FINAL REPORT

[UGC PROJECT]
(01.07.15 To 31.06.18)

MICELLAR, INTERFACIAL AND SPECTROSCOPIC STUDIES OF ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS

UGC SANCTION LETTER NO. 43-183/2014(SR)
Dated 30.10.15

Submitted by
Prof. Kallol K. Ghosh
Principal Investigator

School of Studies in Chemistry,
Pt. Ravishankar Shukla University,
Raipur, (C.G.) 492010
FINAL PROGRESS REPORT 
[UGC PROJECT] 
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STUDIES OF ANTIDEPRESSANT-DRUG-
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Antidepressant drugs are widely used in treating psychiatric and neurologic disorder. Over the year many classes of antidepressants have been used in Chhattisgarh region. The excess amount of drugs can cause overstimulation, psychotic illness and other disorders. So the targeted drug delivery in body organs with surfactants and other system is necessary. In order to use these systems as drug carrier, a detailed study of drug-surfactant interaction as well as the effect of microenvironment is prerequisite. This field is very much important for biochemical application and drug delivery system and as this field is still in the infancy, needs proper examination.

The project seeks to understand in detail the nature of interactions between antidepressant drugs and novel surfactants.
ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication: Please see Encl. 2

iii. Has the progress been according to original plan of work and towards achieving the objectives. if not, state reasons: Yes

iv. Please indicate the difficulties, if any, experienced in implementing the project: NIL

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: Completed

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission: Please see Encl. 3

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

(a) Manpower trained : 3

(b) Ph. D. Awarded: Toshikee Yadav, Date : 24.11.2017

Title : Studies on Antidepressant Drug-Surfactant Systems and Hydrophobic Organic Compound Surfactant Interaction

(c) Publication of results : 2 (Please see Encl. 2)

(d) Other impact, if any : NIL

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Dr. Kallo! K. Ghosh
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UGC Project
School of Studies in Chemistry,
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REGISTRAR/ PRINCIPAL
(SEAL)
Pt. Ravishankar Shukla University
RAIPUR (Chhattisgarh)
Annexure-IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI –110 002

PROFORMA FOR THE SUBMISSION INFORMATION AT THE TIME OF SENDING
THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. TITLE OF THE PROJECT: Micellar, Interfacial and Spectroscopic Studies of Antidepressant-Drug-Surfactant Systems

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR:
   Prof. Kallol K Ghosh
   School of Studies in Chemistry
   Pt. Ravishankar Shukla University,
   Raipur, 492010 (CG)

3. NAME AND ADDRESS OF THE INSTITUTION:
   Pt. Ravishankar Shukla University,
   Amanaka, GE Road,
   Raipur, 492010 (CG)

4. UGC APPROVAL LETTER NO. AND DATE:
   F. No. 43-183/2014(SR) MRP-MAJOR-CHEM-2013-14435  Dated 30.10.15

5. DATE OF IMPLEMENTATION
   30/10/2015

6. TENURE OF THE PROJECT:
   30/10/2015 to 31/10/2018

7. TOTAL GRANT ALLOCATED
   Rs. 14,58,600=00

8. TOTAL GRANT RECEIVED
   Rs. 10,20,600=00

9. FINAL EXPENDITURE
   Rs. 7,83,860=00

10. TITLE OF THE PROJECT:
    MICELLAR, INTERFACIAL AND SPECTROSCOPIC STUDIES OF ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS

11. OBJECTIVES OF THE PROJECT:

    Most of the drugs are used in combination with additives specially surfactants. Therefore, it is necessary to have knowledge of the additive effect on the cmc of amphiphilic drugs. The micellar and interfacial properties are very useful for the development of new drugs as well as
drug-delivery system. This project seeks to understand the nature of interactions between some antidepressant drugs and novel surfactants. The objectives of the project are as follows:

I. To study the surface and micellar properties of some amphiphilic antidepressant drugs by conductometric, tensiometric and fluorimetric methods.

II. To determine the interaction parameter of amphiphilic drugs in the presence of single and mixed surfactants.

III. To characterize the solubilization of drugs in the presence of surfactants.

IV. To study the micellar growth and surfactant as drug-surfactant interaction.

12. WHETHER OBJECTIVES WERE ACHIEVED: Yes

(a) The surface and micellar properties of some amphiphilic antidepressant drugs have been determined by conductometric, tensiometric and fluorimetric methods.

(b) Various interaction parameters of amphiphilic drugs in the presence of single and mixed surfactants have been examined.

(c) Solubilizations of poorly soluble antidepressants drugs have been done using UV-visible spectrophotometer.

(d) Study of the micellar growth has been done as drug-surfactant interaction using dynamic light scattering.

13. ACHIEVEMENTS FROM THE PROJECT:

(i) We have determined the appropriate surfactant systems to increase the activity of antidepressant drugs.

(ii) We obtained the appropriate results for the antidepressant drug-surfactant systems which enhance the bioavailability of amphiphilic antidepressant drugs.

(iii) We have found the increasing solubility of poorly soluble antidepressant drugs by surfactant systems.

14. SUMMARY OF THE FINDINGS: Please see Encl. 3
15. CONTRIBUTION TO THE SOCIETY:

Depression is a major problem in our society. Antidepressants drugs have proved useful for the treatment of depression. They are also used for the treatment of other depressive disorders like obsessive compulsive disorder, anxiety disorders, migraine, dysthymia, chronic pain, dysmenorrhea, snoring, addiction, neuropathic pain, attention-deficit hyperactivity disorder (ADHD) and sleep disorders. But they have some side effects and we can decrease their side effects using surfactants. Several problems arise in the formulation of hydrophobic drugs and major inconvenience is their solubilization in body fluids and interaction with biological membrane.

Surfactants have wide application in various fields such as environmental, biomedical, pharmaceuticals, industrial, detergency, cosmetics and nano-science. They play a key role in the pharmaceutical field because they have numerous unique properties like low viscosity, small aggregation size, long shelf life, simple preparation, narrow size distribution and bioactivity. Owing to these properties they have increase the bioavailability and solubilization of slightly soluble substances in aqueous medium. Micelles are used as vehicles for the sparingly water-soluble drugs.

Thus, the study of physicochemical properties of surfactants and the interaction of drugs with surfactants can provide valuable information for the development of novel drug molecules. These important studies are useful to enhance the solubility of drugs and to increase the bioavailability and prevent from side effects. Drug-Surfactant interaction remains an important research to improve the drug delivery systems.

16. WHETHER ANY PhD ENROLLED/ PRODUCED OUT OF THE PROJECT : Yes
PhD Awarded : 01, PhD Enrolled :

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : 02

SIGNATURE OF THE PRINCIPAL INVESTIGATOR
Dr. Kallol K. Ghosh
Principal Investigator,
UGC Project
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RAIPUR (C.G.)

REGISTRAR/ PRINCIPAL
(SEAL)
Pt. Ravishankar Shukla University
RAIPUR (Chhattisgarh)
REPORT OF THE MAJOR RESEARCH PROJECT

MICELLAR, INTERFACIAL AND SPECTRO-SCOPIC STUDIES OF ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS

Submitted to the
University Grants Commission, New Delhi

(UGC Ref. No. 43-183/2014(SR) Dated 30.10.15)
MRP ID : MRP-MAJOR-CHEM-2013-14435

By
Prof. Kallol K Ghosh
Principal Investigator
School of Studies in Chemistry
Pt. Ravishankar Shukla University
Raipur- 492010 CG
MICELLAR, INTERFACIAL AND SPECTROSCOPIC STUDIES OF ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS

The project aims to study the physicochemical aspects of interaction of drug to surfactant which is very important factor in drug-delivery systems. Various methods have been used to study the antidepressant-drug surfactant systems:

METHODOLOGY USED FOR THE STUDY

Surface Tension Measurement

The tensions ($\gamma$) at the air/solution interface of the drug/surfactant solutions were measured with a calibrated Jencon tensiometer (Kolkata, India) by the du Nouy ring detachment method. The surface properties viz. critical micelle concentration (CMC), maximum surface excess concentration at the air/water interface ($\Gamma_{\text{max}}$), minimum area per surfactant molecule at the air-water interface ($A_{\text{min}}$) and the surface pressure at the CMC ($\pi_{\text{CMC}}$) have been determined.

Conductivity Method

Conductance measurements will be taken by direct reading conductivity meter using cell constant of unity. The critical micelle concentration (CMC) and degree of counter ion binding will be measured.

Solubilization Experiment

The solubilization experiments were performed by spectroscopic measurement on Varian Cary-50, UV-visible spectrophotometer. Quantification of solubilization capacity were undertaken in terms of the molar solubilization ratio (MSR), the micellar water partition coefficient ($\ln K_m$) and Gibb’s free energy of solubilization ($\Delta G^\circ_s$) by employing spectrophotometric method.
Fluorimetric Method

The fluorescence measurements were performed on a Cary Eclipse Fluorescence Spectrophotometer. 1-Pyrene carboxaldehyde was used as a probe. Critical Micelle Concentration (cmc), binding constant and Stern-Volmer ($K_{sv}$) constant have been measured in mixed miceller system.

Absorption Measurements

The absorption measurements were performed on Varian Cary-50, UV-visible spectrophotometer. The titrations were performed by successive additions of 0.01 M stock solutions of surfactants directly into the cuvette containing 3 mL of 0.33 mM drug solution.

(a) Study on surface and micellar properties of some amphiphilic antidepressant drugs by conductometric, tensiometric and fluorimetric methods:

The micellar and surface properties of some antidepressants (amitriptyline hydrochloride (AMT), imipramine hydrochloride (IMP) and chlorpromazine hydrochloride (CPZ)) (Scheme 1) have been studied by surface tension and fluorescence methods in aqueous solution at 300 K.

Scheme 1. Structures of Antidepressant Drugs.
Determination critical micelle concentration (CMC) of antidepressant drugs:

The CMC values for some antidepressants were determined by surface tension and fluorescence measurements. A representative plot of the surface tension versus log molar concentration of drugs AMT, IMP and CPZ in aqueous solution is shown in Fig. 1, 2 and 3 respectively. Fluorescence spectra for the determination of CMC of CPZ have shown in Fig. 4, in which 1-pyrenecarboxaldehyde is used as a probe. Fig. 5 is showing the plot of intensity ($I_1$) vs concentration of drug (CPZ). The CMC values and other parameters (surface tension at cmc ($\gamma_{\text{cmc}}$), surface excess concentration ($\Gamma_{\text{max}}$), minimum area per molecule ($A_{\text{min}}$)) of antidepressants obtained from both the techniques are given in Table 1.

![Fig. 1. Plot of log C versus Surface Tension for Amitriptyline hydrochloride](image1)

![Fig. 2. Plot of log C versus Surface Tension for Imipramine hydrochloride](image2)
Fig. 3. Plot of log C versus Surface tension for Chlorpromazine hydrochloride

Fig. 4. Fluorescence spectra of chlorpromazine hydrochloride for the determination of CMC

Fig. 5. Plot of intensity (I₁) vs concentration of drug Chlorpromazine hydrochloride
Surface Properties

From the surface tension measurements, several interfacial parameters can be determined such as the surface excess concentration (Γ\text{max}) and minimum area per molecule at the air-water interface (A\text{min}), using following eqs.:

\[ \Gamma_{\text{max}} = \frac{1}{2.303nRT} \left[ \frac{d\gamma}{d \log C} \right]_{T,P} \]

\[ A_{\text{min}} = \frac{1}{N\Gamma_{\text{max}}} \]

where, R is the ideal gas constant (8.314 Jmol\(^{-1}\) K\(^{-1}\)), T is the absolute temperature in Kelvin, C is the surfactant concentration, (d\gamma/d\log C) is the slope of the surface tension versus log C plot taken below the CMC, and N is Avagadro’s number (6.022 X 10\(^{23}\) mol\(^{-1}\)).

The value of Γ\text{max} generally decreases and that of A\text{min} increases with increasing amounts of drug (shown in Table 1). The value of the surface pressure at the CMC (π\text{CMC}) was obtained from this eq.,

\[ \pi_{\text{CMC}} = \gamma_0 - \gamma_{\text{CMC}} \]

where, \(\gamma_0\) is the surface tension of solvent and \(\gamma_{\text{CMC}}\) is the surface tension at the CMC. The values of the π\text{CMC} of drug solutions are listed in Table 1. This parameter indicates the maximum reduction of surface tension caused by the dissolution of drug molecules; hence, it becomes a measure of effectiveness of the surface tension reduction, and the greater the π\text{CMC} values, the higher the effectiveness of the drugs.

Table 1: Interfacial Parameters: surface tension at cmc (γ\text{cmc}), surface excess (Γ\text{max}), minimum area per molecule (A\text{min}) of antidepressants AMT, IMP and CPZ

<table>
<thead>
<tr>
<th>Antidepressant Drugs</th>
<th>CMC x 10(^{-4}) mol dm(^{-6})</th>
<th>γ\text{cmc}</th>
<th>Γ\text{max} (10^6) mol.m(^{-2})</th>
<th>A\text{min} (10^{20}) m(^2)</th>
<th>π\text{cmc} mNm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMT</td>
<td>33.0</td>
<td>58.0</td>
<td>0.81</td>
<td>203</td>
<td>14.0</td>
</tr>
<tr>
<td>IMP</td>
<td>41.0</td>
<td>58.5</td>
<td>0.71</td>
<td>233</td>
<td>13.5</td>
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<tr>
<td>CPZ</td>
<td>16.0</td>
<td>42.0</td>
<td>0.30</td>
<td>546</td>
<td>30.2</td>
</tr>
</tbody>
</table>

S.T.- Surface Tension, Fluo- Fluorescence Method
Determination of various interaction parameters of amphiphilic drugs in the presence of single and mixed surfactants

Determination of interfacial parameter of antidepressants in the presence of surfactants:

Surface tension measurement

Surface tension measurements were carried out using ring detachment method on Jencon Surface tensiometer (Kolkata) at room temperature. Adsorption of amphiphiles at the air-water interface changes the surface properties of solutions. For the determination of amount of adsorbed surfactants at air/water interface, Gibb’s adsorption equations are applied. Critical micelle concentration (cmc), surface tension at cmc (γ_{cmc}), Surface excess concentration (Γ_{max}), minimum area per molecule (A_{min}) and surface pressure at cmc (π_{cmc}) have been determined and given by the following relations:

Surface excess concentration (Γ_{max})

\[
Γ_{max} = -\frac{1}{2.303 nRT} \left[ \frac{d\gamma}{d \log C} \right]_{T,P}
\]

Minimum area per molecule (A_{min})

\[
A_{min} = \frac{1}{N Γ_{max}}
\]

where, R is the ideal gas constant (8.314 Jmol\(^{-1}\) K\(^{-1}\)), T is the absolute temperature in Kelvin, C is the surfactant concentration, \(\frac{d\gamma}{d \log C}\) is the slope of the surface tension versus log C plot taken below the CMC, and N is Avagadro’s number (6.022 \(\times\) 10\(^{23}\) mol\(^{-1}\)).

Surface pressure at cmc (π_{cmc})

\[
π_{CMC} = γ_0 - γ_{CMC}
\]

where \(γ_0\) and \(γ_{CMC}\) refers to the surface tension of solvent and the surfactant solution at the CMC, respectively. This parameter indicates the maximum reduction of surface tension caused by the dissolution of surfactant molecules; hence, it becomes a measure of effectiveness of the surface tension reduction, and the greater the π_{CMC} values, the higher the effectiveness of the surfactants. π_{CMC} value of pure surfactants are lower than pure drug and mixed systems in all cases. π_{CMC} values decrease with an increasing mole fraction of gemini surfactants.
Thermodynamic quantity for the evaluation of synergism in mixing, i.e., the free energy of the given air/water interface $G_{\text{min}}^s$ which is defined as follows:

$$G_{\text{min}}^s = A_{\text{min}} \gamma_{\text{CMC}} \cdot N_A$$

$G_{\text{min}}^s$ is regarded as the work needed to make an interface per mole or the free energy change accompanied by the transition from the bulk phase to the surface phase of the solution components. In other words, the lower the values of $G_{\text{min}}^s$, the more thermodynamically stable surface is found. The $G_{\text{min}}^s$ values are decreased with increasing the additive concentration/mole fraction.

A representative plot of the surface tension versus log molar concentration of drugs AMT and CPZ with gemini surfactants i.e. alkanediyl-$\alpha,\omega$-bis(dimethylhexadecylammonium bromide) ($C_{16-10}-C_{16,-2Br}$, $C_{16-12}-C_{16,-2Br}$) are shown in Fig. 6, 7, 8 and 9 respectively. The surface tension ($\gamma$) of solutions was measured for a range of concentration above and below the critical micelle concentration (CMC). A linear decrease in the surface tension was observed with increase in concentrations for all the surfactants above the CMC value. The values of all interfacial parameters are listed in Table 2. The CMC values of drugs decrease with an increasing mole fraction of gemini surfactant.

**Fig. 6.** Plot of log C versus Surface Tension for Chlorpromazine hydrochloride in the presence of 16-10-16

**Fig. 7.** Plot of log C versus Surface Tension for Chlorpromazine hydrochloride in the presence of 16-12-16
Table 2 Interfacial parameters: surface pressure ($\pi_{\text{cmc}}$), surface excess ($\Gamma_{\text{max}}$), minimum area per molecule ($A_{\min}$) and free energy at air/water interface ($G_{\min}^{s}$) of drugs (AMT/CPZ) in presence of surfactants

<table>
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<tr>
<th>Systems</th>
<th>cmc $\times 10^{-4}$ mol dm$^{-3}$</th>
<th>$\alpha_{\text{Surf}}$</th>
<th>cmc</th>
<th>$\gamma_{\text{cmc}}$ mNm$^{-1}$</th>
<th>$\Gamma_{\text{max}}$ 6 10 mol.m$^{-2}$</th>
<th>$A_{\min}$ 20 m</th>
<th>$\pi_{\text{cmc}}$ mNm$^{-1}$</th>
<th>$\Delta G_{\min}^{s}$</th>
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<tr>
<td>CPZ+16-10-16</td>
<td>0.000</td>
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<td>1.89</td>
<td>87.49</td>
<td>28.0</td>
<td>22.13</td>
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<td>160.15</td>
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<td>87.49</td>
<td>36.0</td>
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<td>58.0</td>
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The interactions of antidepressant drugs chlorpromazine hydrochloride (CPZ) and desipramine hydrochloride (DSP) with bile salts (Sodium cholate (NaC) and sodium deoxycholate (NaDC)) (Scheme 2) have been investigated by employing the UV-visible spectroscopy and steady state fluorescence. The aromatic rings of these drugs are responsible for their significant absorption and fluorescence properties, which vary with its local environment.

\[
\text{Sodium Cholate} \quad \text{Sodium deoxycholate}
\]

**Scheme 2: Structure of Bile salts**

**Absorption measurements**

This technique is useful for studying the interaction between drug and surfactants. The titrations were performed by successive additions of 0.01 M stock solutions of surfactants (NaC and NaDC) directly into the cuvette containing 3 mL of 0.33 mM drug solution. The absorption spectra of CPZ and DSP in aqueous solutions with varying concentrations of NaC and NaDC are shown in Figs. 10 and 11. The spectra of CPZ presented two characteristic peaks at 245 nm and 305 nm wavelengths. In which the shorter wavelength band is due to \(\pi-\pi^*\) transition and longer wavelength is due to \(n-\pi^*\) transition and also the presence of lone pair of electron on sulfur atom in tricyclic region of antidepressant drug CPZ. In the case of absorption spectra of DSP it appears at 250 nm. On the addition of surfactants the absorption intensity of antidepressants increases (red shift). It is also observed from the Figs. 10 [(a), (b)] CPZ shows spectral shift of 5 nm at \(\lambda_{max}\) 245 nm but the second spectra at 305 nm not shows spectral shift after addition of bile salts. Similarly from Figs. 11 [(a), (b)] DSP shows the spectral shift of 5 nm at \(\lambda_{max}\) 250 nm. These spectral shifts show the interaction of drug and bile salts which further indicate the new complex formation between antidepressants and bile salts. Binding of bile salts with drug molecules calculated by Benesi–Hildebrand equation:

\[
\frac{1}{A-A_0} = \frac{1}{K(A_{max-A_0}) [\text{Bile Salt}]} + \frac{1}{A_{max-A_0}}
\]
Where, $A_0 =$ absorbance in the absence of bile salts 
$A =$ absorbance at intermediate concentration of bile salts 
$A_{\text{max}} =$ absorbance at infinite concentration of bile salts 
$K =$ binding constant

Fig.10 Absorption spectra of CPZ with increasing concentration of (a) NaC and (b) NaDC.

Fig.11 Absorbance spectra of DSP with increasing concentration of (a) NaC and (b) NaDC.

When we plot the graph between $1/(A-A_0)$ and $1/[$Surfactant$]$, it gives a straight line shown in Fig. 12, which reveals that antidepressants (CPZ, DSP) and surfactants (NaC, NaDC) formed the 1:1 complex between them. The binding constants $K$ were calculated from the ratio of intercept and slope of Benesi–Hildebrand plot are $0.063 \times 10^{-3}$ mol dm$^{-1}$, $0.883 \times 10^{-3}$ mol dm$^{-1}$, $0.027 \times 10^{-3}$ mol dm$^{-1}$ and $0.040 \times 10^{-3}$ mol dm$^{-1}$ for CPZ+ NaC, CPZ+NaDC, DSP+NaC and DSP+ NaDC.
respectively. The values of binding constant tell that NaDC shows more binding affinity towards the antidepressants drugs.

Fig 12 Benesi–Hildebrand plot using changes in absorption spectra of (a) CPZ, (b) DSP for NaC and NaDC

Fluorometric Measurements

To understand the interaction between antidepressants and bile salts the spectroscopic techniques such as steady state fluorescence have been employed. The fluorescence emission spectra (Fig 13 and Fig 14) of CPZ and DSP show the addition of bile salts quenched the spectra of CPZ and DSP at 474 nm which shows the new complex formation between antidepressants and bile salts. The addition of constant volume of quencher (i.e. .001 mL of 10 mM bio-surfactant solutions) to the drug solution avoids complications due to dilution effects within titration type experiments. Process of fluorescence quenching is explained by Stern–Volmer equation.

\[ \frac{I_0}{I} = 1 + K_{sv} [Q] \]

where, \( I_0 \) = fluorescence intensity of CPZ and DSP without quencher
\( I \) = fluorescence intensity of CPZ and DSP with quencher
\( K_{sv} \) = Stern-Volmer constant
\( [Q] \) = concentration of quencher

Figs 15 (a) and 16 (a) show the plot of \( \frac{I_0}{I} \) versus \([Q]\)) and give the value of Stern-Volmer constant shown in Table 3. By applying the following equation we can calculate the value of binding constant \( K_a \) and binding sites \( n \),
\[ \log \left( \frac{I_0 - I}{I} \right) = \log K_a + n \log [ \text{Surfactant} ] \]

Here, \( K_a \) = binding constant

\( n \) = binding sites

The values \( K_a \) and \( n \) are given in Table 3.

All systems show the value of binding capacity (\( n \)) is greater than unity. CPZ + NaDC system shows higher binding capacity while other systems (CPZ + NaC, DSP + NaDC and DSP + NaC) show less binding capacity indicating that they do not show significant binding to each other.

Using the value of \( K_a \) the Gibb’s free energy changes for binding obtained for this process from following equation,

\[ \Delta G_{\text{Binding}} = -RT \ln K_a \]
The negative value of Gibb’s free energy changes for binding ($\Delta G_{\text{Binding}}$) assure that the binding process is spontaneous and it is helpful for studying the interaction of drugs with biosurfactants. The NaDC shows higher value of $K_a$ for both antidepressants than NaC due to hydrophobicity which leads to their different binding abilities. It is also examined that between CPZ + NaC and DSP + NaC systems, the binding is stronger for former case showing higher binding affinity which also explains about the more hydrophobic nature of CPZ than DSP. In the case of CPZ + NaDC and DSP + NaDC, the previous one shows higher binding affinity. CPZ contains phenothiazine ring and positively charged group shows a better binding with negatively charged bile salt. NaDC possesses more hydrophobic nature which promotes the absorption as compare to NaC. The binding constants ($K_a$) showed a considerable hydrophobic contribution modulated by electrostatic interactions of the positively charged drug with the head group of biosurfactants. Fig 17 shows the schematic representation of drug-surfactant interaction.
Fig. 15 (a) Stern-Volmer plot of fluorescence quenching of DSP by NaC
(a) A plot of $\log \left( \frac{(I_0 - I)}{I} \right)$ vs. log [Surfactant] for NaC

Fig. 16 (a) Stern-Volmer plot of fluorescence quenching of CPZ by NaDC
(b) A plot of $\log \left( \frac{(I_0 - I)}{I} \right)$ vs. log [Surfactant] for NaDC
Fig 17 Schematic representation of drug-surfactant interaction

Table 3  Stern–Volmer quenching constants ($K_{sv}$), binding constants ($K$), number of binding sites ($n$), free energy change for binding ($\Delta G_{\text{Binding}}$) for the drug-bile salt complexation of CPZ + NaC/NaDC and DSP + NaC/NaDC using fluorescence technique

<table>
<thead>
<tr>
<th>Drug-bile salts complex</th>
<th>$K \times 10^{-3}$ (mol dm$^{-1}$)</th>
<th>$K_{sv} \times 10^{-3}$ (mol dm$^{-1}$)</th>
<th>$n$</th>
<th>$\Delta G_{\text{Binding}}$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPZ + NaC</td>
<td>2.221±0.04</td>
<td>0.0591±0.003</td>
<td>1.68</td>
<td>-19.76±0.7</td>
</tr>
<tr>
<td>CPZ + NaDC</td>
<td>5.543±0.05</td>
<td>0.1647±0.002</td>
<td>2.02</td>
<td>-42.42±0.4</td>
</tr>
<tr>
<td>DSP + NaC</td>
<td>1.343±0.08</td>
<td>0.0710±0.002</td>
<td>1.65</td>
<td>-7.30±0.2</td>
</tr>
<tr>
<td>DSP + NaDC</td>
<td>2.228±0.06</td>
<td>0.4746±0.003</td>
<td>1.71</td>
<td>-19.84±0.7</td>
</tr>
</tbody>
</table>
Mixed Micellization of Gemini Surfactant with Pluronic Block Copolymer and Their Interaction with Tricyclic Antidepressants

Pluronics are amphiphilic tri block co-polymers. These are water soluble nonionic macromolecular surfactants plays an important role for solubilizing the poorly soluble drugs and as a drug carrier in drug delivery system. Gemini surfactants possess strong self-assembly ability. Interaction of polymeric micelles with gemini surfactants have gained paramount significance in pharmaceutical field. This study involves the mixed micellization of pluronic F-127 and gemini surfactant C_{12-4(OH)}_{2}-C_{12,2Br^-} (Scheme 3) and their interaction with antidepressant drugs chlorpromazine hydrochloride (CPZ) and desipramine hydrochloride (DSP) using surface tension, fluorescence spectroscopy and dynamic light scattering studies at 300 K.

![Scheme 3. Structures Polymeric and Gemini Surfactant.](image)

Mixed Micellization of Gemini Surfactant with Pluronic Block Copolymer

The study of copolymer-surfactant interaction is very essential due to their importance in industrial, biomedical and pharmaceutical applications. The surface tension values were measured for mixed system C_{12-4(OH)}_{2}-C_{12,2Br^-} + F-127 at various mole fraction (0.2-0.8) at 300 K. The values of critical micelle concentration (CMC) are listed in Table 4.

Fig 18 shows the plots of surface tension versus log [surfactants] for binary mixture of C_{12-4(OH)}_{2}-C_{12,2Br^-} + F-127. With the increasing mole fraction of gemini surfactant the value of surface tension decreases. The CMC values obtained for binary systems increases by increasing the mole fraction of gemini surfactant shown in Table 4 The experimental CMC values of binary systems were found to be less than the ideal CMC values calculated using Clint equation, which indicates negative deviation from ideal behaviour for mixed micelle formation.

$$\frac{1}{cmc_{ideal}} = \frac{\alpha_1}{cmc_1} + \frac{1 - \alpha_1}{cmc_2}$$
Various interfacial parameters such as maximum surface excess concentration ($\Gamma_{\text{max}}$), minimum area per molecule at the interface ($A_{\text{min}}$), effectiveness of the surface tension reduction measured by the surface tension at the CMC ($\gamma_{\text{CMC}}$) have been evaluated using following eqs. respectively,

$$\Gamma_{\text{max}} = -\frac{1}{2.303 nRT} \left[ \frac{d\gamma}{d \log C} \right]_{T,P}$$

where $R$ is the gas constant (8.314 Jmol$^{-1}$ K$^{-1}$), $T$ is the absolute temperature, $C$ is the surfactant concentration, and $(d\gamma / d \log C)$ is the slope of the $\gamma$ versus log $C$ plot taken at the CMC.

$$A_{\text{min}} = \frac{1}{N \Gamma_{\text{max}}}$$

where $N$ is Avogadro’s number

$$\gamma_{\text{CMC}} = \gamma_o - \gamma_{\text{CMC}}$$

where $\gamma_o$ and $\gamma_{\text{CMC}}$ refers to the surface tension of solvent and the surfactant solution at the CMC, respectively.

**Fig. 18** Plots of surface tension vs log C of C$_{12}$-4(OH)$_2$-C$_{12}$,2Br$^-$ + F-127 binary system.

Interaction parameters ($\beta$) for mixed systems of C$_{12}$-4(OH)$_2$-C$_{12}$,2Br$^- +$ F-127 have been calculated by applying the Rosen model. Activity coefficient ($f_1^o$ and $f_2^o$) have been evaluated by using the equation given below. All the interaction parameters are listed in Table 4. The negative value of interaction parameter ($\beta^o$) indicates the deviation from ideality which indicates the degree of interaction between two surfactants in mixed micelle.
\[
\frac{(X^\sigma)^2 \ln \left( \frac{\alpha_1 C_{\text{mix}}}{X^\sigma C_1^0} \right)}{(1 - X^\sigma)^2 \ln \left( \frac{(1 - \alpha_1) C_{\text{mix}}}{(1 - X^\sigma) C_2^0} \right)} = 1
\]

where \( C_{\text{mix}}, C_1^0 \) and \( C_2^0 \) are the concentrations of the mixture, pure surfactant 1 and 2 respectively at a fixed \( \gamma \) value, \( \alpha_1 \) is the stoichiometric mole fraction of surfactant 1 in solution.

\[
\beta^\sigma = \frac{\ln \left( \frac{\alpha_1 C_{\text{mix}}}{X^\sigma C_1^0} \right)}{(1 - X^\sigma)^2}
\]

Interaction parameter \( \beta^\sigma \) indicates the degree of interaction between the two components as well as the deviation from ideality.

\[
f_1^\sigma = \exp [\beta^\sigma \cdot (1 - X^\sigma)^2]
\]

\[
f_2^\sigma = \exp (\beta^\sigma X^\sigma)^2
\]

The excess free energy of mixing has calculated from using eq. given below. The negative value of \( \Delta G_{\text{ex}} \) indicates more attractive interaction between molecules in mixed micelles.

\[
\Delta G_{\text{ex}} = RT \left[ X_1 \ln f_1 + (1-X_1) \cdot \ln f_2 \right]
\]
Table 4 Critical micelle concentration ($C_{\text{exp}}, C_{\text{ideal}}$), maximum surface excess concentration ($\Gamma_{\text{max}}$), minimum area per molecule at the interface ($A_{\text{min}}$), the surface tension at the CMC ($\gamma_{\text{CMC}}$), micellar mole fraction ($X_1$ and $X_{\text{ideal}}$), interaction parameter ($\beta^\sigma$), activity coefficients ($f_1^\sigma$ and $f_2^\sigma$) and excess Gibbs free energy ($\Delta G^E$) for binary mixture (C$_{12}$-4(OH)$_2$-C$_{12}$2Br$^-$ + F-127) system at 300 K.

<table>
<thead>
<tr>
<th>$\alpha_{\text{gemini}}$</th>
<th>$C_{\text{exp}}$ (mM)</th>
<th>$C_{\text{ideal}}$ (mM)</th>
<th>$\Gamma_{\text{max}}$ $10^6$ mol.m$^{-2}$</th>
<th>$A_{\text{min}}$ $10^{20}$ m$^2$</th>
<th>$\pi_{\text{cmc}}$ mN.m$^{-1}$</th>
<th>$X_{\text{ideal}}$</th>
<th>$X_1^\sigma$</th>
<th>$\beta^\sigma$</th>
<th>$f_1^\sigma$</th>
<th>$f_2^\sigma$</th>
<th>$\Delta G_{\text{exp}}$ kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.043</td>
<td>0.053</td>
<td>303</td>
<td>30</td>
<td></td>
<td>0.011</td>
<td>0.11</td>
<td>-2.78</td>
<td>0.111</td>
<td>0.967</td>
<td>-678.4</td>
</tr>
<tr>
<td>0.2</td>
<td>0.048</td>
<td>0.053</td>
<td>0.096</td>
<td>302</td>
<td>30</td>
<td>0.011</td>
<td>0.11</td>
<td>-2.78</td>
<td>0.111</td>
<td>0.967</td>
<td>-678.4</td>
</tr>
<tr>
<td>0.5</td>
<td>0.062</td>
<td>0.081</td>
<td>0.204</td>
<td>307</td>
<td>30.2</td>
<td>0.041</td>
<td>0.21</td>
<td>-2.70</td>
<td>0.185</td>
<td>0.888</td>
<td>-1114.9</td>
</tr>
<tr>
<td>0.8</td>
<td>0.069</td>
<td>0.176</td>
<td>0.386</td>
<td>316</td>
<td>32.7</td>
<td>0.048</td>
<td>0.39</td>
<td>-4.50</td>
<td>0.187</td>
<td>0.504</td>
<td>-2673.8</td>
</tr>
<tr>
<td>1.0</td>
<td>0.780</td>
<td></td>
<td></td>
<td>173</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The fluorescence spectroscopic technique have been applied to study the interaction between CPZ and DSP with mixed system of 12-4(OH)2-12 + F- 127. Figs 19 to 24 show that the addition of binary surfactant system (C12-4(OH)2-C12,2Br- + F-127) quenched the fluorescence emission spectra of antidepressant drugs (CPZ and DSP) at 474 nm when excited at 368 nm at different mole fractions (0.2, 0.5 and 0.8). Figs 25 and 26 show the Stern-Volmer plots of fluorescence quenching of CPZ and DSP by C12-4(OH)2-C12,2Br- + F-127 system respectively at various mole fractions (0.2 to 0.8), explains the quenching of antidepressant drugs by binary surfactant system Fig 27 shows the plots of log [(I0 -I) / I] vs. log [Surfactant] for CPZ.

The Stern-Volmer constants of studied system have been calculated. Binding constants have also been calculated. All the parameters are listed in Table 5. The higher values of binding constant have been found at 0.8 mole fraction for both of the antidepressant drugs. With the increasing mole fraction the interaction between drugs and mixed surfactant system have increase.

Fig. 19 Fluorescence spectra of CPZ at increasing concentration of binary system C12-4(OH)2-C12,2Br- + F-127 (α_gemini = 0.2)
**Fig. 20** Fluorescence spectra of CPZ at increasing concentration of binary system C12-4(OH)2-C12,2Br- + F-127 ($\alpha_{\text{gemini}} = 0.5$)

**Fig. 21** Fluorescence spectra of CPZ at increasing concentration of binary system C12-4(OH)2-C12,2Br- + F-127 ($\alpha_{\text{gemini}} = 0.8$)
**Fig. 22** Fluorescence spectra of DSP at increasing concentration of binary system C12-4(OH)2-C12,2Br- + F-127 (αgemini = 0.2)

**Fig. 23** Fluorescence spectra of DSP at increasing concentration of binary system C12-4(OH)2-C12,2Br- + F-127 (αgemini = 0.5)
Fig. 24 Fluorescence spectra of DSP at increasing concentration of binary system $\text{C}_{12}-4(\text{OH})_2\text{C}_{12},2\text{Br}^- + \text{F}-127$ ($\alpha_{\text{gemini}} = 0.8$)

Fig. 25 Stern-Volmer plots of fluorescence quenching of CPZ by $\text{C}_{12}-4(\text{OH})_2\text{C}_{12},2\text{Br}^- + \text{F}-127$ systems
**Fig. 26** Stern-Volmer plots of fluorescence quenching of DSP by $C_{12-4}(OH)_{2}C_{12}2Br^- + F\text{-}127$ systems

**Fig. 27** Plots of $\log \left( \frac{I_0 - I}{I} \right)$ vs. $\log$ [Surfactant] for CPZ
Table 5 Stern–Volmer quenching constants ($K_{sv}$), binding constants ($K$), correlation coefficient ($R$) and free energy change of binding ($\Delta G_{\text{Binding}}$) for antidepressant drug–mixed surfactant systems at 300 K using fluorescence technique.

<table>
<thead>
<tr>
<th>$\alpha_{\text{gemi}}$</th>
<th>Chlorpromazine hydrochloride (CPZ)</th>
<th>Desipramine hydrochloride (DSP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K \times 10^4$ (mol dm$^{-1}$)</td>
<td>$K_{sv} \times 10^4$ (mol dm$^{-1}$)</td>
</tr>
<tr>
<td>0.0</td>
<td>0.20</td>
<td>1.17</td>
</tr>
<tr>
<td>0.2</td>
<td>0.51</td>
<td>0.94</td>
</tr>
<tr>
<td>0.5</td>
<td>0.76</td>
<td>1.18</td>
</tr>
<tr>
<td>0.8</td>
<td>1.59</td>
<td>1.29</td>
</tr>
<tr>
<td>1.0</td>
<td>0.96</td>
<td>1.77</td>
</tr>
</tbody>
</table>

(c) Solubilization of antidepressant drugs in the presence of surfactants

Solubilization of poorly soluble drugs using surface active agents is a very useful technique in pharmaceutical science. This study reports the solubilization of phenothiazine (Scheme 4). Phenothiazines are the drugs possessing a hydrophobic tricyclic ring system and hydrophilic side chain. These drugs are generally employed as antihistamines, antipsychotics and neuroleptics.

Scheme 4. Phenothiazine

1-4 bis (dodecyl-N,N-dimethylammonium bromide) butane ($C_{12}-4-C_{12}, 2Br^-$) 1-4 bis (dodecyl-N,N-dimethylammonium bromide)-2-butanol ($C_{12}-4(OH)-C_{12}2Br^-$)
Scheme 5. Structure of cationic gemini surfactants

Solubilization of antipsychotic drug phenothiazine by single system of cationic gemini surfactant using cationic gemini surfactants (C\textsubscript{12}-4-C\textsubscript{12}, 2Br\textsuperscript{-}), 1,4 bis(dodecyl-N,N-dimethylammonium bromide)-2-butanol (C\textsubscript{12}-4(OH)-C\textsubscript{12}, 2Br\textsuperscript{-}), 1,4 bis(dodecyl-N,N-dimethylammonium bromide)-2,3-butanediol (C\textsubscript{12}-4(OH)\textsubscript{2}-C\textsubscript{12}, 2Br\textsuperscript{-}) (Scheme 5) were evaluated and compared. The absorbance of the solubilize of specified concentration was determined by measuring the molar extinction coefficient ($\varepsilon$) in micellar solutions. Straight line was obtained by plotting the absorbance versus the solubilizes concentrations. The solubility of phenothiazine were greatly enhance increased by all surfactant systems where solubility increased with increasing surfactant concentrations above the CMC. A molar solubilization ratio (MSR) is given to quantify the effectiveness of a surfactant in solubilizing a given solubilize. It can be defined as the number of compound solubilized per moles of surfactant added to the solution and can be calculated as per eq.

$$\text{MSR} = \frac{(S - S_{\text{CMC}})}{(C_s - \text{CMC})}$$

where, $S =$ Apparent solubility of organic compound at surfactant concentration $C_s$ ($C_s > \text{CMC}$) and $S_{\text{CMC}} =$ Apparent solubility of organic compound at CMC.

In addition to MSR, the effectiveness of solubilization can also be expressed in terms of the partition coefficient, $K_m$, of the compound between micelles and the aqueous phase. The value of $K_m$ is a function of temperature and the nature of surfactant and solubilize. The partition coefficient can be written as

$$K_m = \frac{X_m}{X_a}$$

where $X_m$ and $X_a$ are the mole fractions of solute in micelles and the aqueous phase, respectively. The value of $X_m$ can be calculated as $X_m = \text{MSR}/(1+ \text{MSR})$, and $X_a$ can be expressed as $X_a = S_{\text{CMC}} V_w$, where $V_w = 0.01805$ L/mol is the molar volume of water. Consequently, eq can be rearranged to yield

$$K_m = \frac{\text{MSR}}{S_{\text{CMC}} V_w (1+\text{MSR})}$$
The MSR and $K_m$ values for all the surfactants are listed in Table 6. The slopes of the curve of solubility of phenothiazine versus surfactant concentration, expressed in molar concentration, represent the MSR, which was determined using least-squares linear regression.

The order of solubilizing strength for gemini surfactants of C$_{12}$ series is found to be: C$_{12}$-4-C$_{12}$, 2Br$^-$ < C$_{12}$-4(OH)-C$_{12}$, 2Br$^-$ < C$_{12}$-4(OH)$_2$-C$_{12}$, 2Br$^-$ for phenothiazine. The CMC value of gemini surfactants decreases with substitutional hydroxyl group on spacer of same chain length. The substituted spacer group could form hydrogen bond with water more readily and reduce the unfavourable hydrocarbon-water contact. So the C$_{12}$-4(OH)$_2$-C$_{12}$, 2Br$^-$ having two hydroxyl group on spacer reflects greater solubilization as compared to others.

![Fig 28 Absorption spectra of Phenothiazine at different concentration of 12-4(OH)$_2$-12](image1)

![Fig. 29 Variation of solubility of Phenothiazine with Gemini Surfactants](image2)

**Thermodynamics of Solubilization**

The knowledge of the thermodynamic parameters controlling solubilization is helpful for better understanding of the mechanism involved in the process. From the thermodynamic point of view, solubilization can be considered as normal partitioning of the drug between micellar and aqueous phases and the Gibb’s free energy of solubilization, $\Delta G^\circ_s$, can be represented by the expression

$$\Delta G^\circ_s = -RT \ln K_m$$

The $\Delta G^\circ_s$ values thus calculated are presented in Table 6. For all the systems, the $\Delta G^\circ_s$ values appear to be negative showing spontaneity of the solubilization process.
Table 6  Molar solubilization ratio (MSR), $\ln K_m$ and the Gibb’s free energy of solubilization ($\Delta G^o_s$) of Phenothiazine in cationic gemini surfactant systems at 300 K.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>CMC  (mM)</th>
<th>Phenothiazine</th>
<th>$\Delta G^o_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{12}$-4-C$</em>{12}$,2Br$^-$</td>
<td>1.17</td>
<td>0.164</td>
<td>11.4</td>
</tr>
<tr>
<td>C$<em>{12}$-4(OH)-C$</em>{12}$,2Br$^-$</td>
<td>0.94</td>
<td>0.235</td>
<td>11.6</td>
</tr>
<tr>
<td>C$_{12}$-(OH)$<em>2$-C$</em>{12}$,2Br$^-$</td>
<td>0.87</td>
<td>0.663</td>
<td>13.9</td>
</tr>
</tbody>
</table>

(d) Study of the micellar growth of drug and surfactant mixture using dynamic light scattering

The hydrodynamic radii ($R_h$), polydispersity index (PDI) and zeta potential values of pure and mixed systems of antidepressant drugs CPZ and DSP with (C$_{12}$-4(OH)$_2$-C$_{12}$,2Br$^-$ + F-127) have been determined by dynamic light scattering measurements at 300 K. CONTIN software has been used to analyze the polydispersity. All the values are shown in Table 7. Hydrodynamic radii of pure and mixed system in aqueous solution were calculated from the diffusion coefficients, $D_0$, using the Stokes–Einstein equation,

$$R_h = \frac{k_B T}{6\pi \eta D_0}$$

where, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the viscosity of water. Figs 30 to 35 show the size distribution graph of DLS spectra of all studied systems as a function of hydrodynamic radii.

The hydrodynamic radii of drug-surfactant mixed systems increase with the increasing mole fraction of mixed surfactant. The mixture of CPZ / DSP- (C$_{12}$-4(OH)$_2$-C$_{12}$,2Br$^-$ + F-127) makes larger aggregates. However, up to 0.5 mole fraction, aggregation is mild and only mixed micelle are formed. With increasing mile fraction of mixed system increases the micellar radii, indicates the formation of larger aggregates. But at higher mole fraction of mixed system (0.8), due to repulsive interaction (dispersion force) the mixed surfactant micelles dissociate and form micelle with smaller size.
**Fig. 30** Size distribution graph of DLS spectra of pluronic F-127

**Fig. 31** Size distribution graph of DLS spectra of C\textsubscript{12}-4(OH)\textsubscript{2}-C\textsubscript{12}, 2Br\textsuperscript{-}

**Fig. 32** Size distribution graph of DLS spectra of chlorpromazine hydrochloride (CPZ)
Fig 33 Size distribution graph of DLS spectra of desipramine hydrochloride (DSP)

Fig 34 Size distribution graph of DLS spectra of F-127 + CPZ

Fig 35 Size distribution graph of DLS spectra of F-127 + DSP
Table 7 Hydrodynamic radii values ($R_h$), polydispersity index (PDI) and zeta potential values of pure and mixed systems of antidepressant drugs + ($C_{12}(OH)_2-C_{12}Br^- + F-127$) at 300 K

<table>
<thead>
<tr>
<th>Systems</th>
<th>CPZ</th>
<th>DSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_h$ (nm)</td>
<td>PDI</td>
</tr>
<tr>
<td>0.0</td>
<td>30.4 (42)$^a$</td>
<td>0.36</td>
</tr>
<tr>
<td>0.2</td>
<td>124.7</td>
<td>0.27</td>
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<tr>
<td>0.5</td>
<td>159.8</td>
<td>0.64</td>
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<tr>
<td>0.8</td>
<td>84.1</td>
<td>0.33</td>
</tr>
<tr>
<td>1.0</td>
<td>239.4</td>
<td>0.60</td>
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## PUBLICATIONS

### Paper Communicated /Accepted in National /International Journal

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<th>S. No.</th>
<th>Title</th>
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<th>Authors</th>
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### Papers Accepted/Presented in Conferences

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<tr>
<td></td>
<td>Interaction between tricyclic antidepressant drugs and human serum albumin: Spectroscopic and molecular docking approach</td>
<td>22\textsuperscript{nd} CRSI National Symposium in Chemistry, Pt. Ravishankar Shukla University, Raipur (CG)</td>
<td>Kallol K. Ghosh, Reshma, Srishti Sinha and Toshikee Yadav</td>
</tr>
<tr>
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</table>

Principal Investigator
Prof. Kallol K Ghosh
School of Studies in Chemistry
Pt. Ravishankar Shukla University
Raipur- 492010 CG
MICELLAR, INTERFACIAL AND SPECTRO-SCOPIC STUDIES OF 
ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS 

Submitted to the 

University Grants Commission, New Delhi 

(UGC Ref. No. 43-183/2014(SR) Dated 30.10.15) 
MRP ID : MRP-MAJOR-CHEM-2013-14435 

By 
Prof. Kallol K Ghosh 
Principal Investigator 
School of Studies in Chemistry 
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Raipur- 492010 CG 

EXECUTIVE SUMMARY OF MAJOR RESEARCH PROJECT 
(UGC Ref. No. 43-183/2014(SR) Dated 30.10.15)
Most of the drugs are used in combination with additives specially surfactants. Therefore, it is necessary to have knowledge of the additive effect on the CMC of amphiphilic drugs. This project gives emphasis the micellar and interfacial properties of drug-surfactant systems which are very useful for the development of new drugs as well as drug-delivery system.

The studies of micelles in drug delivery yield the necessary information to minimize drug degradation and loss, to prevent harmful side effects and to increase drug bioavailability. The use of micelle in pharmacy is an important tool that finds numerous applications. Special emphasis is given to the more recent use of polymeric micelles.

So this project gives the following information about antidepressant drug-surfactant systems:

(a) The physicochemical behavior and solubilization of tricyclic antidepressant drugs viz. amitriptyline hydrochloride (AMT) and Imipramine hydrochloride (IMP) with cationic surfactants i.e. alkyltriphenylphosphonium bromide (R = 14, 16) and alkylidethylethanolammonium bromide (R = 14, 16) have been investigated by surface tension. The surface properties viz. CMC, maximum surface excess concentration at the air/water interface (Γ_{\text{max}}), minimum area per surfactant molecule at the air/water interface (A_{\text{min}}), surface pressure at the CMC (\pi_{\text{CMC}}) have been evaluated. The mixtures of drugs with cationic surfactants show non-ideal behaviour. The mixture of drug and surfactants are more stable compared to pure drug and pure surfactants.

(b) This study deals with the spectroscopic investigation of interaction between antidepressants and bile salts which give the valuable and plentiful information about uses of bile salts in pharmaceutics. The spectroscopic techniques such as UV-visible and fluorescence have been employed for the determination of binding constant (K, K_a), Stern–Volmer constant (K_n), binding sites (n) and free energy changes for binding (\Delta G_{\text{Binding}}). The value of binding constant (K, K_a) is found to be maximum for CPZ + NaDC mixtures from both the spectroscopic methods. More hydrophobic nature of NaDC is responsible for better interaction with antidepressants drugs. The negative values of Gibb’s free energy changes reveal the spontaneity of all the systems. The order of Gibb’s free energy changes of the studied systems is found to be : CPZ + NaDC (-42.42) < DSP + NaDC (-19.84) < CPZ + NaC (-19.76) < DSP + NaC (-7.30).

(C) Interaction of antidepressant drugs with binary system (C_{12-4(OH)}-2C_{12}2Br^- + F-127) have also been studied by fluorescence technique. The more binding affinities have been found at higher mole fraction of mixed surfactant system for both of the drugs. Size
distribution, polydispersity and zeta potential values have also been evaluated by
dynamic light scattering (DLS) studies.

Principal Investigator
Prof. Kallol K Ghosh
School of Studies in Chemistry
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Raipur- 492010 CG
Evaluation Report of UGC Project

1) UGC Major Project Title: MICELLAR, INTERFACIAL AND SPECTRO-SCOPIC STUDIES OF ANTIDEPRESSANT-DRUG-SURFACTANT SYSTEMS

2) Name and address of the PI:  Professor Kallol K Ghosh
School of Studies in Chemistry
Pt. Ravishankar Shukla University

3) UGC Sanction Letter Number: F. No. 43-183/2014(SR) Dated 30.10.2015

4) Duration: 1.7.2015 to 31.6.2018

5) Comments / Suggestions of the Expert:

Since some important drugs are used in combination with additives specially surfactants, this project is focused towards understanding the nature of interactions between antidepressant drugs and surfactants. The PI has selected few drugs Desipramine hydrochloride, Chlorpromazine hydrochloride, Imipramine hydrochloride for this study. This study provides some preliminary understanding towards identifying appropriate surfactant systems i) to increase the activity of antidepressant drugs ii) to enhance the bioavailability of amphiphilic antidepressant drugs and iii) towards increasing the solubility of poorly soluble antidepressant drugs. These studies have resulted in two publications in international journals (and one paper is communicated for publication). One student has obtained Ph.D. degree working in this project.

I therefore feel that PI has done an excellent work in this project working in Pt. Ravishankar Shukla University with limited research facilities.

Hyderabad
Date: March 12, 2019

Signature of the expert

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Report

The project entitled “Micellar, Interfacial and Spectroscopic Studies of Antidepressant Drug-Surfactant Systems” deals with study of the physicochemical aspects of interaction of drug to surfactant. Conductometric, tensiometric and fluorometric techniques have been used to study the several antidepressant-drug surfactant systems. The interfacial parameters of antidepressant drugs in the presence of single and mixed surfactants have been determined. The principal investigator made a significant contribution as the results of the projects directly related to antidepressant drugs used in the Chhattisgarh state. Special emphasis has been given on the enhancement of the solubility and bioavailability of these drugs using novel surfactants. The results could be useful in acquiring the knowledge of drug-delivery. The results and findings are significant and conclusions derived can be useful to researchers in future. The principal Investigator and his group have published two research papers in the referred journals having an international repute. The Principal investigator and project fellow also presented their work in various scientific gathering of national conferences, seminars and workshops. The proposed objectives of the project are achieved.
Biophysical studies on the interactions between antidepressant drugs and bile salts

Toshikee Yadav, Deepti Tikariha, Srishi Sinha, Kallol K Ghosh

Abstract

The mechanism of the interaction of drugs with other foreign materials is of paramount importance in the drug delivery. The excess amount of drugs can cause overstimulation, psychotic illness and other disorders. In recent years the research on targeted drug delivery in body organs and the role of surfactants is primarily focused. Surfactants have been broadly used in pharmaceutical industries due to their unique micellar solubilization properties. Here we report the characterization of binding of two antipsychotic drugs chlorpromazine hydrochloride (CPZ) and desipramine hydrochloride (DSP) with bio-surfactants sodium cholate (NaC) and sodium deoxycholate (NaDC) which belongs to the class of bile salts. UV-visible and steady state fluorescence have been employed to study the interaction of drugs with bile salts. Various interaction parameters such as binding constant ($K_b$), Stern-Volmer constant ($K_S$), binding sites ($n$) and thermodynamic parameters Gibbs free energy changes ($\Delta G_{bind}$) have been evaluated at 300 K. The observed results show changes in spectral intensities of antipsychotic drugs on the addition of bile salts. Highest binding affinity and most promising activity are shown by CPZ and NaDC system.

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1. Introduction

Several therapeutically active compounds are amphiphilic in nature and generally undergo variety of association with the target site in plasma membrane of organisms [1-6]. Micellar and interfacial properties of amphiphilic drugs are highly useful in the pharmaceutical sciences [7-9]. Interactions between surfactants and drugs are extensively studied in search of efficient drug-delivery system. Due to the presence of an almost planar tricyclic ring system and a short hydrocarbon chain carrying a terminal nitrogen atom they show surfactant like behavior [10-11]. This surface-active behavior among many diverse classes of drugs has been reported and research have been carried out to correlate their surface and biological activities [12-15]. The aggregation of the antidepressant drugs follows the same principles as of conventional surfactants [16]. The self-association of drug depends on the experimental conditions e.g., temperature, pH, salt concentration etc., molecular structure and concentration of drugs [17-18].

Bile salts are naturally occurring amphiphilic molecules. These are a class of potential bio-surfactants which are present in gastrointestinal tract (GIT) and play an important role in drug delivery and their solubilization process [19-20]. Bile salts aggregate to form micelles and their size continuously increases with increment in concentration. These are distinguished class of biological surfactants found in bile and synthesized in the liver as derivatives of cholesterol mixture of sodium salt. They are also used as penetration enhancers which help in the gastrointestinal membrane permeability for oral route administration of drugs, considered as most convenient path for effective action of drug in body [21]. These bi-surfactant has low surface tension and contribute to emulsification of fats, lipids, fat soluble vitamins in our body. They have widely used as transporters in drug delivery, as they have low viscosity, small aggregate size, simple preparation, long shelf-life and non-toxic in nature [22].

Antidepressants are drugs used to relieve or prevent psychic depression and neurologic disorder. Over the years many classes of antidepressants have been used for the treatment of psychiatric disorders. But the tricyclic antidepressant suffers from several side effects like cardiovascular, anticholinergic and anti-histamine effects [23-24]. So the targeted drug delivery in body organs with surfactants and other system is necessary. In order to use these systems as drug carrier, a detailed study of drug–surfactant interaction as well as the effect of microminvironment is very important [25]. Various studies have been made in this context [26-22].

Chlorpromazine hydrochloride (CPZ, Scheme 1) [3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine] is an antipsychotic medication which is used to treat psychotic disorder like schizophrenia [33-36]. This is most widely used antipsychotic drug throughout the world as compared to other neuroleptics. Desipramine hydrochloride
(DSP, Scheme 1) also known as desmethylimipramine [3-(10,11-Dihydro-5H-dibenzo[b,g]azepin-5-yl)-N-methylprop-1-amine] used in the treatment of depression. It inhibits the reuptake of noradrenaline. These are amphetamine tricyclic antidepressants drugs (TCA) containing nitrogen atom [37-38].

Mahajan et al. [32] studied the interaction of two phenothiazine drugs promazine hydrochloride (PMZ) and promethazine hydrochloride (PMT) with bile salts sodium cholate and sodium deoxycholate by conductivity, surface tension, UV-visible and fluorimetric measurements and evaluated that PMZ + NaDC system shows highest value of binding constant. They also reported the physicochemical investigation of interactions between pyridinium gemini surfactants and PMZ using conductivity, surface tension, UV-visible, steady state fluorescence and NMR measurements [33]. This group [34] also explained the binding ability of long chain surfactants with trifluoperazine dicyrchochloride by means of surface tension, electronic absorption and fluorescence measurements. In this investigation they found that cmc values decrease with increase in mole fraction of drug for all the drug-surfactant mixtures. Kahni-Odin et al. [35] studied the micellization of an amphiphilic drug promethazine hydrochloride (PMZ) in the presence of two conventional surfactants CTAB and TTAB along with cationic gemini surfactants conductometrically at different temperatures. They found the attractive interaction in mixed system of drug and surfactants. Caetano and Tabak [36] studied the characteristic of binding of chlorpromazine and trifluoperazine with sodium dodecyl sulphate using electronic absorption and fluorescence spectroscopy by changing the pH. Recently Naqvi et al. [37] reported the mixed micellization of dicarboxylic acids surfactants with amphoteric drug imipramine hydrochloride.

To the best of our knowledge there is no report in the literature that explains the interaction of antidepressants chlorpromazine hydrochloride (CPZ) and desipramine hydrochloride (DSP) with bile salts sodium cholate (NaC) and sodium deoxycholate (NaDC). For our study we have chosen these two antidepressants chlorpromazine hydrochloride (CPZ), desipramine hydrochloride (DSP) (Scheme 1). Their interaction with bile salts i.e. sodium cholate and sodium deoxycholate (Scheme 2) have been determined by UV-visible and fluorescence spectroscopy at 300 K.

2. Experimental

2.1. Materials

Antidepressant drugs chlorpromazine hydrochloride (CPZ) (purity ≥98%), desipramine hydrochloride (DSP) (purity ≥98%), bile salts sodium cholate (NaC) (purity ≥97%), sodium deoxycholate (NaDC) (purity ≥97%) were procured from Sigma Aldrich and used without further purification. 1-pyrene carboxaldehyde (1-PyCHO) (purity ≥99%) used as a probe received from Sigma Aldrich. The solutions were prepared in millipore water.

2.2. Apparatus

The spectrophotometric measurements were made by using a Varian Cary 50 UV-visible spectrophotometer equipped with a pellet temperature controller unit and a computer connected to a spectrophotometer. For the determination of binding constant and Stern-Volmer

### Scheme 1 - Structure of antidepressants drugs

Sodium cholate (NaC) and Sodium deoxycholate (NaDC) shown in Scheme 2. Structure of bile salts.

2.3. Procedure

2.3.1. Absorption measurements

This investigation helps to understand the antidepressants-bile salt interactions. The titrations were performed by successive additions of 0.01 M stock solutions of bile salts (NaC and NaDC) directly into the cuvette containing 3 ml of 0.33 mM drug solution [38]. To reduce dilution effects within titration type experiments the volume of bio-surfactant [Q] were added constantly to drug solution.

2.3.2. Determination of pH

pH values of drug and bio-surfactant solutions were determined using a Eutech (pH 700), pH meter equipped with an InLab® Expert

### Scheme 2 - Structure of bile salts

2.4. Fluorescence measurements

The absorption spectra of CPZ with increasing concentration of (a) NaC and (b) NaDC are shown in Fig. 1.

### Scheme 3 - Structure of bile salts

Chlorpromazine hydrochloride (CPZ) and Desipramine hydrochloride (DSP) shown in Scheme 3.
Pro glass electrode with an accuracy of ±0.01 units. The pH meter was calibrated at 27°C using the two-point calibration method with commercially available standard buffer solutions at pH 7.00 and 9.20. During pre-aggregation pH values of CPZ and DSP were observed as 6.55 and 6.39 respectively. While in post-aggregation pH values for CPZ + NaC and CPZ + NaDC systems were found as 6.19 and 6.72 respectively. In case of DSP with NaC/NaDC, pH values were 6.10 and 6.80 respectively.

2.3.3 Fluorescence measurements

The fluorescence spectroscopy has been applied to determine interactions between drugs and bile salts. We have investigated the interaction parameters for drug-bile salt mixtures by using external probe 1-pyrene carboxaldehyde (1-PyCHO). The emission spectra of drugs recorded in the range of 400 to 600 nm when excited at 368 nm using an emission slit width of 5 nm.

2.3.3.1 Fluorescence quenching measurement of antidepresents. The fluorescence quenching measurements are very useful to know the role of interactions taking place between antidepressants and bile salts (NaC and NaDC). Owing to two types of singlet excited state i.e. n–π* and π–π*, 1-pyrene carboxaldehyde (1-PyCHO) has been used as a fluorescent probe for the detection of moiré, keeping fixed concentration of 1-PyCHO (4.1 × 10⁻⁷ mol L⁻¹). The fluorescence spectra were recorded.

Figs. 2 and 4 shows the fluorescence quenching of CPZ and DSP respectively, recorded over the wavelength of 400 to 600 nm keeping the excitation at 368 nm with slit width of 5 nm. The fluorescence titrations were done with increasing concentration of bile salts added while the concentration of drugs was kept fixed (0.33 mM) at 300 K.

3. Result and discussion

3.1 UV-visible spectroscopy

This technique is useful for studying the interaction between drug and bile salts. The absorption spectra of CPZ and DSP in aqueous solutions with varying concentrations of bile salt NaC and NaDC shown in Figs. 1 and 3. The spectra of CPZ presented two characteristic peaks at 248 nm and 305 nm wavelengths, in which the shorter wavelength band is due to n–π* transition and longer wavelength is due to π–π* transition and also the presence of lone pair of electron on sulfur atom in tricyclic region of antidepressant drug CPZ [8]. In case of absorption spectra of DSP it appears at 250 nm. On the addition of bile salts the absorption intensity of antidepressants increases (red-shift). It is also observed from the Figs. 1(a), (b) and 3(a), (b) CPZ shows spectral shift of 5 nm at λmax 248 nm but the second spectra at 305 nm doesn't show spectral shift after addition of bile salts. Similarly DSP shows the spectral shift of 5 nm at λmax 250 nm. These spectral shifts show the interaction of drug and bile salts which further indicate the new complex formation between antidepressants and bile salts. When the drug enters to more hydrophobic micellar environment from the aqueous solution, it shows red shift in their absorption maxima. Changes in spectral
**3.2. Fluorometric Measurements**

To understand the interaction between antiretrovirals and bile salts, spectroscopic techniques such as steady-state fluorescence have been employed. The fluorescence emission spectra (Figs. 2 and 4) of CP2 and DSP show the addition of bile salts quenched the spectra of CP2 and DSP at 4/4 nm when shows the new complex formation between antiretrovirals and bile salts. The addition of constant volume of quencher (10 mM bio-surfactant solution) to the drug solution avoids complications due to dilution effects within titration experiments. Process of fluorescence quenching is explained by Stern-Volmer equation [41]

\[
\frac{I_0}{I} = 1 + K_q[Q]
\]

(2)

where \(I_0\) = fluorescence intensity of CP2 and DSP without quencher

\(I\) = fluorescence intensity of CP2 and DSP with quencher

\(K_q\) = Stern-Volmer constant

\(Q\) = concentration of quencher.

Figs. 6(a) and 7(a) show the plot of \(I_0/\langle Q\rangle\) and give the value of Stern Volmer constant shown in Table 1.

By applying eq. (2) we can calculate the value of binding constant \(K_b\) and binding sites \(n\)

\[
\log(I_0/I) = \log K_b + n\log[\text{Surfactant}]
\]

(3)

where, \(K_a\) = binding constant

\(n\) = binding sites.

The values \(K_a\) and \(n\) are given in Table 1. All systems show the value of binding capacity \(n\) is greater than unity. CP2 + NaC system shows higher binding capacity while other systems (CP2 + NaDC, DSP + NaDC and DSP + NaC) show less binding capacity indicating that they do not show significant binding to each other.

Using the value of \(K_a\) the Gibbs's free energy changes for binding obtained for this process from the Eq. (4).

\[
\Delta G_{\text{binding}} = -RT \ln K_a
\]

(4)
The negative value of Gibb’s free energy changes for binding ($\Delta G_{\text{binding}}$) ensure that the binding process is spontaneous and it is helpful for studying the interaction of drugs with bio-surfactants. The NaDC shows higher value of $K_b$ for both antidepressants than NaCl due to hydrophobicity which leads to their different binding abilities. It is also examined that between CPZ + NaCl and DSP + NaCl systems, the binding is stronger for former case showing higher binding affinity which also explains about the more hydrophobic nature of CPZ than DSP. In the case of CPZ + NaDC and DSP + NaDC, the previous one shows higher binding affinity. CPZ contains phenothiazine ring and positively charged group shows a better binding with negatively charged bile salt [36]. NaDC possesses more hydrophobic nature which promote the absorption as compared to NaCl. The binding constants ($K_b$) showed a considerable hydrophobic contribution mediated by electrostatic interactions of the positively charged drug with the head group of bio-surfactants [42].

4. Conclusions

This study deals with the spectroscopic investigation of interaction between antidepressants and bile salts which give the valuable and plentiful information about uses of bile salts in pharmaceutics. The spectroscopic techniques such as UV-visible and fluorescence have been employed for the determination of binding constant ($K_b$, Stern-Volmer constant ($K_v$), binding sites ($n$) and free energy changes for binding ($\Delta G_{\text{binding}}$). The value of binding constant ($K_b$, $K_v$) is found to be maximum for CPZ + NaDC mixtures from both the spectroscopic methods. More hydrophobic nature of NaDC is responsible for better interaction with antidepressants drugs. The negative values of Gibb’s free energy changes reveal the spontaneity of all the systems. The order of Gibb’s free energy changes of the studies systems is found to be: CPZ + NaDC ($-42.42$) < DSP + NaDC ($-19.84$) < CPZ + NaCl ($-19.76$) < DSP + NaCl ($-7.30$).

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References

Self-aggregation of bio-surfactants within ionic liquid 1-ethyl-3-methylimidazolium bromide: A comparative study and potential application in antidepressants drug aggregation

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ABSTRACT

Aggregation behavior of bio-surfactants (BS) sodium chloride (NaCl) and sodium deoxycholate (NaDCC) within aqueous solution of ionic liquid (IL) 1-ethyl-3-methylimidazolium bromide [emim][Br] has been investigated using surface tension, conductivity, steady state fluorescence, FT-IR and dynamic light scattering (DLS) techniques. Various interfacial and thermodynamic parameters are determined in the presence of different wt% of NaCl/NaDCC. Information regarding the local microenvironment and size of the aggregates is obtained from the fluorescence and DLS, respectively. FT-IR spectral response is used to reveal the interactions taking place within the aqueous NaCl/NaDCC solution. It is noteworthy to mention that increasing wt% of NaCl/NaDCC results in an increase in the spontaneous micelle formation and the hydrophilic IL shows more affinity for NaCl as compared to NaDCC. Further, the micelle solutions of BS-[emim][Br] are utilized for studying the aggregation of antidepressants drug promazine hydrochloride (PR). In-vivo spectroscopic investigation reveals interesting outcomes and the results show changes in spectral absorbance of PR drug on the addition of micellar solution (BS-[emim][Br]). Highest binding affinity and most promising activity are shown for NaCl as compared to NaDCC.

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1. Introduction

The scientific potential for research on ionic liquids (ILs) is virtually unlimited and ILs have opened up a new face of chemistry [1]. ILs is a fledging science and knowledge about the physical, chemical and biological properties of ILs is limited compared to conventional organic solvents although it is growing at a phenomenal rate [2-4]. The dual nature of ILs as electrolytes (co-solvents) [5] or as co-surfactants [6] encouraged us to study the impact of ILs on the aggregation behavior of bio-surfactants, surfactants, proteins, cyclodextrins, amino acids and drugs etc. [7-9]. One of the impressive features of ILs is that these are non-toxic, possess high conductive, non-volatile, non-flammable and high thermally stable etc. [10-12]. Therefore, these designer solvents can be exploited for various applications i.e., separation, extraction, aggregation, electrochemical and coating etc. [12-15].

Bio-surfactants (BS) are biodegradable surface-active molecules (or amphiphilic molecules) which are produced by micro-organisms such as bacteria, fungi and yeasts [16]. The hydrophobic moiety is a large chain fatty acid like hydroxyl fatty acid or α-alkylglycerol fatty acid [17] while the hydrophilic moiety can be a carbohydrate, an amino acid, cyclic peptide, phosphate and carboxylic acid among others. They show well-recognized surface and emulsifying properties [18,19]. They have similar properties of surfactants such as lowering interfacial tension, foam formation, surface and better solubilization or emulsification of hydrophobic organic compounds [20]. Due to their advantages over synthetic surfactants e.g., low toxicity, high degradability, environmental compatibility, high efficiency, they received significant interest from research worldwide for numerous applications such as chemical manufacturing, pharmaceuticals and contamination remediation, among many others [20-22].

Binary mixtures of ILs with BS show improved surface properties and they show remarkable physico-chemical properties than compared to conventional surfactants [23]. Study on the molecular interactions (electrostatic and hydrophobic) of “NaCl/IL” systems can help us to improve understanding on these biological systems and their potential applications in various fields [24]. The micelle formation between BS and ILs is currently a topic of immense importance [25]. There are some good articles devoted to investigating the micellization of imidazolium-based ILs with BS salts [26-28]. This now, the most
commonly investigated BS are mainly of glycolipids and phospholipids in nature. Wang et al. [27,28] studied the interaction between sodium cholate (NaC) and phospholipid vesicles using surface tension, TEM analysis and spectroscopic measurements. They observed the presence of phospholipid vesicles using the hydrodynamic interactions. These results reveal that the NaC induces solubilization of phospholipid vesicles into phospholipid/NaC mixed micelles. The transition to the micelle transition significantly affects the behavior of phospholipid vesicles, phospholipids/NaC mixed vesicles and phospholipids/NaC mixed micelles with curcumin. Hereuz et al. [29] have investigated these BS-based systems using two-dimensional diffusion order (2D-DOY) NMR spectroscopy to probe the micellar structure of sodium dodecyl sulfate and NaC in aqueous solution in the presence of organic solvent and metallic single walled carbon nanotubes. Further, Molk and coworkers [30] have shown the aggregation behavior of sodium cholate (NaC) and zwitterionic surfactants CMAP [24,2-ethylhexyl] and CMAP [24,2-hexyldecyl] dimethylammonium bromide (DAB) by using tensiometry, fluorometry, and DLS. The results are explored on the physicochemical properties of CHAPS. NaC and their mixtures. Mahajan et al. [31] have reported the interactions between NaC and NaC/NAB micelles using surface tension, steady state fluorescence and DLS measurements, where the interactions have been found to be highly synergistic. The evaluated various solubilization parameters using NMR measurements.

In the present investigation, the interaction of imidazolium-based 1-ethyl-3-methylimidazolium bromide [EMim][Br] is added to aqueous solutions of the two BS sodium cholate (NaC) and sodium deoxycholate (NaDC) with and without addition of [EMim][Br] were studied by surface tension, conductivity, steady state fluorescence, FT-IR and DLS techniques. We have also studied the effect of BS on solubilization process and surface properties i.e., critical micelle concentration (CMC), maximum surface excess concentration (F\text{max}) surface pressure at cmc (\gamma_{\text{cmc}}), minimum surface area per molecule (A_{\text{min}}) and efficiency of solubilization (\gamma_{\text{sol}}) using tensiometric method. The various thermodynamic parameters i.e., the standard Gibbs free energy of micellization (\Delta G_{\text{mic} }), the Gibbs energy of transfer (\Delta G_{\text{tr}}), the Gibbs energy of hydration per tail (\Delta G_{\text{h}}) of the BS were also measured by conductivity measurements. The cmc, aggregation number (N_{\text{agg}}), Stern-Volmer constants (K_{\text{sv}}) have also been studied by steady state fluorescence quenching method. DLS is used as a complementary technique, since it measures the diffusion of the particles which is also related to the structure and interaction present in the system. FT-IR spectroscopy was used to study the interactions that led to modification in the structure and function of the BS assemblies with BS. The investigation of the wave number and band width of different vibrational modes are used to illustrate the alkyl chain and intermolecular bond and group regions of BS molecules. These compounds are environmentally friendly since they are biodegradable, potential for industrial and environmental applications. The chemical structure of BS 1-ethyl-3-methylimidazolium bromide, cetyl pyridinium chloride, sodium cholate, sodium deoxycholate, antidepressant drug promazine hydrochloride and fluorocarbon pyrene are represented in Scheme 1.

2. Experimental Section

2.1. Materials

The bio-surfactants, sodium cholate, sodium deoxycholate, ionic liquid 1-ethyl-3-methylimidazolium bromide, promazine hydrochloride, cetyl pyridinium chloride and pyrene were purchased from Sigma-Aldrich Pvt. Ltd, Bangalore, India and were used without further purification. All the solutions were prepared in Milli-Q water.

2.2. Methods

2.2.1. Critical Micelle Concentration

The cmc values of NaC and NaDC were obtained from surface tension, conductivity and fluorescence studies. The cmc of the binary mixtures NaC+[EMim][Br] and NaDC+[EMim][Br] were determined by conductivity, surface tension and fluorescence studies as well.

2.2.2. Surface Tension

Surface tension measurements were carried out using a tensiometer (Kruss K12) by the ring detachment technique. For each experiment, the instrument was calibrated with Milli-Q water (72.0 ± 0.5 mN/m at 25°C) for the calibration. BS concentration was varied by adding corresponding surfactant stock solution in a beaker and reading was noted after thorough mixing. The cmc value was obtained from the shape change in the slope of the surface tension vs cmc (\gamma_{\text{cmc}}) is calculated at \gamma_{\text{cmc}} = \gamma_{\text{CMC}} - \gamma_{\text{CMC}}, where \gamma_{\text{CMC}} and \gamma_{\text{CMC}} are the surface tension of pure water and binary mixture NaCIL/NaDCIL, respectively.

2.2.3. Conductivity

The specific conductance was measured on an electrical conductivity meter (Synergy Type-369) equipped with a conductivity cell (cell constant=1). The cmc was determined from the break point of conductance vs [BS] concentration curve. The degree of counterion binding (\beta) is calculated as (1+\alpha), where \alpha = \gamma_{\text{CMC}}/\gamma_{\text{CMC}}\text{sp.}, i.e., the ratio of the slope after and before cmc.

2.2.4. Fluorescence Measurements

Fluorescence spectra of pyrene within aqueous NaC and NaDC systems (pure and mixed) were recorded using a Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies). An excitation wavelength (\lambda_{\text{exc}}) of 334 nm for pyrene, slit width (excitation slit 5 mm and emission slit 2.5 mm) and scan range 350-600 nm. The concentration of pyrene was \text{1} \times 10^{-4} \text{ M} throughout in our investigations. The concentration of quencher cetyl/trimethyl chloride (cpc) was varied in the range of 0 to 7 \times 10^{-5} \text{ M}.

2.2.5. Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instrument, Japan) with a He-Ne laser (633 nm) at 90° scattering angle at 25°C. An appropriate amount of BS was added to water or water-[EMim][Br] BS mixtures and taken in a quartz cuvette for DLS measurements.

2.2.6. Fourier Transform Infrared Spectroscopy

Infrared spectroscopic investigations of the BS systems (pure and mixed) were carried out using a Perkin-Elmer Spectrum BX FTIR spectrophotometer (Perkin Elmer, USA). The instrument was calibrated as all spectra were obtained by averaging 32 scans at 4 cm−1 resolution over the spectral range of 4000–400 cm−1 using the auto gain function and slit set at 100 with ATR/DRS modification for wavelength dependence.

2.2.7. UV-vis Spectroscopy

UV-vis absorption spectra of the drug were recorded at 300 K using a UV-visible spectrophotometer (Varian Cary-50). The concentrations of promazine hydrochloride drug (0.01 mol dm−3), NaC (2.33 mol dm−3) and NaDC (2.30 mol dm−3) were taken for spectral measurement in water and 0.10 wt% [EMim][Br]. A quartz cell having an optical cell length of 10 mm was used for the measurements. The wavelength range for the drug was recorded at 280 nm to 600 nm and absorption spectrum in the vicinity of 330 nm was observed caused by the complex of drug with BS.
3. Results and Discussion

Micellization of BS NaC/NaDC in aqueous solution at varying amount of [Emim][Br] has been investigated using surface tension, conductivity, fluorescence measurements.

3.1. Surface Tension

The surface tension (γ) of aqueous BS solutions in the presence of various wt% of IL [Emim][Br] is studied at 298 K as shown in Fig. 1. The cmc and other parameters obtained from surface tension measurements are reported in Table 1. Fig. 1 clearly shows the γ value decreases with increase in the wt% of IL, which reaches a break point known as cmc. Due to interfacial adsorption of BS molecules that allows them to orient at the air/IL solution interface, it results in a decrease in the surface tension of aqueous IL solution. In the present investigation, the observed cmc values are satisfactory to that reported in the literature [22-33]. Deset et al. [22] have studied the micellar properties of hydroxy bile salts and the observed cmc values of NaDC and sodium taurodeoxycholate (NaTDC) are lower than those of trihydroxy salts, NaC and NaTC. Patel et al. [33] have investigated the mixed micellization behavior of sodium taurocholate 12-18 and bile salts (NaDC and NaC)

![Graph A] (A) Surface tension (γ) versus log NaC (M) in the presence of different wt% [Emim][Br]. (B) Surface tension (γ) versus log NaDC (M) in the presence of different wt% [Emim][Br].
using surface tension measurement. They have shown that these surface active molecules significantly lower the surface tension and at low bile salt concentration micelles have low aggregation number due to hydrophobic interaction. Further, Wang et al. [28] have studied the interaction between NaC aggregates and quercetin in pH 7.4 sodium phosphate buffer solutions and the cnc increases due to the electrostatic repulsion.

The Gibbs adsorption isotherm is defined as the efficiency of adsorption at the boundary on the micellar surface [34]. These are calculated (Eq. (1)) from surface tension (γ) vs. logarithm of total concentration for amphilipic molecules (slope dγ/d log C) (Fig. 1):

\[ T_{\text{max}} = \frac{1}{2.303 n M} \int d\gamma / d \log C \]  

where, \( R \), \( T \) and \( C \) are gas constant, temperature, the concentration of BS, \( n \) is the constant (pre factors) value and has been taken as 2. The value of \( T_{\text{max}} \) decreases with increasing the concentration of [Emim][Br] as shown in Table 1. The \( T_{\text{max}} \) value for the mixture of NaC + [Emim][Br] is observed to be slightly larger compared to NaC + [Emim][Br]. At low concentration of IL, \( T_{\text{max}} \) value for all the binary system is lower except pour NaC Da due to formation of the micelle. The IL is hydrophilic in nature as well as surface inactive due to smaller alkyl chain length, hence they do not contribute to the surface properties. However; at high concentration of IL, \( T_{\text{max}} \) values for all systems are lower (NaC > NaC3), because once micelle formation (NaC3/NaC + IL) takes place the low amount required of BS. This is further confirmed by the value of minimum area per molecule (\( A_{\text{min}} \)) calculated using the Eq. (2),

\[ A_{\text{min}} = \frac{1}{T_{\text{max}}} \frac{n}{N_{A}} \]  

where, \( N_{A} \) is the Avogadro number. The calculated values of \( A_{\text{min}} \) are shown in Table 1. The \( A_{\text{min}} \) value of pure BS molecule is larger compared to a binary mixture of BS with [Emim][Br]. As probable, the \( A_{\text{min}} \) value for NaC + [Emim][Br] mixture is larger excluding NaC + [Emim][Br]. As the concentration of IIL is increased, the value of \( A_{\text{min}} \) is decreased which suggest that molecules are closely packed at the air-water interface. Due to increase in the repulsive interaction between IL and BS, it is satisfactory to explain the Eqs. (1) and (2) tend to obtain for \( T_{\text{max}} \) and \( A_{\text{min}} \) value must be opposite and the same phenomena have been observed. Higher values of \( A_{\text{min}} \) for BS than compared to conventional surfactants systems intended smooth orientation of the bile salt at the boundary.

The surface pressure at the cmc (\( \gamma_{\text{cmc}} \)) is calculated by using Eq. (3),

\[ \gamma_{\text{cmc}} = \frac{\gamma_{0} - \gamma_{\text{cmc}}}{2} \]  

where, \( \gamma_{0} \) and \( \gamma_{\text{cmc}} \) are the surface tension of the pure water and binary system of BS + IIL, respectively. The value of \( \gamma_{\text{cmc}} \) increases and \( \gamma_{\text{cmc}} \) decreases with an increase in the wt% of IIL [Emim][Br] presented in Table 1. [Emim][Br] is surface inactive in nature, therefore they do not contribute to the \( \gamma_{\text{cmc}} \) but they play an significant role in the complex process. Hence, the IL affects indirectly on the surface pressure, because the formation of micelle. Table 1 shows that an increasing wt% of IL results in reduction of \( \gamma_{\text{cmc}} \) value, which suggests the decrease in their efficiency. Also, the \( A_{\text{min}} \) value of NaC + [Emim][Br] complex is found to be lower compared to NaC + [Emim][Br] complex.

The \( p_{\text{Cuf}} \) is the efficiency of adsorption of surfactant at the air/ water interface; \( p_{\text{Cuf}} \) was calculated from the Eq. (4),

\[ p_{\text{Cuf}} = -\log C_{\text{uf}} \]  

The \( p_{\text{Cuf}} \) values of pure BS and their mixture with IIL are listed in Table 1. It has been observed that \( p_{\text{Cuf}} \) values increase with increase in wt% of [Emim][Br] in binary system NaC + [Emim][Br]NaC + [Emim][Br]. This is due to the reduction in surface adsorption of BS system. The \( p_{\text{Cuf}} \) values show an overall increase with increase in the wt% of IL, which is due to the reduction in surface adsorption of BS system. This is because IL [Emim][Br] is surface inactive in nature and does not contribute significantly to the surface properties.

3.2. Electrical Conductivity Measurement

The specific conductance (c) of the bile salt solutions in the presence of various concentrations of IIL/water mixtures was measured at 298 K. The conductance at various concentration of IIL (0.02, 0.05, 0.07 and 0.10 wt%) are shown in Fig. 2 and calculated parameters are given in Table 1. Fig. 2 shows a sharp intersection of two linear regimes i.e., (i) pre micelle and (ii) post micelle, corresponding to the monomeric form and micellar phase of the surfactants, respectively. For all the systems investigated, the specific conductivity increases with increasing the wt% of IL and the increase in the slope gradually decreases after formation of micelles. It is well established in the literature that from conductance measurements at the cmc region, degree of counter ion
dissociation (α) and counterion binding of the micelle (β) can be easily obtained for BS. Recently, Das et al. [32] have studied the cmc of dicyclohexyl sodium taurodeoxycholate (NaDc) at different temperatures. The obtained results are in accordance with the higher hydrophobic nature of dicyclohexyl sodium taurodeoxycholate to that of tributylhexyl sodium.

The degree of counter-ion dissociation (α) has been defined as the ratio of post to pre micelle slope (α = S2/S1 i.e., are below and above the cmc (Sm mol⁻¹)), and it is presented in Table 2. It is noticed that on increasing the wt% of IL, the α values gradually decrease for NaDc because the imidazolium ring of IL is bonded to the hydrophobic part of surfactant, but high concentration (0.10 wt%) of [Emim][Br] results in an increase in the α value as compared to pure bile salt BS NaC in the presence of counter ions (OH⁻) is more repulsive to the surface area of the micellar system than NaDc. Chen et al. group [33] has studied the thermodynamics and structural evolution of a vitamin-derived bolaamphiphile induced by NaC. This investigation may improve the thermodynamic mechanism after the structure transition of the macro-aggregates formed by amphiphiles in the gut. Various thermodynamic parameters have been calculated and listed in Table 2 to evaluate the interaction between imidazolium-based IL with BS at the air/water interface, as well as in bulk medium [38]. Sugihara et al. [36] have proposed a thermodynamic quantity for the given air/water interface (ΔG°(mic)) followed as Eq. (5):

$$\Delta G_{\text{mic}}^0 = \Delta H_{\text{mic}}^0 - T \Delta S_{\text{mic}}^0$$  

(5)

The value of ΔG°(mic) are listed in Table 2 as a measure of the synergism. The free energy change is defined as accompanied by the transition from bulk to the surface area of the solution or work needed at the interface per mole. NaDc has ΔG°(mic) values lower compared to NaC BS that means, the lower value of ΔG°(mic) is more thermodynamically stable. Similar behavior has been observed in the mixture (NaC/NaDc + IL), which suggests an enhancement in the spontaneity of the process. Moulik et al. [30] have studied the ΔG°(mic) value which is the lowest for pure 3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfate (CHAPS) and highest for NaC mixtures and explain both negative and positive deviations with a cross-over point at XCHAPS = 0.30. The positive variation that maintains from above 0.30 up to XCHAPS = 0.90 supports non-syrnergistic mixing.

Mean Errors α = ±0.03, β = ±0.03, ΔG°(mic) = ±0.01 kj mol⁻¹, ΔG°(mic) = ±0.01 kj mol⁻¹, ΔG°(mic) = ±0.01 kj mol⁻¹, ΔG°(mic) = ±0.01 kj mol⁻¹, ΔG°(mic) = ±0.02.

The standard Gibbs free energy of micellization (ΔG°mic) has been calculated using following Eq. (6);

$$\Delta G_{\text{mic}}^0 = (2-\alpha) R T \ln X_{\text{Cyc}} = (2-\alpha) \ln \frac{C_{\text{mic}}}{55.40}$$  

(6)

where α is the degree of counter-ion binding, Cmic are in mol dm⁻³, Xmic is the CMC in molar fraction unit and 55.40 comes from l dm³ of water which corresponds to 55.40 mol of water at 298 K. In all binary (NaC/NaDc + IL) system, the values of ΔG°(mic) are more negative, which indicates that the process of micellization is spontaneous and values are presented in Table 2. On increasing the concentration of [Emim][Br] in the binary system, an overall increase in the negative value of ΔG°(mic) has been observed. Table 2 shows that ΔG°(mic) values for the NaC binary system are larger than NaDc. The standard Gibbs energy of adsorption (ΔG°(ads)) was calculated according to following Eq. (7);

$$\Delta G_{\text{ads}}^0 = \Delta G_{\text{mic}}^0 - \frac{T_c}{T_{\text{mic}}}$$  

(7)

where, ΔG°(ads) is the standard Gibbs free energy of the adsorbed BS molecules and Tc/Tmic in Eq. (7), is expressed to convey the energy of amphiphile from a monolayer at a zero surface pressure in the micelle form. Here, calculated ΔG°(ads) values are listed in Table 2, for all the binary mixtures (BS + [Emim][Br]) are observed to be very large compared to ΔG°(mic). Which suggest that the work involved in transferring the free energy of BS from a monomer at zero surface pressure to micelle is more considerable and the value is negative suggesting the process is spontaneous. In comparison, both values of ΔG°(mic) and ΔG°(ads) of the binary mixture of NaC + [Emim][Br] are larger compared to NaDc + [Emim][Br]. The Gibbs free energy of micellization per allyl tail (ΔG°(mic)tail) is calculated according to Eq. (8);

$$\Delta G_{\text{mic}}^0 = \frac{\Delta G_{\text{mic}}^0}{2}$$  

(8)

Table 2 shows, the ΔG°(mic) values of NaC + [Emim][Br] are larger as compared to NaDc + [Emim][Br] binary mixtures, since the amphiphile tail group transfer the Gibbs free energy due to the fact that amphiphile...
tail is removed from the contact with IL mixture and transferred to the hydrophobic core of micelle. These are contribution to the transfer of Gibbs free energy of pure water and interaction between II with BS, it accounts for the solvophobic effect.

3.3. Fluorescence Study

The various micellar parameters of interest, such as cmc, aggregation number ($N_{agg}$), dipolarity, among others are obtained using fluorescence method (using pyrene as the probe). Fluorescence probe, pyrene is utilized to gain information on the cmc and dipolarity of aqueous NaCl/NaDC solution in the presence of different wt% of [Emim][Br]. Pyrene is one of the most widely used fluorescence probe as an aromatic hydrocarbon which is used for polarity studies, which shows significant vibrational band in its fluorescence spectrum in solution. The intensities ratio ($F_2/F_3$) of the first vibronic peak (373 nm) and third vibronic peak (384 nm) is highly sensitive to the polarity of the surrounding medium. Fig 3 shows the variation of $F_2/F_3$ vs log[NaCl] in the presence of different wt% of [Emim][Br]. At concentration, the ratio of $F_2/F_3$ remains constant and then decreases rapidly, which again attains an almost constant value with further increase in BS concentration.

Table 1 shows the cmc values of BS in the presence and absence of different wt% of IL. Fluorescence technique is used to calculate the cmc value which is similar to those observed from surface tension and conductivity techniques. In the present study, II shows more impact on aggregation of BS in aqueous solution. NaDC is strongly aggregated IL due to the hydrophobic interaction compared to NaCl. Wang et al. [27] have studied the phospholipid/NaCl mixed micelles by fluorescence techniques using pyrene as a probe and observed that micelles are formed above 20 mM NaCl. Nearly equal $I_2/I_3$ values are observed for phospholipid/NaCl mixed micelles and pure NaCl micelles indicating similar micro polarities for the two kinds of micelles. Wang et al. [28] have shown that the NaCl monomers gradually aggregate into dimers and quencher mixed with NaCl often gives higher fluorescence intensities than free quencher, which is confirmed in expressions of the hydrophobic binding of quencher with NaCl.

3.3.1. Aggregation Number

The aggregation number of NaC/NaDC in the presence and absence of [Emim][Br] were obtained by fluorescence quenching (PCP 10 mM) method according to the following Eq. (9):

$$\ln \left( \frac{F_0}{F} \right) = \left[ \frac{\text{[PCP]}_{\text{micelles}}}{\text{[BS]} - \text{[CMC]}_{\text{BS}}} \right] N_{\text{agg}}$$

where, $F_0$ and $F$ are an intensity of fluorescence emission spectra of pyrene in the absence and presence of quencher CPC, respectively. $[\text{PCP}]_{\text{micelles}}$, [BS] are the concentration of quencher CPC and BS. Aggregation number ($N_{agg}$) was calculated by using the fluorescence quenching method at different wt% of [Emim][Br] with BS mixture [26] and given in Table 3. The plots of $\ln (F_0/F)$ versus concentration of quencher (CPC) in 100 mM BS solution in the presence of various wt% of [Emim][Br] is presented in the Fig. 4 and S1. A good linear correlation is obtained at each concentration form 0 to 0.10 wt% of [Emim][Br]. The aggregation number for the mixed systems was obtained from the slope of the in $F_0/F$ versus concentration of [CPC] according to Eq. (9), is presented in Table 3.

The Table 3 shows smaller value of $N_{agg}$ for pure system compared to the binary system of BS+[Emim][Br], which is due to the intense packing of these II and hence more closely crowded micelle structure is formed. The electrostatic and hydrophobic interaction between the cation of the imidazolium ring and the head group of the BS is explained to be the reason for the increase in $N_{agg}$. The results suggest that hydrophobic interaction dominates over the electrostatic repulsion, this leads to the formation of a closely packed micellar structure. Table 3, clearly showed $N_{agg}$ value of the NaCl is larger compared to NaDC because in NaCl OH groups are responsible to easily form aggregates in [Emim][Br] than NaDC [38]. Literature reports specified the lower aggregation number of bile salts than common surfactants which has been supported here [30]. Pasi et al. [31] studies the microstructure evaluation of cationic Gemini surfactant, butanediol-1,4-bis(2-ethylhexyl)-trimethylammonium bromide (12–4–12) within NaCl and NaDC by SANS measurement. NaDC is seen to be more capable of altering the aggregation behaviour compared to NaCl. The micelles are formed with $N_{agg} = 78$. An electrostatic interaction is observed between 12 and 14–12 micelles and negatively charged bile salts.

The Stern-Volmer quenching constant ($K_{sv}$) was calculated using the following Eq. (10):

$$\ln (F_0/F) = 1 + K_{sv}[Q]$$

The Stern-Volmer quenching constant ($K_{sv}$) can be estimated from the achieved slope values of the plot in $F_0/F$ versus [CPC]. The $K_{sv}$ values are depicted in Table 3. The difference in the calculated data of $K_{sv}$ is explained in terms of the hydrophobicity of micellar solutions. Table 3 clearly shows that an increase in the wt% of II increases the $K_{sv}$ value (NaCl > NaDC).

3.4. Dynamic Light Scattering

Dynamic light scattering (DLS) is used to obtain the size of the micellar aggregates within aqueous BS (NaCl/NaDC) in the presence of different concentration of [Emim][Br]. Fig. 5, shows the scattered intensity for the hydrodynamic diameter of an aqueous solution of the two BS (NaCl and NaDC) at 0.10 M in the presence of [Emim][Br] under ambient condition. Mono modal distribution is obtained for NaCl in the presence of different concentration of [Emim][Br]. The peak diameter for aqueous NaCl/NaDC in the presence of a varying amount of [Emim][Br], respectively is presented in Table 3. The average radius of pure BS NaCl shows a bimodal distribution with hydrodynamic radii larger $D_h = 430.6, 136.8$ (nm) and a polydispersity index PDI = 0.642. The increased wt% of II reduces the monomodal distribution (a) for 0.02 wt% [Emim][Br], $R_h = 401.6$ nm, PDI = 0.590, (b) for 0.10 wt% [Emim][Br], $R_h = 253.6, 854.6$ nm and a PDI is 0.682. The increased wt% of [Emim][Br] reduces the hydrodynamic radii (a) for 0.02 wt% [Emim][Br], $R_h = 368.7, 1.578, 5.415$ nm, PDI = 0.630 and (b) for 0.10 wt% [Emim][Br], $R_h = 157.9, 630.3, 1.542$ nm, PDI is 295.1. These results suggest that [Emim][Br] forms aggregates with BS and the BS-IL complex is formed. On the whole studies, the data show formation of micelle-like...
<table>
<thead>
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<th>[Emim][Br] wt %</th>
<th>NaC</th>
<th>NaDC</th>
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<tbody>
<tr>
<td></td>
<td>cmc (mM)</td>
<td>$N_{agg}$</td>
</tr>
<tr>
<td>Water</td>
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</tr>
<tr>
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<td>64</td>
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<tr>
<td>0.10</td>
<td>2.91</td>
<td>89</td>
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Mean Errors $N_{agg} = \pm 0.5$, $K_w = \pm 0.02$, $R_a = \pm 2.00$ (nm) PDI = $\pm 2.20$.

aggregates even in the presence of [Emim][Br]. Zhang et al. [37] have studied the aggregation of CHAPS (3-[3-cholamidopropyl]dimethyl ammonio)-1-propanesulfonate, a zwitterionic surfactant and reported an $D_h = 3$ and 2.8 nm by NMR and TEM measurements. Blume et al. [40] reported the aggregation of sodium cholate (NaC) and sodium deoxycholate (NaDC) in water and 0.1 M NaCl at pH 7.5, 1 nm at 298 K. Our results fairly agreed with literature reports. Anionic BS NaC and the zwitterionic non-ionic surfactant CHAPS (3-[3-cholamidopropyl]dimethyl ammonio)-1-propanesulfonate is aggregation number (N), hydrodynamic diameter ($D_h$) values were 1-1 and 3-4 nm for 75 mM NaC and 50 mM CAHPS, respectively in 200-323 K reported by Moulk et al. [30] The overall average trend in peak diameter of micellar aggregates is similar for both the BS; diameter appears to increase at 0.10 wt% IL additions. It is observed that increase in the wt% of [Emim][Br] IL increases the peak diameter (NaC, NaDC). IL-BS interaction, which plays the significant role in changing the characteristic of BS cited with vertically polarized light. Change in the micelle aggregate within BS-IL (0 to 0.10 wt %) is shown by electrostatic attraction. As explained earlier, electrostatic attraction between OH⁻ on NaC moieties with the micelles in a manner that renders the average micellar size fairly large. Within NaC, however, the lack of such interaction combined with the anionic nature of the surfactant along with the presence of Na⁺ counter ion resulted in much more compact micelles. Mahajan et al. [39] have studied the size of P84 and P84-SDC mixed micelles and the size is found to be 13 and 5 nm, respectively. However, after the CLZ loading, $D_h$ values were observed to be 18 and 7 nm, respectively.

3.5. Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy is a valuable tool in order to obtain molecular information of BS, classify hydrogen bonds between interfacial water molecules and head groups of BS. Furthermore, since FT-IR is well suitable for the studies of the change in the structure of micelle in the presence of imidazolium-based IL.

NaC and NaDC were irradiated using [Emim][Br] IL and characterized with FT-IR spectroscopy before and after irradiation. Their FT-IR spectra are shown in Figs. 6–7. The frequencies before and after irradiation of NaC and NaDC were listed in Table 4. Significant changes have been observed due to the irradiation process. The strong absorption bands range at 3700–3000 cm⁻¹ were found which correspond to the presence of OH⁻ stretching for NaC and NaDC. The C–H stretching bands shift and become an even broader band after irradiation. However, the OH⁻ stretching vibrations band shift from 3382 cm⁻¹ to a broad band situated at 3486 cm⁻¹ for NaC. The C–H symmetry stretching bands were shifted from 2978 cm⁻¹, 2930 cm⁻¹ and 2868 cm⁻¹ to 3090 cm⁻¹, 2984 cm⁻¹ and 2942 cm⁻¹. Hao et al. [40] study a biological amphiphile NaDC in aqueous solution by addition of inorganic salts and changing pH by FT-IR measurements and small angle XRD. The major differences of peak positions before and after irradiation are about 4 cm⁻¹, which shows that the packing of C–H chain is changed after irradiation. The possible peak of NaC and NaDC of C–O and COO⁻ groups was less outstanding due to the C–O stretching. This is a sharp band at 1630 cm⁻¹, while after irradiation it becomes a broad band at 1649 cm⁻¹, which also indicates that hydrogen bond associations were rearranged and more hydrogen bonds were formed.

The vibrational frequencies in the O–H stretching vibration region for the pure [Emim][Br] IL molecules pure form can be observed in Fig. 6. The asymmetric $v_{asym}$ was 3283 cm⁻¹ and symmetric $v_{sym}$ (CH₃) of C–H stretching vibrational frequencies are located at 2939 cm⁻¹ and 2865 cm⁻¹ respectively. The CH₃ signal intensities are lower than those for the CH₂ stretching features are probable. The strong and sharp peak was obtained in the range 1572 cm⁻¹ (C–O stretching) and 173 cm⁻¹ (CH₂ stretching) for pure [Emim][Br] IL. For NaDC, the

![Fig. 4. Pyrene (1 µM) fluorescence quenching by CPC in 120 mM aqueous bio-surfactants in the presence of different wt% of [Emim][Br] respectively, i.e., (A) 0.10 wt% [Emim][Br] + NaC (B) 0.10 wt% [Emim][Br] + NaDC. Solid line represent the result of the linear regression analysis.](image-url)
O—H stretching vibrations bands were obtained at 3567 cm⁻¹, 3553 cm⁻¹ and 3329 cm⁻¹. The CH₂ asymmetric and symmetric vibrational feature (v₁ and v₃) is the most intense band in the NaDC spectrum, 1572 cm⁻¹ (C—O stretching) and 1173 cm⁻¹ (CH₂ stretching) were also found in the NaDC molecules Fig. 6(B). These are some peaks, which is identical for [Emim][Br] IL and NaDC.

Xu et al. [41] have studied the NaDC and NaCl and NaBr in sodium phosphate buffer, the addition of two kinds of amino acids (l-lysine and L-arginine) to NaDCNaX hydro gels, the gel becomes solution at room temperature is characterized by TEM, SEM, X-ray powder diffraction, FT-IR and rheological measurements. Fig. 7(C) presents the IR absorption spectra for mixed NaDC-[Emim][Br] IL, where the absorption band reduce in the similar region. The band shift from higher 2939 cm⁻¹, 2865 cm⁻¹ and 2939 cm⁻¹, 2865 cm⁻¹ to 2981 cm⁻¹, 2868 cm⁻¹ lower frequency for [Emim][Br] IL and NaDC that the number of gauche conformers reduces and the number of particularly structured all trans conformers of alkyl chain increases. The differences between the spectra of [Emim][Br] IL and NaDC is a hydroxyl group (OH⁻) and C=O, COO⁻ group, therefore, they have comparable structure modification after irradiation. The results show that the structure
of [Emim][Br]IL and NaDC has been changed. The results also show that their binding with NaDC and [Emim][Br]IL after irradiation. The calculation spectra of NaC and NaC after and before irradiations were exposed in Scheme 1. The changes of the FT-IR spectra of the carboxyl acids and carboxylates samples comprise salicylic acid, sulfosalicylic acid, cholic acid, deoxycholic acid, sodium cholate and sodium deoxycholate, it proved that the spectral variations of the example induced by free electron lasers facilities (FEL) are closely associated with their hydrogen bond systems is reported of Wu et al. [42] Mahajan et al. [38] and our group has been studied the complexation of drug Clozapine (CIZ) and piracetam, SDC and confirmed using Powder X-ray diffraction (PXRD) and FT-IR techniques.

In conclusion, for NaC and NaDC, the interaction of [Emim][Br]ILs can induce the dissociation and rearrangement of hydrogen bond structure. There are many hydrogen bonds formed by H$_2$O, OH$^-$, COO$^-$ in the structures of NaC/NaDC, which have energy delivery, are simple to be significant with H$_2$O, these are dissociated/rearranged. When the ligands synchronize to the metal ion (Na$^+$), Na$^+$ is synchronized to OH$^-$ and COO$^-$, the hydrogen bond association. The C=O of COO$^-$ stretching band is two sharp peaks at 1714 cm$^{-1}$ and 1695 cm$^{-1}$, while after irradiation they show a broadband at 1653 cm$^{-1}$. Two sharp peaks of C=O vibrations become an expansive band; generally, complexation with Na$^+$ can bring this kind of changes. Here, the irradiations of [Emim][Br]$^+$ also cause the large changes of C=O vibrations, which show that hydrogen bond networks containing C=O were distorted after irradiation. These results specify that the hydrogen bond arrangement was dissociated and rearranged after irradiation for the two samples. Considering the obvious variation in skeleton vibration, it is suggested that the molecular skeleton of these two bile salts vary after irradiation.

For example, for NaC, the peak positions of many bands have shifted and comparative intensities were changed in the fingerprint region; the comparative intensity at 1472–1339 cm$^{-1}$ bands is increased; the 1085 cm$^{-1}$ and 1049 cm$^{-1}$ bands have shifted to 1176 and 1088 cm$^{-1}$ frequency region.

4. UV-visible Spectroscopy

In this study, the absorption spectra of drug promazine hydrochloride (pH within [Emim][Br]$^+$ added aqueous NaC and NaDC solutions are taken to confirm the formation of complexes. Since NaC and NaDC have almost no absorption band through the wavelength range 300–500 nm (Figs 8 and S2), the absorption band for the drug was observed at $\lambda_{max} = 300$ nm. Changes in the intensities are the indication of an interaction of drugs with bile salts. Binding of bile salts to drug molecules was calculated using the Benesi-Hildebrand Eq. (11).

$$\frac{1}{A-A_0} = \frac{1}{[\text{Bile Salt}]} + \frac{1}{A_{max}-A_0} \times \frac{1}{[\text{drug}]}$$  \hspace{1cm} (11)

where, $A$ is the absorbance at an intermediate concentration of bile salts, $A_{max}$ is an absorbance at the infinite concentration of bile salts and $K$ is the binding constant. When, we plot the graph between $1/(A - A_0)$ and $1/[\text{drug}]$, it gives a straight line shown in Figs 8 and S2, which reveals that antidepressants drug (pH) and bile salts (NaC and NaDC) formed the 1:1 complex between them. The binding constants ($K$) calculated from the ratio of intercept and slope of Benesi-Hildebrand plot is 4.5 mol dm$^{-3}$ for NaC and 2 mol dm$^{-3}$ for NaDC respectively. The values of binding constants illustrate that NaC shows the more binding affinity towards the antidepressants drugs.

Rub et al. [43] have investigated that the mixed micelle formation of hydrotopes (p-aminothiobutyl hydrochloride and orto-thiobutyl hydrochloride) with promazine hydrochloride (pH) in absence and presence of NaCl at different temperature. The evaluated values of cmc were established to be inferior to cmc$^{34}$ values suggesting attractive

| Table 4: The characteristic vibrational bands of FT-IR spectra of NaC/NaDC before and after [Emim][Br] IL in liquid irradiation. |
|-----------------|----------------|----------------|----------------|
| Preliminary Assignments | NaC Original (cm$^{-1}$) | NaC After [Emim][Br] IL (cm$^{-1}$) | NaC Original (cm$^{-1}$) | NaC After [Emim][Br] IL (cm$^{-1}$) |
| C=O | 3567, 3550, 3329 | 3578, 3565, 3342 | 3382 | 3488 |
| CH | 2978, 2950 | 2978, 2936, 2868 | 2978, 2930, 2868 | 2978, 2932, 2842 |
| CH$^+$ | 1716, 1695 | 1643 | 1630 | 1649 |
| CH$^+$ | 1440 | 1460 | 1578 | 1598 |
| CH$^+$, C=O, C-O, C=O, C=O | 1402, 1300, 1130, 1048 | 1454, 1338, 1170, 1094 | 1401, 1271, 1085, 1049 | 1472, 1338, 1170, 1098 |
interactions linking both components in the solutions. NaCl effectively reduces the cmc of pure amphiphiles and their mixed systems as a result of electrostatic interactions. The negative values of free energies of combination confirm the stability of the mixed system of drug and hydrospores. Patil et al. [44] have studied the interaction of two drug PH and chlorpromazine hydrochloride (CPZ) molecules with 
cyclodextrin showing important differences in the mode of interaction. The activity coefficients are higher (for 72) and lower (for 22) than that expected on the basis of the Debye-Hückel limiting law for a 1:1 electrolyte. The effect of complexation is established to be more in the case of CPZ than PH.

5. Conclusions

In this work, the aggregation behavior of BS (NaCl and NaDC) in presence of imidazolium-based IL [Emim] [Br] is investigated with the help of surface tension, conductivity, fluorescence, FT-IR spectroscopy, and dynamic light scattering. The overall results indicate the partitioning of [Emim] [Br] into the micellar phase of NaCl/NaDC. Addition of [Emim] [Br] results in the considerable change in the properties i.e., cmc, interfacial parameter, thermodynamic properties, N_{agg} micellar size, R spectra of aqueous NaCl/NaDC A significant decrease in cmc and increase in N_{agg} upon addition of [Emim] [Br] to aqueous NaCl/NaDC indicates a favorable micellization process in the presence of IL. The negative values of δX_{Agg} and δX_{ion} confirms that micelle formation and adsorption of NaCl/NaDC at air/water interface is energetically favorable. NaDC shows the lowest value of G_{mic}, which indicates the formation of the more thermodynamically stable surface. In agreement with both FT-IR and DLS results, it is shown that IL interacts with NaCl/NaDC BS and induces compositional/structural changes. UV-Visible spectroscopy exposes that PH drug has a more binding affinity and most capable action are shown by NaCl than NaDC. These studies point towards a new dimension to the research on IL-BS systems and their various applications in biomedical science as well as bioindustries.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2018.01.079.

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