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Synthesis and characterization of cationic surfactants and their interactions with drug and metal complexes

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ABSTRACT

Two new cationic surfactants, n-hexadecyl-3-methylpyridinium bromide and n-heptadecyl-3-methylpyridinium bromide have been synthesized and characterized in solid state by FT-IR, and in solution by ^{1}H- and ^{13}C-NMR spectroscopy. The values of critical micelle concentration (CMC) were determined by UV-visible spectroscopy and conductometry. Interaction of synthesized surfactants with two anionic drugs, i.e., diclofenac sodium {[2-(2, 6-Dichloroanilino) phenyl] acetic acid} and ketoprofen [(RS)-2-(3-benzoylphenyl) propionic acid] was studied by UV-visible spectroscopy. Binding constant (K), Gibb's free energy (ΔG) and number of drug molecules (n) per micelle were also calculated. These synthesized surfactants were proved to be efficient in increasing the solubility and bioavailability of drug molecules. In order to check the carrier efficiency of synthesized surfactants against bioactive coordinate, on complexes, interaction of recently reported bioactive zinc complexes was tested with synthesized cationic surfactants by conductometric measurements. Mole fractions (X_{cmc}) and Gibbs free energy (ΔG_{cmc}) values were also calculated. Both surfactants were further screened for anti-fungal and anti-bacterial activities.

1. Introduction

Surface active agents are amphipathic compounds possessing polar and non-polar moieties as head and tail respectively. Depending on the charge present on head moieties they can be classified as; anionic, cationic, amphoteric and non-ionic surfactants [1]. One of the exciting features of surface active agents is self-aggregation which results in the formation of micelles which is considered to be an alternative mechanism to interfacial adsorption. Micellization is accompanied with reduction in free energy of system, increment in entropy which has a spontaneous mechanism [1]. The critical micelle concentration (CMC) is defined as the minimum concentration at which surfactant molecules starts to aggregate that can be spotted as an inflection point when any of the physicochemical property is plotted against concentration of surfactant molecules [2, 3]. The CMC value can be influenced by many factors including structure, chemical nature of hydrophobic and hydrophilic moieties, temperature, presence of electrolyte, solvent, nature of counter ions, pH etc [2].

In bio-medical field the limited therapeutic efficiency of drugs, which may be in metal complexes, is generally related to their low solubility in aqueous media. Surfactants have potential to act as drug delivery systems in which micelles not only solubilize but also increase bioavailability of drug molecules. They can stay in body for a longer time and protect drug molecules from adverse effects of biological surroundings [4]. The further use of micelle in drug delivery systems is also to reduce degradation rate of drug molecules. Micelles interact with drug molecules via either through their outer surfaces or by incorporation of drug molecules in their core [5]. Ionic surfactants being an important group of surfactants have also established their importance due to their interactions with different inorganic and organic bio-active, DNA, and proteins. The path of interaction is governed by the interaction of hydrophobic or hydrophilic sites of drugs and that of surfactants [5].

Surfactants have multitude of industrial applications and are used in cosmetics, corrosion inhibition, biocides, fabric softeners, emulsifier,

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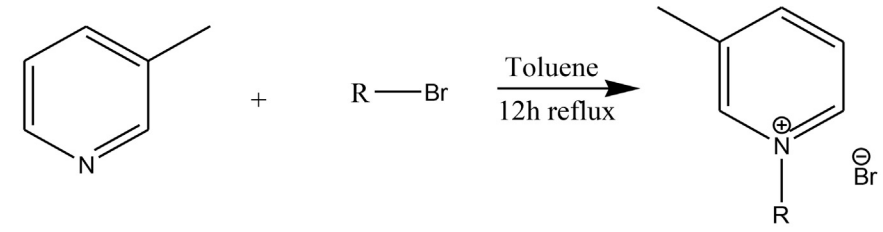
detergents etc. Moreover they are effective bactericides and fungicides [6, 7, 8, 9, 10, 11, 12, 13].

In the present work we have reported the synthesis of two N-(n-alkyl)-3-methylpyridinium based cationic surfactants that are characterized in solid state by FTIR spectroscopy whereas in solution by ^{1}H- and ^{13}C-NMR. The critical micelle concentration (CMC) has been measured by UV-visible spectroscopy and conductometry. Interaction of synthesized surfactants with two anionic drugs has been studied by UV-visible spectroscopy. Moreover, in order to prolong the bioavailability and hence increasing the bio-active potential of zinc metal complexes, their interaction with synthesized surfactants has also been investigated. The anti-microbial activities of surfactants have also been studied.



3-methyl pyridine Alkyl bromide 3-methyl pyridinium halide

where R= **(I)** $C_{16}H_{33}$, **(II)** $C_{17}H_{35}$

2. Experimental

2.1. Materials and methods

3-methylpyridine and 1-bromoalknane was procured from Alfa-Aesar (USA). Ethanol, methanol, chloroform, toluene were obtained from Merck Chemicals (Germany). All these chemicals were of analytical grade and used without further purification. Toluene was used after drying for further synthesis. Melting points were recorded by Sanyo electro thermal melting point apparatus. ^{1}H and ^{13}C-NMR were recorded in $CDCl_3$ as solvent on Bruker AC spectrometer at 300.13 MHz for ^{1}H and at 75.47 MHz for ^{13}C. FT-IR spectra were recorded on thermo Nicolet-6700 spectrophotometer in the frequency range of 4000–400 cm^{-1}. To determine CMC of the surfactant molecules and to study drugs interaction with surfactants, Shimadzu double beam Spectrophotometer 1800 was used. Inolab 720 precision conductivity meter was used to determine electrical conductivity of surfactant molecules at room temperature and was also used to study their interaction with metal complexes.

2.2. General procedure for the synthesis of surfactants

Equimolar quantities (10 mM) of 3-methylpyridine and 1-bromoalkane were dissolved in 35 mL of toluene in 250 mL round bottom flask. The reaction mixture was refluxed for 12 h with continuous stirring. Diethyl ether (20–25 mL) was added to cold solution and stirred. Separating funnel was then used to separate the solvents and the desired product. The product was washed with diethyl ether. The general reaction is:

The structures and numbering for interpretation of ^{1}H- and ^{13}C-NMR are given in scheme 1.

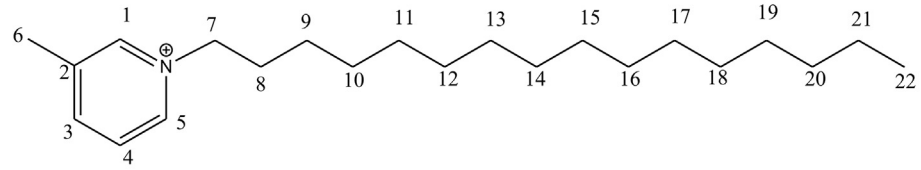
^{1}H and ^{13}C-NMR data of surfactants (I & II) are given as:

N-(n-hexadecyl)-3-methylpyridinium bromide (I)

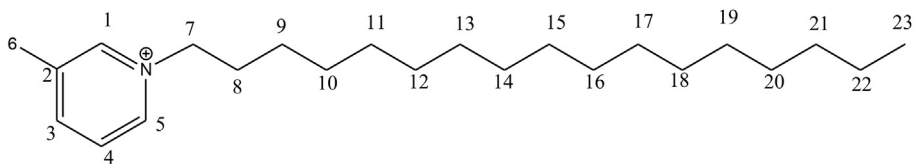
^{1}H-NMR ($CDCl_3$, δ-ppm, 300 MHz): 9.36 (1H, H^1, s), 8.28 (1H, H^3, d), 8.05 (1H, H^4, dd), 9.23 (1H, H^5, d), 2.63 (3H, H^6, s), 4.90 (2H, H^7, t), 1.20–2.31 (28H, H^{8-21}, m), 0.87 (3H, H^{22}). **^{13}C-NMR ($CDCl_3$, δ-ppm, 75.5 MHz):** 145.6 (C^1), 139.5 (C^2), 142.2 (C^3), 127.8 (C^4), 144.4 (C^5), 26.1(C^6), 61.8 (C^7), 18.7–34.0 (C^8–C^{22}). **FT-IR (υ cm^{-1}):** 1636 (C=N), 1149 (C–N), 1506 (C=C), 2919 (Aromatic –CH), 2852 (-CH_3).

N-(n-heptadecyl)-3-methylpyridinium bromide (II)

N-(n-hexadecyl)-3-methylpyridinium bromide **(I)**



N-(n-heptadecyl)-3-methylpyridinium bromide **(II)**



Scheme 1. Numbering of carbon and proton atoms of surfactants (I & II) for interpretation of ^{1}H- and ^{13}C-NMR.

^{1}H-NMR ($CDCl_3$, δ-ppm, 300 MHz): 9.31 (1H, H^1, s), 8.28 (1H, H^3, d), 8.04 (1H, H^4, dd), 9.22 (1H, H^5, d), 2.66 (3H, H^6,s), 4.94 (2H,H^7, t), 1.26–2.03 (30H, H^{8-22},m), 0.88 (3H, H^{23}, t). **^{13}C-NMR ($CDCl_3$, δ-ppm, 75.5 MHz):** 145.6 (C^1), 139.6 (C^2), 142.2 (C^3), 127.8 (C^4), 144.5 (C^5), 26.1 (C^6), 62.0 (C^7), 18.8–34.1 (C^8–C^{23}). **FT-IR (υ cm^{-1}):** 1644 (C=N), 1151 (C–N), 1506 (C=C), 2917 (Aromatic –CH), 2848 (-CH_3).

2.3. Drug-surfactant interaction study

Two anionic drug solutions were prepared and divided into two parts. One part was used as reference solution and other was used to prepare different drug-surfactant solutions having concentration range from pre-micellar to post-micellar. To run auto zero, both cells were filled with deionized water. Then surfactant solutions of different concentrations having constant drug concentration were examined to study the influence of varying surfactant concentrations on the absorption spectra of drug. In order to avoid errors same stock solutions were used [14]. All the reported values are the average of at least triplicate independent measurements with relative standard deviation of ±3.5%.

2.4. Metal complex-surfactant interaction study

The conductivity measurements of complex-surfactant solutions were carried out to study the effect of complex-surfactant interaction on CMC value at 25 ± 0.1 0C. Conductivity measurements were performed by keeping the complex concentration constant and varying the concentration of surfactant solutions. All the reported values are the average of at least triplicate independent measurements with relative standard deviation of ±3.5%.

2.5. Antimicrobial activities

For antimicrobial studies synthesized surfacntants were tested against three fungal strains; *Aspergillus flavous, Aspergillus fumigatus, Aspergillus niger,* and four bacterial species, *Bacillus subtilis (+), Staphylococcus aureus (+), Klebeseilla pneumonae (-), Escherichia coli (-).* Disc diffusion method was used for antimicrobial study. At 45 °C broth culture of test sample was homogeneously mixed with 75 mL of nutrient agar medium, then decant into 14 cm antiseptic petri plate. The agar media was solidified. Then sterilized metallic borer was used to make 8 mm wells in media. Afterwards, respective wells were filled with 100 μL of DMSO solution of test sample at the amount of 1 mg mL^{-1}. DMSO acted as negative (-) control while antibacterial and antifungal drugs Azithromycine (1 mg mL^{-1}), Ciprofloxacine (1 mg mL^{-1}) and Terbenafine (1 mg mL^{-1}) were served as positive (+) control. At 37 °C, duplicate plated of each fungal strain and triplicate plates of each bacterial strain were incubated for 24 h.

3. Results and discussions

3.1. Characterization

The two surfacntants i.e., N-(n-hexadecyl)-3-methylpyridinium bromide (**I**) and N-(n-heptadecyl)-3-methylpyridinium bromide (**II**) were synthesized by simple addition reactions. The important physical properties of the compounds are reported in Table 1.

The structural confirmation of synthesized surfactants was done by FT-IR, ^{1}H- and ^{13}C-NMR spectroscopy. In FT-IR spectra of both the surfactants, the characteristic peaks are at 1636-1644 cm^{-1} (C=N), 1149-1151 cm^{-1} (C–N), 1506 cm^{-1} (C=C), and 2917-2917 cm^{-1} (Aromatic –CH). In ^{1}H-NMR the signal for H^7 shifted from 3.39 ppm to 4.90 ppm (**I**) and 4.94 ppm (**II**) while in ^{13}C-NMR the signal for C-7 shifted from 33.71 ppm (in alkyl bromides) to 61.8 ppm (**I**) and 62 ppm (**II**), respectively.

3.2. CMC determination

Conductivity measurements and UV-visible spectroscopy was used to investigate the CMC values of both surfactants. Firstly, standard solutions of surfactants were prepared in ethanol solvent and then conductivity measurements of varied surfactant concentrations were performed at 298 K. The data of conductivity obtained was plotted against concentration and CMC value was recorded. For CMC calculations, conductivity plots against varying surfactant concentrations are shown in Fig. 1. The CMC corresponds to the inflection point in the specific conductivity-concentration plot. In order to eliminate vagueness in results, differential conductance was plotted against concentration of surfactant and a reverse sigmoid shaped curve is formed which gave more precise CMC value [15, 16]. In pre micellar region, there is significant increase in conductance due to presence of free anions and cations but not in post micellar region probably due to micelle formation. CMC values for surfactants (**I**) and (**II**) were calculated as 0.243 and 0.239 mmol dm^{-3}, respectively.

CMC values obtained from conductivity measurements were cross-checked by using UV-Visible spectroscopy. Firstly λ_{max} was determined by plotting graph between absorbance and wavelength. Then absorbance values of surfactants (**I**) and (**II**) were recorded at λ_{max}. The plotted graphs between absorbance and surfactant concentration are shown in Fig. 2. CMC values determined from both techniques are in close agreement.

It has been observed that by increasing chain length (even by one carbon), the value of CMC decreases [17].

3.3. Drug interaction studies

The drugs used for interaction studies are diclofenac sodium [{2-(2, 6-Dichloroanilino) phenyl} acetic acid] and ketoprofen [{(RS)-2-(3-benzoylphenyl) propionic acid}]. Both drugs are members of NSAIDs which are used as anti-pyretic and analgesics [18, 19]. These drugs have been interacted with surfactants to increase their bioavailability and controlled release rate in body fluids. Two types of interactions are possible, one involves the interaction with head of surfactants and in other drug molecules incorporated into hydrophobic core of micelles [20]. UV-Visible spectroscopy was used in order to study surfactant drug interactions.

The λ_{max} for diclofenac sodium was observed at 282 nm (Fig. 3). As drug was mixed with pre-micellar concentration of desired surfactants (**I and II**), a considerable blue shift of 8 nm was observed in its λ_{max}. By approaching the post-micellar concentration, the rise in absorption of drug molecules was witnessed showing the increased bioavailability of drug molecules. By increasing surfactant concentration, absorption of drug increases [21] and this surfactant-drug interaction follows a straight line pattern as shown in Fig. 4. The value of Gibb's free energy (ΔG) and binding constant (K) were calculated from the Eqs. (1) and (2) [22]. Negative sign of Gibb's free energy (ΔG) shows that the reaction was

Table 1
Physical properties of surfacntants (**I** and **II**).

Surfacntant	MolecularFormula	Molecular Weight	Physical State	Melting Point (°C)	Color	CMC (m mol dm^{-3})	Solubility
I	$C_{22}H_{40}NBr$	398	Solid	51–52	White	0.243	Water, Methanol, Ethanol, Acetone, DMSO, Chloroform
II	$C_{23}H_{42}NBr$	411.9	Solid	53–54	White	0.239	As above

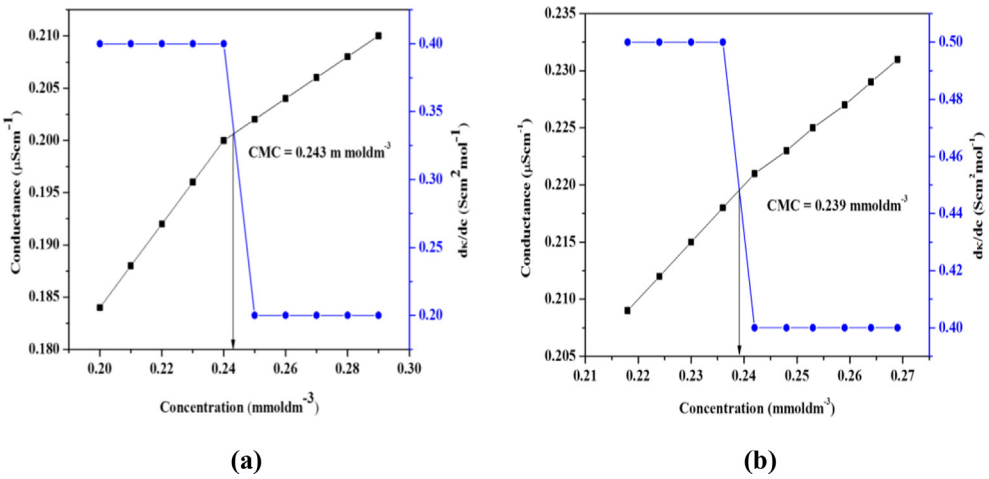


Fig. 1. Plots of conductivity and differential conductivity vs concentrations of a) surfactant (I) and b) surfactant (II).

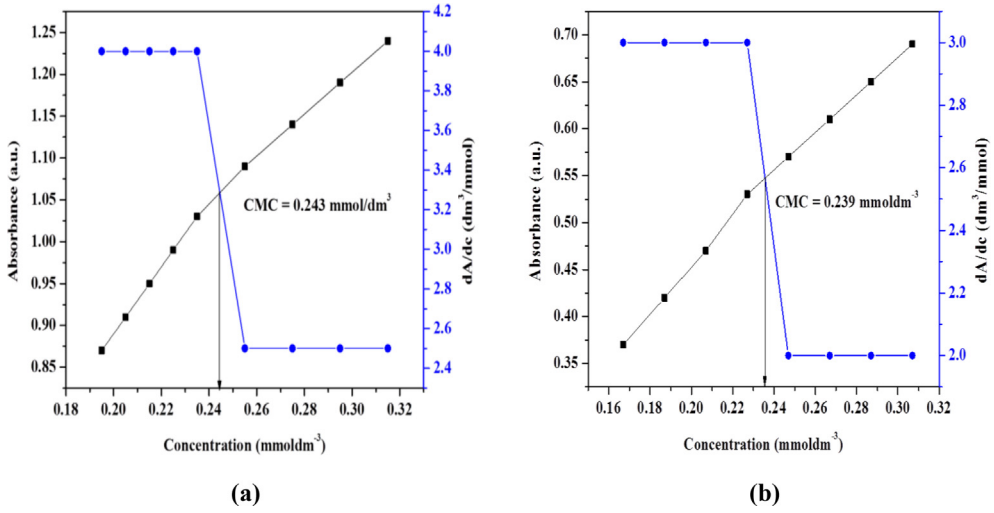


Fig. 2. Plots of absorbance and differential absorbance vs concentrations of a) surfactant (I) and b) surfactant (II).

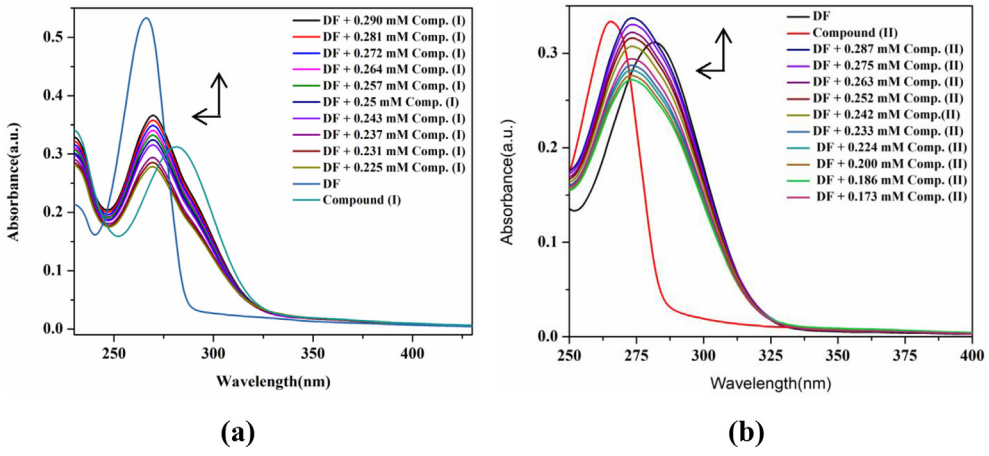


Fig. 3. Absorption spectra of diclofenac sodium with a) surfactant (I) and b) surfactant (II).

spontaneous between drug and surfactant.

$$\frac{1}{\Delta A} = \frac{1}{K} \cdot \frac{1}{\Delta A\infty (Ca + Cs^{mo})} + \frac{1}{\Delta A\infty} \quad (1)$$

$$\Delta G = -RTlnK \quad (2)$$

Ketoprofen gives λ_{max} at 254 nm. When surfactants **(I)** and **(II)** was homogenized with KP, a bathochromic shift of 6 nm was observed in the

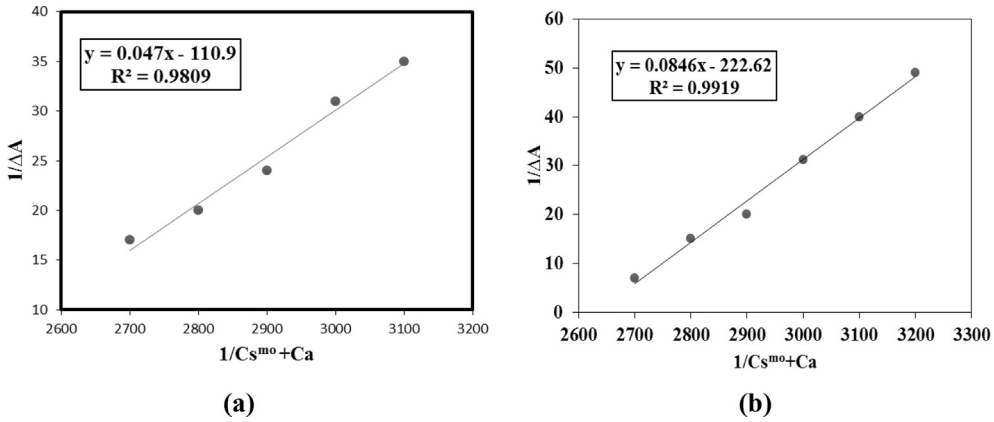


Fig. 4. Plots of $1/\Delta A$ vs $1/Cs^{mo} + Ca$ for a) compound (I) and b) compound (II).

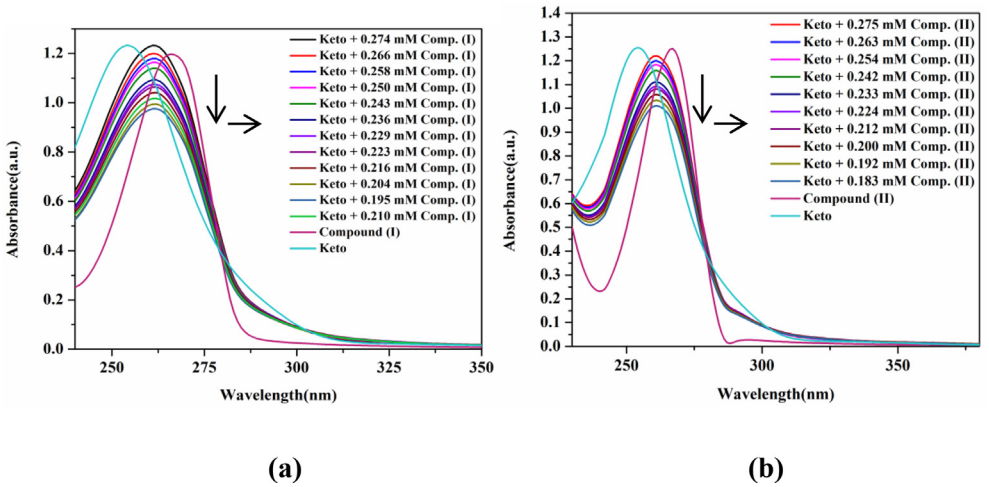


Fig. 5. Absorption spectra of KP with a) compound (I) and b) compound (II).

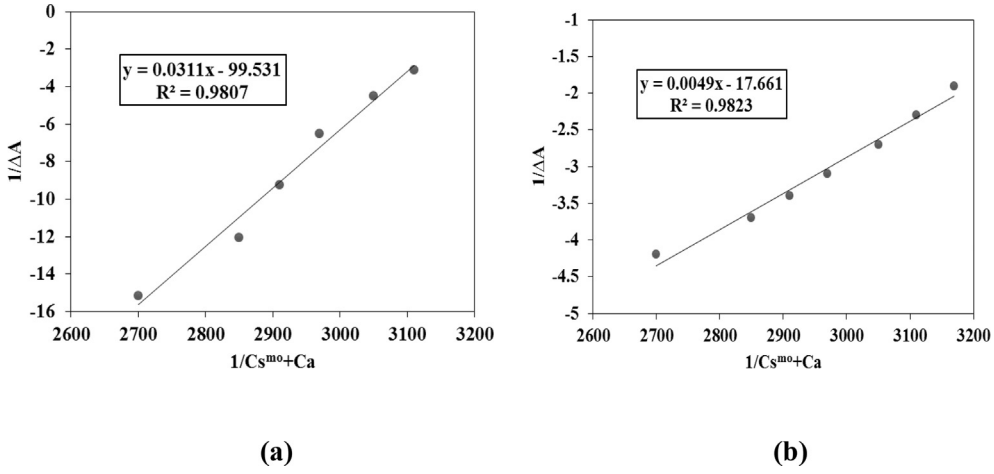


Fig. 6. Plots of $1/\Delta A$ vs $1/Cs^{mo} + Ca$ for a) compound (I) and b) compound (II).

λ_{max}. Moving from pre micellar to post micellar concentration, a decrease in drug absorption was spotted as an indication that the drug is being incorporated into micellar core, thus the outside harmful environment cannot poison it and it can be safely transferred to its target (see Figs. 5 and 6).

In order to calculate the number of drug of drug molecules (n) per micelle following expressions were used [14].

$$n = \frac{C_m}{M} \tag{3}$$

In Eq. (3), 'n' gives number of drug molecules attached to micelles, 'M' represents micelle concentration and 'C_m' is concentration of drug solubilized in micelles.

$$M = \frac{C_s - CMC}{N} \tag{4}$$

Table 2
Calculated parameters for drug interaction studies.

Compounds no.	A_o	A	E_o x 10^5	E_m X 10^5	C_m x 10^{-5} mol/dm^3	Mx 10^{-5} mol/dm^3	N	K dm^3/mol	-ΔG kJ/mol
I + DF	0.317	0.332	3.17	3.32	0.100	0.025	4	2359	19.2
II + DF	0.317	0.333	3.17	3.33	0.100	0.035	3	2631	19.5
I + KP	1.267	1.252	18.1	17.8	0.070	0.14	0.5	3200	20.0
II + KP	1.267	1.003	18.1	14.7	0.077	0.17	0.4	3604	20.3

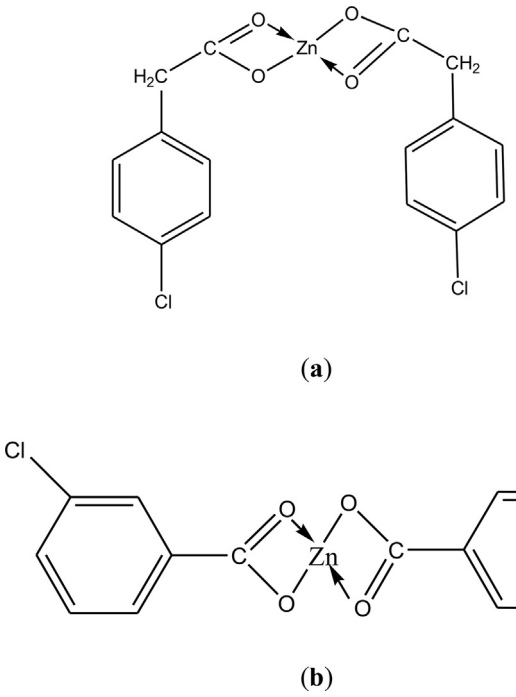


Fig. 7. Zn (II) Complexes a) [Zn (L^1)$_2$] b) [Zn (L^2)$_2$].

'Cs' is concentration of surfactants. 'N' gives aggregation number. 'CMC' is critical micelle concentration of surfactants.

$$Cm = \frac{A_o - A}{E_o - E_m} \tag{5}$$

Where, 'A_o' and 'A' are absorbance of drug without and with surfactant concentration, respectively. 'E_o' and 'E_m' gives corresponding absorptivities calculated from Beer lambert law. Table 2 gives the binding constant (K), Gibb's free energy (ΔG) and number of drug molecules (n) attached per micelle.

3.4. Complex interaction studies

Zinc (II) complexes have wide range of biological applications. In order to increase controlled release rate and bioavailability of metal complexes based drugs in body fluids, interactions of synthesized surfactants were studied with our two recently reported synthesized zinc (II) complexes [23] i.e., (a) = [Zn (L^1)$_2$] and (b) [Zn (L^2)$_2$], through conductivity measurements. Here L^1 = 3-chlorobenzoic acid and L^2 = 2-(4-chlorophenyl) acetic acid. Solutions of 1 mM of each complex were prepared in ethanol and their specific conductance was measured in the presence of compound (I) and (II) to study the complex–surfactant interaction. The concentration of the complex was kept constant while that of the surfactants was varied from 0.05 mmol dm^{-3} to 0.75 mmol dm^{-3}, in order to monitor the change in CMC of each surfactant upon the formation of Zn complex-surfactant adduct (see Fig. 7).

On interaction with complexes, the conductivity of surfactants increases hence their value of CMC. The increase in conductance was observed because of presence of free positive and negative ions of surfactants. The concentration of complex was kept constant. By increasing the concentration of compound (**I**) the value of CMC increases showing the strong interaction between surfactant and complex. In pre micellar region, there is significant increase in conductance due to presence of free anions and cations and surfactant molecules existed as monomers. In post micellar region, there is less increase in conductance due to micelle formation. However, the observed conductance is due to presence of bromide ions. Due to strong bonding between complex and cations of surfactants the CMC value increased. The role of complex is to reduce repulsion between nitrogen atoms of cationic head by decreasing its charge density. The complex decreases the micellization and increases the entropy [24]. If the complex is administered orally with surfactant of higher CMC, then on dilution the micelles will rupture and complex

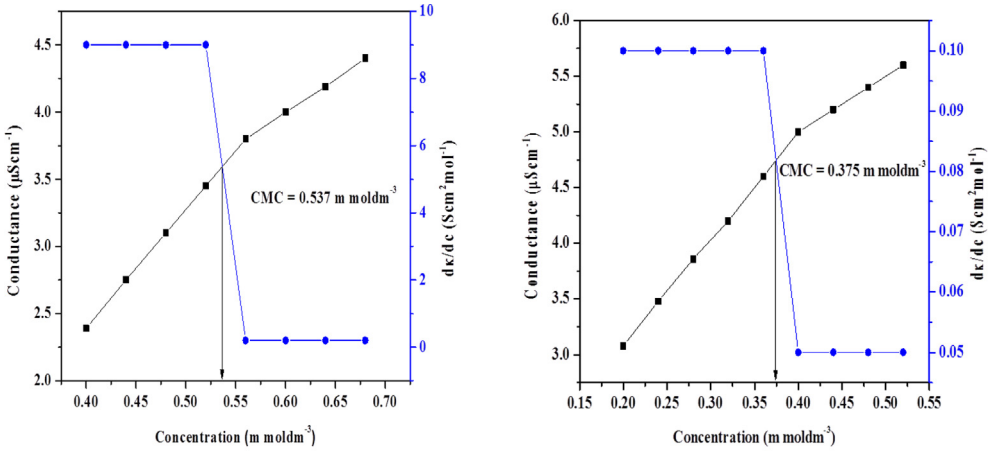
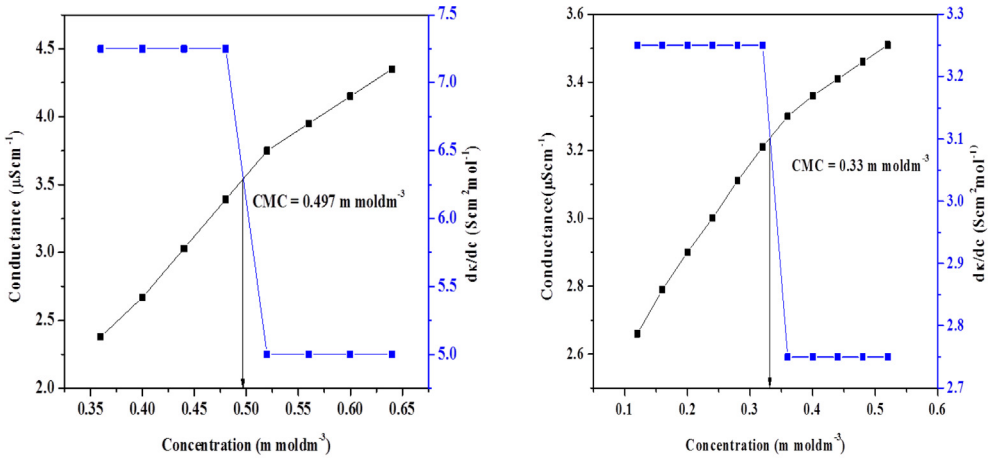


Fig. 8. [Zn (L^1)$_2$] interactions with a) compound I and b) compound II.



(a) **(b)**

Fig. 9. [Zn $(L^2)_2$] interactions with a) compound I and b) compound II.

Table 3
Calculated parameter for complex interaction studies.

Compounds no.	Complexes	CMC [mM]	$-\Delta G_m$ (kJ/mol)
I	$Zn(L^1)_2$	0.537	36.46
II	$Zn(L^1)_2$	0.375	40.51
I	$Zn(L^2)_2$	0.497	39.87
II	$Zn(L^2)_2$	0.33	42.41

(drug) will be released to perform its activity but in case of surfactant of low CMC, the complex will reside in micelle and result in reduced bioavailability. This reduction in activity of complex reveals stronger interaction with micelles and this phenomenon is particularly very useful for prolonged delivery of pharmaceutical agents in biological systems. So CMC regulates release rate of drug in biological system [25] (see Figs. 8 and 9).

To calculate the ΔG_m values of complex surfactant interactions, following expressions was used [25, 26].

$$\Delta G_m = (1 + \beta)\, RT \ln X_{CMC} \quad (6)$$

Where β = degree of counter ion binding and can be calculated as:

$$\beta = 1 - \alpha \quad (7)$$

α = degree of dissociation and calculated from the ratio of slopes before and after the CMC value:

$$\alpha = S_2/S_1 \quad (8)$$

S_1 and S_2 are slopes before and after the CMC value

$$X_{CMC} = cmc/55.55 \quad (9)$$

Above expression is for the calculation of CMC in terms of mole fraction.

Following table gives the ΔG_m values of interaction of surfactants with different zinc complexes (see Table 3).

Negative sign of ΔG_m shows that complex surfactant interactions are spontaneous. Surfactant-complex interaction plays a significant role in augmentation of complex solubility.

3.5. Anti-microbial activity

Ant-bacterial and anti-fungal activities of all compounds are shown in Tables 4 and 5.

Compound **I** showed significant activity with *Staphylococcus aureus* and *Escherichia coli.* Compound **II** showed effective anti-bacterial activity against *Bacillus subtilis* and *Staphylococcus aureus.* All compounds are significant bactericides.

Compound **I** displayed efficient anti-fungal activity against *Aspergillus niger* and *Aspergillus flavous* while compound **II** exhibited substantial activity against *Aspergillus niger* and *Aspergillus fumigatus.* Hence both compounds are good fungicides.

Table 4
Anti-bacterial activity of compounds (**I** & **II**).

Compounds no.	*Bacillus subtilis*	*Staphylococcus aureus*	*Klebeseilla pneumonae*	*Escherichia coli*
	(+)	*(+)*	*(-)*	*(-)*
Zone of inhibition (mm)				
I	15	21	12	17
II	17	19	9	14
Azithromycine	28	24	21	22
Ciprofloxacine	22	24	26	29

Table 5
Anti-fungal activity of compounds (**I** & **II**).

Compounds no.	*Aspergillus fumigatus*	*Aspergillus niger*	*Aspergillus flavous*
% inhibition			
I	43	57	55
II	57	59	49
Terbenafine	100	100	100

4. Conclusion

Cationic surfactants, N-(n-hexadecyl)-3-methylpyridinium bromide (I) and N-(n-heptadecyl)-3-methylpyridinium bromide (II) have been synthesized and characterized by FT-IR, ^{1}H and ^{13}C-NMR spectroscopy. Critical micelle concentration (CMC) values are determined by UV-Visible spectroscopy and results are further verified by conductometry. CMC values determined for compound I and II are 0.243 and 0.239 mM, respectively. Interaction of cationic surfactants with two anionic drugs i.e., diclofenac sodium and ketoprofen is studied by UV-Visible spectroscopy. The Binding constant (K), Gibb's free energy (ΔG) and drug molecules attached per micelle (n) are also reported. The synthesized cationic surfactants are found effective in increasing the solubility and bioavailability of drugs. By varying alkyl chain length, we can vary CMC

so we can control retention time of drugs in body. In order to check the effective interaction of synthesized surfactants towards bio-active coordination complexes, two bio-active zinc complexes are interacted and studied with surfactants by conductometric technique. Metal complex-surfactant interaction results in increase in the CMC value of surfactants. The negative value of ΔG in case of interactions of surfactants with drugs and complexes show spontaneous type of interactions. Synthesized compounds are proved to be potentially anti-bacterial and anti-fungal agents.

Declarations

Author contribution statement

Kalsoom Akhter, Kaleem Ullah: Performed the experiments; Wrote the paper.

Rabia Talat, Faizan Ullah: Performed the experiments.

Ali Haider, Saqib Ali: Conceived and designed the experiments.

Nasir Khalid: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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