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Research Article

The effects of short chain alcohols and temperature on the surfactant-protein interactions: A conductometric and spectroscopic study

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ABSTRACT

This study investigates the self-aggregation of dodecyltrimethylammonium bromide (DTAB) and triton X-100 (TX-100) with bovine serum albumin (BSA) in absence and presence of monohydroxy organic compounds such as ethanol (EtOH) and 1-propanol (1-PrOH) by means of conductivity and UV-visible spectroscopic methods. The binding and partitioning of BSA with TX-100 micellar media were also explored through UV-visible spectroscopic tool. The critical micelle concentration (*CMC*) values of the studied surfactants were found to be strongly dependent on the BSA concentration, chain length of alcohols, solvent composition as well as on temperature. The *CMC* values of DTAB + BSA and TX-100 + BSA mixtures were notably higher in aqua-alcohols media compared to those values of the mixtures in aqueous medium. The greater values of binding constant (K_b), partition constant (K_c) and partition coefficient (K_x) of TX-100 and protein mixture revealed the stronger binding and significant partitioning of BSA in TX-100 micellar media. The values of free energy changes (ΔG_m^o/ ΔG_b^o/ΔG_p^o) were achieved to be negative which indicated the spontaneous micellization/binding/partitioning of the studied systems. The values of enthalpy (ΔH_m^o) and entropy changes (ΔS_m^o) for the DTAB + BSA mixture in both aqueous and aqua-alcohols media indicated that the micellization of the system was predominantly an entropy-driven process at lower temperatures, which became both enthalpy and entropy controlled process at higher temperatures. The results of the study showed significant effects of the composition of aqua-alcohol mixed solvents and temperatures on the investigated mixtures for optimizing micellar systems in pharmaceutical and biochemical applications.

Introduction

The exploration of the interactions amongst the proteins and surfactants has been gained special attention to the scientists owing to wide ranges applications in different applied fields (Wei et al., 2003; Liu et al., 1998). The organic amphiphilic molecules, with a hydrophobic tail and a hydrophilic head, that have the ability to reduce surface tension are known as surfactants (Rosen, 2004; Rub et al., 2022; Fendler, 1982). In solution phase, surfactants undergo self-aggregation beyond a definite concentration, which is termed as the critical micelle concentration (*CMC*) and the aggregated forms of surfactants

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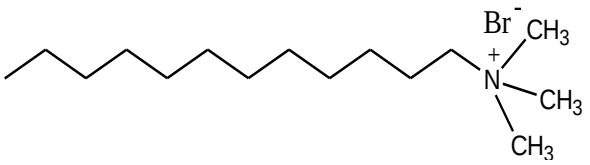
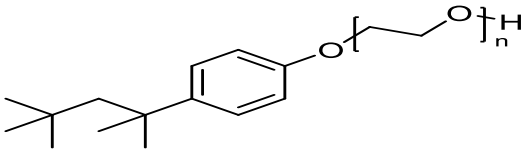
in such processes are defined as micelles (Joy et al., 2021; Hossain et al., 2022; Kumar and Rub, 2018a; Rub et al., 2021; Kumar, and Rub, 2018b; Rub et al., 2018). The dodecyltrimethylammonium bromide (DTAB) (Scheme I) is a cationic surfactant, which has been utilized as a foaming agent, stabilizer, dispersing agent etc. DTAB is used in bactericidal lotions, dying of fabrics and as well as waste water treatment (Joy et al., 2022). Triton X-100 (Scheme I) (simply abbreviated as TX-100), a typical nonionic surfactant, is used in detergency, virus inactivation, SiO_2 nanoparticle synthesis, and to remove toxic-colored materials from water (El-Aila et al., 2009; Pourreza and Elhami, 2010; Farcet et al., 2019). It is also used in co-immunoprecipitation assays, where a 0.5% TX-100 solution aids in detecting and studying protein-protein interactions (El-Hariry et al., 1999).

The serum albumin, a plasma protein, is accountable for preservation of the blood pH (Figge et al., 1991) and maintenance of osmotic blood pressure (about 80%) (Carter and Ho, 1994). Bovine serum albumin (BSA), having 583 amino acid residues along with 17 disulfides bonds, exhibits about 70% similarity to the sequence of human serum albumin (HSA) (Valstar et al., 2001; Karush, 1950).



DTAB



Triton X-100

Scheme I: Schematic illustration of DTAB and Triton X-100 (n= 9-10).

Thus, BSA has been broadly utilized in the research purposes to detect the interactions between ligands and proteins. From nuclear magnetic resonance and electron spin resonance investigations, it has been suggested that both the nonpolar and polar parts of DTAB interact with the BSA (John, 1973). The concentration of DTAB, pH, and temperature are some of the factors that affect the strength and kind of the interaction between BSA and DTAB (Suryawanshi et al., 2016). Understanding protein-ligand interaction is useful in protein purification and comprehending the biological impacts of surfactants. Thus, the study of the interaction between DTAB and BSA in variable experimental conditions is still important. In the current investigation, the interaction of BSA with DTAB and TX-100 has been inspected through the BSA mediated micellization of DTAB and TX-100 through conductivity and UV-Visible spectroscopic techniques in H_2O as well as aqua-alcohol mixed media. Also, the present study explores the binding and partitioning of BSA with TX-100 micellar solution using UV-Visible spectroscopic technique in water and aqua-alcohol mixed solvents. The *CMC*, micelle ionization, counterion binding, binding constant (K_b), partition constant (K_c), partition coefficient (K_x) and thermodynamic parameters of micellization/binding phenomena of surfactant-BSA mixtures were determined and illustrated with literature comparison and appropriate explanation.

Experimental

Materials

All the chemicals (BSA, DTAB, EtOH, and 1-PrOH) were of analytical grades and used without purification. The entire solutions were formulated using distilled-deionized water having conductivity (κ) of less than 2 μS cm^{-1}.

Conductivity measurements

The measurement of the conductivity (κ) values of DTAB + BSA solution was performed using a METTLER Toledo conductivity meter (Five Go F3, Switzerland) with cell constant of 0.54 cm^{-1} (Mahbub et al., 2020; Mahbub et al., 2021). The calibration of the conductivity meter was carried out using KCl (0.01M) solution. At the initial stage of the experiments, the DTAB stock solution (100

mmol·kg⁻¹) was prepared using the target solvents (aqueous solution of BSA in with and without alcohols where the concentrations of BSA and alcohol were kept fixed as required). The mother DTAB solution was slowly introduced by means of micropipette to 20 mL of corresponding solvent taken in a test tube, then mixed the system through stirring and the resultant mixture was allowed to stand for some time to achieve the equilibrium situation. The κ values of the solution was then measured and recorded. A thermostatted water bath (RM6 Lauda) having thermal accuracy of ±0.2 K was applied to obtain the target temperature of resulting solution. The *CMC* of the study system was evaluated from the distinct breakpoint of the plot κ versus concentration of surfactant.

UV-Visible spectroscopic technique

The absorbances of the TX-100+ BSA mixture in water or aq. alcohol solutions were achieved using a UV-Visible spectrometer (Shimadzu 1800 PC, Japan). For the present study, a series of TX-100 solutions with concentrations from 0.05 mmol kg^{-1} to 0.8 mmol kg^{-1} were prepared using desired solvent (aqueous BSA or aqueous BSA + alcohols (EtOH and 1-PrOH)) solutions at fixed concentration of BSA and alcohol). The resulting TX-100 solutions were shaken in a shaker at 80 rpm for 1 hour. The aqueous BSA or aqueous BSA + alcohols (EtOH and 1-PrOH) solutions with fixed concentration of BSA and alcohol was used as reference. The absorption spectra were recorded in the wavelength range of 200 – 800 nm in all the cases. For TX-100 + BSA mixture, the absorbance was recorded at the maximum wavelength (λ_{max}) of 276 nm for TX-100. The *CMC* of TX-100 with BSA was obtained from absorbance versus log [TX-100] plots. The experiments of the micellization and binding phenomena were developed following the literature described procedure (Irshad et al., 2021; Shah et al., 1998).

Results and discussion

Conductivity study

Assessment of micelle formation of DTAB in presence of BSA in aqueous and aqua-alcohols media

The conductivity (κ) values of DTAB solution initially increase with the increasing concentration of DTAB due to the formation of free DTA^+ and Br^- ions in solution. However, beyond a certain concentration, the incremental increase in κ values becomes slower because DTAB molecules begin to aggregate into micelles. As a result, the slope of the plot of κ versus concentration of DTAB changes, producing a distinct breakpoint. The concentration corresponds to the breakpoint is called the *CMC* [Rub et al., 2021]. Two sharp breakpoints were noticed from the plot of κ vs. DTAB concentration (e.g. Fig. 1) and the concentrations corresponding to the breakpoints were designated as CMC_1 and CMC_2 respectively (Ali et al., 2023). The three linear regions are detected in the κ *vs* C_{DTAB} plots which reveal three points: (i) the first straight line indicates a monotonic increase in conductivity with increasing DTAB concentration, where no aggregation is observed; (ii) for the second linear part of the plot, the value of slope experiences a drop in magnitude compared to that of first linear region which causes the development of first breakpoint (corresponds to CMC_1); and (iii) the third linear region indicates the constancy of conductivity due to the binding of counter ions to the head group of DTA^+ ions. The CMC_1 is attributed to the initial cooperative association of surfactant monomers with specific sites on the protein surface. Initially positively charged DTA^+ ions bind with negatively charged amino acid residue of BSA through electrostatic interaction and after reaching a critical concentration the surfactant molecules form micelle-like aggregates on the protein chain, indicating the formation of localized sub-micellar assemblies before appearance of free micelles in solution (Liu and Guo, 2007; Sood, 2019). The CMC_2 refers to the formation of free micelles in the bulk solution after saturation of BSA binding sites (Liu and Guo, 2007; Sood, 2019).

It is also noticeable that the variation in κ is steeper in pre-micellar region as compared to post-micellar region (Fig. 1). The ratio of slopes in the post-micellar (S_2) and pre-micellar area (S_1) yields the degree of ionization (α) i.e $\alpha_1 = \frac{S_2}{S_1}$ and $\alpha_2 = \frac{S_3}{S_1}$ (Akhtar et al., 2008; Bhuiyan et al., 2022; Sood, 2019), which can be used further to estimate the counterion binding (β) solving the equation, β_1 = 1- α_1 and β_2 = 1- α_2 (Rub and Azum, 2021). In the present study, both *CMC₁* and *CMC₂* values of DTAB were found to be decreased with increasing BSA concentration (Table 1). Rafati et al. (Rafati et al., 2004) described the two critical concentrations for the assembly of DTAB in aq. 0.1% BSA solution where first one is for the starting of interaction between DTAB and BSA, while second one corresponds to the aggregation of surfactant ions and thus the formation of micelles. They reported the *CMC* value of 14.5 mmol kg^{-1} (second *CMC*) for the assembly of DTAB in aq. 0.1% BSA in phosphate buffer solution (pH= 7) at 300.15 K (Rafait et al., 2004). Chakraborty et al. (Chakraborty et al., 2009) reported a single *CMC* of 11.9 mM for the micellization of DTAB in 10 mM phosphate buffer solutions (pH = 7) at 303 K using microcalorimetric titration technique. Using same technique, they also reported two *CMC* of 3.44 and 10.43 mM for DTAB in the presence of 0.025% BSA in 10 mM phosphate buffer solution (pH = 7) at 303 K (Chakraborty et al., 2009). The *CMC* values in the range of 14.5 to 16 mmol kg^{-1} for the assembly of DTAB were reported (Amin et al., 2022). For the micellization of DTAB in 0.1% BSA solution, the *CMC₁* and *CMC₂* values of 4.84 and 14.82 mmol kg^{-1} respectively were found at 303.15 K in the current investigation. As a result, the *CMC* values of current study show the close agreement with the literature reported *CMC* values. The decrease of *CMC* values in the incidence of BSA discloses the earlier micellization of DTAB. The continual changes of *CMC* for DTAB assembly with increasing content of BSA also indicates the presence of interaction between DTAB and BSA.

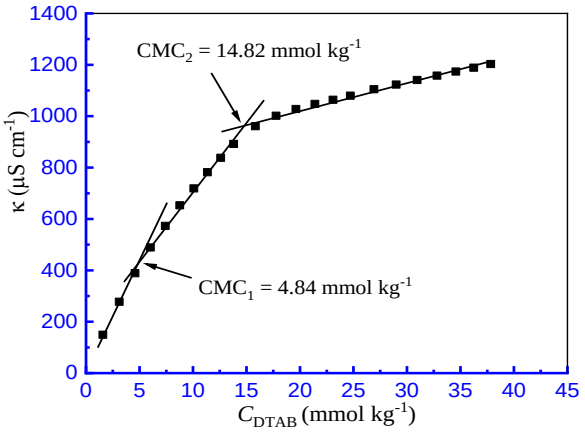


Fig. 1. The variation of κ with the enhancement of the concentration of DTAB (C_{DTAB}) during micellization of DTAB in aq. BSA (0.1%, w/w) solution at 303.15 K.

To explore the impact of alcohol on the micelle development of DTAB + BSA mixture, two monohydroxy alcohols (EtOH and 1-PrOH) were chosen. In the presence of alcohols, the *CMC* values for DTAB + BSA mixture were remarkably higher than that found in aq. solution for pure DTAB (Table 1). The presence of studied alcohols (EtOH and 1-PrOH) increases the *CMC* values which demonstrates that the micellization in these media is less favorable than that in aqueous media. Li et al. reported the rise of *CMC* values for the cationic amphiphiles as a function of concentration of lower carbon chain alcohols (Li et al., 2006). The values of *CMC* increase upon inclusion of alcohol is primarily due to the changes in dielectric constant (ε) and cohesive energy density of medium (Liepinsh and Otting, 1994; Kumar et al., 2012). Dielectric constant and cohesive energy density of water are likely to be lessened with introduction of alcohols. A decrease of dielectric constant as well as cohesive energy density of the water-organic mixed solvents causes an upsurge of *CMC* values (Liepinsh and Otting, 1994; Kumar et al., 2012). The observed *CMC* values follow the trend: $CMC_{H_2O} < CMC_{EtOH} < CMC_{1-PrOH}$. Li *et al.* found the increased *CMC* values for the association of octadecyltrimethylammonium bromide in the existence of monohydroxy organic compounds (Li et al., 2020).

Table 1. The values of *CMC* for DTAB + BSA mixture in H_2O and short-chain alcohol media at 303.15 K.

Medium	C_{BSA}	$C_{Alcohol}$	CMC_1	CMC_2
	(%, w/w)	(mmol kg^{-1})	(mmol kg^{-1})	(mmol kg^{-1})
H_2O	0.01	0	5.82	15.41
	0.03	0	5.33	15.27
	0.06	0	5.16	14.99
	0.10	0	4.84	14.82
	0.15	0	4.46	14.56
H_2O + EtOH	0.10	200	5.14	14.75
	0.10	400	5.44	14.86
	0.10	600	5.99	15.05
	0.10	1000	6.21	16.91
	0.10	1500	6.85	17.72
	0.10	2000	7.58	19.37
H_2O + 1-PrOH	0.10	200	5.31	14.89
	0.10	400	5.95	15.02
	0.10	600	6.85	15.41
	0.10	1000	7.03	15.48
	0.10	1500	7.65	15.97
	0.10	2000	8.32	16.43

Effect of temperature

To examine the influence of temperatures on the aggregation of DTAB + BSA mixture in H_2O and lower carbon chain alcohols solution, a range of temperatures from 288.15 to 318.15 K were chosen. The conductivities of DTAB + BSA mixture in H_2O and lower alcohols was increased with the rise of temperature. The acquired *CMC* and β values for the studied system at variable temperatures are presented in Fig. 2 and Table 2. The *CMC* values demonstrate the U- shaped change with *T* for the DTAB + BSA mixture in H_2O and short-chain alcohols media. Thus, the micellization of DTAB + BSA mixture in studied media is attained favorable condition at lower temperatures while the micelle formation becomes delayed at elevated temperatures investigated. The results of the current study displayed a good similarity with literature reports for the association of ionic amphiphiles as well as ionic liquids at variable temperatures (Oremusova, 2012).

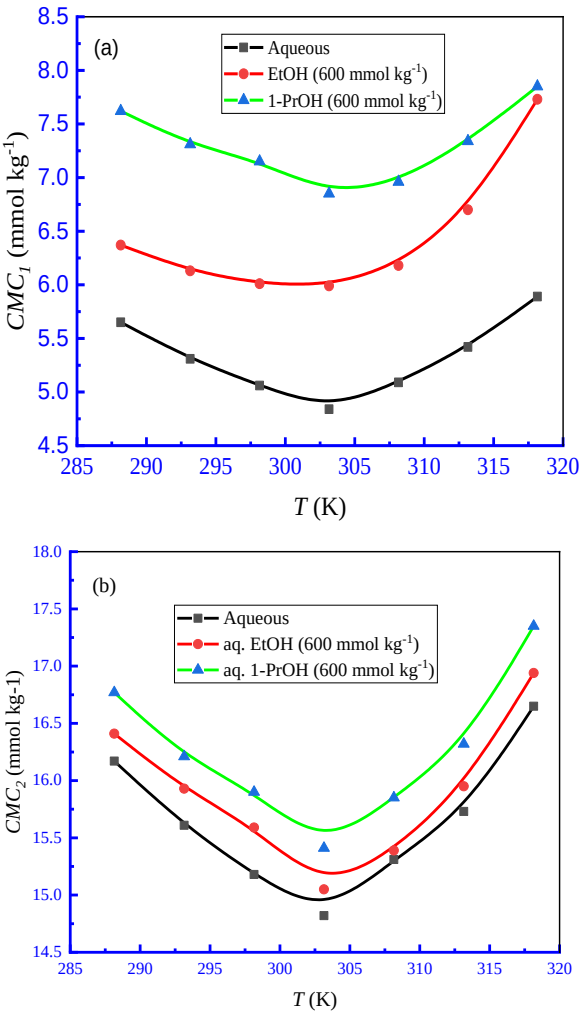


Fig. 2. The temperature dependence of (a) CMC_1 and (b) CMC_2 values for the assembly of DTAB with BSA in aqueous and 600 mmol kg^{-1} alcohol solutions.

The temperature dependence of *CMC* can be explained by the changes of arrangement of water surrounding the nonpolar part (hydrophobic hydration) and hydrophilic head groups (hydrophilic hydration) of amphiphiles with the escalation of temperature. The rise in experimental temperature causes the decrease of both categories of hydrations; while the lessening of hydrophilic hydration facilitates the development of micelles (i.e. decrease in *CMC* value) and the lessening in hydrophobic hydration delays the assembly process (i.e. upsurge of *CMC* value) (Chauhan and Sharma, 2014; Rosen and Kunjappu, 2012). Consequently, the factors mentioned above jointly control the variation of *CMC*. In the current study, the hydrophilic dehydration dominates over hydrophobic dehydration at lower temperatures while the opposite effect becomes effective at elevated temperatures investigated.

Thermodynamic parameters of association process

The free energy (ΔG_m^o), enthalpy (ΔH_m^o) and entropy (ΔS_m^o) changes of the micellization of DTAB + BSA mixture were evaluated through solving following standard thermodynamic equations (1-3) (Gurung and Pulikkal, 2019; Kumar et al., 2018c; Rub et al., 2023).

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

$$\Delta H_m^o = -(1+\beta)RT^2\left(\frac{\partial ln X_{CMC}}{\partial T}\right) \quad (2)$$

$$\Delta S_m^o = (\Delta H_m^o - \Delta G_m^o)/\mathrm{T} \quad (3)$$

Here, X_{CMC}, R, and T connote the mole fraction of *CMC*, gas constant, and experimental temperature, accordingly.

The relation of $ln X_{CMC}$ with temperature can be stated by equation (4):

$$ln X_{CMC} = A + BT + CT^2 \quad (4)$$

The constants A, B, and C in equation (4) were assessed from the second order polynomial fitting of $ln X_{CMC}$ versus T plots (Fig. 3) and the values were provided in Table 3. Then the quantity, $\frac{\partial ln X_{CMC}}{\partial T}$ in equation (2) was obtained using the term: $B + 2CT$ and finally, the ΔH_m^o was evaluated applying equation (5):

$$\Delta H_m^o = -(1+\beta)RT^2[B + 2CT] \quad (5)$$

Table 2. The values of β for the micellization of DTAB + BSA mixture in H_2O and lower carbon chain **alcohols from 288.15 to 318.15 K.**

Medium	$C_{Alcohol}$ (mmol kg^{-1})	T (K)	β_1	β_2
H_2O	0	288.15	0.46	0.88
	0	293.15	0.46	0.87
	0	298.15	0.44	0.87
	0	303.15	0.45	0.88
	0	308.15	0.44	0.89
	0	313.15	0.43	0.88
	0	318.15	0.42	0.89
H_2O + EtOH	600	288.15	0.47	0.85
	600	293.15	0.46	0.85
	600	298.15	0.46	0.86
	600	303.15	0.45	0.88
	600	308.15	0.46	0.88
	600	313.15	0.45	0.88
	600	318.15	0.44	0.90
H_2O + 1-PrOH	600	288.15	0.57	0.81
	600	293.15	0.58	0.83
	600	298.15	0.56	0.82
	600	303.15	0.56	0.84
	600	308.15	0.56	0.84
	600	313.15	0.56	0.85
	600	318.15	0.54	0.87

Table 3. The values of regression constants (*A*, *B,* and *C*) obtained for DTAB + 0.1% (w/w) BSA using equation (4).

Medium	A_1	B_1	C_1	A_2	B_2	C_2
H_2O	54.66	-0.4234	7×10^{-4}	28.652	-0.2441	4×10^{-4}
H_2O + EtOH	53.866	-0.4213	7×10^{-4}	28.849	-0.2451	4×10^{-4}
H_2O + 1-PrOH	36.25	-0.299	5×10^{-4}	28.21	-0.2409	4×10^{-4}

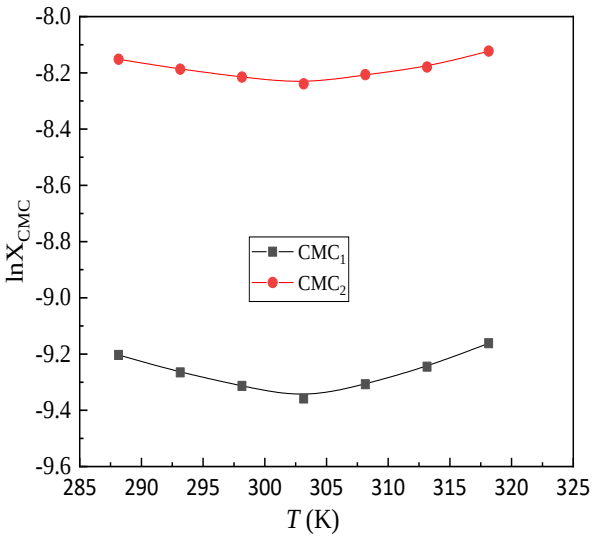


Fig. 3. The temperature dependence of lnX_{CMC} for DTAB + 0.1% (w/w) BSA association in aqueous medium.

The values of thermodynamic parameters for the association of DTAB + BSA mixture in H_2O and in short-chain alcohols media are shown in Table 4. The $\Delta G^{o}_{m,1}$ and $\Delta G^{o}_{m,2}$ values (negative in all the cases) connote the micellization of DTAB + BSA mixture is spontaneous in nature. A little escalation of $-\Delta G^{o}_{m}$ values was observed with the augmentation of temperature. The ΔH^{0}_{m} values were positive (i.e. endothermic process) at lower temperatures and negative (i.e. exothermic process) at higher temperatures. The energy is required for the destruction of hydrogen bonded network structure of water surrounding nonpolar chains of monomeric surfactant as well as partial dehydration of hydrophilic head group and reorganization of the water arrangement nearby counter ions during micellization (Mehta et al., 2005; Zieliński 2001). When surfactant molecules aggregate into micelles, then energy is released due to the (i) favorable van der Waals forces experienced by the hydrocarbon chains in the micelle core and (ii) counterion binding to the charged micelle surface which reduces electrostatic repulsion between head groups (Mehta et al., 2005; Zieliński 2001; Nusselder and Engberts, 1992; Usman et al., 2013). At lower temperatures, the energy required for the aforementioned processes is greater than the energy released by the later processes, leading to the positive enthalpy changes. However, as the temperature increases, thermal motion weakens water's hydrogen-bond network, making hydration shells less stable and reducing the energy needed to disrupt them. At sufficiently high temperatures, the exothermic effects thus become significant enough to overcome the endothermic effects, causing the enthalpy of micellization to become negative ($\Delta H^{0}_{m} < 0$).

The literature description also demonstrates the similar pattern of the changes of enthalpy values for the association of ionic surfactants (Das and Hazra, 2005; Zieliński 2001). The $\Delta S^{o}_{m,1}$ and $\Delta S^{o}_{m,2}$ values are positive in the entire cases of the investigation where the magnitudes of the entropy changes dwindle with the augmentation of temperature. The positive enthalpy and entropy changes at lower temperature indicate that the micellization of DTAB with BSA is entirely entropy controlled whereas the negative enthalpy and positive entropy changes demonstrate that the process becomes both enthalpy and entropy controlled at higher temperatures.

Evaluation of enthalpy-entropy compensation parameters

The plot of ΔH^{o}_{m} vs ΔS^{o}_{m} exhibits excellent linearity in all solvent cases and this behavior is also referred to as enthalpy-entropy compensation. The following equation (6) can be used to illustrate the compensation parameters for the mixture of DTAB + BSA in aqua- alcohol mixed solvents (Jolicoeur and Philip, 1974)

$$\Delta H^{o}_{m} = \Delta H^{o,*}_{m} + T_c \Delta S^{o}_{m} \quad (6)$$

In Eq. (6), T_c represents the compensation temperature and used to describe the existence of interaction between surfactants and employed solvent. T_c values can be obtained from the slope of the plot of ΔH^{o}_{m} vs ΔS^{o}_{m}. The $\Delta H^{o,*}_{m}$ parameter is known as intrinsic enthalpy gain and obtained from the intercept of the corresponding plot. This parameter illustrates the surfactant-surfactant interactions. The obtained compensation parameters (T_c and $\Delta H^{o,*}_{m}$) including R^2 are tabulated in Table 5.

Table 4. The values of thermodynamic parameters for the micellization of DTAB + BSA mixture in H_2O and in short-chain alcohols media.

Medium	$C_{Alcohol}$	T	$\Delta G^o_{m,1}$	$\Delta H^o_{m,1}$	$\Delta S^o_{m,1}$	$\Delta G^o_{m,2}$	$\Delta H^o_{m,2}$	$\Delta S^o_{m,2}$
	(%, w/w)	K	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1} K^{-1}	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1} K^{-1}
H_2O	0	288.15	-32.09	20.09	181.1	-36.70	17.62	188.5
		293.15	-33.05	13.58	159.1	-37.39	12.83	171.3
	0	298.15	-33.31	6.39	133.2	-38.11	7.717	153.7
	0	303.15	-34.12	-1.12	108.9	-39.10	2.273	136.5
	0	308.15	-34.31	-9.10	81.83	-39.69	-3.607	117.1
	0	313.15	-34.43	-17.50	54.04	-40.14	-9.866	96.68
	0	318.15	-34.49	-26.36	25.55	-40.65	-16.59	75.62
H_2O + EtOH	600	288.15	-31.92	18.11	173.6	-36.00	18.59	189.4
	600	293.15	-32.36	11.326	149.0	-36.91	14.02	173.7
	600	298.15	-33.18	4.209	125.4	-37.84	9.07	157.3
	600	303.15	-33.37	-3.440	98.73	-38.97	3.706	140.8
	600	308.15	-34.04	-11.64	72.70	-39.57	-2.111	121.6
	600	313.15	-34.03	-20.19	44.21	-40.06	-8.327	101.3
	600	318.15	-33.80	-29.17	14.57	-40.77	-15.07	80.76
H_2O+ 1-PrOH	600	288.15	-33.51	11.77	157.1	-35.28	13.00	167.6
	600	293.15	-34.38	6.591	139.8	-36.43	8.36	152.8
	600	298.15	-34.72	0.981	119.7	-36.85	3.201	134.3
	600	303.15	-35.46	-4.951	100.7	-38.04	-2.278	118.0
	600	308.15	-35.84	-11.23	79.84	-38.62	-8.183	98.80
	600	313.15	-36.26	-17.97	58.41	-39.28	-14.53	79.03
	600	318.15	-36.20	-24.85	35.67	-39.88	-21.38	58.14

Table 5. Enthalpy-entropy compensation parameters for the mixture of DTAB + BSA in aqueous and aq. alcohols media.

Medium	$C_{Alcohol}$	$\Delta H^{o,*}_{m,1}$	$\Delta H^{o,*}_{m,2}$	$T_{c,1}$	$T_{c,2}$
	(mmol kg^{-1})	(kJ mol^{-1})	(kJ mol^{-1})	(K)	(K)
H_2O	0	-33.68	-39.32	298.20	304.01
H_2O + EtOH	600	-33.33	-39.87	298.59	309.79
H_2O + 1-PrOH	600	-35.49	-39.35	302.03	313.72

Both T_c values were obtained in the range of 298.2-302.03 K for this study. The T_c values in the range of 270-350 K were reported for aqueous solutions of protein and small solutes (Lumry and Rajender, 1970). The negative $\Delta H_m^{o,*}$ values explicitly illustrate the stability of micelles. In the present work, negative $\Delta H_m^{o,*}$ values were observed in aqueous and aq. alcohols media. The negative $\Delta H_m^{o,*}$ values indicate the greater stability of the micelles, and the process can take place from enthalpy contribution even at zero entropy contribution.

UV-Visible spectroscopic study

Estimation of *CMC* of TX-100 + BSA mixture in aqueous and aq. alcohol media

UV-Visible spectroscopy is an effective tool to assess the interactions between surfactant and protein. Generally, pure TX-100 shows maximum absorbance at 275 nm (Shestopalova and Fomina, 2024). In this spectral study, the maximum wavelength for the pure TX-100 was determined to be 276 nm, while the maximum absorption of TX-100 in BSA solution was observed at 278 nm. Khan and coworkers reported two absorption wavelengths (λ_{max}= 215, 280 nm) for BSA protein in a universal buffer (Khan and Al-Thabaiti, 2018). The absorbance at λ_{max} = 215 nm is attributed to the peptide backbone of BSA, whereas absorption at λ_{max} = 280 nm arises from the presence of aromatic amino acids tyrosine, tryptophan (Khan and Al-Thabaiti, 2018). Other researchers determined two absorption peaks at λ_{max} = 192 and 278 nm for BSA protein (Bronze-Uhle and Costa BC, 2016). In the current examination, the absorbances of TX-100 + BSA mixture were measured at 276 nm. The validity of Beer-Lambert law was checked in this study to select the concentration range of TX-100. The absorbance of the TX-100 + BSA mixture was found to be changed with the surge of TX-100 concentration.

The *CMC* values of TX-100 + BSA mixture in the aqueous as well as aq. alcoholic solutions at specific temperature were determined applying UV-visible spectroscopic technique. The concentrations of TX-100 were selected based on the values below and above the *CMC* of TX-100. The *CMC* of 0.28 mM for pure TX-100 in water medium at 298.15 K was reported by Acosta et al. (Acosta et al., 2005), whereas Wang et al. revealed 0.33 mM of *CMC* value of TX-100 in same medium at 298.15 K (Wang et al., 2005). Non-ionic surfactants are considered as suitable candidate for drug delivery system due to low *CMC* value. The water insoluble drugs are easily dissolved in the micellar core of non-ionic surfactant. In the optimized drug delivery system, it is necessary to exist favorable interactions between drugs and non-ionic surfactants, which are markedly influenced by the presence of foreign materials, alteration in the amount of solvent and temperatures (Schreier et al., 2000; Strickley, 2004). The plot of absorbance vs. log [TX-100] gives sudden inflection point (as for example, Fig. 4), which points out the determination of *CMC* value for the respective mixture (Bhuiyan et al., 2024). Table 6 shows the *CMC* values of TX-100 + 0.08% BSA mixture in aqueous and aq. alcohols media. In the aqueous system, the *CMC* values of TX-100 are affected by the presence of BSA; while the *CMC* values initially decreased with increasing BSA concentration from 0.03 to 0.08 % (w/w) and the *CMC* increased at 0.15 % (w/w) BSA. For TX-100 + 0.08 % BSA mixture in aqua-EtOH mixed solvent, the *CMC* values decreased when EtOH concentration augmented from 1000 mmol kg^{-1} to 1500 mmol kg^{-1} and after that the *CMC* value increased at 2000 mmol kg^{-1} aq. EtOH solutions. In the cases of aq. 1-PrOH solutions, the growing trend in the *CMC* values was detected with the enhancing contents of 1-PrOH in solutions. Shah et al. also observed the rise in *CMC* values of TX-100 with increasing volume fraction of short chain alcohols (Shah et al., 2025). The increase in *CMC* values indicate the delayed micellization requiring higher extent of surfactant for the development of micelles. Alcohols decrease in cohesive energy density, breakdown of hydrogen bonding of water structure and weakens the hydrophobic effect which leads to the rise in *CMC* values (Shah et al., 2025).

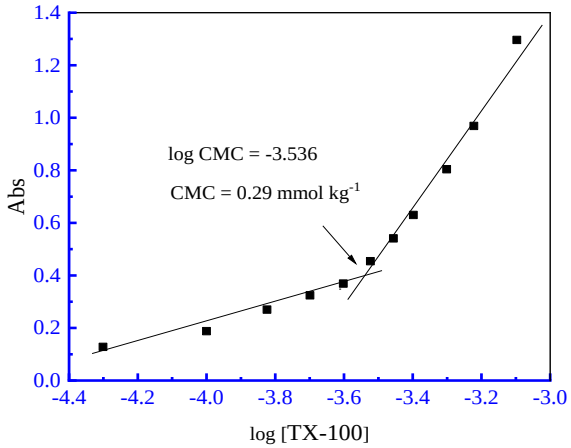


Fig. 4. Absorbance versus log [TX-100] plot for TX-100 + 0.08% BSA mixture in aqueous medium at 298.15 K.

Table 6. Values of *CMC* of TX-100 + BSA mixture in aqueous and aq. alcohols media at 298.15 K.

medium	C_{BSA} (%, w/w)	$C_{alcohol}$ (mmol kg^{-1})	*CMC* (mmol kg^{-1})
H_2O	0.03	0	0.414
	0.08	0	0.290
	0.15	0	0.518
H_2O + EtOH	0.08	1000	0.410
		1500	0.348
		2000	0.366
H_2O + 1 - PrOH	0.08	1000	0.360
		1500	0.537
		2000	0.578

Determination of binding and partition parameters

UV-Visible spectroscopy was used to evaluate the binding and partition parameters of surfactant-protein mixture. Partition constant (K_c) indicates the extent of solubilization of solutes (e.g. drugs, polymers, proteins etc.) in micellar medium compared to that in the bulk water phase. The extent of interaction between surfactant and protein can be estimated by assessing the binding constant (K_b) and the partition coefficient (K_x) at variable temperatures (Toader et al., 2020). The absorbance data were used to calculate the binding constant and partition properties of the TX-100 + BSA mixture in aqueous and aqueous alcohols media.

The binding constant (K_b) for the binding of TX-100 with BSA (at the constant concentration of BSA (0.08 %, w/w)) was determined in pre-micellar concentrations of TX-100. Benesi–Hildebrand equation (7) was used to compute the K_b for the binding of TX-100 with 0.08% BSA at premicellar states (Ahmed et al., 2025).

$$\frac{[D]}{\Delta A} = \frac{1}{\Delta\epsilon [C] K_b} + \frac{1}{\Delta\epsilon} \quad (7)$$

In the mathematical expression, [D] signifies the concentration of BSA and [C] demonstrates the concentration of TX-100. The symbol ΔA expresses the differential absorbance and $\Delta\epsilon$ refers to the differential absorption coefficient. The binding constant (K_b) was computed from the plot of $\frac{[D]}{\Delta A}$ vs. $\frac{1}{[C]}$ (Fig. 5) using the intercept $\frac{1}{\Delta\epsilon}$ and slope $\frac{1}{\Delta\epsilon K_b}$. The obtained K_b values of the TX-100 + 0.08% BSA mixture in aqueous and aqueous alcohols media were compiled in Table 7. The binding constant (K_b) values of TX-100 + 0.08 % BSA mixture at premicellar level were found to be increased with enhancing temperature.

Kawamura equation (8) was employed to determine the partition constant (K_c) for the mixture of TX-100 + 0.08% BSA in aqueous and aqueous alcohols media.

$$\frac{1}{\Delta A} = \frac{1}{K_c \, \Delta A_\infty \, (C_d + C_s^{m_o})} + \frac{1}{\Delta A_\infty} \quad (8)$$

The differential absorbance values within experimental concentration ranges and at infinite condition represent by the symbol ΔA and ΔA_∞ respectively. The C_d parameter refers to the concentration of BSA and $C_s^{m_o}$ represents the concentration of TX-100 used in this experiment. The $C_s^{m_o}$ parameter can be derived using the following relationship (9) (Irshad et al., 2021; Noor et al., 2022):

$$C_s^{m_o} = C_s - CMC_0 \quad (9)$$

Where, C_s expresses the concentration of TX-100 and CMC_0 is the *CMC* of surfactant without additives. The plot of $\frac{1}{\Delta A}$ vs. $\frac{1}{C_d + C_s^{m_o}}$ (Fig. 6) produces the linear graph from which partition constant (K_c) was derived from the slope and intercept of the corresponding graph.

The partition coefficient (K_x) was derived from the magnitudes of partition constant (K_c) using the following equation (10) (Shah et al., 1998):

$$K_x = K_c n_w \quad (10)$$

Where, n_w corresponds to the number of water species expressed in mol kg^{-1}. The values of K_c and K_x for the of TX-100 + 0.08 % BSA mixed system in aqueous and aqueous alcohols media are represented in Table 7. The greater values of partition coefficient (K_x) indicates larger amount of protein molecules are moved from aqueous phase to micellar phase. Nevertheless, the values of both K_c and partition coefficient (K_x) were found to be increased with the rise in temperature in aqueous medium, whereas the K_c and K_x values initially increased and finally decreased within the studied range of temperature in aqua-alcohol mixed media.

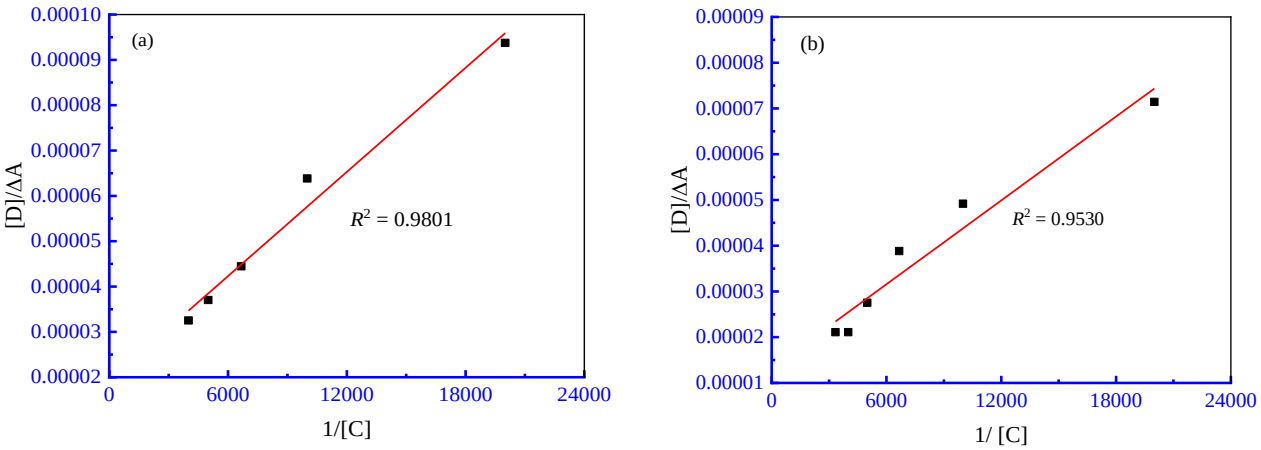


Fig. 5. Benesi–Hildebrand plot of $\frac{[D]}{\Delta A}$ vs. $\frac{1}{[C]}$ for TX-100 + 0.08 % BSA mixture at 298.15 K in (a) water and (b) aq. 1000 mmol kg^{-1} EtOH media.

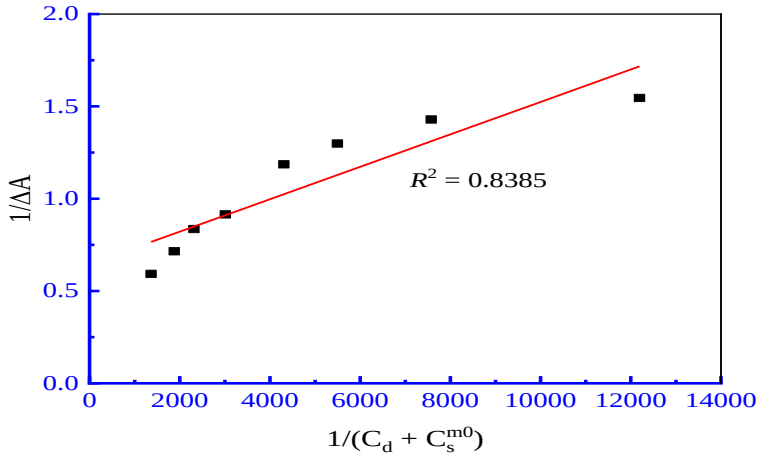


Fig. 6. The plot of $\frac{1}{\Delta A}$ vs. $\frac{1}{C_d + C_s^{m_o}}$ for TX-100 + BSA system in aq. 1000 mmol kg^{-1} EtOH medium at 298.15 K.

Table 7. Binding and partition parameters for TX-100 + 0.08 % mmol kg^{-1} BSA mixture in water and 1000 mmol kg^{-1} alcohols at 298.15 K.

Medium	$C_{alcohol}$ (mmol kg^{-1})	T (K)	K_b [a] (L mol^{-1})	K_c (×10^{-4})	K_x (×10^{-5})	ΔG_P^o (kJ/mol)	ΔG_b^o (kJ/mol)
H_2O	0	293.15	4000	2.269	12.59	-34.52	-25.10
		298.15	5000	2.483	13.78	-35.62	-25.81
		303.15	7000	2.571	14.27	-36.62	-26.24
H_2O + 1 -EtOH	1000	293.15	3000	0.709	3.94	-31.39	-22.15
		298.15	3333	0.718	3.98	-31.955	-22.53
		303.15	3500	0.699	3.88	-31.43	-22.91
H_2O + 1 -PrOH	1000	293.15	1500	0.394	2.18	-32.43	-21.24
		298.15	2000	0.555	3.08	-31.84	-22.19
		303.15	10000	0.240	1.33	-29.73	-20.44

[a] K_b values were determined at premicellar state

The values of Gibbs free energy of partition (ΔG_P^o) and free energy binding (ΔG_b^o) for the TX-100 and BSA mixed system in different studied media were evaluated using following equations (11 and 12) respectively (Nadeem et al., 2024; Rub et al., 2025).

$$\Delta G_P^o = -RT \ln K_x \quad (11)$$

$$\Delta G_b^o = -RT \ln K_b \quad (12)$$

The values ΔG_P^o and ΔG_b^o are found to be negative (Table 7) that specifies spontaneous partitioning and binding phenomenon (Nazar et al., 2010). In our earlier investigation (Ahmed et al., 2025), the observed values of ΔG_P^o for the sodium dodecyl sulfate (SDS) and ofloxacin drug in aqueous and aqueous KCl media are -32.78 kJ mol^{-1} and -29.30 kJ mol^{-1} respectively at 298.15 K, whereas the values of ΔG_b^o were found -22.86 kJ mol^{-1} and -19.30 kJ mol^{-1} for the same system at the same temperature in aqueous and aqueous KCl media respectively. Olaseni and coworkers (Olaseni et al.,2018) acquired ΔG_P^o and ΔG_b^o values of −33.2 kJ mol^{-1} and −8.42 kJ mol^{-1} respectively for the CV dye and SDS mixture in water environment. Consequently, the findings of the study exposed close similarity with the reported values of ΔG_P^o and ΔG_b^o (Ahmed et al., 2025; Mahata et al., 2010).

Conclusions

Herein, the study presents a detailed investigation of the aggregation of DTAB and TX-100 with BSA as well as binding of TX-100 with BSA in H_2O and aqua-alcohol mixed solvents. Conductometric analysis demonstrated that micellization of DTAB + BSA mixture is facilitated with the increase in BSA concentration. The *CMC* values were progressively augmented (delaying of micellization) within the entire concentrations of EtOH and 1-PrOH used. The

temperature-dependent *CMC* exhibited a U-shaped profile in water and aqua-alcohols media. The thermodynamic analysis discloses that the micellization takes place spontaneously. However, the micellization process of the system is mainly entropy-driven at lower temperatures and that becomes both enthalpy and entropy-controlled process at higher temperatures investigated. In aqueous environment, the *CMC* of TX-100 primarily declined with growing concentration of BSA and introduction of alcohols cause the rise of *CMC* with the increase of alcohol concentration. The observed greater values of binding constant (K_b), partition constant (K_c) and partition coefficient (K_x) values suggest the stronger binding and effective transport of BSA from the solvent medium to the micellar phase. The findings of present investigation offer a thorough understanding of the employed system, which is indispensable for optimizing micellar formulations in pharmaceutical, biochemical and drug delivery utilizations where solvent environment and thermal conditions are critical.

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Authors contribution

Tanni Sarker: Investigation, Formal analysis, Methodology, Writing – original draft; Jahanara Alam Jumur: Investigation, Formal analysis, Methodology, Writing – original draft; Shamim Mahbub: Writing – original draft, Writing – review & editing; Md. Rafikul Islam: Writing – original draft, Writing – review & editing; Md. Anamul Hoque: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing; D. M. Shafiqul Islam: Supervision, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that they have no conflict of interest.

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