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A Comparative Study of Interactions between Protein (Lysozyme) and Ionic Surfactants (SDS, CTAB) in Aqueous Rich Mixtures of Dmso At Different Temperatures.

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ABSTRACT

The present work emphasize on the effect of DMSO on solution behavior of SDS and CTAB in aqueous solutions of lysozyme.The CMC values of SDS and CTAB expressed in mole fraction unit (X_{cmc}) have been determined by the usual conductivity method.The dependence of X_{cmc} as a function of lysozyme concentration has been traced to protein – surfactant interactions. A comparison of X_{cmc} data for these two protein – surfactant systems reveals the existence of stronger intermolecular interactions between SDS and lysozyme than CTAB and lysozyme. The expected effect of DMSO i.e., structural consequences of intermolecular interactions and screening of electrostatic effect between protein and lysozyme have also been inferred from the experimental data.Other thermodynamic parameters i.e. (ΔH^{O}_{m} , ΔS^{O}_{m} , ΔG^{O}_{m}) have also been derived in support of findings.

Keywords: CTAB, Dimethylsulfoxide (DMSO), Lysozyme and SDS

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INTRODUCTION

The study on protein –- surfactant interaction has always been fascinating to the researchers in academics due to the heterogeneity of surfactant binding sites in protein molecule and denaturing effect of the surfactant, various attempts have been made to understand the interaction at molecular level in terms of the conceptual models, which take account of the observed solution behaviour of such systems. Protein-surfactant interactions occur in many applications within the food, laundry and pharmaceutical sector however surfactants inhibit protein aggregation, essential for long-term storage of pharmaceutical products [1,2]. In the food industry surfactants act as antioxidant agents, stabilizers and anti-adhesives. The basic difference between SDS- Lysozyme system and CTAB-Lysozyme system is that the former in an oppositely charged protein –- surfactant system, whereas the later is similar charged protein– surfactant system.Comparison between the results for these two systems reveal the importance of surfactant headgroups, the alkyl chain length of the surfactant and the groups on the protein exposed to the medium. In case of SDS – Lysozyme system, at low concentration, individual molecule binds to the discrete binding sites of lysozyme in a non–cooperating way, which is followed by cooperative binding of surfactant to lysozyme, as the surfactant concentration approached the CMC value. On the other hand, the data for CTAB–Lysozyme system indicate that CTAB has no interaction with lysozyme, describing that micelle formation of CTAB impedes the binding to lysozyme. However, the derived parameters have been reported in the form of tables, and presented in figures to correlate the findings.

MATERIALS AND METHODS

Lysozyme (lyophilized $M_W \approx 14,600 g\ mol^{-1}$) obtained from Chicken egg white was procured from HiMedia (India). The protein was however stored at (4 –5)^{O}C and used without giving any additional treatment. Sodium dodecyl sulfate (SDS) (Biochemical grade from BDH) was further purified as suggested by Duynstee and Grunwald [2]. Cetyltrimethyl ammonium bromide (CTAB) was of AR grade and purity > 99%. It was also obtained from s.d. fine - chem. Ltd. However, a pure sample of CTAB was obtained by several recristallization from ethanol as suggested by Ionescu et al. [3]. However, there is no significant differences could be detected in the critical micelle concentration (cmc) value of these purified surfactants at 25^{O}C.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. A high precision water thermostat of capacity ~ 30L fitted with a digital temperature controlled device was used for conductance measurements. It was supplied by NSW - New Delhi. The temperature of the thermostat was maintained within ± 0.1^{o}C over the entire temperature range studied i.e., 20 - 35 ^{o}C. However, the temperature of the bath was continuously monitored with the help of a 1/100 ^{o}C calibrated thermometer. Conductivity measurements were carried out with a digital conductometer operating at 1 KHz, supplied by Naina Electronics Chandigarh (India).

RESULTS AND DISCUSSION

Table 1: X_{cmc} values of SDS and CTAB in aqueous solution of lysozyme.

lysozyme(% w/v)	20^{O} C	X_{cmc} , 10^4(SDS) 25^{O}C	30^{O}C	35^{O}C	20^{O}C	X_{cmc} , 10^5(CTAB) 25^{O}C	30^{O}C	35^{O}C
0	1.43	1.45	1.47	1.49	1.71	1.82	1.91	1.98
0.05	1.32	1.36	1.40	1.26	6.08	6.30	6.48	6.62
0.10	1.22	1.26	1.30	1.19	6.48	6.64	6.86	7.06
0.15	1.17	1.21	1.26	1.15	6.77	6.97	7.15	7.31
0.20	1.16	1.20	1.24	1.15	6.84	7.04	7.22	7.40
0.375	1.15	1.19	1.23	1.15	6.91	7.15	7.31	7.52
0.625	1.15	1.18	1.23	1.15	6.98	7.13	7.36	7.56

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Table 2: X_{cmc} values of SDS and CTAB in aqueous mixtures of DMSO containing lysozyme

lysozyme(%w/v) w/v)	X_{cmc} , 10^4(SDS)				X_{cmc} , 10^5(CTAB)			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
	1.10 mol% DMSO				1.10 mol% DMSO			
0	1.45	1.47	1.49	1.51	1.80	1.87	1.94	2.02
0.05	1.39	1.43	1.46	1.44	2.07	2.25	2.39	2.54
0.10	1.34	1.39	1.43	1.44	2.32	2.52	2.66	2.79
0.15	1.32	1.37	1.40	1.44	2.52	2.66	2.81	2.91
0.20	1.30	1.35	1.38	1.44	2.59	2.72	2.82	2.93
0.375	1.30	1.35	1.38	1.44	2.63	2.73	2.86	2.95
	2.20 mol% DMSO				2.20 mol% DMSO			
0	1.48	1.51	1.60	1.68	1.83	1.91	1.82	2.05
0.05	1.44	1.48	1.54	1.51	2.12	2.25	2.36	2.46
0.10	1.41	1.44	1.48	1.51	2.43	2.54	2.64	2.75
0.15	1.39	1.42	1.45	1.51	2.61	2.70	2.79	2.90
0.20	1.38	1.40	1.42	1.51	2.66	2.79	2.90	2.97
0.375	1.37	1.39	1.42	1.51	2.72	2.84	2.97	3.06
	4.40 mol% DMSO				4.40 mol% DMSO			
0	1.65	1.78	1.94	2.06	1.85	1.92	1.99	2.05
0.05	1.58	1.62	1.69	1.80	2.30	2.53	2.68	2.91
0.10	1.58	1.62	1.69	1.80	2.66	2.96	3.16	3.43
0.15	1.58	1.62	1.69	1.80	2.96	3.23	3.45	3.68
0.20	1.58	1.62	1.69	1.80	3.07	3.38	3.67	3.86
0.375	1.58	1.62	1.69	1.80	3.14	3.45	3.79	4.01
lysozyme(% w/v)	X_{cmc} , 10^4(SDS)				X_{cmc} , 10^5(CTAB)			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
	6.60 mol% DMSO				6.60 mol% DMSO			
0	1.89	1.98	2.05	2.33	1.88	1.94	2.01	2.06
0.05	2.14	2.25	2.35	2.33	2.84	3.14	3.59	3.91
0.10	2.27	2.39	2.47	2.33	3.32	3.68	4.22	4.51
0.15	2.30	2.44	2.52	2.33	3.55	3.93	4.47	4.76
0.20	2.31	2.46	2.55	2.33	3.70	4.07	4.61	4.94
0.375	2.33	2.47	2.58	2.33	3.90	4.25	4.76	5.06
	10.30 mol% DMSO				10.30 mol% DMSO			
0	2.09	2.20	2.33	2.78	1.94	2.01	2.06	2.13
0.05	2.37	2.51	2.66	2.57	3.58	4.57	5.38	6.09
0.10	2.48	2.65	2.79	2.57	4.21	5.29	6.45	7.17
0.15	2.51	2.72	2.86	2.57	4.48	5.73	6.72	7.62

0.20	2.53	2.75	2.91	2.57	4.75	5.82	6.90	7.79
0.375	2.53	2.71	2.91	2.57	4.93	6.00	6.99	7.88
	12.58 mol% DMSO				12.58 mol% DMSO			
0	2.20	2.40	2.45	2.96	1.99	2.04	2.11	2.05
0.05	2.23	2.81	2.88	2.58	4.03	4.98	6.07	7.02
0.10	2.63	2.93	2.98	2.58	4.81	6.00	7.11	8.07
0.15	2.66	2.97	3.03	2.58	5.57	6.62	7.66	8.50
0.20	2.68	3.03	3.07	2.58	5.82	6.80	8.05	8.98
0.375	2.70	3.06	3.09	2.58	6.00	7.25	8.41	9.49

The specific conductance measurements of SDS (0.2 mM –10 mM) and CTAB (0.2 mM –6.5 mM) are carried out in the concentration range 0 – 0.375% w/v of lysozyme at 20, 25, 30 and 35^{O}C in water as well as in aqueous mixtures of 1.10 – 12.58 mol % DMSO. The pH of SDS – lysozyme system is found to lie in the range 6.22 – 6.42 and that of CTAB – lysozyme in the range 5.25 – 5.53. Specific conductance values thus obtained have been plotted against SDS concentration. It is clear from graphs that, increases almost linearly with [SDS] with definite break points. The break points were quite significant as observed in , therefore critical micellar concentrations (CMC) were evaluated for SDS from these plots Chauhan et.al. [4]. In all the cases, cmc value is found to be much lower than the cmc of pure surfactant solution. This may arise from a lowering of repulsion between surfactant head group and also the hydrophobic nature of lysozyme which provides surface for the micellization of SDS.

Thus the extra hydrophobicity offered by Lysozyme, seems to reduce the cmc value of SDS Valstar et. al. [5]. Further, these cmc values decrease with rise in temperature unlike as CTAB . This observation is suggested to indicate the saturation of polymer at low concentration, by virtue of polymer-surfactant binding which is similar to micellization. A decrease in cmc with temperature, therefore supports the appearance of more binding sites due to unfolding of lysozyme. However, this anomalous behavior may be attributed to self association or net-work structure formation between the polymer chains. From these cmc values, various thermodynamic parameters have also been obtained to derive information of about protein – surfactant interaction.

Effect of DMSO: The X_{cmc} behavior of SDS and CTAB is in striking contrast up to 4.40 mol % DMSO. In the case of SDS, X_{cmc} falls with the addition of lysozyme, whereas in the case of CTAB it increases. Above 6.60 mol % of DMSO the X_{cmc} of both SDS and CTAB behave in a similar manner. The data further indicate that above 0.15% w/v concentration of lysozyme, the X_{cmc} of these surfactants become relatively insensitive to the lysozyme concentration. Another common feature of these data is the apparent increase in X_{cmc} value with the increase in temperature. However, each run was carried out with two different stock solutions of the surfactant, SDS and CTAB with a precision of $\pm$ 0.2 %. The CMC values of SDS and CTAB in water were found to be equal to 8 mM and 1 mM respectively at 25 ^{o}C. These values were in excellent agreement with those reported in literature Das et.al. [6]

Similar procedure was adopted for the determination of CMC of these surfactants in aqueous mixtures of 1.11, 2.21, 4.43, 6.63, 10.32 and 12.58 mol% DMSO containing 0.05, 0.1, 0.15, 0.2, and 0.375 % w/v lysozyme at different temperatures. The estimated error in the CMC value was found to lie between 1 - 2%. The CMC values thus obtained were however converted into mol fraction unit (X_{cmc}) in order to estimate the various thermodynamic parameters of micellization.

THERMODYNAMICS OF SDS–LYSOZYME AND CTAB-LYSOZYME INTERACTIONS:

The standard enthalpy change for micellization was determined from the slope of the van't Hoff plots based on the equation [8].

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

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where X_{cmc} is the CMC (in mole fraction), R is gas constant, T is temperature in Kelvin

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

Similar argument was put forward by Rio et al. [7] while estimating the ΔH^{o}_{m} values of various surfactants in buffer solutions at different temperatures. However, before subjecting the X_{cmc} data to Eqn. (2), the temperature dependence of $\ln X_{cmc}$ was examined. It was found that in the case of CTAB there exists a good linear relationship between $\ln X_{cmc}$ and temperature over the entire temperature range studied, whereas in the case of SDS, such a linear relation was found to hold good only up to 30° C. It can also be depicted from the X_{cmc} data reported above, the X_{cmc} of SDS was found to decrease as we approach 35 °C. Similar observation has been reported by Chauhan et al. [8] in SDS – gelatin system. The van't Hoff slope, d ($\ln X_{cmc}$) /dT of these plots were determined from the least – squares fitting of data. The standard entropy change for micellization (ΔS^{o}_{m}) for SDS and CTAB was determined from Eqn. (3) [9].

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

where ΔG^{o}_{m} is known as standard Gibbs free energy change associated with the formation of micelle. A perusal of Table 1 and 2 reveals that all ΔH^{o}_{m} values are negative, which is indicative of attractive force.

Table 3: Thermodynamic parameters for Lysozyme-SDS interactions in aqueous rich mixtures of DMSO at 25°C.

Lysozyme (%w/v)	(mol %) DMSO						
	0	1.1	2.2	4.4	6.6	10.3	12.58
	ΔH^{o}_{m} (*Estimated uncertainty is ± 0.1 kJ mol^{-1})						
0	-1.18	-1.91	-2.25	-7.08	-8.51	-11.70	-12.12
0.05	-5.72	- 4.46	-4.46	-7.19	-8.22	-11.35	-11.91
0.10	-6.62	- 4.92	-4.37	-6.93	-7.92	-11.08	-11.64
0.15	-6.62	- 6.61	-4.60	-7.60	-10.49	-12.68	-13.68
0.20	-6.46	- 5.65	-3.48	-7.52	-11.01	-13.86	-14.25
0.375	-6.57	- 5.50	-3.17	-6.82	-10.05	-13.78	-14.24
	ΔS^{o}_{m} (*Estimated uncertainty is ± 5 J K^{-1}mol^{-1})						
0	62	65	59	48	42	30	30
0.05	54	58	58	58	48	30	22
0.10	52	58	58	49	42	31	30
0.15	35	51	58	49	34	19	24
0.20	53	55	62	46	32	21	21
0.375	53	52	63	49	35	21	25
	ΔG^{o}_{m} (*Estimated uncertainty is ± 0.1 kJ mol^{-1})						
0	-21.80	-21.86	-21.78	-21.39	-21.11	-20.87	-20.64
0.05	-22.06	-21.93	-21.86	-21.61	-20.79	-20.54	-20.25
0.10	-22.23	-22.01	-21.91	-21.61	-20.64	-20.40	-20.15
0.15	-22.35	-22.03	-21.96	-21.61	-20.62	-20.35	-20.12
0.20	-22.38	-22.08	-21.98	-21.61	-20.59	-20.30	-20.10
0.375	-22.40	-22.08	-22.01	-21.61	-20.57	-20.35	-20.05

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Table 4: Thermodynamic parameters for Lysozyme-CTAB interactions in aqueous rich mixtures of DMSO at 25°C.

lysozyme (% w/v)	mol % DMSO						
	0	1.1	2.2	4.4	6.6	10.3	12.58
	ΔH^{o}_{m} (*Estimated uncertainty is ± 0.1 kJ mol^{-1})						
0	-16.98	-9.74	-9.24	-8.49	-7.80	-7.90	-7.50
0.05	-6.90	-17.10	-16.80	-20.17	-27.90	-41.90	-43.10
0.10	-7.50	-15.10	-16.10	-19.90	-26.10	-41.50	-42.80
0.15	-6.60	-12.30	-13.10	-17.10	-24.40	-39.80	-41.60
0.20	-6.70	-10.90	-11.01	-15.70	-23.70	-36.80	-38.20
0.375	-7.10	-9.10	-10.11	-16.60	-23.20	-35.50	-36.70
	ΔS^{o}_{m} (*Estimated uncertainty is ± 5 J $K^{-1}mol^{-1}$)						
0	96	120	121	124	126	125	126
0.05	115	98	98	86	51	-5	-13
0.10	114	97	98	77	52	-10	-21
0.15	116	110	108	84	57	-8	-21
0.20	115	112	114	88	57	-8	-10
0.375	115	118	114	84	4	-7	-6
	ΔG^{o}_{m} (*Estimated uncertainty is ± 0.1 kJ mol^{-1})						
0	-27.00	-26.90	-26.90	-26.90	-26.90	-26.80	-26.70
0.05	-23.90	-26.50	-26.50	-26.20	-25.70	-24.80	-24.50
0.10	-23.80	-26.20	-26.20	-25.80	-25.30	-24.40	-24.10
0.15	-23.70	-26.10	-26.10	-25.60	-25.10	-24.20	-23.80
0.20	-23.70	-26.10	-25.90	-25.50	-25.00	-24.20	-23.80
0.375	-23.60	-26.00	-25.90	-25.50	-25.00	-24.10	-23.60

These results are however, presented in Figure 1 for SDS – lysozyme and in Figure 2 for CTAB – ly sozy me indicating the dependence of ΔH^{o}_{m} values of SDS and CTAB respectively on lysozyme concentration. From these figures it is noted that the effect of lysozyme on ΔH^{o}_{m} is very different in aqueous solutions of lysozyme; at low protein concentrations, ΔH^{o}_{m} value of SDS decreases, whereas in the case of CTAB it increases. At higher concentrations, the ΔH^{o}_{m} of these surfactants become independent of lysozyme concentration.

Figure 1 and 2 also show the changes in ΔH^{o}_{m} value of SDS and CTAB upon addition of DMSO. It should be noted that ΔH^{o}_{m} value of CTAB – lysozyme system is relatively more strongly dependent on DMSO than SDS in SDS – lysozyme system. The ΔH^{o}_{m} value of CTAB decreases sharply to a minimum at around 0.05 – 0.1 % w/v lysozyme and then increases relatively slowly at higher concentration region of lysozyme. Another interesting feature of these plots is a systematic decrease in ΔH^{o}_{m} value with the increase in DMSO concentration; largest decrease is observed to occur in 12.58 mol% DMSO. On the other hand, Figure 1 shows that ΔH^{o}_{m} of SDS is almost independent of lysozyme concentration in 2.20 mo%DMSO.

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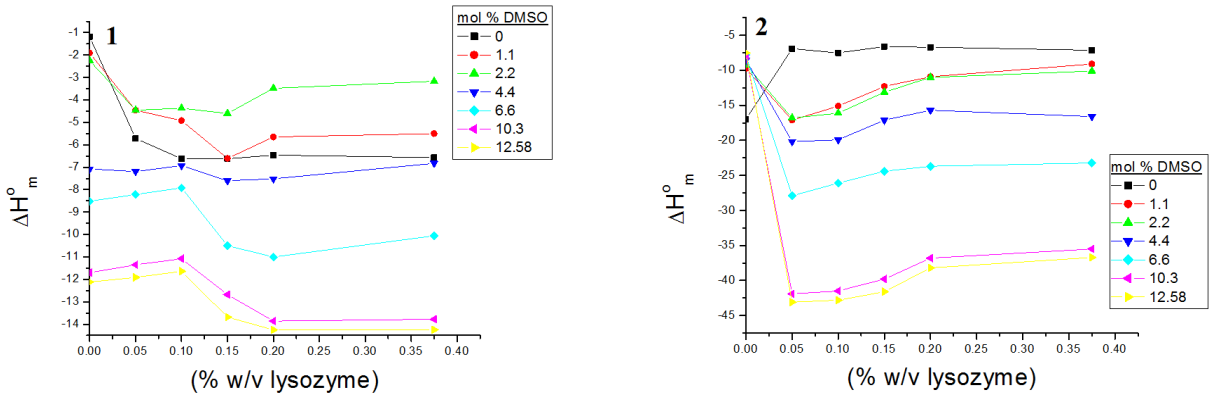
This constancy however, holds good only up to 0.10 %w/v lysozyme as we increase DMSO concentration beyond 2.20 mo% DMSO i.e., it behaves in a manner similar to that observed in absence of DMSO.



Turning to the effect of DMSO, one notable feature is that ΔH^{o}_{m} is virtually constant up to ~ 1.10 % w/v lysozyme in the region 4.40 – 12.58 mol% DMSO. Interestingly, the subsequent increase in DMSO concentration affects the ΔH^{o}_{m} value in a manner that is completely consistent with CTAB – lysozyme system. [10], it appears permissible to suggest that lysozyme is involved in two different complex processes in the solution region of SDS – lysozyme system. In the region < 0.01 %w/v, the surfactant binding is expected to be cooperative because cationic binding sites being limited in this region get saturated with DS^{-} anion, but enhances the subsequent binding of surfactant molecule due to cooperative interaction, and thereby the net charge of the SDS/lysozyme becomes negative and repulsion set in, explaining ΔH^{o}_{m} to be nearly constant.

Moreover, it has been reported that in very dilute solutions lysozyme exists as monomers, but at higher concentrations, it shows an associative behavior and forms dimmers and higher oligoners [11]. Thus a possible explanation of the behaviour pattern observed at lysozyme concentrations > 0.1 % w/v might be due to the stacking interaction between lysozyme molecules involving a significant measure of hydrophobic bonding. Thus, making ΔH^{o}_{m} slightly more negative. As suggested by Moren and Khan [12], it is also possible that small surfactant aggregates formed on the protein in this region bind mainly hydrophobically to protein molecules and form networks similar to hydrophobically modified polymer – surfactant gel [13].

Thus, the observed anomalous behaviour of X_{cmc} at 35 ^{o}C can be attributed to the unfolding of lysozyme as a result, many more hydrophobic binding sites are exposed to the medium and micellization is favoured, explaining the decrease in X_{cmc}. On the basis of precipitation and redissolution effects noted above, it may be deduced that two – points (hydrophobic and electrostatic) binding of surfactant to protein is required for strong interaction. However, since no such effects were observed in the case of CTAB – lysozyme system, the observed behaviors of ΔH^{o}_{m} as a function of lysozyme concentration (Figure 2) appears to represent weak micellar interaction of CTAB with lysozyme. It is expected to attach its hydrophobic groups to the hydrophobic part of the surfactant [14], inhibiting the hydrophobic interaction between CTAB and lysozyme. A large negative value of ΔH^{o}_{m} in the case of CTAB – lysozyme system, therefore reflects the contribution of strong intermolecular interactions between water and DMSO with the concomitant electrostatic binding of counterion, Br^{-} with lysozyme.

The ΔS^{o}_{m} value for SDS – lysozyme and CTAB – lysozyme systems have been plotted as a function of lysozyme concentration in Figure 3 and 4 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 observation is also in agreement with the thermodynamic data of Chauhan et al. [15] on SDS – gelatin system.

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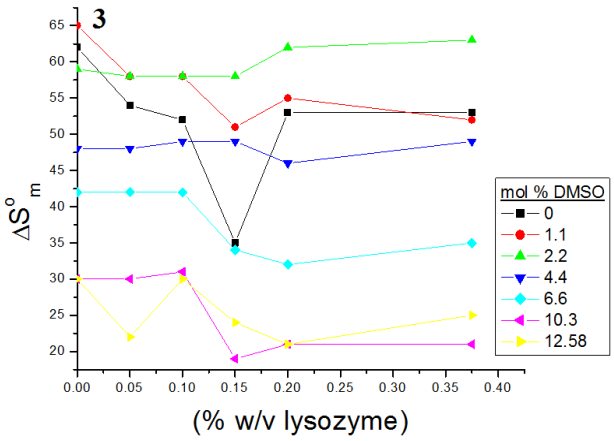
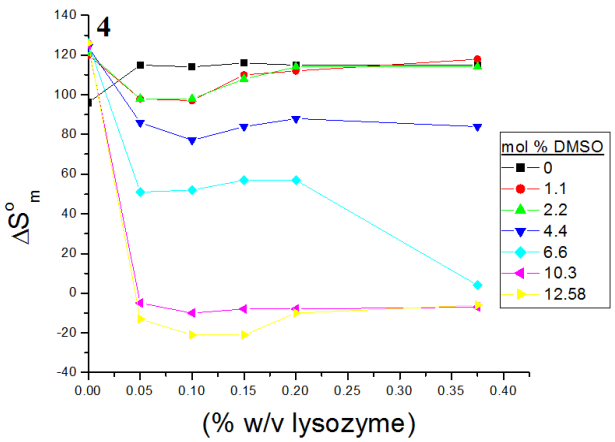
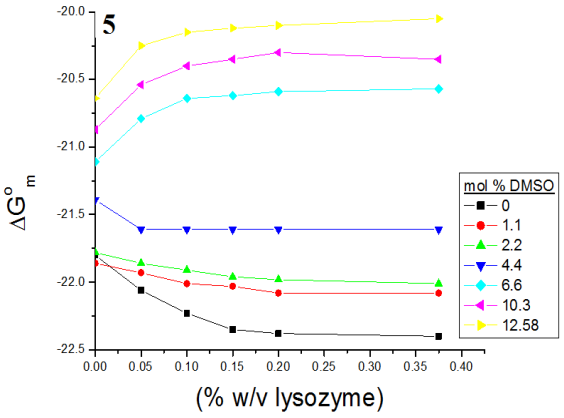
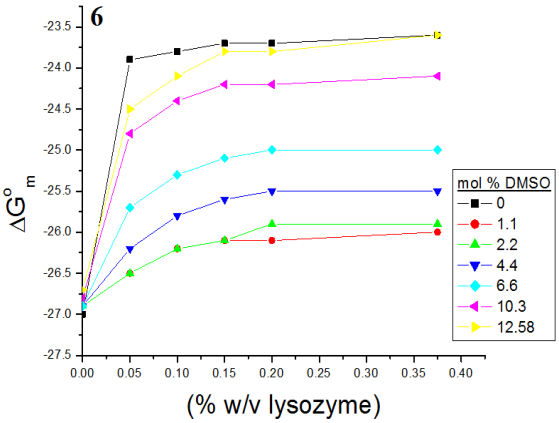




It is concluded from this observation that there are very prominent effects on the thermodynamics of protein – surfactant interaction brought about by the addition of DMSO, which can be very probably attributed to structural changes in the salvation of hydrophobic side chains, irrespective of any other effects, DMSO might have on protein – surfactant interaction. In addition, since ΔS^{o}_{m} showed a similar trend with the DMSO concentration (Figure 3 and 4) in both SDS – lysozyme and CTAB – lysozyme systems, 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.

CONCLUSION

Comparison between the results for these two system reveal the relative importations of surfactant headgroups, the alkyl chain length of the surfactant and the groups on the protein exposed to the medium. In case of SDS – lysozyme system, at low concentration, individual molecule binds to the discrete binding sites of lysozyme in a non – cooperating way, which is followed by cooperative binding of surfactant to lysozyme, as the surfactant concentration approached the CMC value. On the other hand, the data for CTAB – lysozyme system indicate that CTAB has no interaction with lysozyme, describing that micelle formation of CTAB impedes the binding to lysozyme

REFERENCES

[1] Otzen DE.Protein unfolding in detergents: Effect of micelle structure, ionic strength, pH, and temperature, Biophys. J. 2002; 83: 2219-2230.
[2] Duynstee EFJ, Grunwald E. *J.Am. Chem. Soc*. 1954;81:4540.
[3] Lonescu LJ,Tadashi T, Benzamin CJ, Eric SS. In *"Solution Behaviour of Surfactant"* Ed.: K.L.Mittal Voll. II Plenum Press New York,1979.
[4] Chauhan MS,Sharma KC, Kumar G,Chauhan S. *Coll. Czech.Chem. Commun.* 2002;67: 1141.

[5] Valstar A, Almgren M, Brown W,Vasilescu M. *Langmuir*.2000;16:922.
[6] Das KP, Chattoraj DK. *Frontiers in Applied Chemistry,*A.K. Biswas ed. Narosa Publication House,1989.
[7] Del Rio JM, Pombo C,Perieto G, Mosquera V, Sarmiento F.*J. Colloid Interface Sci.* 1995;172:137.
[8] Chauhan MS,Kumari N,Pathania S,Sharma K,Kumar G.*Colloids and Surface A*.2007;293: 157.
[9] Ruso JM,Taboada P,Mosquera V,Sarmiento F. *J. Colloid Interface Sci.* 1999; 214: 292.
[10] moren AK,Khan A. *Langmuir* .1995;11:3636.
[11] Lampreave F, Piniero A, Brock JH, Castillo H, Calvo L. M. *int. J. Biol.Macromol.* 1990;12: 2.
[12] Moren AK,Khan A. *Langmuir* .1998;14:6818.
[13] Chauhan MS,Sharma K,Kumar G, Chauhan S. J*Colloids and Surfaces* .2003;221: 135.
[14] Chauhan MS, Sharma KC,Gupta S,Sharma M, Chauhan S. *Acoustics Letts.* 1995;18: 233.
[15] Chauhan S,Chauhan MS,Sharma P,Rana DS,Ahmad U.*J Fluid Phase Equilibria*.2012;337:39-46.