Indian Journal of Chemistry
Vol. 59A, August 2020, pp. 1128-1135

# Interfacial and thermodynamic approach of surfactants with α-chymotrypsin and trypsin: A comparative study

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*Received 24 April 2020; revised and accepted 15 June 2020*

This work deals with the interactions among cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) with α-chymotrypsin (α-CT) and trypsin in aqueous medium on pH 7.75 by conductivity and surface tension measurements. The critical micelle concentrations (CMC), surface parameters *i.e.,* the maximum surface excess concentration ($\Gamma_{max}$), minimum area per surfactant molecule ($A_{min}$), the surface pressure at CMC ($\pi_{CMC}$) and thermodynamic parameters *i.e.,* degree of ionization (α), Gibbs free energy of micellization ($\Delta G°_m$), the standard Gibbs energy of adsorption ($\Delta G°_{ads}$), the free energy at air-water interface ($\Delta G^s_{mim}$) have been evaluated. The CMC has increased and surface tension of CMC ($\gamma_{CMC}$) values have decreased (at maximum μL of α-CT and trypsin), significantly in the presence of different μL of the added α-CT and trypsin. In this study, $\Delta G^0_{ads}$ value is established to be greater than $\Delta G^0_m$, showing that adsorption is more favored in aqueous surfactants systems. Thermodynamic parameters show that enzyme-CTAB/SDS monomeric aggregation started to form micelles at a higher concentration of surfactant to compare with the CMC of pure CTAB/SDS micelles. It is significant that increasing the μL of α-CT and trypsin results in an increase in the spontaneity CMC on surfactants, α-CT and trypsin have more affinity for SDS compared to CTAB.

**Keywords:** α-Chymotrypsin, Trypsin, Surfactant, Micellization Behavior, Conductivity, Surface tension

Surfactants are amphiphilic molecules and these shows the aggregation behavior of various molecules viz, DNA, enzyme, ionic liquids and amino acid under different condition[1-4]. Surfactant consist of a hydrophilic (water soluble) and non-polar hydrophobic part, usually a straight / branched hydrocarbon chain (containing 8-18 carbon atoms)[5,6]. Surfactants have shown the different physicochemical properties, *i.e.* high detergency, high viscoelasticity, high surface wetting capability, high solubilization, a better tendency to lower the oil–water interfacial tension than their single chain analogues[7-9]. Surfactants have shown various applications *viz.* wetting agents, cleaning agents, dispersants, foaming agents, emulsifiers, soaps, shampoos, antiseptics and corrosion inhibitors[5,6,10]. Trypsin and α-chymotrypsin (α-CT) are significant mechanism of the enzymatic barrier[11]. They can mortify the beneficial proteins and peptides, as a result inhibit their activity and thus decrease their oral bioavailability[12]. Individual kind of indirect protease inhibitors have shown proof of concept in clinical trials[13].

Surfactant has varied intensive properties in the solution such as 'self-assembly', this called micelles, and development occurs to denote the critical micelle concentration (CMC)[14-17]. Verma et al.[18] has studied the interactions among cetyltriphenylphosphonium bromide (CTPB) with α-chymotrypsin (α-CT) and trypsin in aqueous medium at pH 7.75. The surface parameter and thermodynamic parameters has been calculated using surface tension and conductivity method. The hydrolysis of p-nitrophenyl acetate and p-nitrophenyl benzoate catalyzed by trypsin in the presence of CTPB, cetyltrimethyl ammonium bromide (CTAB) and SB3-12. Also, Verma and Ghosh[19] studied the interaction between tetradecyl triphenyl phosphonium bromide, CTPB, CTAB, cetyltrimethyl ammonium chloride (CTACl), cetyldiethylethanol ammonium bromide (CDEEAB), tetradecyltrimethyl ammonium bromide (TTAB), and ($C_{16}$-3-$C_{16}$, 2Br-) gemini surfactant at different pH (3.1, 7.0, and 7.75) by conductivity and surface tension measurements. The CMC, interfacial and thermodynamic parameters have also been determined. Verma et al.[19] studied the interaction between sodium dodecylsulphate (SDS), CTAB, and polyoxyethylene lauryl ether (Brij-35) with 1-ethyl-3-methylimidazolium bromide [Emim][Br]. Various

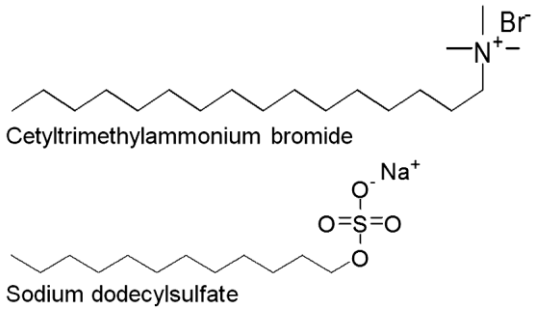
interfacial properties and thermodynamic parameters were determined by the surface tension and conductivity method. Adachi et al.[21] studied the careful separation of trypsin from a mixture in proteins, i.e., pancreatin, using trypsin inhibitor immobilized in the reverse micelles. The immobilization efficiency of trypsin inhibitor and also the forward and backward extractions of trypsin were done. Yu et al.[22] studied the hydrolysis of peptide bonds in Trypsin, it is play a central role in catalyzing. Liquid crystals (LCs) residential by utilize as the enzyme substrate bovine serum albumin (BSA) and dodecyltrimethyl ammonium bromide (DTAB) as the manager for association of LC. The DTAB could form a self-assembled monolayer at the aqueous/LC interface to produce the dark optical images of LCs.

In present investigation, the interactions among CTAB, SDS with α-CT and trypsin in aqueous medium on pH 7.75. The effect of α-CT and trypsin on micellization behavior and surface properties, i.e., CMC, surface excess concentration ($\Gamma_{max}$), surface pressure at CMC ($\pi_{CMC}$), minimum area per molecule ($A_{min}$), the efficiency of adsorption ($pC_{20}$) by tensiometric method. The various thermodynamic parameters, i.e., the standard Gibbs free energy of micellization ($\Delta G°_m$), Gibbs energy of adsorption ($\Delta G°_{ads}$), Gibbs energy of transfer ($\Delta G°_{trans}$), Gibbs energy of micellization per alkyl tail ($\Delta G°_{tail}$), air-water interface ($\Delta G^s_{min}$) have also been evaluated. The chemical structure of CTAB and SDS are shown in Scheme 1.

## Materials and Methods

### Materials

CTAB (BioXtra, ≥ 99%), SDS (ReagentPlus®, ≥9 8.5% (GC)), potassium chloride (BioXtra, ≥ 99.0%), α-CT (≥40 units/mg protein, vial of 5 mg) and trypsin (powder, ≥7,500 BAEE units/mg solid) were purchased from Sigma Aldrich Pvt. Ltd. Bangalore, India. All the solutions were prepared by double distilled water.




Scheme 1 — Chemical structure of cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS).

### Methods

#### *Conductivity*

The conductance was measured on the electrical Systronics Type-306 conductivity meter operational with a conductivity cell. Before the measurements, the conductivity cell was calibrated with the 0.01 M and 0.1 M aqueous KCl solutions. The cell constant was determined to be 1 $cm^{-1}$. At least three measurements were performed for all concentration. The conductance measurement was taken after stirring the solution with each addition. The graph plot between conductivity versus concentration of surfactants (M) since observed the break point of each curve is known is CMC.

#### *Surface tension*

Surface tension measurements were done with a Tensiometer (Jencon Kolkata) using a platinum ring by the ring detachment method at 300 K. The surface tension of double distilled water i.e. 72 $mNm^{-1}$ was used for the calibration purpose. The both anionic and cationic surfactants concentration (M) was diverse by adding concentrated surfactants solution in small installment. Analyses data were noted after thorough mixing. A measured surface tension value was noted and graph plot between surface tension versus logarithm of surfactants concentration by using Origin Pro 6.1 software.

## Results and Discussion

### Determination of critical micelle concentration

CMC was determined for two selected conventional cationic i.e., CTAB and anionic i.e., SDS surfactants with enzyme (CTAB+ trypsin/α-CT and SDS+ trypsin/α-CT) with the help of conductivity and surface tension methods at 300 K. Table 1, shows the CMC value of all CTAB/SDS and mixture of α-CT and trypsin. The calculated CMC values by both surface tension and conductivity techniques are good agreement with reported in the literature value[7].

Zdziennicka et al.[22] studied the effect of methanol, ethanol, 1-propanol on CTAB by using surface tension, density, viscosity and conductivity measurements in aqueous solutions. Alcohols significantly affect the CMC of CTAB and the degree of counter ions bound to its micelles. The mixed micelles of CTAB with methanol are most likely formed in the entire range of alcohol concentration. Wang et al.[23] investigated the effects of CTAB on imidazolium-based IL (CnmimBr, n = 10, 12, 16) and interfacial parameters, aggregation of these surfactants

Table 1 — The critical micelle concentration (CMC) of cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) in the presence of different μL of α-CT and Trypsin at 7.75 pH media at 300 K

| Type of Proteins | Volume of Proteins (μL) | CMC (mM) | | | |
|---|---|---|---|---|---|
| | | Conductivity | Surface tension | Conductivity | Surface tension |
| | | CTAB | | SDS | |
| α-CT | Water | 1.2±0.03 | 1.0±0.03 | 8.1±0.05 | 8.1±0.05 |
| | 13 | 2.0±0.04 | 2.0±0.05 | 10.0±0.07 | 11.0±0.06 |
| | 33 | 2.6±0.05 | 2.4±0.07 | 13.8±0.08 | 13.5±0.07 |
| Trypsin | 13 | 1.8±0.05 | 1.6±0.06 | 12.2±0.06 | 12.8±0.06 |
| | 33 | 2.4±0.06 | 2.2±0.07 | 14.5±0.08 | 14.2±0.08 |

explored by surface tension and conductivity measurements. The increase of the CMC with temperature was observed, while $pC_{20}$, $\Gamma_{max}$, and standard entropy of aggregation were decreased. Safari et al.[24] investigated the aggregation behavior of SDS and CTAB aqueous solutions and water-ethylene glycol by using surface tension, conductometry, cyclic voltammetry, zeta potential measurements, transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. The degree of counter ion dissociation (α), CMC, aggregation numbers, interfacial properties, interparticle interaction parameters, and morphology of aggregates were determined. Zeta potential and size of the aggregates were indomitable using dynamic DLS and established the models recommended for the process, taking place in each system.

**Conductivity measurement**

The plots between conductivity versus surfactants concentration (mM) at different μL of α-CT/trypsin at 300 K are shown in Fig. 1a and b. Here, a characteristic performance was observed which can be described by the reality of two linear regimes with different slopes: (i) pre-micellar region and (ii) the post-micellar region. The cross intersection of these two linear regimes is known as the CMC. Lower the CMC and the conductivity values are increases with concentration due to increasing number of free ions in the solution, as no micelles presented in the surfactant systems. It increases gradually above the CMC due to attraction of fraction on counter ions to the micellar surface thus reducing the number of present carrier. Also, due to lower mobility of micelles they give to a lesser extent to conductance. The CMC value of pure aqueous SDS solution obtained by us is in good agreement with the literature value[7]. The micellization behavior depends on the electrostatic interactions between the hydrophobic interactions and charged head groups among the hydrocarbon tail groups.

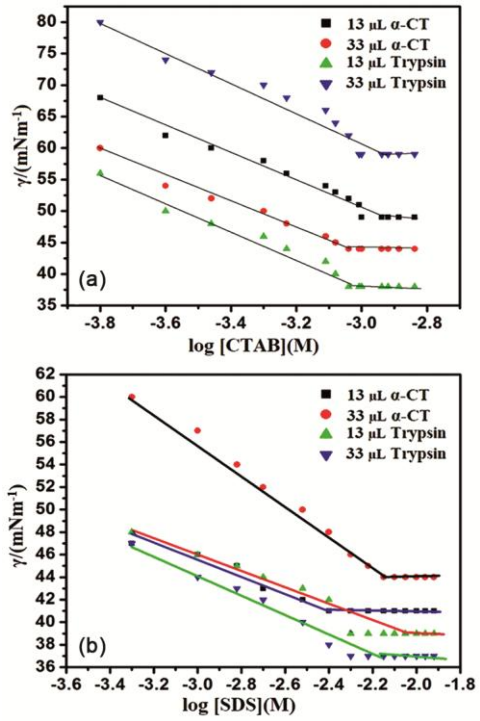



Fig. 1 — (a) Specific conductance (κ) versus concentration of CTAB (M) in the presence of different μL of α-CT and Trypsin at 300 K and (b) Specific conductance (κ) versus concentration of SDS (M) in the presence of different μL of α-CT and Trypsin at 300 K.

Wang et al.[25] studied the effect of acetonitrile on the CMC of SDS by conductometry and also the effect of phosphate buffer on the CMC of SDS in acetonitrile-water binary solvent was studied by fluorometry with used of pyrene as a probe. As results observed the CMC of SDS first decreased up to 3 % (v/v) and then increased with increasing of the volume ratio of acetonitrile to water up to 5 % (v/v). Mosquera and co-workers[26] investigated the interaction between n-dodecyl sulfate, n-DTAB, and chlorpromazine hydrochloride by using the conductivities and dielectric constant measurements in water at 25 °C. In this new technique, the CMCs are directly obtained as singular points in the dielectric constant/concentration

curves, and thus, this technique is an alternative to the determine CMC's from conductivities.

**Surface tension measurements**

The important aspect of surfactants is the ability to lower the interfacial tension between aqueous solutions. In the present study, we examined the surface tension of aqueous CTAB/SDS surfactants system in the presence and absence of different μL of α-CT/trypsin. The both surfactant concentration required to oversupply the air/solution interface is the CMC which results in a break point in the surface tension versus logarithm surfactants (M) plot as shown in Fig. 2. A careful examination of the surface tension versus logarithm surfactants (CTAB/SDS) plots after addition of different μL of α-CT/trypsin (Fig. 2a) reveals that as the concentration and counter ions of the surfactants surface tension of the aqueous CTAB/SDS increases. Table 1, shows the CMC values of both cationic and anionic surfactants, i.e., CTAB and SDS in the presence and absence of different μL of α-CT/trypsin. Surface tension technique is simple and sensitive method to calculate the CMC value. The CTAB+Trypsin/α-CT and SDS+Trypsin/α-CT interaction measured by tensiometric method as shown in Fig. 2. A lineally

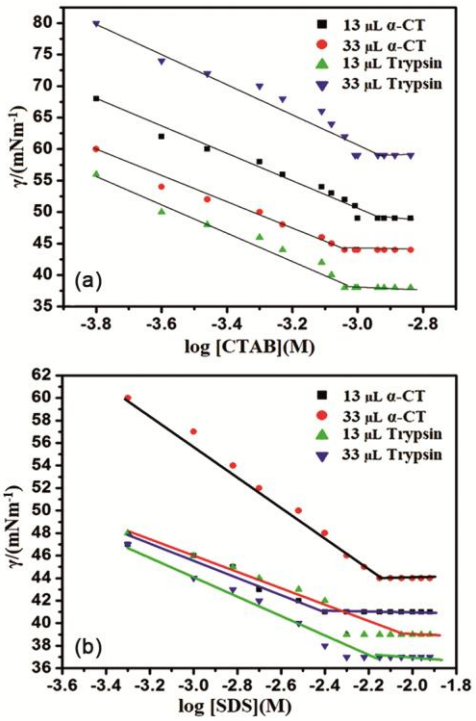



Fig. 2 — (a) Plots of surface tension versus logarithm of [CTAB] concentration (M) in the presence of different μL of α- CT / Trypsin and (b) Plots of surface tension versus logarithm [SDS] concentration (M) in the presence of different μL of α- CT /Trypsin.

decrease in surface tention (γ) is observed with increase in CTAB/SDS concentration for all the systems. The anionic surfactants have more interaction in both enzyme (trypsin/ α-CT) because of the presents in anions ($SO_4^{-2}$), which is the most responsive to the hydrophobic interaction except CTAB. The evaluated CMC values are in good agreement with those obtained from conductivity analysis.

Benny et al.[27] studied the CMC of SDS by surface tension and conductivity measurements and also dye micellization, by coumarin-6 as a fluorescent probe for CMC determination. Pal et al.[28] investigated the complexation between poly(acrylic acid sodium salt) [NaPAA] and lauryl isoquinolinium bromide [C12iQuin][Br] in aqueous solution by using surface tension, isothermal titration calorimetry, and conductance. They evaluated CMC, surface parameters and the thermodynamic parameters. The results obtained from DLS and turbidity measurements show that size of the aggregates first decreases, and then increases in presence of polyelectrolyte. Tsubone and Ghosh[29] investigated the micellization behavior of (GA) $(CH_2)_2[N(COC_{11}H_{23})\ CH(CO_2H)CH_2(CO_2H)]_2{\cdot}2NaOH$ gemini surfactant having N,N-dialkylamide, carboxyl, and carboxylate groups, in NaCl at pH 5.0 by surface tension and fluorescence methods. The higher CMC value was close to that observed by the surface tension method.

Mahajan et al.[30] studied the effect of the SDS, dioctylsulphosuccinate sodium salt (AOT), DTAB and didodecyldimethylammonium bromide (DDAB), on trifluoperazine dihydrochloride (TFP) by using surface tension, fluorescence and electronic absorption measurements. Various interfacial, micellar, spectroscopic and corresponding thermodynamic parameters were calculated from these techniques. The values of the interaction parameter (β) recommend that cationic surfactants show less synergistic interactions with TFP compared to anionic surfactants.

**Degree of micellar ionization (α)**

Degree of micellar ionization (α) can be obtained from the slopes of the two linear curves form conductivity using the following Eqn (1),

$$\alpha = \frac{S_2}{S_1} \qquad \ldots. (1)$$

where, $S_1$ and $S_2$ are the particular belief below and above the CMC. α adsorbed at their interface, concentration of activist charged particles and decreased the conductivity. α calculated from the ratio

Table 2 — Surface excess parameter ($\Gamma_{max}$), surface pressure at CMC ($\pi_{CMC}$), minimum surface area per molecule ($A_{min}$) for cationic (CTAB) and anionic (SDS) surfactant in the presence of α-CT/ Trypsin at 7.75 pH media at 300 K

| Type of Proteins | Volume of Proteins (μL) | Interfacial Parameters | | | | |
|---|---|---|---|---|---|---|
| | | $\Gamma_{max}$ ($10^4$ mol·m$^{-2}$) | $A_{min}10^{20}$ (m$^2$ mol$^{-1}$) | $\pi_{CMC}$ (mN·m$^{-1}$) | $\gamma_{CMC}$ | $pC_{20}$ |
| CTAB | | | | | | |
| α-CT | water | 1.89±0.04 | 87.5±0.07 | 34.0±0.06 | 38±0.06 | 3.0±0.04 |
| | 13 | 0.89±0.02 | 67.2±0.05 | 26.0±0.04 | 46±0.07 | 2.69±0.03 |
| | 33 | 1.26±0.06 | 47.7±0.04 | 38.0±0.08 | 34±0.04 | 2.61±0.02 |
| Trypsin | 13 | 0.76±0.03 | 78.6±0.06 | 27.0±0.05 | 45±0.06 | 2.79±0.04 |
| | 33 | 0.94±0.04 | 63.7±0.05 | 37.0±0.07 | 35±0.05 | 2.65±0.03 |
| SDS | | | | | | |
| α-CT | water | 1.19±0.06 | 1.39±0.06 | 39.0±0.05 | 33±0.04 | 2.09±0.05 |
| | 13 | 1.03±0.05 | 16.05±0.07 | 33.0±0.04 | 39±0.06 | 1.95±0.04 |
| | 33 | 7.76±0.08 | 21.37±0.09 | 31.5±0.02 | 40.5±0.07 | 1.86±0.02 |
| Trypsin | 13 | 1.49±0.06 | 72.27±0.08 | 28±0.06 | 44±0.08 | 1.89±0.03 |
| | 33 | 7.14±0.07 | 43.04±0.06 | 31±0.07 | 41±0.06 | 1.84± 0.02 |

of post micelles to pre-micelle slope. Since, the μL of α-CT/trypsin increases, due to the α-CT/trypsin are bonded to counter ions of both cationic and anionic surfactants, which enter jointly to polar shell of the micelle and results is slighter α values.

The counter ions binding of the micelle (β) calculated the following Eqn (2),

$$\beta = 1 - \alpha \quad \ldots (2)$$

The summarized the calculated values of α and β listed in Table 3. Rehman et al.[31] studied the diblock copolymer to the surfactant solutions increase the values of α and β. To decrease in the values of CMC signifying that the process of micellization is more constructive and spontaneous. Sinha et al.[32] investigated the micellization behavior in aqueous solution affected EG and DEG in the mixture. The CMC and a values increase with increasing the volume % of EG and DEG in solution and increasing ethereal oxygen in the glycol.

**Interfacial properties of cationic and anionic surfactants in enzyme**

***Maximum surface excess concentration ($\Gamma_{max}$)***

Cationic and anionic surfactants consist at the air/water/solution interface as well as air/protein solution interface and decreased surface tension (γ) of water or protein solution. The surface parameters calculated by literature reported by Banjare et al.[33,34]. The interfacial adsorptions per unit area of surface at a various concentration of CTAB/SDS surfactant were calculated with the help of Gibbs adsorption isotherm. The maximum surface excess ($\acute{\Gamma}_{max}$), at CMC has been evaluated the following Gibbs adsorption Eqn (3);

$$\Gamma_{max} = -\frac{1}{2.303nRT}\left[\frac{d\gamma}{d \log C}\right]_{\Gamma, P} \quad \ldots (3)$$

where R, T and C are gas constant, temperature and concentration, respectively. The constant 'n' (pre-factor) is 2. At the air/water interface, the minimum are of the per surfactant molecule ($A_{min}$) (Eqn (2)) and the surface pressure at the CMC ($\pi_{CMC}$) (Eqn (3)) value presented in Table 2. It's observed the $\acute{\Gamma}_{max}$ values are increased with increases concentration of the enzyme (Trypsin/α-CT) in the mixture, Table 2, shows that the $\acute{\Gamma}_{max}$ value are higher for SDS except CTAB.

***A Minimum area per molecule ($A_{min}$)***

The values of $A_{min}$ of surfactant at the air-liquid interface[7] have been calculated by Eqn (4),

$$A_{min} = 1/\Gamma_{max} N_A \quad \ldots (4)$$

where, $N_A$ is the Avogadro's number (*i.e.*, 6.022 × $10^{23}$ mol$^{-1}$), $A_{min}$ is the minimum area per molecule (m$^2$ mol$^{-1}$) and $\Gamma_{max}$ is the maximum surface excess concentration (mol m$^{-2}$). The calculated values $A_{min}$ of both catinic and anionic surfactants, i.e., CTAB/SDS with α-CT/trypsin systems is presented in Table 2. The results specify that the value of $\Gamma_{max}$ and $A_{min}$ differ with the molecular formation, performance $A_{min}$ with the scenery of surfactants which release that the molecules are less efficiently packed at the air/water interface for the elasticity. As the results, the $A_{min}$ is: SDS > CTAB (α-CT > trypsin). The values of $\Gamma_{max}$ decrease where, $A_{min}$ increases due to reduction of forces between the head group of surfactants with enzyme.

Table 3 — Degree of ionization (α), Gibbs free energy of micellization ($\Delta G°_m$), the standard Gibbs energy of adsorption ($\Delta G°_{ads}$), the free energy at air-water interface ($\Delta G^s_{mim}$), Gibbs free energy of micellization per alkyl tail($\Delta G°_{m,tail}$), Gibbs energy of transfer ($\Delta G_{trans}$) for cationic (CTAB) and anionic (SDS) surfactant in the presence of α-CT/ Trypsin media at 300 K

| Type of Proteins | Volume of Protein (μL) | Thermodynamic Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $\Delta G^s_{min}$ (kJ/mol) | $\Delta G^o_m$ (kJ/mol) | $\Delta G^o_{ads}$ (kJ/mol) | α | β | $\Delta G°_{m,tail}$ (kJ/mol) | $\Delta G_{trans}$ (kJ/mol) |
| CTAB | | | | | | | | |
| α-CT | Water | 2.17±0.07 | -15.60±0.035 | -29.46±0.034 | 0.44±0.3 | 0.56±0.5 | -7.8±0.036 | - |
| | 13 | 0.23±0.04 | -11.56±0.030 | -28.94±0.028 | 0.90±0.6 | 0.10±0.2 | -5.78±0.032 | 4.04±0.3 |
| | 33 | 0.27±0.05 | -7.43±0.025 | -74.37±0.040 | 0.72±0.4 | 0.28±0.4 | -3.71±0.028 | 8.47±0.5 |
| Trypsin | 13 | 0.28±0.06 | -11.10±0.023 | -29.60±0.030 | 0.98±0.6 | 0.02±0.2 | -5.55±0.034 | 4.5±0.4 |
| | 33 | 0.34±0.05 | -8.23±0.020 | -43.49±0.036 | 0.58±0.3 | 0.42±0.4 | -4.11±0.030 | 7.37±0.6 |
| SDS | | | | | | | | |
| α-CT | Water | 2.76±0.04 | -61.38±0.045 | -94.05±0.045 | 0.66±0.3 | 0.34±0.6 | -30.84±0.040 | - |
| | 13 | 37.72±0.07 | -8.66±0.036 | -43.56±0.041 | 0.98±0.5 | 0.02±0.3 | -4.33±0.030 | 52.72±0.7 |
| | 33 | 53.69±0.09 | -4.58±0.028 | -45.83±0.043 | 0.84±0.4 | 0.16±0.5 | -2.29±0.025 | 56.8±0.5 |
| Trypsin | 13 | 0.36±0.05 | -8.01±0.031 | -18.06±0.034 | 0.88±0.4 | 0.22±0.3 | -4.00±0.032 | 53.38±0.8 |
| | 33 | 0.57±0.07 | -5.56±0.025 | -35.79±0.038 | 0.74±0.3 | 0.26±0.5 | -2.78±0.025 | 55.82±0.6 |

***Surface pressure at the CMC ($\pi_{CMC}$)***

Surface pressure at the CMC ($\pi_{CMC}$)[32] is calculated by following Eqn. (5),

$$\pi_{CMC} = \gamma_o - \gamma_{CMC} \quad \ldots (5)$$

where, $\gamma_o$ is surface tension of pure water and $\pi_{CMC}$ is the surface tension at CMC. The calculated values of $\pi_{CMC}$ at 300 K are given in Table 2. Extent of $\pi_{CMC}$ for both surfactants with α-CT/trypsin system is: SDS > CTAB and α-CT > trypsin. The highest values of $\pi_{CMC}$ in SDS signify more valuable adsorption at the interface of enzyme. The $\pi_{CMC}$ values depend on the interfacial area occupied by cationic/anionic surfactants with their precise position and the structure at the interface[35]. The decrease of the $\gamma_{CMC}$ of the aqueous solution by suspension of the surfactants indicates more effective adsorption at the interface of enzyme[36]. Increasing concentration of enzyme decreases the $\pi_{CMC}$, because of the goods of surfactants.

***Efficiency of adsorption ($pC_{20}$)***

The efficiency of adsorption ($pC_{20}$)[33] is calculated by using the Eqn (6):

$$pC_{20} = -\log C_{20} \quad \ldots (6)$$

The calculated values $pC_{20}$ of the surfactants are given away in Table 2. CTAB has less efficiency for the adsorption at interface as compared to SDS since, it is more shielded. The overall falling order of efficiency of adsorption ($pC_{20}$) is: SDS > CTAB.

**Thermodynamic properties of cationic and anionic surfactants in enzyme**

Trypsin/ α-CT modified the thermodynamic properties of both CTAB and SDS surfactants. Various types of intermolecular forces involved for the micellization behavior of surfactants such as van der Waals forces[37], dipole-dipole interaction and hydrogen bonding involved for interaction of trypsin/ α-CT with CTAB/SDS surfactants. Gracie et al.[38] investigated the micellization behaviour of SDS and EG using conductivity, density, EMF, surface tension, viscosity, ultrasonic velocity, and fluorescence. Various thermodynamic parameters by conductivity and aggregation numbers obtained from static fluorescence quenching methods. CMC values were consistent with a decrease in the micropolarity surrounding the probe molecule as the EG content in the solvent mixture increased.

**The standard Gibbs free energy of micellization ($\Delta G^o_m$)**

The ($\Delta G°_m$)[34] calculate by Eqn (7),

$$\Delta G^0_m = (2 - \alpha)\, RT \ln X_{CMC} = (2-\alpha) \ln \frac{C_{CMC}}{55.4} \quad \ldots (7)$$

where, α is the micellar ionization and $X_{CMC}$ is the CMC in mole fraction unit.

The calculated $\Delta G°_m$ values are listed in Table 3. Since, addition of α-CT/trypsin makes to transfer of the hydrophobic tail from the bulkiness phase to micellar phase has less favorable, hence $\Delta G°_m$ value increases. In both surfactants CTAB/SDS with α-CT/trypsin systems, $\Delta G°_m$ values are highly negative with increased the μL of α-CT/trypsin content in their mixture. This shows that the micellization behavior was more spontaneous with increased μL of α-CT/trypsin.

**The standard Gibbs free energy of adsorption ($\Delta G^{o}_{ads}$)**

The $\Delta G^{o}_{ads}$[34] is calculated by following Eqn (8);

$$\Delta G^{\circ}_{ads} = \Delta G^{\circ}_{m} - \pi_{CMC} / \Gamma_{max} \quad \ldots (8)$$

where, $\Delta G^{\circ}_{m}$ is the Gibbs free energy of micellization, $\pi_{CMC}$ is surface pressure at the CMC and $\Gamma_{max}$ is the maximium surface excess concentration values listed in Table 2. CTAB/SDS both are hydrophobic in nature and these are easy to bind with α-CT/trypsin and form a micelles. As a result, $\Delta G^{\circ}_{ads}$ value is greater than that of $\Delta G^{\circ}_{m}$ value for both micelle systems. Therefore, the head group of surfactant plays a noteworthy character in the surface behaviors and adsorption method is moderately stronger than the bulk procedure of micellization. The maximum $\Delta G^{\circ}_{ads}$ observed for the SDS in 33 μL α-CT. As a result, the both $\Delta G^{\circ}_{m}$ and $\Delta G^{\circ}_{ads}$ values are found to negative and both parameters which signify a spontaneous micellization procedure in the CTAB/SDS system, these calculated values are listed in Table 3.

**Gibbs free energy at air/water interface ($\Delta G^{(s)}_{min}$)**

Sugihara et al.[7] have planned a thermodynamic amount for opinion of synergism in the mixture, ($\Delta G^{(s)}_{min}$) calculated by Eqn (9),

$$\Delta G^{(s)}_{min} = A_{min}. \gamma_{CMC.} N_A \quad \ldots (9)$$

The calculated value of $\Delta G^{(s)}_{min}$ is determined of valuation of synergism and clear as Gibbs free energy vary convoy by transition from the bulk phase of the surface part of the solution. The lowly free energy is more thermodynamically stable. The value of $\Delta G^{(s)}_{min}$ is larger for SDS except CTAB.

**Gibbs free energy of micellization per alkyl tail ($\Delta G^{\circ}_{m,tail}$)**

The $\Delta G^{\circ}_{m,tail}$ calculated following Eqn (10);

$$\Delta G^{\circ}_{m,tail} = \Delta G^{\circ}_{m}/2 \quad \ldots (10)$$

Table 3, shows $\Delta G^{\circ}_{m,tail}$ value of both surfactants and SDS which is higher value as compared to CTAB. The CTAB/SDS surfactants tail transfers Gibbs free energy from trypsin/ α-CT mixture to hydrobhobic core of micelles. Tail parts of surfactant apart owing to solvophobic effects. As compared with the pure medium, the additions of trypsin/ α-CT compose more favorable for CTAB/SDS surfactants molecule and the hydrophobic group to move from the bulky phase into micellar phase.

**Gibbs energy of transfer ($\Delta G^{o}_{trans}$)**

The impact of α-CT/trypsin on the micellization behavior was feasible during the ($\Delta G^{\circ}_{trans}$)[33] calculated by using Eqn (11),

$$\Delta G^{o}_{trans} = \Delta G^{o}_{m\,(enzyme\ mixed\ media)} - \Delta G^{o}_{m\,(pure\ water)} \quad \ldots (11)$$

Calculate $\Delta G^{\circ}_{trans}$ values listed in Table 3. $\Delta G^{\circ}_{trans}$ value depends on $\Delta G^{o}_{trans}$ from pure water and the α-CT/trypsin in addition to their reciprocated interaction. Addition of different μL of α-CT/trypsin changes the bulk phase assembly it more preferable except pure water. As a result, Table 3, shows the $\Delta G^{\circ}_{trans}$ increases with the increase in the CMC values of CTAB/SDS surfactants system.

## Conclusions

The micellization behaviours of cationic cetyltrimethylammonium bromide and anionic sodium dodecyl sulfate surfactants in trypsin/ α-CT at 7.75 pH media at 300 K have been systematically determined using conductivity and surface tension techniques. The present study shows surfactant micellization behavior in 7.5 pH media solution is affected by the presence of μL of trypsin/ α-CT in the mixture. The CMC values increase with increasing the μL of trypsin/ α-CT in solution.The values of $\Gamma_{max}$ decrease where, $A_{min}$ increases due to reduction of forces between the head group of surfactants with enzyme. The decrease of the $\gamma_{CMC}$ of the aqueous solution by suspension of the surfactants indicates more effective adsorption at the user interface of enzyme. Negative values of $\Delta G^{\circ}_{m}$ and $\Delta G^{\circ}_{ads}$ show that the micelles formation and adsorption of amphiphiles at the air/water interface is energetically favorable. Various thermodynamic parameters of micellization show that increasing μL of trypsin/ α-CT make micellization more favorable. Low value of $\Delta G^{s}_{min}$ ensures stability of mixed micelles.

## Acknowledgement

The authors are grateful to Dr. Ashish Saraf, Head, MATS School of Sciences, MATS University, Pagariya Complex, Pandari, Raipur (C.G.) for provided that laboratory facility.

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