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Aggregation and Adsorption Behaviors of Sodium Deoxycholate in Water–Ethylene Glycol Medium

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Critical micelle concentrations (*cmc*s) of sodium deoxycholate (SDC) were determined at 25 °C in water + ethylene glycol (EG) medium (mass fraction of EG, $w_{\mathrm{EG}} \times 10^2 = 0$, 10, 20, 30, 40, 50, and 60) by measuring surface tension, fluorescence emission intensities of pyrene probe, and conductance. *Cmc* was also determined in the presence of NaCl from the surface tension isotherms, and counter ion binding constants (β) of SDC were evaluated by applying the Corrin–Harkins equation and slope-ratio method. The value of β in water is about 0.18 and becomes almost half of this value in water + EG. Surface excess at the *cmc* decreases as the EG amount increases, but increases on adding NaCl. Gordon parameter and ratio of surface tension of the medium to that of the surfactant solution at the *cmc* are used to represent solvophobicity.

The molecular structure of bile salts, having hydrophobicity due to the steroid ring system and hydrophilicity due to the hydroxy and carboxy groups, provides them aggregating ability. The aggregation behavior of bile salts is, however, different from that of conventional head-and-tail type surfactants. Bile salts form primary aggregates due to hydrophobic interactions in which the hydrophobic surfaces face each other and the hydrophilic groups point outward toward the aqueous phase. As the bile salt concentration increases, secondary aggregates are formed due to hydrogen bonding between the hydroxy groups.[1] This is a generally accepted model[1] for bile salt aggregates and these aggregates are also called facial micelles.[2] In the second model, a helical structure of bile salt micelles in polar solvents has been proposed,[3] in which the hydrophobic surfaces of the bile salts are oriented outward toward the aqueous phase.

Aggregation of bile salts is responsible for their important physiological functions. Therefore, different aspects of aggregation process of bile salts are being investigated by using various experimental[4–24] and computational techniques.[25–27] All these investigations on bile salts are carried out in aqueous medium for the obvious reason that water is the most important biological liquid.

Solvent plays key role in determining the adsorption and aggregation behaviors of amphiphiles, since aggregation is a solvent-induced phenomenon. For instance, Seguin et al.[28] showed that aggregation of nonionic surfactants in a mixture of ethylene- and propylene glycols can be switched on or off by adjusting the composition of the mixed glycol media. It is also known that depending upon the polarity of solvent a surfactant may form regular or reverse micelles.

The influence of varying solvent type on adsorption and micellization properties of conventional surfactants has been studied extensively. Hardly any study has been made on the micellization behavior of bile salts in mixed or nonaqueous solvents. To the best of our knowledge, only two such studies have been reported. Park and Chung[11] studied the effect of adding a maximum of about 5% mole fraction of *N*-methylacetamide, dimethylformamide, acetamide, and formamide to water on the critical micelle concentration (*cmc*) of sodium deoxycholate (SDC). Recently, Skórka and Asztemborska[29] applied formamide solutions of sodium cholate and SDC as stationary phases in gas chromatography and for this purpose determined the *cmc* of these two bile salts in formamide.

Our aim was to obtain basic knowledge about the influence of varying solvent type or solvophobicity on the adsorption and micellization characteristics of bile salts, since they behave differently from conventional surfactants due to formation of facial micelles. With this aim in mind, we studied the aggregation as well as adsorption behaviors of SDC in water + ethylene glycol (EG) media. EG was chosen because (i) it is widely used in the studies of conventional surfactants (hence comparison can be made) and in protein conformation studies, and (ii) it is considered to be a water-like solvent in terms of hydrogen-bonding ability.

Experimental

SDC (Fluka, 98%), EG (Rankem, AR grade), NaCl (Merck, GR grade, 99.5%), and pyrene (Fluka; as fluorescence probe) were used as received. Solutions were prepared using Milli-Q grade water. Surface tension measurements were made by the Wilhelmy plate method using a K11 Krüss tensiometer. Fluorescence emission intensities (excitation wavelength = 335 nm) of pyrene were recorded at 373 (I_1) and 384 (I_3) nm using a Hitachi F4500 FL spectrophotometer. In water + EG medium amount of EG is expressed in mass fraction × 10^2. Density of the water + EG mixtures was measured by using an Anton Paar DMA 5000 density meter. Conductance measurements were made using a Wayne Kerr B905 automatic precision bridge and a dip-type cell. Studies on bile salts were generally carried out either in unbuffered or buffered media. In

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the present study we used unbuffered media and the pH of the SDC solutions was found to vary between 7.2 and 8.5 (measured using Jenway 3345 ion meter and combined glass electrode), which indicates that SDC undergoes hydrolysis. All measurements were carried out at 25 °C.

Results and Discussion

Representative plots of surface tension (γ) isotherms of SDC (structure shown in Figure 1) in water + EG medium in the absence of NaCl are shown in Figure 2. Surface tension isotherms in the presence of NaCl are found to be similar to those in Figure 2. The values of *cmc* of SDC determined from the surface tension isotherms in media containing 0 to 60% EG and different amounts of NaCl are given in Table 1.

In the absence of NaCl, we also determined the *cmc* values of SDC from the I_1/I_3 values of pyrene (Figure 3). As described by Matsuoka and Moroi,[16] *cmc* value is chosen as the SDC concentration at which the I_1/I_3 value of pyrene starts decreasing sharply. As the micelle is formed, most of the pyrene molecules get solubilized in the micelle and hence I_1/I_3 value of pyrene suddenly decreases. The value of I_1/I_3 above *cmc* is found to be 1.0 ± 0.1 in 0 to 40% EG media, and hence in these media pyrene molecules, which are solubilized inside the micelle, seem to experience similar environment. With increase in the percent amount of EG the medium becomes less solvophobic and hence more and more pyrene molecules can remain in the bulk. Such a change in the distribution of pyrene in the micellar and bulk phases affects the sigmoidal shape of the plot of I_1/I_3 versus surfactant concentration. In 50% EG the value of I_1/I_3 above *cmc* increased to about 1.4 indicating that in this medium pyrene resides at the micellar–solution interface and not inside the micelle as in 0 to 40% EG media. Eventually, in 60% EG the plot of I_1/I_3 versus SDC concentration did not show sigmoidal shape. Hence *cmc* of SDC in 60% EG could not be determined correctly from the I_1/I_3 data of pyrene. When the EG amount in the mixed solvent exceeded 60%, correct *cmc* values could not be determined even from surface tension data. Good agreement is found between the *cmc* values obtained from surface tension and fluorescence methods (Table 1). One noteworthy observation in water medium is that in the region below *cmc* I_1/I_3 of pyrene decreases sharply with increase in SDC concentration. A similar trend is observed in the I_1/I_3 data reported by Matsuoka and Moroi[16] (Figures 1 and 2 of Ref. 16). We are unable to explain this observation, but the hydrolysis of SDC causing change in the pH of the aqueous medium may be a probable reason.

With increasing NaCl concentration *cmc* of SDC decreases (Figure 4), a normal trend for ionic surfactants. Addition of NaCl increases the concentration of counter ions in the surfactant solution, which causes decrease in the repulsive interaction between the head groups favoring thereby micelli-

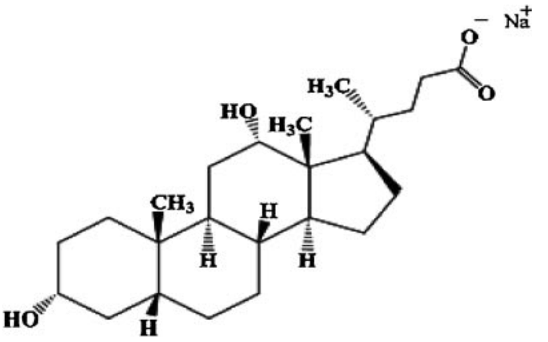

Figure 1. Structure of sodium deoxycholate.

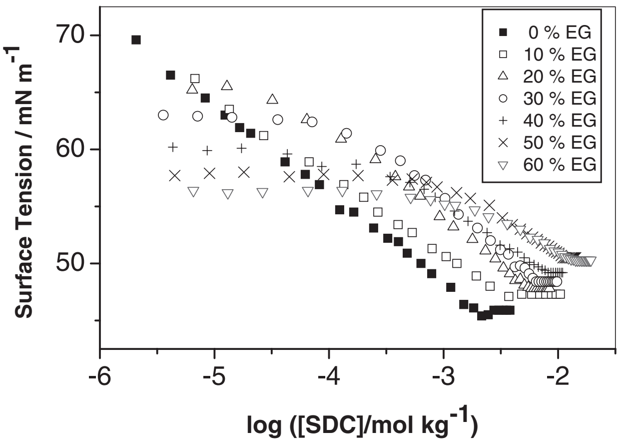

Figure 2. Variation of surface tension of SDC + water + EG with SDC concentration when the medium contains no NaCl and different mass fraction × 10^2 (given in inset) of EG.

Table 1. The *Cmc* Values of SDC in Water + EG Media at Different NaCl Concentrations Determined from Surface Tension, Fluorescence, and Conductance Measurements

[NaCl] /mol kg^{-1}	*Cmc*/mmol kg^{-1}							
	$w_{EG} \times 10^2$:	0	10	20	30	40	50	60
0		2.34	3.63	5.50	6.31	8.13	10.2	14.1
		(2.04)[a)]	(3.6)	(5.2)	(6.5)	(8.3)	(10.9)	—
		—	[3.7][b)]	[5.2]	[6.8]	[9.0]	[10.9]	[13.7]
0.005		1.93	3.35	5.20	6.00	7.83	9.90	13.8
0.007		—	3.27	5.13	5.94	7.74	9.79	13.7
0.010		1.73	3.19	5.00	5.80	7.60	9.63	13.5
0.018		—	3.07	4.80	5.65	7.42	9.44	13.3
0.020		1.57	—	—	—	—	—	—
0.022		—	3.00	4.77	5.60	7.36	9.38	13.2
0.1		1.18	2.65	4.20	5.00	6.67	8.60	12.2

a) From fluorescence. b) From conductance.

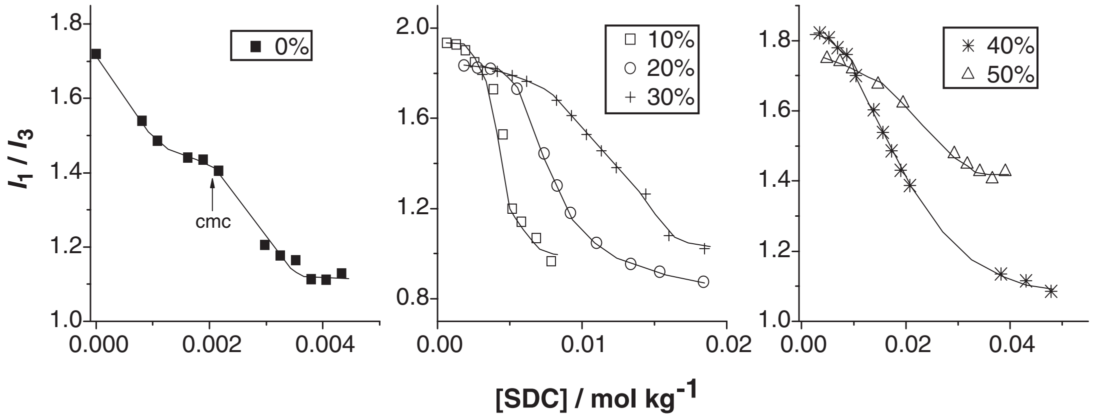

Figure 3. Variation of I_1/I_3 values of pyrene with SDC concentration in water + EG media. The mass fraction × 10^2 values of EG are indicated in the insets.

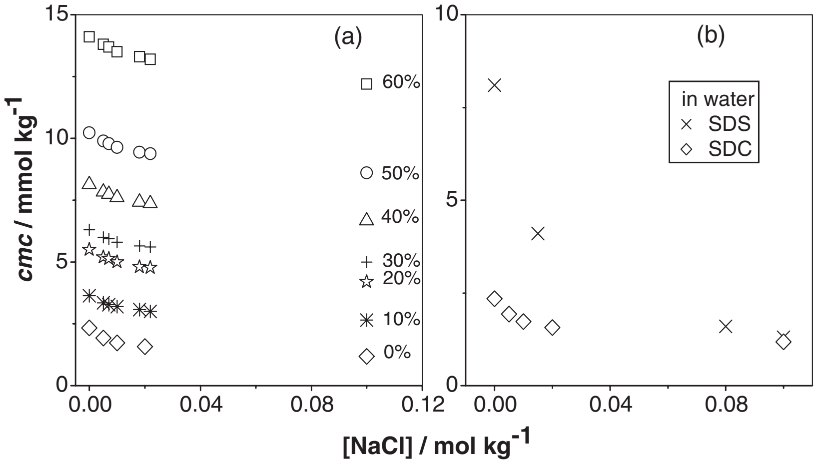

Figure 4. Variation of *cmc* of SDC with NaCl concentration in water + EG media. The *cmc* values of SDS in water are from Ref. 30.

zation and hence *cmc* decreases. In Figure 4, we have shown the reported[30] *cmc* values of sodium dodecyl sulfate (SDS) in water as a function of NaCl concentration for the sake of comparison. It is clear from Figure 4 that in water the dependence of *cmc* of SDC on ionic strength of the solution is much less than that of SDS. The dependence of *cmc* on the concentration of added electrolyte (c_e) having the same counter ion as that of the surfactant is described by the Corrin–Harkins (CH) equation of the form

$$\ln(cmc/\mathrm{mol\,kg^{-1}}) = A - \beta \ln[(cmc + c_e)/\mathrm{mol\,kg^{-1}}] \quad (1)$$

where A is a constant related to the standard free energy of micellization and β is the binding constant of sodium counter ion. The linear plots of ln(*cmc*) versus ln(*cmc* + c_e) for SDC in water + EG media are shown in Figure 5.

SDC undergoes hydrolysis in aqueous medium to produce deoxycholic acid (HDC), which is a weak acid. The influence of hydrolysis species on the micellization behavior of SDC has generally been ignored. Sugihara and co-workers[4,5] developed a treatment to describe the behavior of aqueous SDC solutions by considering that HDC and undissociated SDC also undergo aggregation along with DC$^-$. Equation 1 was modified by Sugihara and co-workers[4,5] to the form

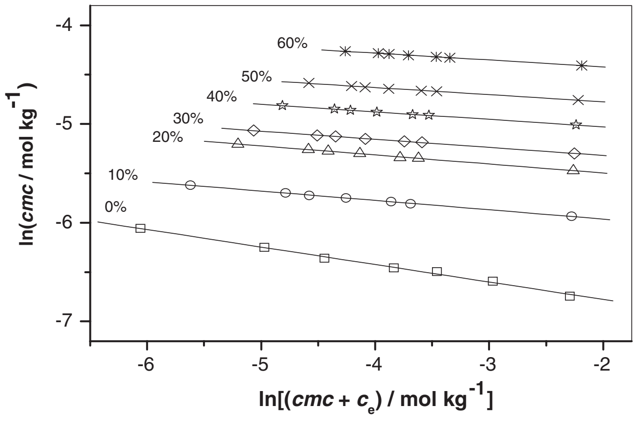

Figure 5. Corrin–Harkins plots for SDC in water + EG media.

$$\log(cmc) = A_1 + \alpha\mathrm{pH} + \beta\mathrm{pNa} \quad (2)$$

where A_1 is a constant and α is the binding constant of hydrogen counter ion. The binding of proton to the micelle can be visualized in two ways: (i) aggregation of HDC along with DC$^-$ (equivalent to binding of one mole of proton to the

Table 2. Least-Squares Fitted Values of the Parameters of eq 1 and of ΔG^0_m for SDC in Water + EG Media[a)]

$w_\mathrm{EG} \times 10^2$	A	β	ΔG^0_m /kJ mol^{-1}
0	−7.131	0.177	−15.46
10	−6.151	0.094 (0.09)	−15.23
20	−5.680	0.092 (0.09)	−14.08
30	−5.482	0.082 (0.10)	−13.58
40	−5.182	0.076 (0.11)	−12.83
50	−4.919	0.072 (0.12)	−12.17
60	−4.559	0.069 (0.15)	−11.29

a) Values of β from conductance data are given in parentheses.

micelle) and (ii) hydrolysis of the micelle. In water + EG media, undissociated SDC would exist in solution which can also aggregate with DC$^-$ and HDC. Thus, in water + EG media sodium ion binding can take place by aggregation of undissociated SDC along with DC$^-$ and by binding of sodium ion to the micelle. The linearity of the plots in Figure 5, however, envisages that eq 1 is applicable in SDC + NaCl + water + EG systems. Therefore in water + EG medium having a particular mass fraction of EG, the αpH term of eq 2 appears to remain approximately constant as the NaCl concentration is varied so that it can be absorbed in the A_1 term, and consequently eqs 1 and 2 become equivalent. The least-squares fitted values of A and β are given in Table 2. In water β for SDC is much less than the value 0.74 reported[30] for SDS. A vast difference in the β values of SDC and SDS in water envisages that structure of SDC micelles is different from that of conventional surfactant micelles. By adding 10% EG the value of β suddenly decreased from 0.177 to 0.094 and thereafter further addition of EG up to 60% caused only small decrease in β. In the case of conventional ionic surfactants also β decreases by the addition of polar organic solvent to water.

In order to confirm the values of β through another method, we measured specific conductance of SDC in water and water + EG media as a function of SDC concentration. The values of specific conductance are presented in Figure 6. We could not determine *cmc* or β of SDC in water from the conductance plot (Figure 6) since no perceivable change in the slope was observed near *cmc*. This has been explained by Matsuoka and Moroi[16] as they also made similar observation about the conductance of SDC in water. In water + EG media, change in the slopes of plots of specific conductance versus SDC concentration was observed near the *cmc*, the change was however not so prominent. From the ratio of the slopes above (S_2) and below (S_1) *cmc*, the value of β was determined by using the relation $\beta = 1 - S_2/S_1$. The values of *cmc* and β derived from the conductance data are listed in Tables 1 and 2, respectively. The values of β obtained from the CH plots and the slope-ratio method are found to be comparable in 10 to 30% EG, the agreement is however poor in 40 to 60% EG (Table 2). Determination of β from the slope-ratio method is based on the assumptions that (i) ionic surfactant is completely dissociated and (ii) ionic conductance of micelle is equal to $n(1 - \beta)\lambda_1$, where n is the aggregation number and λ_1 is the molar ionic conductance of the monomer (in the present case deoxycholate ion). As mentioned above, in water + EG media undissociated SDC would exist and the degree of dissociation of SDC is expected to decrease with increasing amount of EG in the medium. Consequently, the two assumptions of slope-ratio method may become less applicable as the amount of EG increases. This may be the reason for the increase in difference between the values of β obtained from the slope-ratio and CH methods with increasing EG content of the medium.

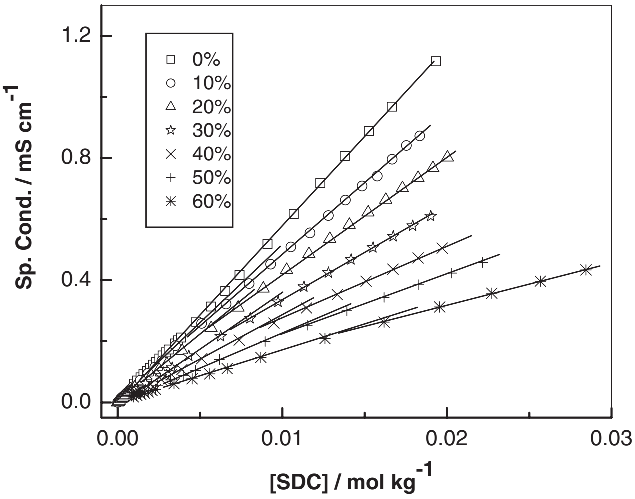

Figure 6. Variation of specific conductance with SDC concentration in water + EG media. The mass fraction × 10^2 values of EG are indicated in the inset.

Two types of counter ion binding have been reported by Pártay et al.[27] for SDC based on molecular dynamics simulations. At 30 mM SDC concentration, the reported[27] values of β are 0.02 and 0.15. According to Pártay et al.[27] the lower value refers to contact counter ion binding, while the higher value refers to binding of both contact and solvent-separated counter ions. β determined from the above two methods therefore refers to binding of contact as well as solvent-separated counter ions.

As the EG amount in the medium increases, the dielectric constant of the medium decreases and this should cause increase in counter ion binding if electrostatic interaction is only responsible for the binding. The decrease of β of ionic surfactants by adding an organic polar solvent to water clearly indicates that interaction other than electrostatic controls the counter ion binding. Therefore, it is considered that solvophobic interaction controls, besides aggregation, counter ion binding also. This implies that during aggregation of monomers counter ions also simultaneously get bound to form an ionic micelle, rather than first aggregation of monomers and then condensation of counter ions on to the micellar surface. Due to these simultaneous processes of aggregation and counter ion binding, β appears to be controlled by solvophobicity as is the case with aggregation. β has also been reported to have a dependence on the shape of ionic micelle.[31–33] Low value of β for facial type of SDC micelle and much higher value of β for spherical type SDS micelle also reveal dependence of β on shape of ionic micelle.

Surface excess of SDC at the *cmc*, Γ_cmc, was evaluated by using the expression[34]

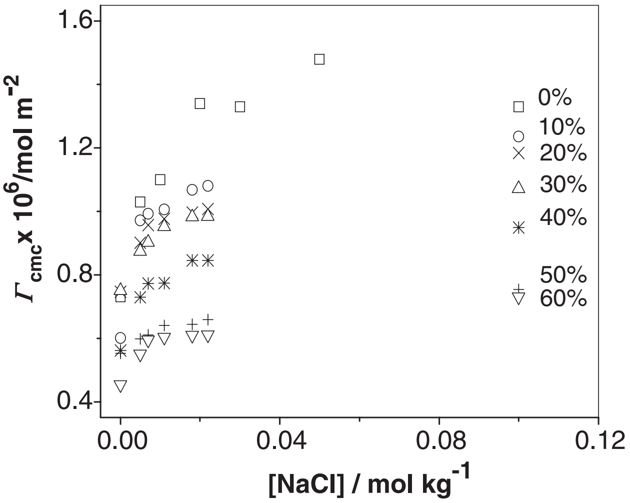

Figure 7. Surface excess of SDC at *cmc* in water + EG media as a function of NaCl concentration.

$$\Gamma_{\rm cmc} = -\frac{1}{RT}\left[\frac{1}{1+\dfrac{cmc}{cmc+c_{\rm e}}}\right]\left(\frac{{\rm d}\gamma}{{\rm d}\ln(c/{\rm mol\,kg^{-1}})}\right)_{\rm cmc} \quad (3)$$

where R and T are the gas constant and absolute temperature, respectively, and c is the surfactant concentration. In deriving eq 3, the ionic surfactant is considered to be completely dissociated and this condition may not be satisfied by the solutions of SDC in water + EG media. Since both dissociated and undissociated SDC will be adsorbed at the air–solution interface, eq 3 can still be assumed to be applicable to the solutions of SDC in water + EG media. The values of $\Gamma_{\rm cmc}$ are shown in Figure 7 and adsorption of deoxycholate ion at the air–water interface decreases as the EG content in the bulk increases. With the addition of EG, the solvophobicity of the medium decreases and hence more and more deoxycholate can reside in the bulk resulting in decrease of $\Gamma_{\rm cmc}$. In water, in the absence of NaCl, eq 3 becomes $\Gamma_{\rm cmc} = -[1/(2RT)]({\rm d}\gamma/{\rm d}\ln c)_{\rm cmc}$ and the value of $\Gamma_{\rm cmc}$ is found to be equal to 0.73 μmol m^{-2}, which matches with the value 0.75 μmol m^{-2} calculated by Jana and Moulik[13] using the same equation. Using the relation $\Gamma = -[1/(RT)]({\rm d}\gamma/{\rm d}\ln c)$, the maximum values of Γ for SDC in water are reported to be 1.66 and 2 μmol m^{-2} by Tiss et al.[35] and by Thomas and Christian,[36] respectively. These reported values of surface excess in water, after correcting for the factor 2 in the denominator of the expression for Γ, show good agreement with the value of $\Gamma_{\rm cmc}$ obtained in this study. By adding NaCl, $\Gamma_{\rm cmc}$ increases in water as well as water + EG media and attains almost constant value when the NaCl concentration becomes more than about 0.03 mol kg^{-1}. The increase in surface excess on adding NaCl may be explained as due to following reasons: (i) When NaCl is added, due to salting-out effect more deoxycholate ions go to the interface and get adsorbed. (ii) Added NaCl increases counter ion concentration in the Stern layer at the air–solution interface, which decreases repulsive interaction between the carboxyl head groups of the adsorbed deoxycholate ions causing area per adsorbed ion (A_0) to decrease or surface excess (proportional to $1/A_0$) to increase. (iii) In eq 3 the value of the terms in the square bracket increases due to increase in NaCl concentration, which also causes increase in surface excess.

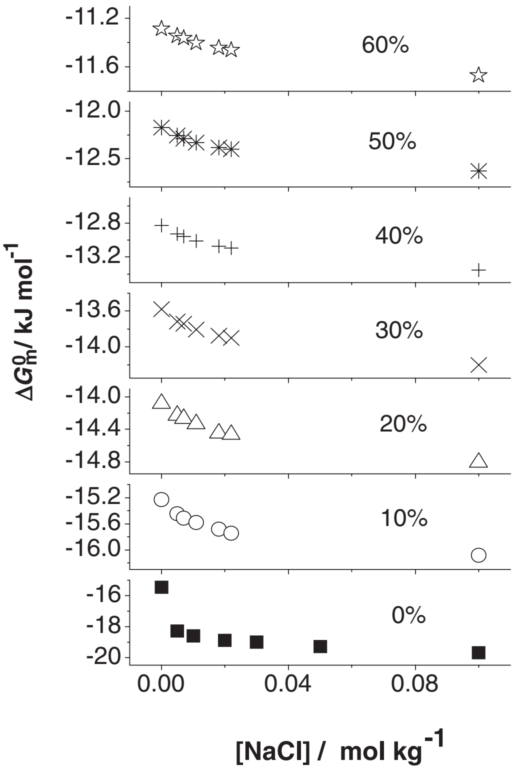

Figure 8. Variation of $\Delta G^0_{\rm m}$ of SDC with NaCl concentration.

Standard free energy of micellization per mole of monomer ($\Delta G^0_{\rm m}$) has been calculated using the expression

$$\Delta G^0_{\rm m} = RT(1+\beta)\ln(cmc/{\rm mol\,kg^{-1}}) \quad (4)$$

The values of $\Delta G^0_{\rm m}$ in water + EG media are given in Table 2. Since *cmc* increases with increase in EG amount, $\Delta G^0_{\rm m}$ also increases with mass fraction of EG. The variation of $\Delta G^0_{\rm m}$ with NaCl concentration is shown in Figure 8. For the increase in $\Delta G^0_{\rm m}$ with increasing amount of EG, the decreasing solvophobicity of the medium is responsible, while for the decrease in $\Delta G^0_{\rm m}$ with increasing concentration of NaCl in a particular medium the reduction in the repulsive interaction between the carboxyl head groups is responsible. The solvophobicity of the medium has been expressed in terms of Gordon parameter (*GP*)[37,38] and the ratio of surface tension of the medium (γ_0) to the surface tension at the *cmc* ($\gamma_{\rm cmc}$).[39,40] *GP* is defined as $\gamma_0/V^{1/3}$, where V is the molar volume of the medium. The values of *GP* and $\gamma_0/\gamma_{\rm cmc}$ are given in Table 3. The values of these parameters decrease with increase in the amount of EG and thus their values directly reflect the solvophobicity of the medium. $\Delta G^0_{\rm m}$ varies with *GP* and $\gamma_0/\gamma_{\rm cmc}$ in a similar fashion as apparent from Figure 9. The value of *GP* depends entirely on the property of the medium irrespective of the surfactant, while the value of $\gamma_0/\gamma_{\rm cmc}$ depends both on the properties of medium and the surfactant. Thus, $\gamma_0/\gamma_{\rm cmc}$ represents solvophobicity of a particular surfactant in a particular medium, and hence is a better parameter to express solvophobicity.

Table 3. Density, Molar Volume, Gordon Parameter, and Surface Tension of Water + EG Media, and Surface Tension of SDC in Water + EG at the *Cmc*

$w_{\mathrm{EG}} \times 10^2$	Density $\times 10^{-3}$ /kg m^{-3}	$V \times 10^6$ /m^3 mol^{-1}	$(\gamma/V^{1/3})$ /J m^{-3}	γ_0 /mN m^{-1}	γ_{cmc} /mN m^{-1}	$\gamma_0/\gamma_{\mathrm{cmc}}$
0	0.99704	18.00	2.75	72.0	46.0	1.56
10	1.01234	19.14	2.51	67.2	47.3	1.42
20	1.02055	20.56	2.37	65.4	47.8	1.37
30	1.04109	21.97	2.24	63.0	48.4	1.30
40	1.05482	23.83	2.06	60.0	49.2	1.22
50	1.06774	26.14	1.95	57.8	50.5	1.14
60	1.07951	29.05	1.81	56.3	50.3	1.12

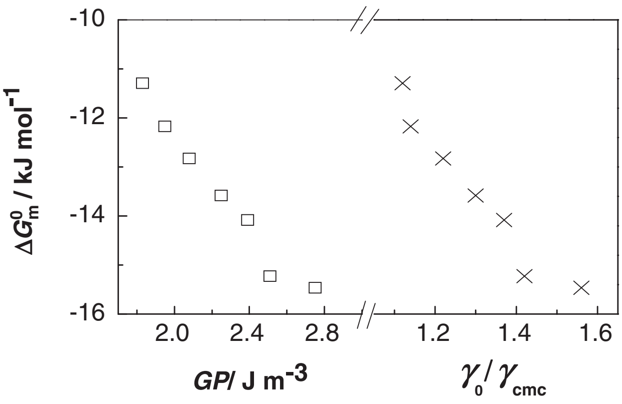

Figure 9. Variation of ΔG^0_{m} of SDC with Gordon parameter and the ratio $\gamma_0/\gamma_{\mathrm{cmc}}$.

Conclusion

The *cmc* of SDC increases similar to that of conventional surfactants by the addition of EG. By adding 50% EG, *cmc* of SDC increased by a factor of about 4.4, while reported[39] *cmc* values of sodium dodecyl sulfate, cetyltrimethylammonium bromide, and tetradecyltrimethylammonium bromide increased by factors of about 2.8, 4.6, and 3.6, respectively. Values of β in water + EG media indicate that counter ion binding behavior of SDC is different from that of conventional surfactants, which is attributable to facial type of deoxycholate micelles. The ratio $\gamma_0/\gamma_{\mathrm{cmc}}$ can be used as an alternative to Gordon parameter to represent solvophobicity.

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