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Influence of bovine serum albumin (BSA) on micellization behaviour of sodiumdodecylsulphate (SDS) in aqueous rich mixtures of dimethylsulfoxide at different temperatures

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ABSTRACT

This paper depicts the nature of interaction between bovine serum albumin (BSA) and a model amphiphile SDS from conductometric measurements, since no information regarding the mode of surfactant binding to protein from this simple technique is available. The effect of dimethyl sulfoxide (DMSO) on these interactions shall be of some fundamental importance to elaborate further the solution behaviour of SDS in Bovine Serum Albumin (BSA). Other thermodynamic parameters of micellization i.e (ΔH^0_m, ΔG^0_m and ΔS^0_m) are also derived in support of the findings.

Key words: Bovine serum albumin (BSA), Dimethylsulfoxide (DMSO), Sodium dodecylsulphate (SDS).

INTRODUCTION

Protein surfactant interactions have been studied from last century. Anson [1] recognized that proteins are denatured by synthetic surfactants. A renewed interest in this field during the past decade can be attributed to the availability of new experimental techniques to study these mixtures and the application of the results in formulation of detergents, food emulsion, pharmaceutical, cosmetic products etc. Also, an increased understanding within this field is found to be of great importance for other related fields such as protein surfactant absorption at interface [2] as well as in relatively complex biological phenomena especially in biological membrane.[3] The understanding of protein - surfactant interactions at molecular level is however complicated since protein are complex biomacromolecules with unique primary structure expressed in terms of their amino acid sequences due to which it is difficult to generalise the consequences of protein surfactant interactions. The specific binding at low surfactant concentration involves both electrostatic and hydrophobic interactions, while the nonspecific binding at high surfactant concentration is dominated by the hydrophobic forces.[4,5] Also surfactant can bind to the protein not only in monomer form but also in aggregated state, and the interaction may result in stabilization or a destabilization of the protein structure, depending on the surfactant concentration and the natural environment of the protein.[6,7] A few studies reported on the effect of surfactants chain length on protein surfactant interaction. [4,8-11] It is found that long tailed surfactants interact strongly with protein and interactions are observed at low surfactants and protein

concentration. To obtain more information on the interplay between the hydrophobic and electrostatic interactions and the phase behaviour of SDS –BSA Bovine Serum Albumin systems have been investigated. It is shown that interactions are dominated by the electrostatic and hydrophobic forces. When the hydrophobic part of the surfactant is increased the capability of the system to form and to redissolve the precipitate increases, since precipitate was neutral, and the composition reveals the net charges of the protein in solution. It was shown that the head group has a much stronger effect on the equilibrium adsorption state than the chain length. Recently, Chauhan et al.[12] have reported the head group effect of surfactant on surfactant - protein interactions in aqueous solutions of dimethylsulphoxide (DMSO). The structural consequences are found to play a very significant role in governing the interactions in addition to the nature of the head group.

EXPERIMENTAL SECTION

Ordinary tap water of conductivity in the range 3 - 5 x 10^{-6} S cm^{-1} at 25°C was distilled twice in the presence of alkaline $KMnO_4$. The distillation was carried out through a 750-mm long vertical fractionating column. The middle fraction of the double distilled water of conductivity 1- 4 x 10^{-7} S cm^{-1} and pH in the range 6.8 - 7.0 all at 25°C was collected for use in all experiments.

Bovine serum albumin (BSA) is a large globular protein (66,000 Da) was supplied by sd fine-chem limited. It was kept at 0–8° C in the refrigerator and was used without further purification. Sodium dodecyl sulfate (SDS) (Biochemical grade from BDH) was further purified as suggested by Duynstee and Grunwald [13].Dimethyl sulfoxide (DMSO) was of AR grade and purity 99.5%. It was supplied by s.d.fine - chem. Ltd. It was however used without further purification. Conductivity measurements were carried out with a digital conductometer operating at 1 KHz, supplied by Naina Electronics Chandigarh (India). The cell constant of this conductivity cell was determined at 25°C from conductance measurements with aqueous solutions of KCl as described by Fuoss et al.[14]. The conductivity measurements at different temperatures and concentration of SDS were repeated at least three times. The reproducibility of the present conductivity measurement was ± 0.01%. The CMCs were determined precise to ± 1% from the apparent discontinuity in the plot of specific conductance κ verses concentration of SDS. However, the concentration of SDS was taken (2.0-30.0 mM).The CMC = 8×10^{-3} mol dm^{-3} for SDS in water at 25 ° C was in excellent agreement with 8×10^{-3} mol dm^{-3}value reported in literature[17].

RESULTS AND DISCUSSION

3.1 THERMODYNAMICS FOR SDS–BSA SYSTEM:

The standard enthalpy change for micellization was determined from the slope of the van't Hoff plots based on the following equations. [12, 17-18]

$$\Delta H^{\circ}_{m} = -RT^{2}\,\frac{d\ln(CMC)}{dT} \qquad (1)$$

Where R is gas constant, T is temperature in Kelvin

$$\Delta G^{\circ}_{m} = -RT\ln CMC \qquad (2)$$

Similar argument was put forward by Rio et al. [15] while estimating the ΔH^{o}_{m} values of various surfactants in buffer solutions at different temperatures. However, before subjecting the CMC data to Eqn. (2), the temperature dependence of CMC was examined. SDS-BSA a linear relation was found to hold good only up to 30° C. It can also be depicted from the CMC data reported above, the CMC of SDS was found to decrease as we approach 35 °C. Similar observation has been reported by Chauhan et al. [12] in SDS – gelatin system. The van't Hoff slope, d (CMC) /dT of these plots were determined from the least – squares fitting of data. The standard entropy change for micellization (ΔS^{o}_{m}) for SDS determined from Eqn. (3) [12, 17-18].

$$\Delta G^{\circ}_{m} = \Delta H^{\circ}_{m} - T\Delta S^{\circ}_{m} \qquad (3)$$

Where ΔG^o_m is known as standard Gibbs free energy change associated with the formation of micelle. A perusal of Table 1 the value of CMC of SDS increases as the DMSO concentration increases which reveals in table 2 having all ΔH^o_m values are negative, which is indicative of attractive force having both specific and nonspecific binding between SDS-BSA interactions.

Table 1: CMC values of SDS in aqueous rich mixtures of DMSO containing BSA at different temperatures

BSA(% w/v)	0 mol % DMSO			1.1 mol % DMSO		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	7.9	8.1	8.2	9.2	9.4	9.6
0.4	8.8	9.1	9.4	9.8	9.9	10
0.8	9.4	9.7	10	10.1	10.3	10.7
1.2	9.8	10.1	10.2	10.4	10.6	11.1
BSA(% w/v)	**2.2 mol % DMSO**			**4.4 mol % DMSO**		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	9.4	9.7	9.8	9.9	10.2	10.3
0.4	10.1	10.7	11.5	11.4	12.2	12.6
0.8	10.5	10.9	11.3	11.9	12.9	13.4
1.2	10.9	11.7	12.1	12.6	13.8	14.3

*(*Estimated uncertainty is ± 1 %)*

Table 2: ΔH^o_m values of SDS in aqueous rich mixtures of DMSO containing BSA at different temperatures

BSA(% w/v)	0 mol % DMSO			1.1 mol % DMSO		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	-1.85	-1.97	-2.10	-1.57	-1.68	-1.79
0.4	-2.44	-2.60	-2.78	-0.75	-0.81	-0.85
0.8	-2.28	-2.44	-2.60	-2.13	-2.28	-2.43
1.2	-1.48	-1.58	-1.68	-2.40	-2.57	-2.74
BSA(% w/v)	**2.2 mol % DMSO**			**4.4 mol % DMSO**		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	-1.54	-1.64	-1.75	-1.47	-1.56	-1.67
0.4	-4.79	-5.12	-5.45	-3.71	-3.96	-4.21
0.8	-2.71	-2.91	-3.09	-4.39	-4.69	-4.99
1.2	-3.86	-4.12	-4.39	-4.67	-4.99	-5.32

*(*Estimated uncertainty is ± 0.1 kJ mol^{-1})*

Table 3: ΔG^o_m values of SDS in aqueous rich mixtures of DMSO containing BSA at different temperatures

BSA(% w/v)	0 mol % DMSO			1.1 mol % DMSO		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	-5.12	-5.36	-5.56	-5.51	-5.74	-5.98
0.4	-5.39	-5.66	-5.93	-5.66	-5.87	-6.08
0.8	-5.55	-5.81	-6.08	-5.73	-5.97	-6.27
1.2	-5.66	-5.93	-6.14	-5.81	-6.05	-6.36
BSA(% w/v)	**2.2 mol % DMSO**			**4.4 mol % DMSO**		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	-5.55	-5.82	-6.03	-5.68	-5.95	-6.17
0.4	-5.73	-6.03	-6.46	-6.03	-6.41	-6.71
0.8	-5.83	-6.12	-6.41	-6.14	-6.55	-6.85
1.2	-5.92	-6.31	-6.59	-6.28	-6.72	-7.03

*(*Estimated uncertainty is ± 0.1 kJ mol^{-1})*

These results are however, presented in plots 1-4 for SDS – BSA interactions the variation ΔH^o_m indicates the strong hydrophobic interactions which further supported by plots 5-8 the variations of ΔG^o_m having similar behavior as mentioned above. The ΔH^o_m value of SDS decreases sharply to a minimum at around 1.1 mol% DMSO to 4.4 mol% DMSO as a function of DMSO. Another interesting feature of these plots is a nonlinear decrease in ΔH^o_m value with the increase in DMSO concentration; largest decrease is observed to occur in 4.4 mol% DMSO.

Table 4: ΔS^{o}_{m} values of SDS in aqueous rich mixtures of DMSO containing BSA at different temperatures

BSA(% w/v)	0 mol % DMSO			1.1 mol % DMSO		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	10.98	10.98	10.87	13.16	13.17	13.16
0.4	9.90	9.91	9.90	16.47	16.47	16.47
0.8	10.97	10.98	10.97	12.06	11.98	12.05
1.2	14.01	14.09	14.01	11.38	11.27	11.38
BSA(% w/v)	**2.2 mol % DMSO**			**4.4 mol % DMSO**		
	25^0C	35^0C	45^0C	25^0C	35^0C	45^0C
0.0	13.47	13.56	13.47	14.15	14.23	14.15
0.4	3.14	3.08	3.14	7.81	7.96	7.81
0.8	10.45	10.46	10.45	5.86	6.04	5.86
1.2	6.92	7.07	6.92	5.38	5.61	5.38

(*Estimated uncertainty is ± 5 J $K^{-1}mol^{-1}$)

A large negative value of ΔH^{o}_{m} in the case of SDS – BSA system, therefore reflects the contribution of strong intermolecular interactions between water and DMSO with the concomitant electrostatic binding of counterion, Na^{+} with BSA. However, the structural consequences of intermolecular interactions appear to be qualitatively independent of protein concentration.

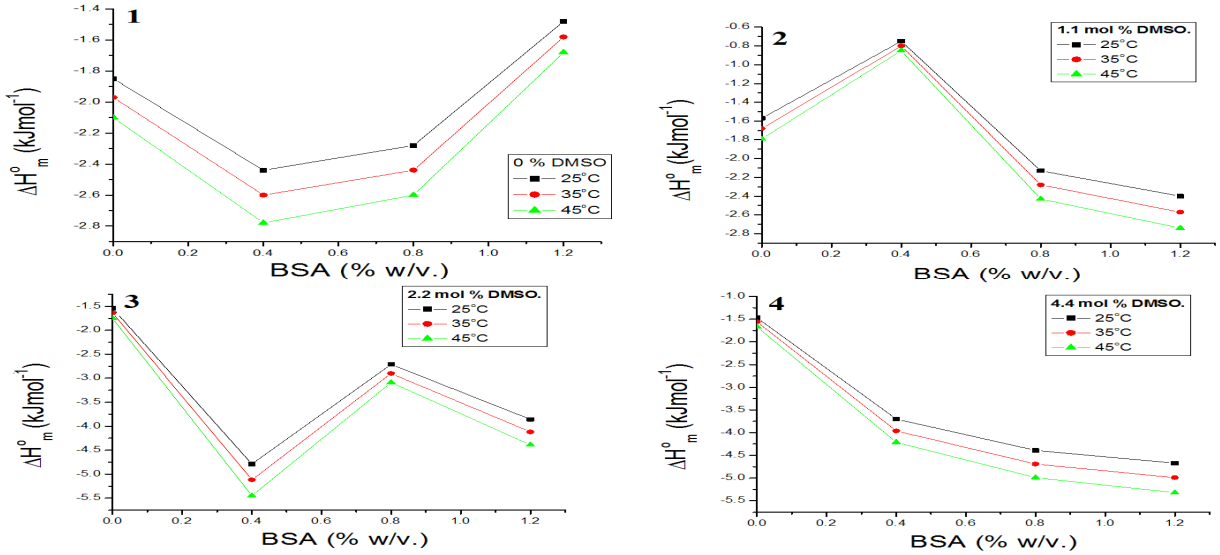

Figure 1: Variation of ΔH°_{m} verses BSA (%w/v) in aqueous rich mixtures of DMSO at different temperatures

On the basis of precipitation and redissolution effects noted above, it may be deduced that both hydrophobic and electrostatic binding of protein-surfactant strong interactions. [16]

The ΔS^{o}_{m} value for SDS – BSA systems have been plotted as a function of BSA concentration in plots 9 to 12 respectively. It is interesting to note that there is a remarkable qualitative similarity between the behavior of ΔS^{o}_{m} and ΔH^{o}_{m}. This corresponds to favorable thermodynamic parameters. This observation is also in agreement with the thermodynamic data of Chauhan et al. [12,17-18] on SDS – gelatin system.

It is concluded from this observation that there are very prominent effects on the thermodynamics of protein – surfactant interaction brought about by the addition of DMSO, which can be very probably attributed to structural changes in the salvation of hydrophobic side chains, irrespective of any other effects, DMSO might have on protein – surfactant interaction.

However, a large change in both ΔH^{o}_{m} and ΔS^{o}_{m} values can be seen to compensate the effect of each other giving rise to relatively small changes in the magnitude of ΔG^{o}_{m} value with protein concentration.

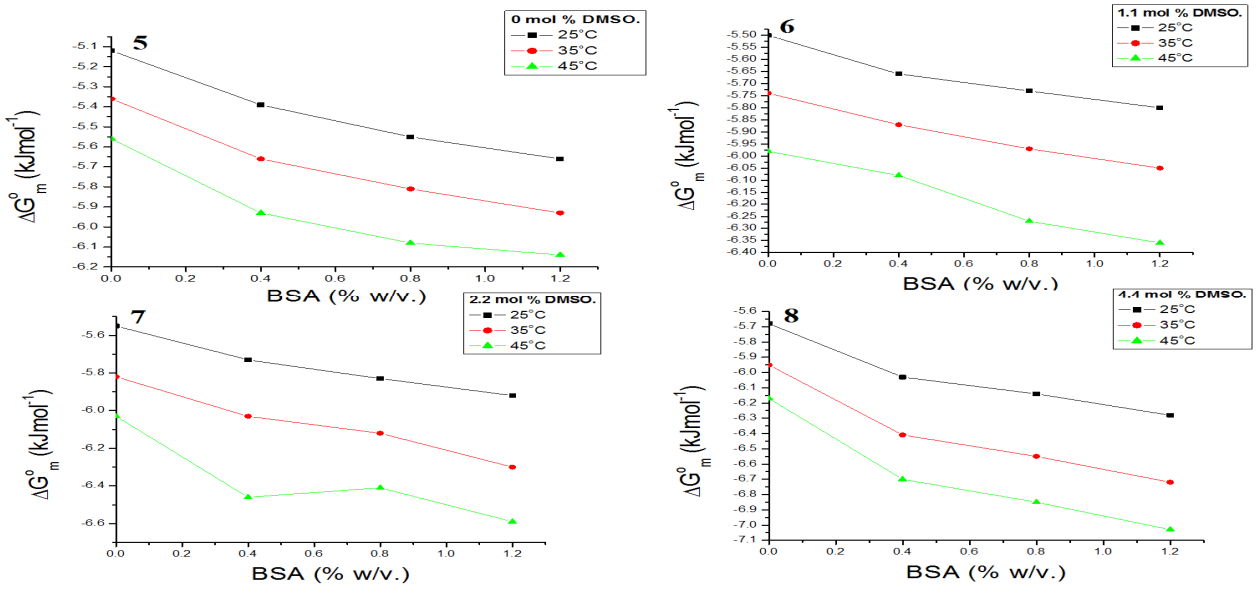

Figure 2: Variation of Δ G°$_m$ verses BSA (%w/v) in aqueous rich mixtures of DMSO at different temperatures

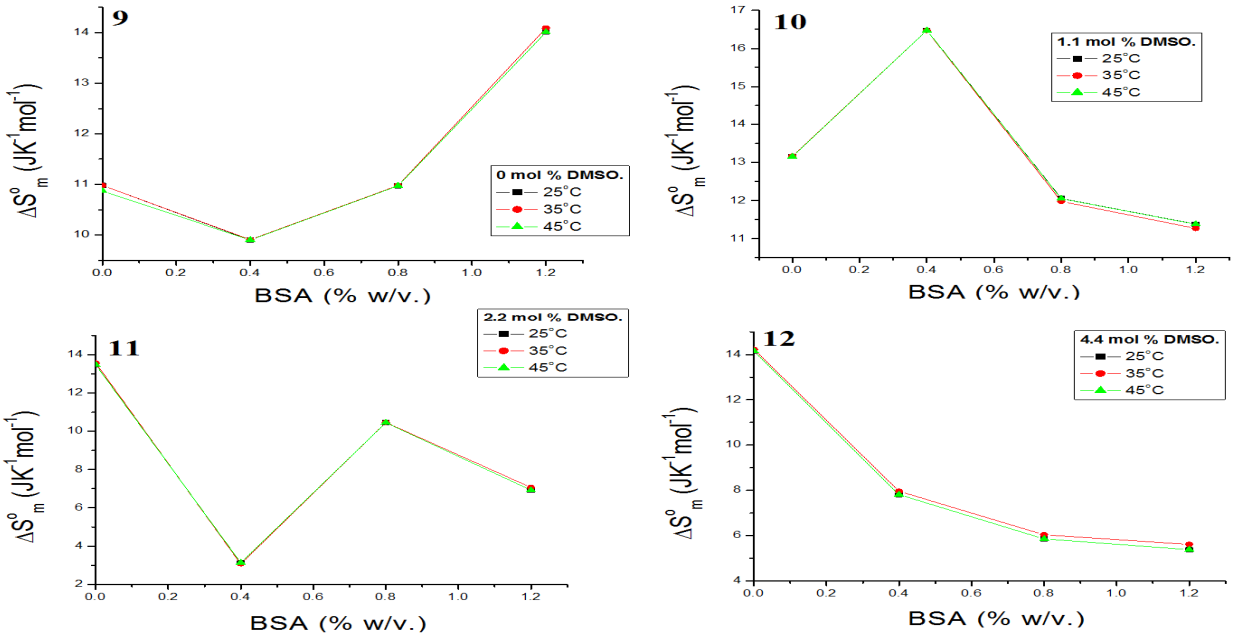

Figure 3 : Variation of Δ S °$_m$ verses BSA (%w/v) in aqueous rich mixtures of DMSO at different temperatures

CONCLUSION

It is concluded from this observation that there are very prominent effects on the thermodynamics of protein – surfactant interaction brought about by the addition of DMSO, which probably attributed to structural changes in the salvation of hydrophobic side chains, irrespective of any other effects, DMSO might have on protein – surfactant interaction. it might be considered to support the conclusions drawn above. However, a large change in both ΔH^{o}_{m}

and ΔS^{o}_{m} values can be seen to compensate the effect of each other giving rise to relatively small changes in the magnitude of ΔG^{o}_{m} value with protein concentration, which is similar but markedly dependent on solvent compositions. However, an interesting correlation is seen to exist between the onset of micellar complexation of SDS with BSA and the concentration of DMSO in the solvent medium.

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