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Research Article

Antimicrobial activity, Spectral studies and CMC determination of some Surfactant-Copper (II) complexes

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ABSTRACT

Some novel surfactant-copper(II) coordination complexes, [Cu(phen)2($C_{16}H_{33}NH_2$)](ClO_4)$_2$ (A) and [Cu(phen)$_2$($C_{12}H_{25}NH_2$)](ClO_4)$_2$ (B (phen = 1,10-phenanthroline) were synthesized from the corresponding halogeno complex by ligand substitution method. The critical micelle concentration (CMC) values of these surfactant metal complexes in aqueous solution were obtained using conductivity method. The surfactant–copper (II) complexes were screened for their antibacterial and antifungal activities against various microorganisms. The results were compared with the standard drugs, Ciprofloxacin and Nystatin respectively.

Keywords: Surfactant-metal complexes, Critical Micelle Concentration, Antimicrobial studies , Micelles

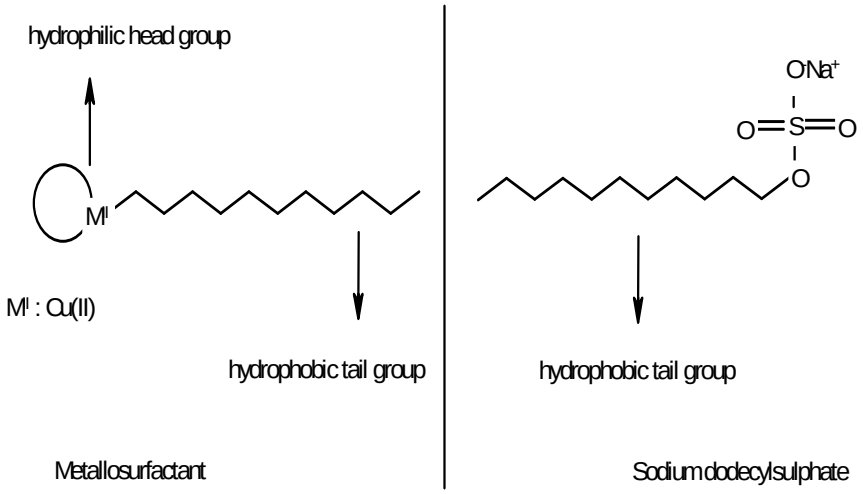
INTRODUCTION

Surfactant- metal complexes have received a sustained level of attention [1–6] due to their relevance in various redox processes in the biological system and are promising agents as anthelmintics[7], antiparasitics[8], antibiotics[9], and because of their multiple applications in fields such as medicine [10], magnetic resonance imaging[11] and drug delivery[12]. Surfactant- metal complexes with chelating ligands are of interest for metallobiomolecules in the search for appropriate systems for binding and activating simple molecules, catalysis, and magnetic interactions[12,13]. I.A.Fallis et al. have studied the synthesis and characterization of novel Ni(II) and Cu(II) based surfactants and also the solid state and solution behaviour of novel transition metal containing surfactants which have been determined by X-ray crystallography[14]

A characteristic feature of transition metals is their ability to form complexes with a variety of neutral molecules such as bipyridine (bpy) and phenanthroline (phen). These are widely used as a classical N,N - bidentate ligand to prepare mixed-ligand complexes in coordination chemistry. Metal complexes of pyridine and phenanthroline chelators are of great interest since they exhibit numerous biological properties such as antitumor, anticandida and antibacterial activity [15-17]. At the same time, metal complex bearing ethylenediamine have also been interest because in the classical antitumor agent cisplatinum, one of the ligands must be an N-donor and posses at least one hydrogen atom attached to the nitrogen [18].

Surfactant–metal complexes are a special type of surfactants, where a coordination complex acts as the surfactant. In these surfactants, the metal complex containing the central metal ion with its primary coordination sphere acting as the head group and the hydrophobic part of one or more ligands acts as the tail. Like other well-known surfactants, these metallosurfactants also form micelles at a specific concentration called critical micelle concentration (CMC) in aqueous solution.We have been interested in synthesis and micelle forming properties of many surfactant-metal complexes for a long-time[19-22].

In all these surfactant-metal complexes the coordination complex containing a central metal ion with surrounding ligands (coordinated to metal) acts as the surfactant **(Scheme 1).** Like any other well-known surfactant, for example, sodium dodecyl sulphate (SDS), these surfactant-metal complexes also form micelles at a specified concentration called critical micelle concentration(CMC) in aqueous solution. In recent times there has been some reports from various research groups on surfactant-metal complexes of various nature and their micelle forming properties [23-25]. In all these surfactant-metal complexes, the metal complex part containing the central metal ion with its primary coordination sphere acts as the head group and the hydrophobic part of one or more ligands acts as the tail part. The present study has realized the synthesis, characterization, CMC determination of some novel surfactant-copper(II) complexes containing single long chain amine as one of the coordinating ligands.



Scheme 1

EXPERIMENTAL

Materials: All the reagents were of analytical grade (Aldrich and Merck). Ultra pure water obtained by deionising distilled water using a milli-Q reagent grade water system was used for the preparative work and to make up solutions for all physical measurements.

Microorganisms: The test bacterial strains Staphylococcus aureas, Bacillus subtilis, E.Coli were grown in nutrient agar medium and incubated at 37 ±1 °C for 24 - 48 h followed by frequent subculture to fresh (Nutrient broth) medium and were used as test bacteria. The fungal strains Pseudomonas aeruginosa,Candida Albicans, Aspergillus Niger cultures grown into Potato dextrose agar (PDA) medium, incubated at 25 ±1 °C for 72 h followed by periodic sub culturing to fresh mycological broth medium and were used as test fungus. All the medias and standard disks were purchased from Hi-Media (Mumbai, India). The test cultures were obtained from NCIM and MTCC.

Synthesis of single chain surfactant-copper (II) Complexes: $[Cu(phen)_2Cl]Cl$ was prepared as described previously[26].The surfactant-copper(II)-dodecylamine complex, $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$was prepared by the following method. [Cu (phen) $_2$Cl]Cl (0.65 g) was dissolved in methanol (5 mL). To this solution dodecylamine (0.23 mL) was added dropwise and was kept as such at room temperature for about 24 hours. Afterwards a saturated solution of sodium perchlorate in very dilute perchloric acid was added. A blue green precipitate was separated out and it was filtered off, washed with small amounts of alcohol followed by acetone, and then it was dried over air. The precipitate was further dried in a drying pistol over fused calcium chloride and stored in a vacuum desiccator (Yield = 1.27 g).

Surfactant-copper(II)-cetylamine complex, $[Cu(phen)_2(C_{16}H_{33}NH_2)](ClO_4)_2$ was prepared using the same procedure described above for complex $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$, but using cetylamine (0.3 g) in the place of dodecylamine (Yield = 1.5 g).

Instrumentation and Physical Methods: The carbon, hydrogen and nitrogen contents of samples were determined at SAIF, Lucknow, India. Absorption spectra were recorded on a UV-VIS-NIR Cary300 Spectrophotomer using cuvettes of 1-cm path length, FT-IR spectra were recorded on a FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. EPR spectra were recorded on Varian E-112 EPR spectrometer at LNT (Liquid nitrogen temperature, 77 K), the field being calibrated with DPPH=1,1′-diphenyl-2-picrylhydrazyl (g = 2.0037) at SAIF, I.I.T., Chennai, India. Conductivity measurements were studied using Elico conductivity bridge type CM 82 and dip-type cells with cell constant of 1.0

CMC Determination: The critical micelle concentation values of the surfactant-copper(II) complexes were measured conductometrically using a specific conductivity meter. Various concentrations of surfactant-copper (II) complexes were prepared in the range of 1×10^{-5} -1×10^{-1} mol dm^{-3} in aqueous solutions. The conductivities of these solutions were measured at 30.0^{o}C. The conductivity cell was calibrated with KCl solutions in the appropriate concentration range.

Microbial assay / Testing of antimicrobial activity: The in vitro antimicrobial screening of the surfactant–copper (II) complexes were tested for their effect on certain human pathogenic bacteria and fungus by disc diffusion method [26]. The complexes were stored dry at room temperature and dissolved in DMSO (1%). Both the Gram positive (Staphylococcus aureus, Bacillus subtilis) and Gram negative (Escherichia coli, Pseudomonas aeruginosa) bacteria were grown in nutrient agar medium and incubated at 37 °C for 48 h followed by frequent subculture to fresh medium and were used as test bacteria. The yeast Candida albicans grown into Sabouraud dextrose agar medium, incubated at 27 °C for 72 h followed by periodic sub culturing to fresh medium and were used as test fungus. Then the petri dishes were inoculated with a loop full of bacterial or fungal culture and spread throughout the petri dishes uniformly with a sterile glass spreader. To each disc the test samples (10 µg/mL) and reference Ciprofloxacin (5 µg/disc for bacteria) or Fluconazole (10 µg/disc for fungus) was added with a sterile micropipette. The plates were then incubated at 35±2 °C for 24–48 h and 27±1 °C for bacteria and fungus, respectively.

Plates with disc containing respective solvents served as control. Inhibition was recorded by measuring the diameter of the inhibitory zone after the period of incubation. All the experiments were repeated thrice and the average values are presented. The results of the antimicrobial activities are summarized in Table -2.

RESULTS AND DISCUSSION

Spectroscopic Characterization: The surfactant-copper (II) complexes synthesized in the present study were characterized by UV-Visible, IR, EPR techniques. The purity of the complex is checked by elemental analysis and was found to be in good agreement with that of the calculated value (**Table -1**).

The uniqueness of the surfactant-copper(II) coordination complexes lies in the fact that the bond between the head group and the tail part of the surfactant-copper(II) complex is a coordinate bond and the surfactant contains a higher charge on the head group unlike common surfactants (sodium dodecyl sulfate). At the same time like the common surfactants, these surfactant-copper(II) coordination compounds form foam in aqueous solution when mechanically disturbed like shaking, and these complexes dissolve slowly in water, though sometimes we have to sonicate the solution to get a homogeneous solution.

Strukl[27] and Schilt et al [28] studied the infrared spectra of several bipyridyl and phenanthroline complexes. In the IR region, the bands around 1518 $cm^{-1}$and 1425 cm^{-1}can be attributed to the ring stretching frequencies [ν(C═C) and ν(C═N)] of 1,10-phenanthroline [29]. The IR values, δ (C-H) 853 cm^{-1} and 737 cm^{-1} observed for phenanthroline are redshifted to 849 cm^{-1} and 725 cm^{-1} (**Fig.1a & Fig. 1b**). These shifts can be explained by the fact that the two nitrogen atoms of phenanthroline ligands donate a pair of electrons each to the central copper metal forming a coordinate covalent bond [30].

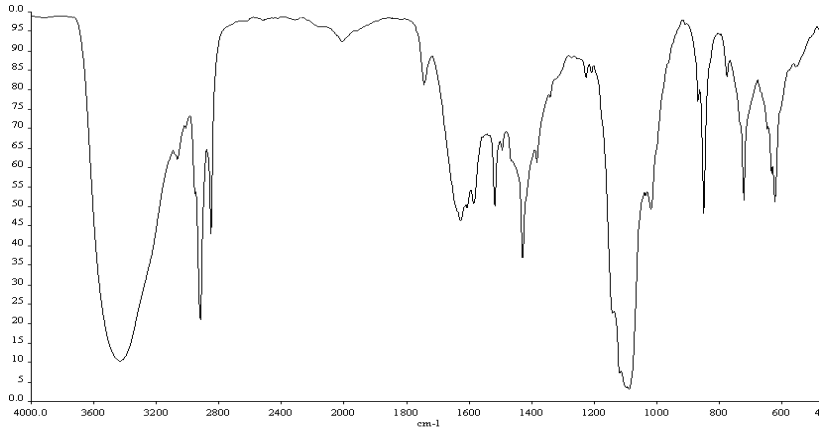


Fig. 1a: IR spectrum of $[Cu(phen)_2(C_{16}H_{33}NH_2)](ClO_4)_2$

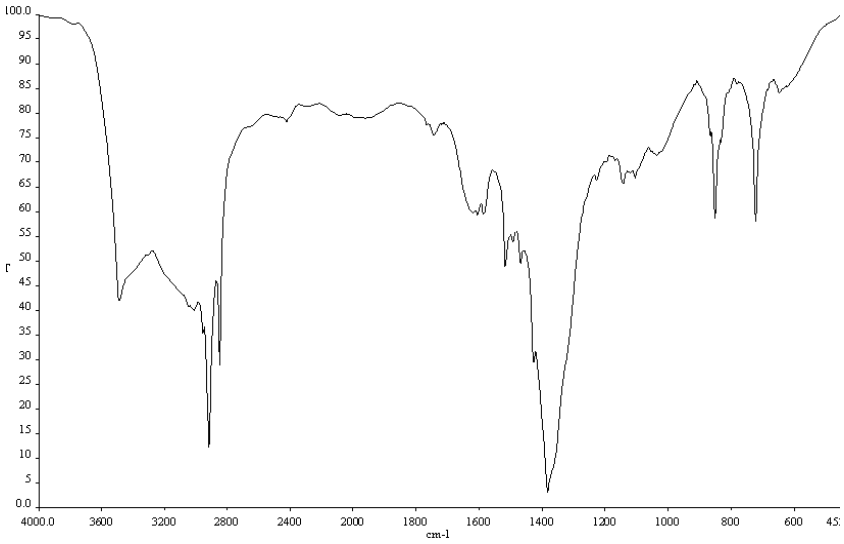


Fig. 1b: IR spectrum of $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$

In the UV-visible region, the intense absorption bands appeared from 200-300 nm is attributed to charge transfer transitions. Another band which appeared around 638 nm is assigned to ligand field transitions.

The solid state EPR spectra of the Surfactant-copper(II) complex (x = 0.203) was recorded in X-band frequencies at room temperature as well as in frozen solution (77 K) (**Fig. 2a and 2b**).

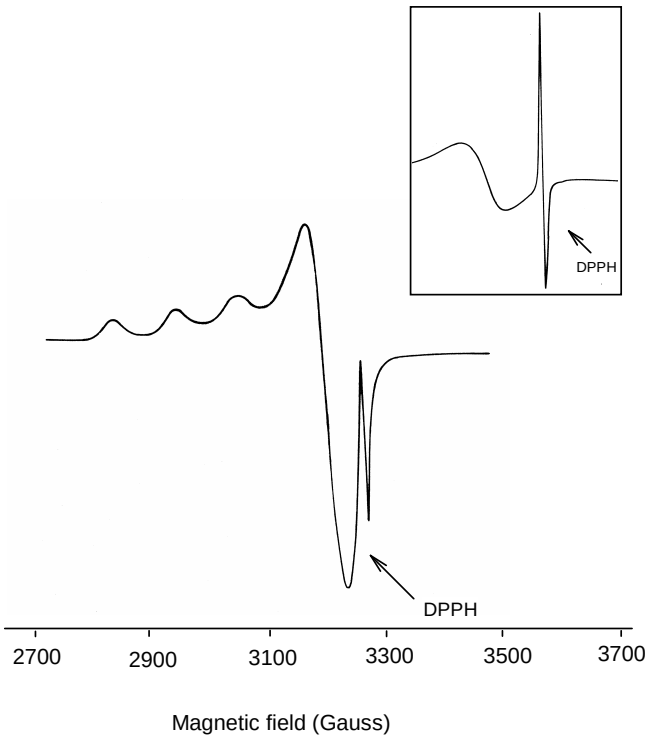


Fig .2a: EPR spectrum of $[Cu(phen)_2(C_{16}H_{33}NH_2)](ClO_4)_2$ (x = 0.203) in DMSO at liquid nitrogen temperature (Inset: solid state EPR spectrum at room temperature).

Table 1: Microanalysis, CMC and Selected IR, electronic, EPR data for surfactant-copper(II) complexes, $[Cu(phen)_2(C_{16}H_{33}NH_2)](ClO_4)_2$ (A)and $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$ (B)

Complex	IR[a], cm^{-1}			λ_{max} (nm)[b]	EPR[c]		(%) Found (Cald)			CMC (M)
	ν (C=C)	ν (C=N)	ν (Cl-O)		$g_{\|\|}$	$g_{\perp}$	C	H	N	
A	1518	1428	1092	679	2.29	2.09	64.38 (64.04)	5.21 (5.66)	8.05 (7.78)	9.75×10^{-5}
B	1520	1430	1090	735	2.28	2.07	62.25 (62.5)	4.81 (5.09)	8.45 (8.30)	1.98×10^{-4}

[a] KBr phase

[b] In MeOH

[c] DMSO, LNT at 77 K

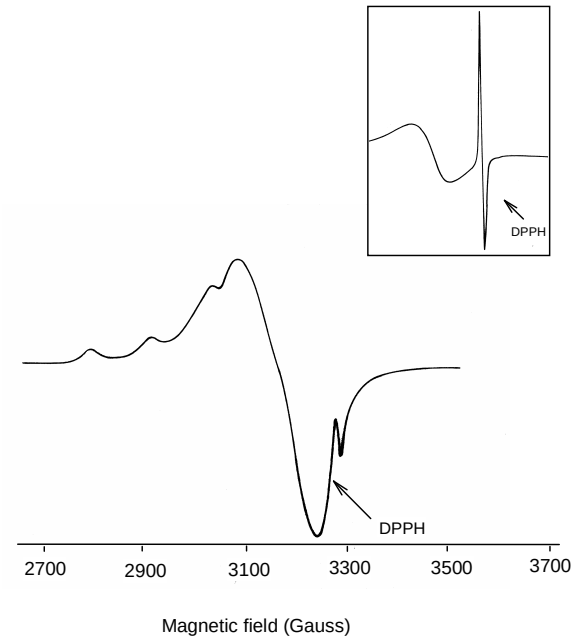
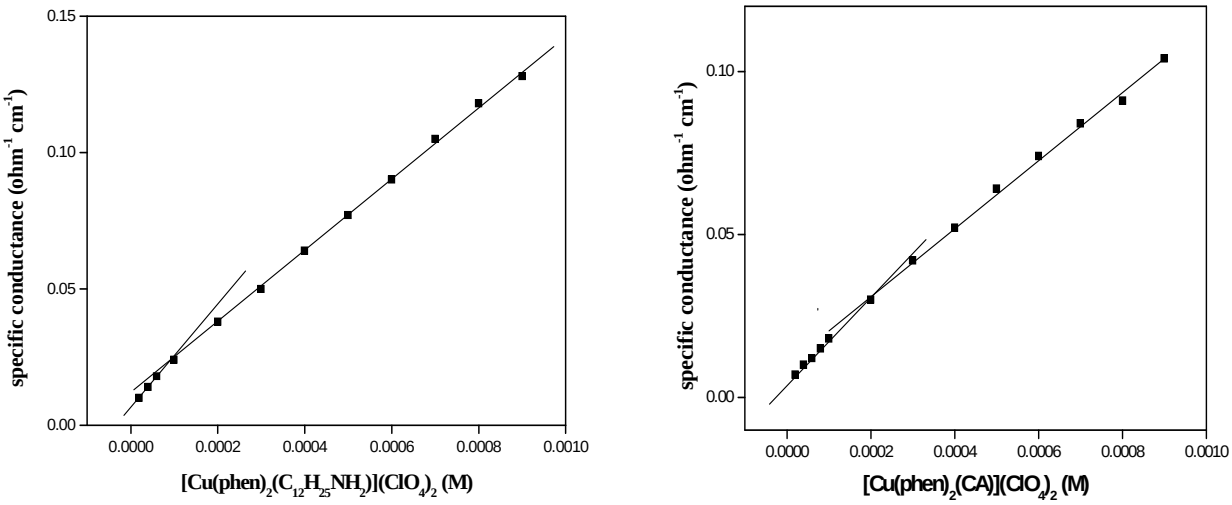


Fig. 2b: EPR spectrum of $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$ (x = 0.203) in DMSO at liquid nitrogen temperature (Inset: solid state EPR spectrum at room temperature).

At room temperature the complex exhibits well defined single isotropic feature near g = 2.053. Such isotropic lines are usually results of intermolecular spin exchange, which broadens the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during a coupling of two paramagnetic species. Spectrum obtained from frozen solution of the complex (**Fig. 2**) has well documented features of axial symmetry. The existence of g (parallel/> g(perpendicular) > 2.0023 suggest that d^2x- d^2y is the ground state with the d^9 (Cu^{2+}) configuration [31]. The bonding coefficient α^2 [32], is a measure of the covalency of the in-plane σ bonding

(a2 = A||/0.036 + (g(para) -2.002) + 3/7(g(perp)-2.002) + 0.04). A value of $a^2 = 1$ indicates complete ionic character, while $a^2 = 0.5$ denotes essentially 100% covalent bonding, assuming negligibly small values of the overlap integral. The value of $a^2 = 0.80$ in our case indicates appreciable in-plane covalent bonding. At 77 K we observed three peaks with third being broad for the surfactant-copper(II) complex containing cetylamine(Fig 2b) . This is because the copper complex units have been mounted on a surfactant chain resulting in some spin-spin coupling between the copper complex units which lead to a small amount of broadening.

Determination of Critical Micelle Concentration: The CMC values of the complexes were determined conductometrically using a specific conductivity meter. The conductivity cell was calibrated with KCl solutions in the appropriate concentration range. The cell constant was calculated using molar conductivity data for KCl [33]. Various concentrations of surfactant-copper(II) complexes were prepared in the concentration range 10^{-5} – 10^{-1} mol dm^{-3} in aqueous solution. The conductivity of these solutions was measured at 298, 308 and 318 K. the temperature of the thermostat was maintained constant to within ±0.01 K. The conductance was measured after thorough mixing and temperature equilibrating at each dilution. The establishment of equilibrium was checked by taking a series of readings after 15-min until no significant change occurred. The CMC values were computed from the slopes of [complex] *versus* specific conductance data (**Fig. 3**).



(a) (b)

Fig. 3 Specific conductivity *versus* [Complex] in aqueous solution

The complex concentration at which the micellization starts is evident from the change in the slope of the plot and this particular concentration is the CMC under the experimental conditions. The CMC values were determined by fitting the data points above and below the break to two equations of the form $y = mx + c$ and solving the two equations simultaneously to obtain the point of interaction.The CMC values of the surfactant-copper(II) complexes thus obtained are given in **Table- 1**. It was found that CMC value of surfactant-copper(II)-cetylamine was lower than that of the corresponding surfactant-copper(II)-dodecylamine. This may be due to an increase in hydrophobic character of the molecule in the coordination sphere in the case of cetylamine.

Antimicrobial activity of surfactant sample against microorganisms: The surfactant–copper(II) complexes (S1 & S2) were screened in vitro for their microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. These complexes were found to exhibit considerable activity against Gram positive(Staphylococcus aureus and Bacillus subtilis) and Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and the pathogenic yeast Candida albicans. The test solutions were prepared in dimethyl sulphoxide (1%) and the results of the antimicrobial activities are summarized in **Table 2**. The surfactant–copper(II) complexes showed significant microbial activity against Gram positive, Gram negative bacteria and fungus. In our biological experiments, using surfactant–copper(III) complexes, we have observed high antibacterial activity against Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) than Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The surfactant–copper (II) complexes are also very active against the yeast Candida albicans. The activity of these surfactant–copper (II) complexes may be due to an efficient diffusion of the metal complexes into the baterial/fungal cells and/or interaction with the bacterial/fungal cells[34]. The antimicrobial activities of these surfactant–copper (II) complexes were also compared with standard drugs Ciprofloxacin (for bacteria) and Fluconazole (for fungi). Out of the four surfactant–copper (II) complexes, the complex, 2 possessed very good activity against all the microorganisms. This may be due to higher hydrophobic character of the complex which can damage the bacterial/fungal cellular membrane/wall. It may be concluded that our surfactant–copper (II) complexes S1 and S2 inhibit the growth of bacteria and fungi.

Table 2 Antimicrobial activities of surfactant–copper(II) complexes $[Cu(phen)_2(C_{12}H_{25}NH_2)](ClO_4)_2$ **(S1)** and $[Cu(phen)_2(C_{16}H_{33}NH_2)](ClO_4)_2$ (S2)

Name of the microorganisms	Zone of inhibition in mm		
	S1(DA)	S2(CA)	Standard
Staphylococcus aureas	25	30	30
Bacillus subtilis	30	30	35
E.Coli	25	30	38
Pseudomonas aeruginosa	25	28	40
Candida Albicans	18	20	35
Aspergillus niger	20	22	32

Standard; Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units/disc for fungi; Solvent - DMSO

CONCLUSION

We have synthesized and characterized two novel surfactant-copper(II) complexes and their CMC values were determined by conductivity method. The interesting and useful aspect is that the critical micelle concentration values observed for the surfactant-copper(II) complexes in the present work are low compared to that of the simple organic amphilic ligand, dodecylammonium chloride (CMC = 1.5 10^{-2} mol dm^{-3})[12]. Thus it is concluded that, these metal surfactant complexes have more capacity to associate themselves forming aggregates compared to those of the ordinary synthetic organic surfactants. This suggests that the introduction of a metal complex to the hydrophilic part of the amphiphile can remarkably enhance the ability of aggregation. The surfactant–copper(II) complexes showed good antimicrobial activity against Gram positive and Gram negative bacteria and fungi. Thus our results show that the surfactant–copper(II) complexes can also be considered for antifungal and antibiotic drugs.

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