1.105 g, 1.87 mmol) and cetylamine (CA) (2.44 g, 10.10 mmol) in ethylene glycol (25 mL) was added and gradually stirred for 10 min, during which the solution turned brown. There after the solution was left undisturbed at room temperature for a 48 h. A dark blackish brown precipitate was obtained by addition of 4-fold excess of a saturated aqueous solution of $NaClO_4$. All the perchlorate salts are potentially explosive and therefore the reaction was handled in small quantity with utmost care. The precipitate was repeatedly washed with acetone and filtered. A light chocolate brown precipitate was obtained and it was dried to remove water particles (yield: 0.3 g). Anal. calcd. (found) % for $C_{44}H_{59}N_5O_4Cl_2Ru$: C, 59.18 (60.09), H, 6.55 (6.46), N, 7.84 (6.93). TOF-MS: *m/z* = 894.318 $[M+2]^+$ [Na] . ^{1}H NMR (500 MHz, DMSO-d_6): 8.53 (4H), 8.11 (4H), 7.75 (4H), 3.57 (2H), 2.62(12H), 1.72 (2H) 1.2-1.4 (26H), 0.86 (3H). IR (KBr, ν_{max}, cm^{-1}): 2918, 2849 (C-H), 1657, 1605 (C=N), 1151 (N-H), 2348, 2315 (-NH_2), 1467 (C=C), 1086, 623 (ClO_4), 452 (Ru-N).

Synthesis of $[Ru(DMP)_2(CA)_2](ClO_4)_2$: A mixture of $Ru(DMP)_2Cl_2$ (1.105 g, 10.10 mmol) and cetylamine (6.38 g, 26.34 mmol) in ethylene glycol (25 mL) was added and gradually stirred for 10 min during which the solution turned brown. After the solution was left undisturbed at room temperature for a 72 h.A dark blackish brown precipitate was obtained by addition of 4-fold excess of a saturated aqueous solution of $NaClO_4$. All the perchlorate salts are potentially explosive and therefore the reaction was handled in small quantity with utmost care. The precipitate was repeatedly washed with acetone and filtered. A brown chocolate precipitate was obtained and it was dried to remove water particles (yield: 0.3 g). Anal. calcd. (found) % for $C_{60}H_{92}N_6O_8Cl_2Ru$: C, 60.19 (59.97), H, 7.74 (7.03), N, 7.02 (6.94). TOF-MS: *m/z* = 1199.846 $[M+2]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 8.26 (4H), 7.76 (4H), 7.21 (4H), 3.60 (4H), 2.62 (12H), 1.72 (4H) 1.2-1.4 (52H), 0.86 (6H). IR (KBr, ν_{max}, cm^{-1}): 3336 (N-H), 2956, 2917,2849 (C-H), 1656, 1579 (C-N), 1121 (N-H), 2333 (-NH_2), 1489, 1486, 1466 (C=C), 1089, 623 (ClO_4), 460 (Ru-N).

Determination of CMC: Typically, process of micellization is effected by various factors like nature of surfactant (length of the chain, hydrophilic head and hydrophobic tail) and temperature [25,26]. Based on micellization, the CMC values of complexes **1** and **2** were studied by the conductometric method using a specific conductivity meter. The conductivity cell was standardized with KCl solutions in suitable concentration range. The cell constant was determined using the molar conductivity data for KCl [27-29]. Different concentrations of ruthenium(II) metallosurfactant complexes were prepared in the range 10^{-2} to10^{-4} mol dm^{-3} in aqueous solution. For ruthenium(II) metallosurfactant complexes, the CMC was determined with conductivity values of solutions *versus* variations in temperatures (25, 35, 45 and 55 ºC). The conductance was measured after complete mixing of solutions and the temperature maintained uniformly at each dilution. The equilibrium was tested by taking the analyses at constant time intervals of 10 min till no significant variation happened. Further the thermodynamic parameters were evaluated using the temperature dependent CMC.

DNA binding study

Absorption spectral titration: The binding of nucleic acids to ruthenium(II) metallosurfactant complexes can be carried out by absorption titration, fluorescence quenching and viscometry measurements. The absorption titration was performed by using a fixed ruthenium(II) metallosurfactant complex concentration to which increasing amount of the DNA solution was added. Ruthenium(II) metallosurfactant complex-DNA solutions were allowed to incubate for 20 min before the absorption spectra were documented. The concentration of DNA solution was determined using astandard extinction coefficient value of 6600 M^{-1} cm^{-1} at a wavelength of 260 nm [30].

The fluorescence emission studies were performed by using DNA formerly treated with ethidium bromide (EB) and put to 30 min for incubation. Ruthenium(II) metallosurfactant complex was then added as a succeeding molecule to this EB-DNA mixture and the outcome on emission intensities were noted. The sample solutions were excited at 500 nm and emission range was set around 500-700 nm. These titration work were carried out in a mixture of 5 mM Tris-HCl and 50 mM NaCl buffer at pH 7.1 in aqueous media.

The viscosity of DNA was measured by an Ubbelodhe type viscometer sustained at 25 ºC in absence and presence Ru(II) metallosurfactant complexes. CT-DNA sample solutions were prepared by sonication in order to reduce the complications arising from DNA flexibility. The time of flow of the solution through the capillary was measured with an accuracy of ± 0.1 s using a digital stopwatch and each sample was measured at least for three times. The resulting data are presented as $(\eta/\eta_o)^{1/3}$ *versus* ratio of [complex]/[DNA], where η is the relative viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone [31].

in vitro Anticancer activity

Cell line culture and growth: The monaural layer HeLa cervical cancer cells were alienated with trypsin combined with ethylenediaminetetraacetic acid (EDTA) to generate single cell suspensions. The existing cells were counted by tryphan blue exclusion using a hemocytometer and diluted with medium which contains 5 % FBS to give adecisive density of 1×10^5 cells/mL. The cell suspension (100 mL/well) were broadcasted into 96-well plates at plating concentration of 10,000 cells/well and incubated to allow for cell attachment at 37 ºC, 5 % CO_2, 95 % air and 100 % relative humidity. After 24 h, the cells were treated with consecutive concentrations of complex samples.

Cytotoxicity assay: Human cervical cancer (HeLa) cells were examined for viability subsequent treatment by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. This method is based on the ability of mitochondrial dehydrogenases in the feasible cells to cleave the tetrazolium

rings of MTT to form dark blue membrane resistant crystals of formazan, which upon solubilization can be measured spectrophotometrically [32,33].

Approximately 1×10^5 HeLa cells were plated in 96-well cultured plate with 10 % fetal bovine serum (FBS) and cultured for 12 h. Ruthenium(II) metallosurfactant complexes were then initially dissolved in 1 % DMSO and was diluted to various required sequential concentrations in the range of 0.25 to 100 µM. Subsequently aliquots of 100 µL of these complex sample dilutions were added to the suitable wells now containing 100 µL of the medium. Ensuring sample addition, the plates were put to incubation for 24 and 48 h at 37 ºC, 5 % CO_2, 95 % air and 100 % relative humidity. The medium covering without sample dilutions served as control and duplicates was maintained for all concentrations.

Post incubation, 15 µL of MTT dye solution (5 mg/mL in PBS) was added to each well, followed by wrapping in aluminum foil and incubated at 37 ºC for 4 h. The yellow tetrazolium salt of MTT was cleaved by succinate dehydrogenase (mitochondrial enzyme) in viable cells leading to the formation of insoluble purple formazan. Hence, amount of formazan formed is directly proportional to the number of living cells [34]. The culture medium was discarded and 100 µL of DMSO was added to solubilize the purple MTT formazan. The intensity of purple colour formed was estimated at an absorbance of 570 nm using micro-plate reader. The cytotoxicity of ruthenium(II) metallosurfactant sample solutions was determined as the percentage ratio of the absorbance of treated cells over the control cells.

The percentage cell inhibition was determined using the following formula:

% Cell inhibition = 100 – Abs (sample)/Abs (control) × 100

Non-linear regression graph was plotted between % cell inhibition and concentration of complex and IC_{50} was determined using GraphPad Prism software. 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 control.

Morphological studies

AO/EB Staining: Nucleic acid dyes acridine orange (AO) and ethidium bromide (EB) were utilized to identify the morphological changes in abnormal cells. The cervical cancer (HeLa) cells were strewn onto compartment slides in the wells at a concentration of 10^5 cells per wells and put for incubation for 24 h. The cells were cultivated in 10 % fetal bovine serum (FBS) followed by incubation for 24 h at 37 ºC in 5 % CO_2. Ru(II) metallosurfactant complex solution samples of different concentrations were supplied in duplicates to the medium. The cells without samples are treated as control. The plates were put to incubation for 24 h. The medium was removed and the cells were washed with ice cold phosphate buffer saline (PBS). The cell nuclei were counterstained with acridine orange (AO) and ethidium bromide (EB) (AO: 100 µL/mL, EB: 100 µL/mL) for 20 min followed by imaging under inverted phase contrast microscope.

Tryphan blue assay: Solitary of colours, mainly realistic for cell viability assessment is tryphan blue. Similar to AO/EB staining the cervical cancer cells were supplemented with fetal bovine serum (FBS) (10 %) and incubated for 24 h. In short, about 10^5 number of cells were broad-casted in well plates and incubated at 37 ºC for 24 h. After incubation, the plates were taken and freshly prepared Ru(II) metallosurfactant complex solutions were added in duplicates and again put for incubation for 24 h. Then, the plates were removed and stained with 0.4 % of tryphan blue for 40 min. The additional dye was washed and removed with phosphate buffer saline (PBS). Finally, the cell images were visualized under inverted phase contrast microscope.

Antimicrobial screening: The antibacterial and antifungal activities of complexes were evaluated using Kirby-Bauer disc diffusion method [35]. This procedure is considered as exceptionally successful for rapid growing microorganisms and the activities of complexes are measured by calculating the diameter of zone of inhibition [36,37]. Ruthenium(II) metallosurfactant complexes were dissolved in 1 % DMSO at different concentrations. The bacterial microorganisms *i.e*. Gram-positive (*B. cereus* and *S. aureus*) and Gram-negative (*E. coli* and *K. pneumonia*) were cultured in a medium of nutrient agar and incubated at 37 ºC for 48 h. The fungal organisms *viz*. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus* and *Penicillium* sp. were cultured in dextrose agar medium and incubated at 27 ºC for 48 h. During this incubation, the medium was frequently subjected to subculture to fresh medium and used as test bacteria and fungi. Subsequently, fresh cultural discs were inoculated with a loop full of bacterial and fungal culture and it was equally applied throughout the discs equivalently using a sterile glass spreader. This was followed by the addition of 100 µL Ru(II) metallosurfactant complexes as sample concentrations of 50 and 100 mM to each disc. The plates were incubated at 35 ºC for 24-48 h. The discs where the solvents were added served as the control. The inhibitory activity was noted by measuring the diameter of visible zone after the period of incubation.

RESULTS AND DISCUSSION

FTIR analysis: The IR bands *viz*. (C-H) 856, 738 cm^{-1}, observed for phenanthroline are shifted to 794 and 720 cm^{-1} in ruthenium(II) metallosurfactant complexes. This shift can be clarified on the fact that nitrogen atoms of phenanthroline ligands contribute a pair of electrons each to central ruthenium metal, creating a coordinate covalent bond [38,39]. In addition, it is also established by the shift of (C-N) of phenanthroline from about 1656 cm^{-1} in the free ligand to 1605 and 1579 cm^{-1} after coordination [40]. Perchlorate bands at 1086 and 623 cm^{-1} belong to an ionic species showing that the counter-ion is not involved in ruthenium-ligand coordination [41].

For ruthenium(II) metallosurfactant complexes **1** and **2**, the bands around 2918 and 2956 cm^{-1}, respectively can be allotted to C-H asymmetric and symmetric stretching vibration of aliphatic CH_2 of cetylamine [38]. A sharp vibration peak assigned to Ru-Cl stretching mode was observed around 380 cm^{-1} for complex **1** which is absent in complex **2** indicating the absence of Ru-Cl bond in complex **2** and a peak assigned to (Ru-N) stretching mode was observed around 452 cm^{-1} for both the complexes.

^{1}H NMR analysis: The methylene protons of extended chain moiety (cetylamine) provided rise to a multiplet at 1.2-1.8 ppm, while the terminal methyl group of hydrocarbon chain

substituent donated a triplet around δ 0.86 ppm. The aromatic protons of phenanthroline ligands of two complexes appeared in the region 7-10 ppm and an intense peak at δ 2.62 ppm is assignable to methylene proton in phenanthroline ligand [42-44].

Critical micelle concentration (CMC): The aggregation attributes of ruthenium(II) metallosurfactant complexes in aqueous medium across a range of temperatures were studied by conductivity titrations. Specific conductance was found to be dependent on the concentration of Ru(II) metallosurfactant complexes [45]. Solid definite inflexion points were gained suggestive of that the specific conductivity data is concentration and temperature dependent, which shows a plodding increase with the volume of Ru(II) metallosurfactant complex added gradually as well as with the increase in temperatures *viz*. 25, 35, 45 and 55 ºC (Fig. 1). From these plots, it is clear that a break in inflexion points gives the critical micellar concentrations (CMC) of Ru(II) metallosurfactant complexes as reported in Table-1. At the concentration of CMC, formation of micelles is greatly favoured, tending changes in the physical properties of complexes.

A sharp increase from pre-micellar to post micellar region was observed in the plots and this is due to restricted mobility of the molecules by aggregation [46]. However, an increase in temperature increases the CMC values accordingly. Comparatively the complexes with higher alkyl amine chain length showed lower CMC values than complexes possessing lower number of alkylamine groups. Specifically, double chain Ru(II) metallosurfactant complex (complex **2**) have lower CMC than single chain complex (complex **1**).

Thermodynamics of micellization: Commencing from the CMC values as a function of temperature, certain thermodynamic parameters have been calculated *viz*. standard Gibbs energy change (ΔG°_m), standard enthalpy change (ΔH°_m) and standard entropy change (ΔS°_m) to acquire evidence on hydrophobic and head group interfaces.

Based on the phase separation model [47,48], standard Gibbs free energy change of micelle formation per mole of monomer, ΔG°_m, is given as:

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

where R, T and α_{ave} are gas constant, absolute temperature and average degree of micellar ionization, which is the micelle ionization degree at the CMC is equal to the ratio between the slopes of nearly linear specific conductance *versus* [complex] plots above and below the CMC [49], respectively.

The enthalpy of micelle formation can be obtained by:

$$\Delta H^{\circ}_m = -RT^2(2-\alpha_{ave})\,d\frac{\ln CMC}{dT}$$

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

$$\Delta S^{\circ}_m = \frac{\Delta H^{\circ}_m - \Delta G^{\circ}_m}{T}$$

Thermodynamic parameters of micellization at different temperatures for single and double chain Ru(II) metallosurfactant complexes are shown together in Table-1.

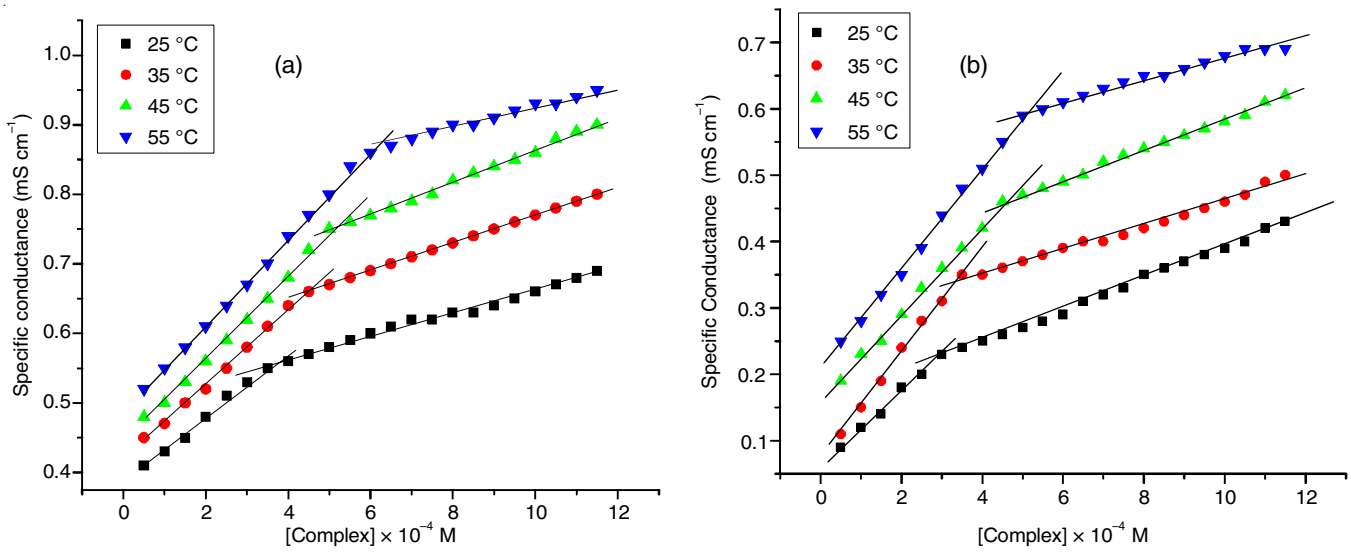

Fig 1. Plots of ruthenium(II) metallosurfactant complex concentration *versus* specific conductance of complexes **1** and **2**

TABLE-1
CMC AND THERMODYNAMIC PARAMETRIC VALUES OF RUTHENIUM(II) METALLOSURFACTANT COMPLEXES IN AQUEOUS SOLUTION

Ruthenium(II) metallosurfactant complexes	Temp. (K)	CMC (× 10^{-4} M)	ΔG^0_m (kJ mol^{-1})	ΔH^0_m (kJ mol^{-1})	ΔS^0_m (kJ mol^{-1})
$[Ru(DMP)_2(CA)Cl](ClO_4)$	298	3.778	-31.81	-21.07	+35.98
	308	4.425	-32.27	-22.59	+31.54
	318	5.272	-33.49	-24.72	+27.54
	328	6.392	-37.06	-28.96	+24.68
$[Ru(DMP)_2(CA)_2](ClO_4)_2$	298	2.877	-34.13	-26.24	+26.46
	308	3.335	-36.16	-29.27	+22.37
	318	4.665	-37.27	-32.50	+14.97
	328	5.187	-38.03	-34.70	+10.17

The data shows that standard Gibbs free energy of micellization is negative for the complexes specifies that the increase in head group polarity helps micellization progression of the system, which is spontaneous and unprompted within the temperature range. Likewise the micelle formation was generally accompanied by a large change in negative ΔG°_{m} showing the aggregation is thermodynamically favoured [50]. Furthermore, the enthalpy of micelle formation (ΔH°_{m}) is also negative for Ru(II) metallosurfactant complexes concluding the micellization process to be exothermic. This is taken as an evidence for the involvement of London dispersion interfaces representing the attractive dynamism of micellization [51]. Finally, positive values of standard entropy changes (ΔS°_{m}) for Ru(II) metallosurfactant complexes points out that the micellization progression in aqueous solution is entropy dominated and ruled by hydrophobic interactions ensuing in getting round the collapse of systematized water molecules adjacent the hydrophobic tail groups [52].

Following the magnitude of values of ΔS°_{m} (Table-1), it is evident that entropy decreases with increase in temperature and the randomness is administered uniquely by hydrophobic interactions between Ru(II) metallosurfactant complexes.The disparity in the thermodynamic parameters with the concentration of Ru(II) metallosurfactant complexes with the variation in temperature recommends the manifestation of hydrophobic interfaces in the considered system.

DNA binding study

Electronic absorption spectral titration: The different modes of binding of molecules to DNA are base pair intercalation, electrostatic and groove binding [53]. The binding between positive charges of the drug moieties and the negative charges of phosphate backbone of the DNA refers to electrostatic binding. Groove binding on the other hand is the binding in due of hydrogen bonds in the major or minor grooves of DNA [54]. Intercalation is the π-π orbital overlapping of molecular complexes with the planar structure and the base pairs of DNA moiety. Based on the above classification binding modes can be predictable by spectroscopy and viscosity dimension. Absorption spectroscopy is one of the most expedient techniques tostudy the binding of any drug to DNA [55-58]. The binding performance of Ru(II) metallosurfactant complexes to DNA helix has been monitored through absorption spectral titrations. The absorption spectra of $[Ru(DMP)_2(CA)Cl](ClO_4)$ (**1**) and $[Ru(DMP)_2(CA)_2](ClO_4)_2$ (**2**), in absence and presence of CT-DNA are displayed in Fig. 2. The exceptional properties of DNA in solution are hyperchromism and hypochromism, observed when an interaction ensues with complexes and the DNA moiety, which is correlated to the extension and compression of DNA double helix [54]. With increasing concentration of calf thymus DNA, the absorption bands of complexes were affected, resulting in the tendency of hyperchromism and a slight blue shift.

Ruthenium(II) complexes can bind to DNA in different binding modes on the basis of their organization and charge and brand of ligands. Since our Ru(II) metallosurfactant complexes contain methylene groups of long aliphatic amine (cetylamine), these complexes can bind to DNA by van der Waals interactions between the methylene groups and thymine methyl groups of DNA [59]. Likewise, since DNA possesses several hydrogen bonding sites in the minor as well as major grooves, and Ru(II) metallosurfactant complexes contain -NH- groups, there could be hydrogen bonding between the complexes and the base pairs in DNA [60-63]. In addition, hydrophobic interaction could also be the possibility due to the presence of alkyl chains of surfactants.

Meanwhile, complexes **1** and **2** contains substituted phenanthroline ligand, providing an aromatic moiety extending from the metal core through which overlapping would occur with the base pairs of DNA by intercalation. The primary ligand of a complex capable of intercalation ought to contain an aromatic heterocyclic ring, which would insert and stack between the base pairs of DNA and the extension lead of intercalative primary ligand planarity may increase the strength of interaction of the complexes with DNA [64,65]. Besides given intercalation into the DNA helix by one ligand, it is expected that accumulating the hydrophobicity of ancillary ligand would lead to an increase in the binding affinity of complex with DNA. Based on the facts and observations, it was found that Ru(II) metallosurfactant complexes exhibits intercalative nature of binding. However, the hyperchromism effects observed in the present study in respect of both the complexes propose that there is a

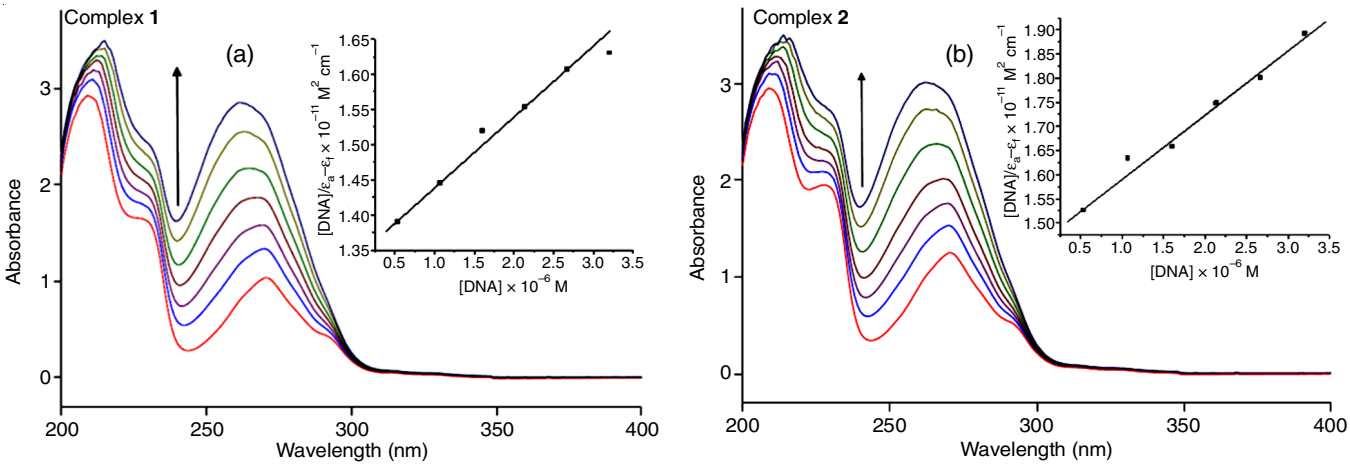

Fig. 2. Absorption spectra of complexes **1** and **2** in the absence (red line) and in the presence of increasing amounts of DNA, [Complex] = 10 mM, [DNA] = 0.5-2.5 μM. Inset: Plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ *versus* [DNA] experimental data points; solid line, linear fitting of the data

strong hydrophobic association between the methylene chain of Ru(II) metallosurfactant complex and the hydrophobic inland of DNA. In order to compare the binding strengths of complexes, the intrinsic binding constant (K_b) was determined using the equation [66].

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

where [DNA] is the concentration of DNA solution in base pairs, ε_a, ε_f and ε_b correspond to A_{obs}/[Ru], the extinction coefficient of free ruthenium complex and the extinction coefficient of the complex in the fully bound form, respectively, and K_b is the intrinsic binding constant. The ratio of slope to intercept in the observed plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA] gives the value of K_b (Fig. 2). The intrinsic binding constant value is calculated for ruthenium(II) metallosurfactant complexes at below CMC value. Values with an order of 10^5 M^{-1} for K_b are reflected to be symbolic of a relatively strong interaction between DNA and metal complexes [67-71]. The binding constants for Ru(II) metallosurfactant complexes **1** and **2** are presented in Table-2. The data shows that the binding constant of complex **2** is higher than complex **1** specifically in due respect of the existence of planar phenanthroline cluster and higher hydrophobicity of alkyl chain, complex $[Ru(DMP)_2(CA)_2](ClO_4)$ (**2**) binds more strongly to DNA than complex **1**.

Competitive binding between ethidium bromide (EB) and Ru(II) metallosurfactant complexes: Beyond the absorption spectral titration, to emphasis binding mode of Ru(II) metallosurfactant complexes to DNA, competitive binding *via* displacement method using ethidium bromide was carried out. Ethidium bromide is one of the best penetrating fluorescent probes that can bind to DNA [72-74]. Ethidium ion demonstrates an intense increase in fluorescence efficiency when it intercalates into the DNA. A competitive binding of the surfactant metal complex to CT-DNA can effect in the decline of emission intensity due to the reduction of binding sites of nucleic acid available for ethidium bromide [75].

The scope of fluorescence quenching of ethidium bromide bound to DNA is used to define the binding of a second molecule to DNA. Positively charged species [76], such as minor cations, polypeptides and macromolecules, with rectilinear or bifurcated structures, may competitively displace the dye from DNA into solution phase, leading to the noticeable quenching of ethidium bromide fluorescence. Bhattacharya and Mandal [77] have testified that addition of cationic surfactants to ethidium bromide bound DNA complex can effect in quenching of the fluorescence due to displacement of ethidium bromide by the surfactants. Similarly, emission spectra of ethidium bromide clung to DNA in absence and presence of Ru(II) metallosurfactant complexes **1** and **2** were determined (Fig. 3). The addition of complex to DNA pretreated with ethidium bromide caused a substantial reduction in the nucleic acid induced emission intensity, specifying the replacement of ethidium bromide fluorophore by the complex.

According to the classical Stern-Volmer equation [78]:

$$\frac{I_o}{I} = 1 + K_{sv}[Sur\text{-}Ru] = 1 + K_q \tau_o [Sur\text{-}Ru]$$

where I_o and I are the fluorescence intensities in the absence and presence of complex, respectively, K_{sv} is the Stern-Volmer

TABLE-2
VALUES OF INTRINSIC BINDING, STERN VOLMER, APPARENT BINDING AND BIMOLECULAR QUENCHING CONSTANTS OF RUTHENIUM(II) METALLOSURFACTANT COMPLEXES WITH CALF THYMUS DNA

Ruthenium(II) metallosurfactant 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})
$[Ru(DMP)_2(CA)Cl](ClO_4)$	6.7828	3.267	3.693	1.485
$[Ru(DMP)_2(CA)_2](ClO_4)_2$	8.8547	3.615	3.963	1.643

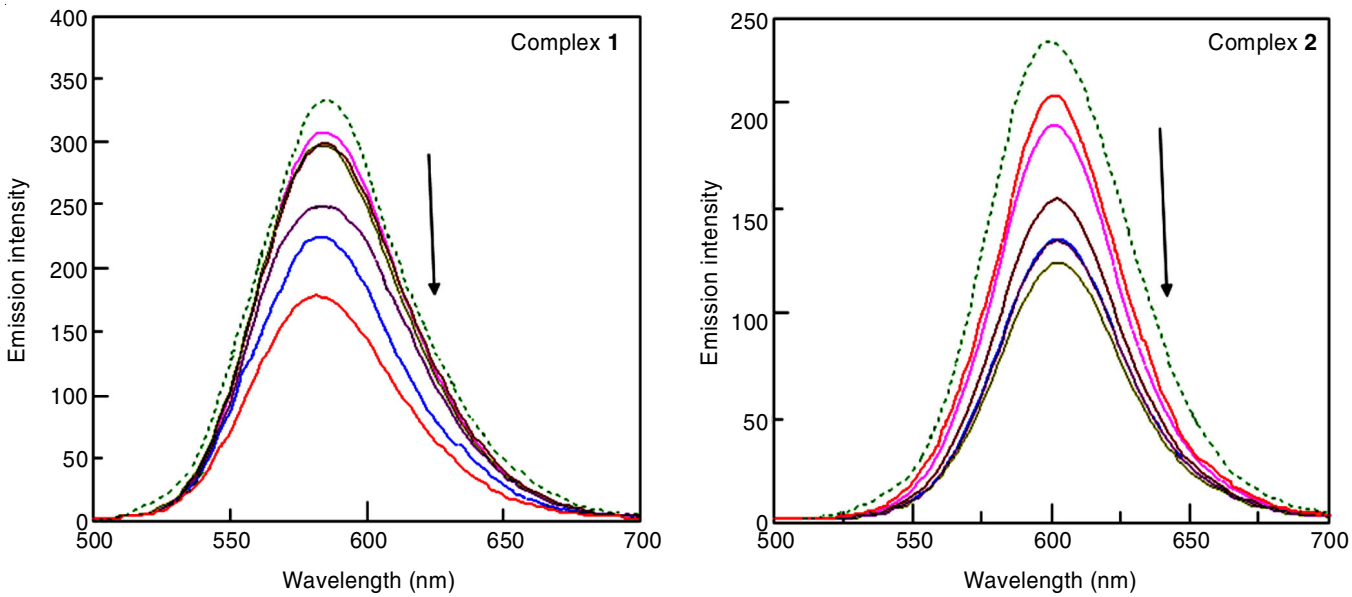

Fig. 3. Emission spectra of EB bound to DNA in the absence and presence of complex **1** and **2**, [EB]= 8×10^{-6} M, [DNA] = 8×10^{-6} M, [Complex] = 0-24 μM. Arrow shows intensity changes upon increasing concentration of the complexes

constant and Q is the concentration of Ru(II) metallosurfactant complexes. A plot of I_o/I *versus* [Q] was drawn, which shows linearity (Fig. 4) and the slope gives K_{sv} values. Stern-Volmer values for Ru(II) metallosurfactant complexes, thus obtained, which are compiled in Table-2. The values with a magnitude order of 10^3 M^{-1} for K_{SV} are reflected to be an indication of strong interaction between DNA helix and metal complexes [67,71,79,80].

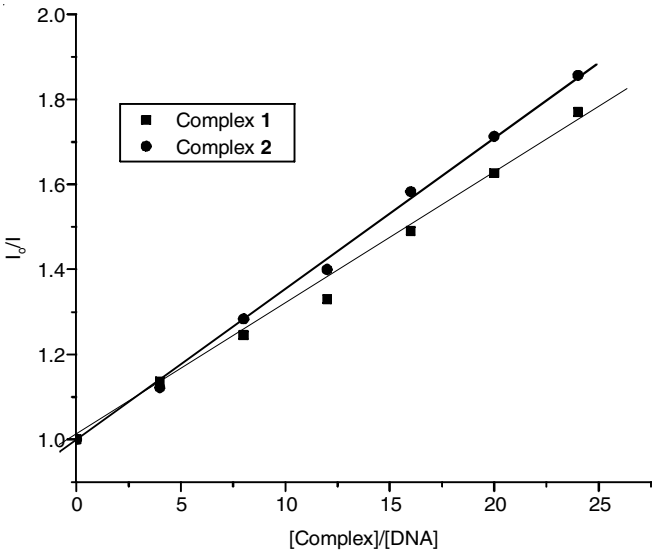

Fig. 4. Stern-Volmer plot for the fluorescence quenching of EB bound to DNA by ruthenium(II) metallosurfactant complexes **1** and **2**

Furthermore to assess the strength of binding, the apparent binding constant (K_{app}) was expressed as

$$K_{app} = \frac{K_{EB}[EB]}{[Ru]_{50\%}}$$

where K_{EB} is the binding constant for ethidium bromide and the value is taken as 1.2×10^6 M^{-1} and $[Ru]_{50\%}$ is the value of concentration of Ru(II) metallosurfactant complex that causes 50 % drop of EB-DNA initial fluorescence (Fig. 5) [81,82].

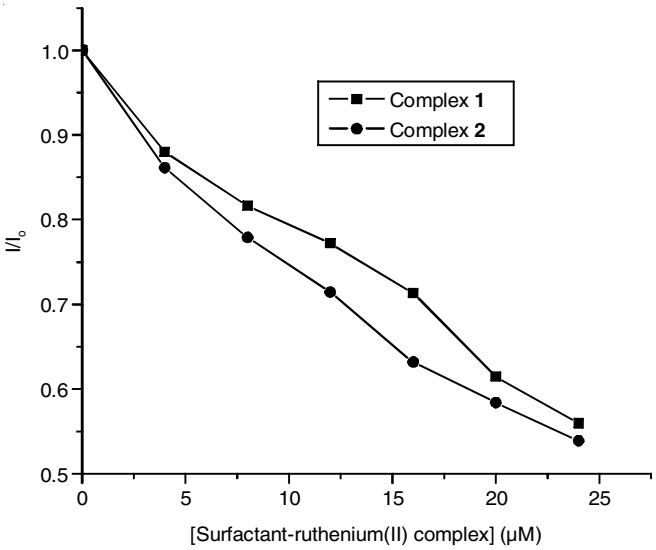

Fig. 5. Effect of the addition of complexes **1** and **2** on the emission intensity of EB bound to DNA. [Ruthenium(II) metallosurfactant] = 0-24 μM, [DNA] = 8×10^{-5} M, [EB] = 8×10^{-5} M

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(II) complexes exhibit good binding affinity. Metal complexes which display a strong interaction with DNA give K_{app} values with a magnitude order of 10^5 - 10^6 M^{-1} [67,71,79,80].

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_o as 22 ns [81,83], which is the average lifetime of EB-DNA complex [59,61]. The values of K_q were found to be in the order of 10^{12} M^{-1} s^{-1}, which is more than the limiting value of 10^{10} M^{-1} s^{-1} [84] which is taken as the highest probable value in aqueous medium [84,85]. Hence, it can be concluded that the complexes show good quenching affinity towards the nucleic acid binding.

The data suggest that the interaction of Ru(II) metallosurfactant complexes in the present study binds strongly with CT-DNA, which is consistent with the spectroscopic results described above. The order of binding is $[Ru(DMP)_2(CA)_2](ClO4)_2$ > $[Ru(DMP)_2(CA)Cl](ClO_4)$. Also, the K_{sv} shows an increase with increase in the hydrophobicity of Ru(II) metallosurfactant complexes. This evidences the growth in the length of surfactant chain, which has an impact on the emission study giving a change in the intercalation of complexes with DNA.

Determination of association constant: Further, association constant (K_b) and numbers of binding sites (n) can be determined by using the following equation:

$$\log\left(\frac{I_o - I}{I}\right) = \log K_b + n \log [Complex]$$

The complexes **1** and **2** possess the values of K_b from the plot of log (I_o – I/I) *versus* log [complex] (Fig. 6) and found to be $7.10^7 \times 10^3$ M^{-1} and 9.295×10^3 M^{-1}, respectively. These results confirms the strong intercalative interaction of both the complexes with CT-DNA, further complex **2** shows more intercalation than complex **1** in due of hydrophobicity. The binding sites 'n' value for both complexes **1** and **2** are nearly equal to 1 (Table-3), indicating both Ru(II) metallosurfactant complexes have one mode of interaction with CT-DNA.

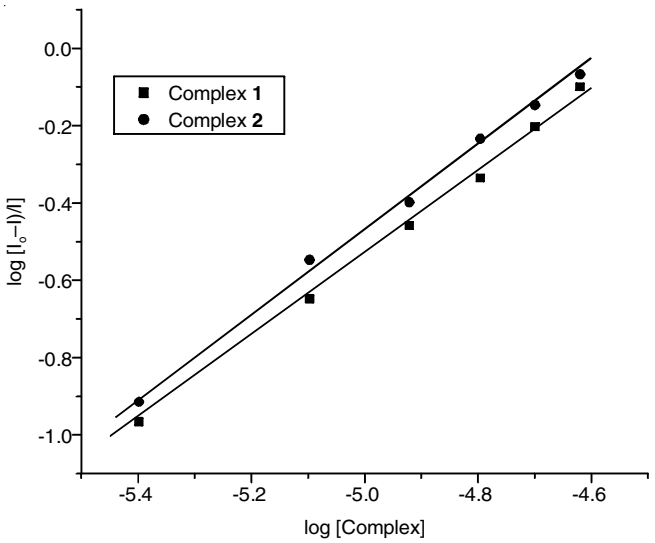

Fig. 6. Double-logarithmic plots for the quenching of CT DNA by ruthenium(II) metallosurfactant complexes **1** and **2**

TABLE-3
VALUES OF BINDING CONSTANT AND BINDING NUMBER FROM log $(I_o - I)/I$ *vs.* log [COMPLEX] OF RUTHENIUM(II) METALLOSURFACTANT COMPLEXES WITH CALF THYMUS DNA

Ruthenium(II) metallosurfactant complexes	$K_b \times 10^3$ (M^{-1})	n
$[Ru(DMP)_2(CA)Cl](ClO_4)$	7.107	0.875
$[Ru(DMP)_2(CA)_2](ClO_4)_2$	9.296	0.877

Viscosity measurements: Conclusively, the intercalative nature of Ru(II) metallosurfactant complexes with CT-DNA were examined by viscosity measurements in the absence and presence of Ru(II) metallosurfactant complexes. In absence of crystallographic structural data, hydrodynamic methods, which are sensitive to increase in DNA length, are observed as the least ambiguous and the most critical assessment of binding in solution [31,86]. Optical or photo-physical probes normally provide essential, but not adequate clues to support an intercalative binding model. Under appropriate conditions intercalation instigates a momentous rise in the viscosity of DNA solutions owing to the breakup of base pairs at intercalation sites and, henceforth, increase the overall DNA contour length whereas ligands that bind absolutely in the DNA grooves or electrostatically interacted ligandsclassicallycause a curvature (or kink) in DNA helix sinking its actuallength and so its viscosity.

The effects of Ru(II) metallosurfactant complexes **1** and **2** on the viscosity of CT-DNA are displayed in Fig. 7. The plots revealed the binding of complex **1** and **2** indicated an increase in the relative specific viscosity of DNA while complex **2** wielded a huge increase of DNA viscosity. Generally DNA viscosity is severely reliant on the length alterations that might take place as a concern of an interaction between double helix and a peripheral molecule. Furthermore, splitting of base pairs is done by a perfectly intercalative metal complex since the complex needs to be housed within the DNA helix, leading to a lengthening of nucleic acid helix and thereby in its viscosity [87]. Likewise the base pairs are spaced out enough to provide accommodation to the incoming unavoidable ligand, followed by intercalation which is liable to lengthen the DNA helix

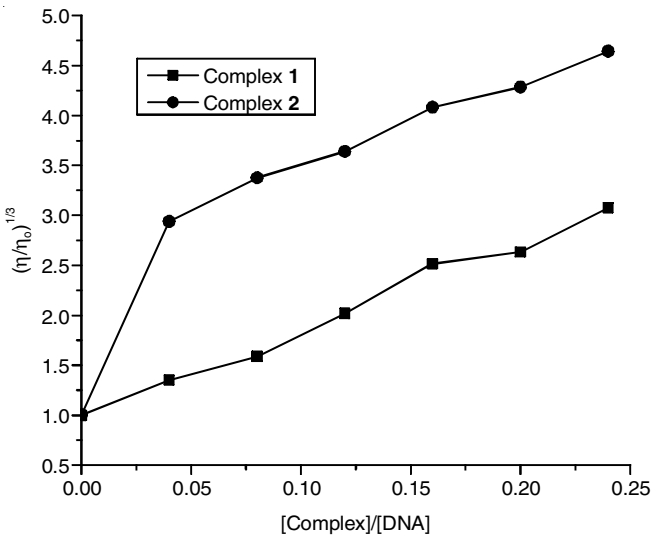

Fig. 7. Effects of increasing amount of complex **1** and **2** on the relative viscosities of the calf thymus DNA

shadowed by an increase in the DNA viscosity. In contrast, when exterior binding arises, viscosity of DNA would not change basically [86]. For two complexes, relative viscosity increases upon increasing the concentration ratio of complex to DNA.

From the plot, it is obvious that present synthesized Ru(II) complexes binds to CT-DNA through intercalative fashion as there is a gradual increase in the viscosity of DNA solutions. Besides it is evident that the order of intercalation of DNA with the complexes follows the order, $[Ru(DMP)_2(CA)_2](ClO_4)_2$ > $[Ru(DMP)_2(CA)Cl](ClO_4)$. The influence of surfactant alkyl chain length is also perceptible in the extension of DNA binding. The surfactant with the longer chain length, binds more readily to DNA leading to the separation of base pairs, thereby increasing the viscosity [88].

***in vitro* Cytotoxicity assay:** With an intention to assess the prospective antitumor nature, the *in vitro* cytotoxic assay of Ru(II) metallosurfactant complexes against human cervical cancer (HeLa) cell lines were carried out to evaluate the cytotoxicity of the tested complexes. The consequences were explored *via* cell inhibitory activity expressed as IC_{50} values and are presented in Table-4 and Fig. 8. Various concentrations of the complexes in the range of 0.25-100 µM were put to treatment with HeLa cells for 24 and 48 h and the inhibitory activity was noted. The complexes decreased the viability of cervical cancer cells substantially in a dose and duration dependent manner. The outcomes of cytotoxic action on human cervical cancer cell lines were determined according to the dosage values of the exposure of complex requisite to lessen the survival of the cell lines to 50 % (IC_{50}).

TABLE-4
IC_{50} VALUE OF RUTHENIUM(II) METALLOSURFACTANT COMPLEXES FOR HeLa CANCER CELLS

Ruthenium(II) metallosurfactant complexes	IC_{50} (µM)	
	24 h	48 h
$[Ru(DMP)_2(CA)Cl](ClO_4)$	31.93 ± 1.77	28.80 ± 1.7
$[Ru(DMP)_2(CA)_2](ClO_4)_2$	15.48 ± 10.52	12.72 ± 3.6

Both Ru(II) metallosurfactant complexes displayed highly active cytotoxic action against the cervical cancer cell lines at low concentrations. Meanwhile, the parallel treatment of the complexes to a normal cell line showed less effects on inhibition of viability. Active cytotoxic action may perhaps be due to the fact that Ru(II) metallosurfactant complexes possess the ability to diminish the energy status in tumor cells, which already require a higher energy for metabolic mechanisms. When there is a deficiency in energy, the cellular functions are deactivated leading to cell death. Further, an extent of cellular damage perpetrated by these complexes depends on the nature of their axial ligands. It is widely accepted that phenanthroline containing metal complexes have a wide range of biological activities such as antitumor, antifungal, apoptosis and interaction with DNA inhibiting replication, transcription and other nuclear functions and arresting cancer cell proliferation so as to arrest tumor growth. Moreover, the higher hydrophobicity of the complexes, higher is the extent of cellular membrane damage. The IC_{50} value for Ru(II) metallosurfactant complexes showed that complex **2** is highly cytotoxic than complex **1** as the complex

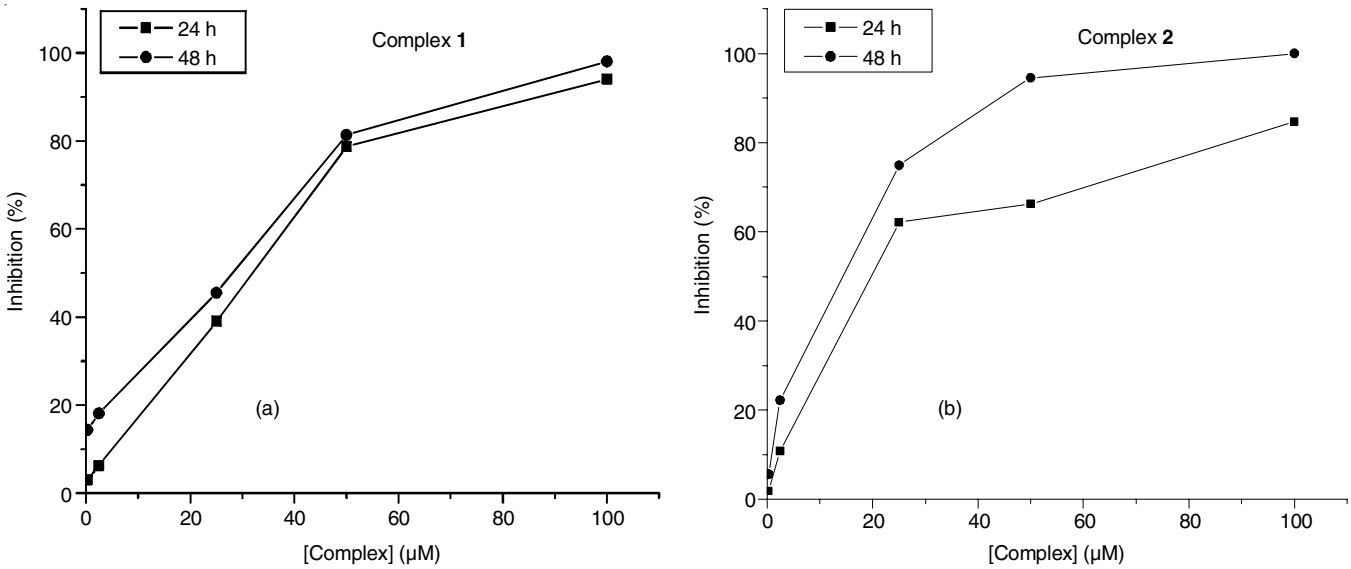

Fig. 8. Plot showing the percentage inhibition *vs.* variable concentrations of the complexes exposed to HeLa cancer cells for 24 and 48 h. The complexes effect the viability of the cells in a duration dependant manner

due to more hydrophobicity and an increase in the number of alkyl chain length favouring comfortable penetration decreasing the energy of the cells in a duration dependent fashion.

Morphological changes through apoptosis assay (AO/EB staining): Acridine orange/ethidium bromide assay was made use to assess the influence of Ru(II) metallosurfactant complexes on the morphology of HeLa cancer cell lines and the outcomes acquired are displayed in Fig. 9.

The morphological alterations detected are categorized into four kinds based on the fluorescence images and structural highlights of chromatin condensation in the AO/EB stained nuclei, firstly viable cells with good organization possessing uniform green fluorescing nuclei, secondly initial apoptotic cells with intact cell membranes and in the way of DNA fragmentation having bright green fluorescing nuclei and but peripheral nuclear chromatin compression is visible as patches or territories, thirdly final apoptotic cells transforming from greenish to orange to red fluorescing nuclei with compressed chromatin, and lastly necrotic cells, swollen in addition to uniform orange red fluorescing nuclei with no sign of chromatin fragmentation [89].

Fluorescing images of Ru(II) metallosurfactant complexes revealed that the cells with apoptotic features underwent morphological modifications further exhibiting different characteristics. Control mode of cell lines predominantly displayed bright green emission highly organized and systematic. These figures implicit distinct changes of apoptosis can be identified by staining approach. Further, this assay would be applicable for the recognition and portrayal of cell structural changes at various levels in the process.

The morphological changes publicize that these Ru(II) metallosurfactant complexes possibly would prompt cell loss by two basic processes *i.e.* clampdown of cell proliferation and initiation of apoptosis on the HeLa cancer cell lines. Meanwhile, it can be concluded 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 exclusion assay is grounded on the interpretation that viable cells retain intact cell membranes that intake dyes like tryphan blue, Eosin or propidium. This exclusion assayis a typical method to evaluate the cell proliferation or death *via* their membrane integration scrutiny [90]. Previous studies have recognized that cell membrane integrity is an elementary benchmark for distinguishing viable cells from non-

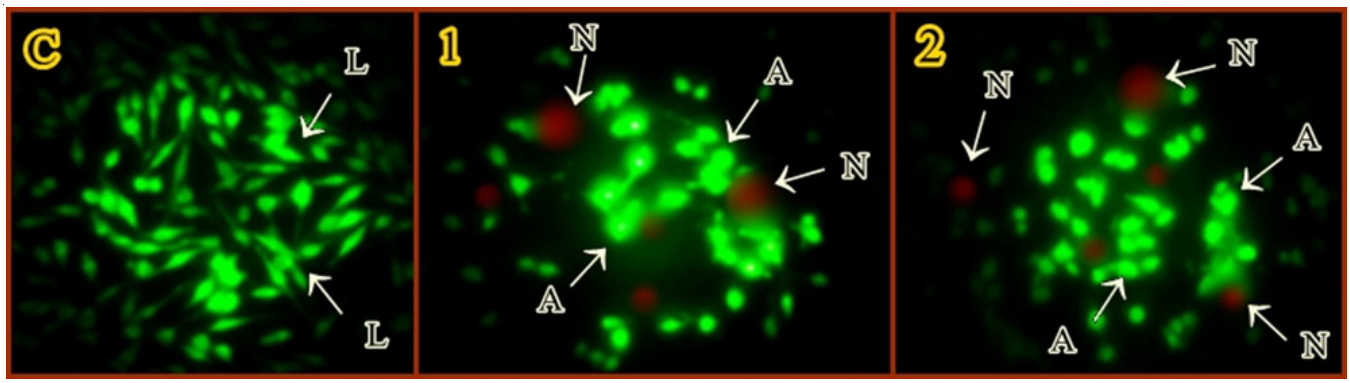

Fig. 9. HeLa cells were stained by AO/EB and observed under fluorescence microscopy. HeLa cells without treatment (C), in the presence of complex **1** (1) and complex **2** (2). The viable cells uniformly green fluorescing L are living, A: apoptotic (bright green clusters, cell blebbing and cell enlargement) and N: necrotic cells (orange to red, cell blebbing) respectively

viable cells. Tryphan blue, owns the propensity to precisely infiltrate the cytoplasm of non-viable cells due to loss of membrane specificity whereas viable cells are unstained.

Accordingly cells with anomalous cytoplasm absorbs the dye and is seen as blue stained and these cells are non-viable. The living cells remain transparent without any morphological colour change. Nevertheless, Ru(II) metallosurfactant complexes when put forward for this staining with HeLa cell line absorbed tryphan blue selectively resulting in cell membrane damage and exhibited thick blue coloration. The morphological changes were observed at low concentrations of complexes **1** and **2**, respectively (Fig. 10).

Screening for antibacterial and antifungal activities: Ruthenium(II) metallosurfactant complexes **1** and **2** were screened *in vitro* for their antimicrobial activity against certain human pathogenic bacterial species using well diffusion method and the results are summarized in Table-5. The proneness of certain strains of bacterial and fungal organisms toward the complexes was mediated by quantifying the size of the emergent inhibition diameter.

There was good antibacterial activity of both the complexes against Gram-positive bacteria *B. cereus* and *S. aureus*, and the Gram-negative bacteria *E. coli* and *K. pneumonia* followed by enhanced activity of fungal species *A. flavus*, *A. niger*, *A. terreus* and *Penicillium* sp. This positive activity may be due to a competent diffusion of metal complexes into the bacterial cell membrane. Ruthenium(II) metallosurfactant complexes being hydrophobic in nature ruins the bacterial and fungal cell wall making the organism inactive. Further Ru(II) metallosurfactant complexes interrupt the respiration process of the cell, as a consequence synthesis of proteins within the cell is obstructed, which restrains additional growth of the organisms. Out of the two complexes, complex **2** exhibited better activity than complex **1** enhanced hydrophobic character of complex **2**, which can destruct the bacterial and fungal cell wall more efficiently. It may be concluded that synthesized ruthenium (II) complexes are in general capable of inhibiting the growth of bacteria to a moderate extent. These factors enable the promotion of antibacterial/fungal activity with the increase in the dosage of the complexes. Zoroddu *et al*. [91] have reported that phenanthroline ligand alone showed null-significant activity against the Gram-positive and Gram-negative bacteria but ruthenium(II) phenanthroline complexes exhibited considerable activity against bacterial fungal species.

Conclusions

In summary, a single and double chain Ru(II) metallosurfactant complexes with substituted phenanthroline moiety as ligands were synthesized and characterized successfully. The vital property of surfactants, CMC were determined and it shows that double chain surfactant complex is lower than single chain due to more aggregation nature of the complexes. The thermodynamic parameters of micellization was evaluated with the CMC confirming that the process is temperature dependent, spontaneous from negative values of standard Gibbs free energy, exothermic from negative values of enthalpy of micelle formation and entropy driven from positive values of entropy of micelle formation. Notable is the variation in the temperature dependent CMC and the proceeding of the reaction by exothermic, spontaneous and entropy driven process.

Considering the mode of binding of these complexes with CT-DNA by spectroscopic and viscosity studies, a favorable conclusion is attained due to the binding constant values proving intercalation mechanism. This result is remarkably

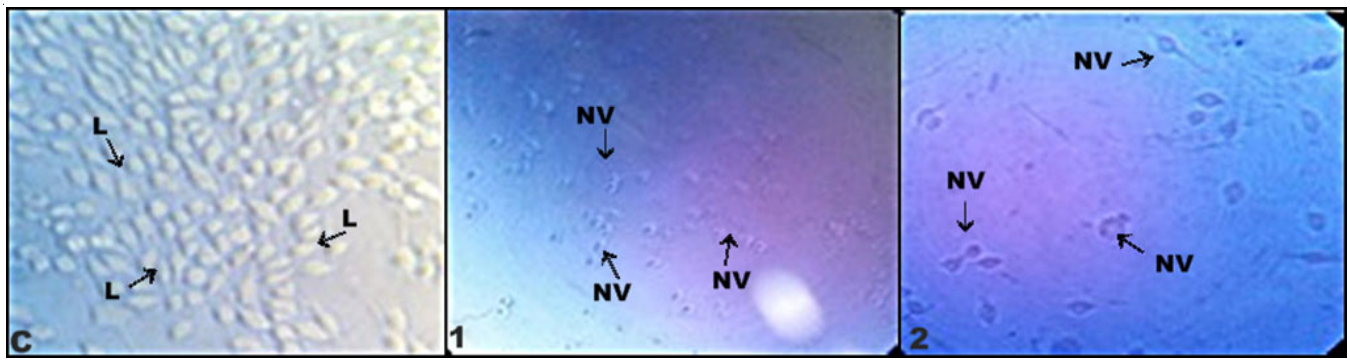

Fig. 10. Cell death was measured by tryphan blue staining for 24 h (1: Complex **1**, 2: Complex **2** and C: Control). L indicates living cells; NV non-viable cells loosing membrane integrity

TABLE-5
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF RUTHENIUM(II) METALLOSURFACTANT COMPLEXES

Test organism	Zone of inhibition (mm)				Test organism				
	Antibacterial activity					Antifungal activity			
	$[Ru(DMP)_2(CA)Cl](ClO_4)$		$[Ru(DMP)_2(CA)_2](ClO_4)_2$			$[Ru(DMP)_2(CA)Cl](ClO_4)$		$[Ru(DMP)_2(CA)_2](ClO_4)_2$	
	50 mM	100 mM	50 mM	100 mM		50 mM	100 mM	50 mM	100 mM
E. coli	6	7	10	14	*A. flavus*	19	24	25	34
K. pneumonia	5	6	6	7	*A. niger*	24	26	25	36
S. aureus	7	9	8	9	*A. terreus*	28	30	24	35
B. cereus	8	10	11	17	*Penicillium* sp	24	29	26	36
Standard: Streptomycin; Solvent: DMSO (showed nil activity against microorganism tested)					Standard: Penicillin; Solvent: DMSO (showed nil activity against microorganism tested)				

analogous to the results from ethidium bromide displacement assay and viscosity dimension. This mode of intercalation is payable to the presence of more drawn-out aromaticity of ligands and elongated aliphatic chain in the complexes. Moreover, the pathway of their antitumor activities vary from attack on CT-DNA and disturbance of the balance in cells leading to a drastic change in inhibitory concentration (IC_{50}) values. The IC_{50} values of double chain complexes are lower than the single chain because of the facilated penetration of more hydrophobic complexes towards the abnormal cells. The establishment of morphological variations by Ru(II) metallosurfactant complexes in cancer cells was detected by AO/EB and tryphan blue staining methods. It is clear that changes in their external membrane structures have dramatic effects on cell activity leading to apoptosis and necrosis. Also, the complexes possess good antimicrobial activity towards the bacterial and fungal species. Hence, this might lead to the discovery of a series of surfactant complexes for more pharmacological exploration.

The transmission of pathways and concepts from the well-known metallic drug mechanism into the field of surfactant based metallic drugs is likely to yield new paradigms for the design of innovative drugs of the future. The evolvement of surfactant metal complexes on the road to clinical trials will improve the recognition of novel surfactant metallic complexes by the pharma industry and move on for further research into anticancer metallodrugs.

CONFLICT OF INTEREST

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

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