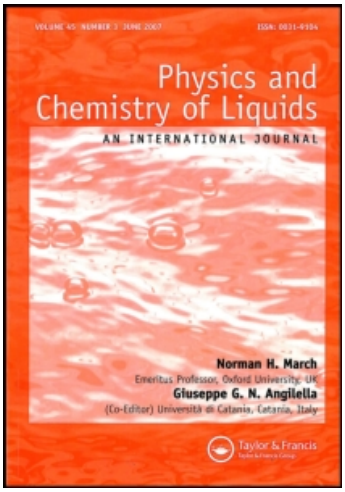

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THERMODYNAMIC STUDIES OF INTERACTION BETWEEN CATIONIC SURFACTANTS AND LYSOZYME USING POTENTIOMETRIC TECHNIQUES

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The interaction between a homologous series of nalkyl trimethyl ammonium bromides and lysozyme was studied by a potentiometric method using a surfactant-selective electrode. The binding isotherms the interaction between a homologous series of nalkyl trimethyl ammonium bromides were measured for different conditions and show a concentration dependence attributable to aggregation of the lysozyme-surfactant complexes. The binding isotherms are discussed in terms of the binding potential concept of Wyman and are used to calculate an apparent Gibbs energy of binding per surfactant cation. The binding isotherms show that the C_1 decreases with increasing chain length of surfactant.

Keywords: Surfactant; Cationic surfactant; Lysozyme; Binding isotherm; Potentiometry

1. INTRODUCTION

This paper has under taken further work on the thermodynamic properties [1–3] associated with the interaction between macromolecules and small charged organic molecules, such as surface-active agents, which have been investigated by several workers [4–8]. Essentially two types of experimental techniques have been used: firstly, those which measure macroscopic quantities, *i.e.*, which are

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influenced by all the components present in the solution (*e.g.*, viscosity, conductivity, emf measurements); secondly, techniques which measure changes in the molecular properties of the interacting species (*e.g.*, spectroscopic changes, NMR, *etc.*).

The result of the experimental measurements can be used to construct a binding isotherm, *i.e.*, a curve representing the amount of bound surfactant per monomer unit of the macromolecule as a function of the concentration or activity of the free surfactant in equilibrium. From these isotherms, an attempt is made to infer a mechanism or model for the binding process. The most widely used model for detergent-macromolecular interactions assumes that the binding of detergent molecules (S) to the macromolecules (L) occurs through a stepwise sequence of several chemical equilibrium (multiple equilibrium) in which each elementary equilibrium process is governed by the law of mass action [5]:

$$L + S \underset{K_{-1}}{\overset{K_1}{\Leftrightarrow}} LS$$

$$LS + S \underset{K_{-2}}{\overset{K_2}{\Leftrightarrow}} LS_2$$

$$LS_{n-1} + S \underset{K_{-n}}{\overset{K_n}{\Leftrightarrow}} LS_n$$

Lysozyme-surfactant interactions can be understood if the balance of forces involved can be estimated. Generally the dominant forces can be listed under the headings of electrical, hydrophobic interaction and dispersion forces. The first includes charge and dipolar interactions, *e.g.*, charge–charge interactions between a polyelectrolyte and surfactant head group and the dipoles of a non-ionic lysozyme.

Tadros obtained clear evidence of the interaction between polyvinyl alcohol (PVOH) and cetyltrimethyl ammonium bromide (CTAB) using surface tension, viscosity and conductivity techniques [9].

In this work the ion-selective electrode (ISE) without liquid junction potential was applied to determine the binding isotherm of cationic surfactants (DTAB = dodecyltrimethyl ammonium bromide and CTAB = cetyltrimethyl ammonium bromide) with lysozyme in the presence of various concentrations of buffer solution.

2. EXPERIMENTAL SECTION

The lysozyme was obtained from Merck and was used as received. It has a nominal molecular weight of 14600. The lysozyme was used to make up solution (0.05 w/V%) with water which had been doubly distilled. All of the cationic surfactants were Aldrich 99% pure grade.

The cationic surfactant-selective electrodes were constructed using a method which has been described previously [10–12]. For cationic surfactants the monomer surfactant activities in various solutions can be obtained from emf measurements from the following cell:

Ag/AgBr/Internal soln. membrane/
PVC membrane/Exp. soln./Ref. electrode

In the experiments the temperature was controlled to within 0.1°C by circulating thermostated water through the jacketed glass cell, and the sample solution was continuously stirred using a magnetic stirrer. The concentration of lysozyme was kept constant as the concentration of surfactant was varied during each experiment. The experiment was repeated with different concentrations of buffer solution.

3. RESULTS AND DISCUSSION

In this work, a system of fixed lysozyme and constant concentration of buffer solution, in the range between C_1 and C_2 of the surfactant concentration was considered. C_1 represents the concentration at which interaction between the surfactant and the lysozyme first occurs, and C_2 the concentration at which proper micelles are formed on the lysozyme. The emf of cell was plotted against total concentration of surfactant, DTAB (Fig. 1) referenced to a saturated calomel electrode and three different region were found:

I – The first region shows the Nernstian slope which indicates there is no binding and hence no measurable interaction between lysozyme and surfactant which is present in the form of free monomer.
II – In the second region a break (shown as C_1) was found in the linear line where the intraction between lysozyme and surfactant

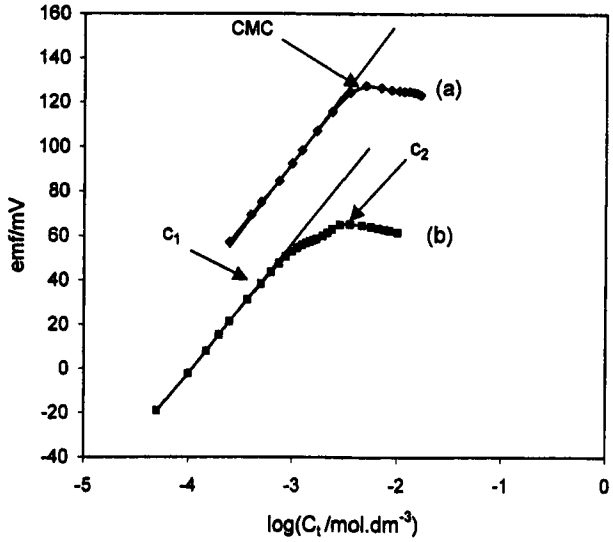

FIGURE 1 Plot of the emf of cell *versus* logarithm of total concentration of DTAB: (a) in water (without lysozyme); (b) in an aqueous solution of lysozyme (0.05 w/V%). Concentration of buffer solution and temperature are constant and equal to 0.05 mol·dm^{-3} and 308.15 K, respectively.

begins. In this region the concentration of both bound and free monomers of surfactant is varying.

III – C_2 is the beginning of the third region where the monomers concentration has reached a maximum. Above this point, the monomer concentration was found gradually to decrease as the total concentration of surfactant was increased.

This is normally considered as due to the appearance of proper micelles presumably occuring on the chain of lysozyme [13, 14]. The emf were used for the construction of the binding isotherm. The ratio of bound amount per number of mole of lysozyme, $\nu = (C_t - m_l)/C_P$, was plotted against ln m_l, where m_l is the monomer concentration of surfactant, CTAB (Fig. 2).

3.1. Determination of Gibbs Energy of Binding

Figure 3, typically, shows the number lysozyme of moles of DTAB bound per mole of lysozyme (ν) measured by the potentiometric technique as a function of the logarithm of the free DTAB

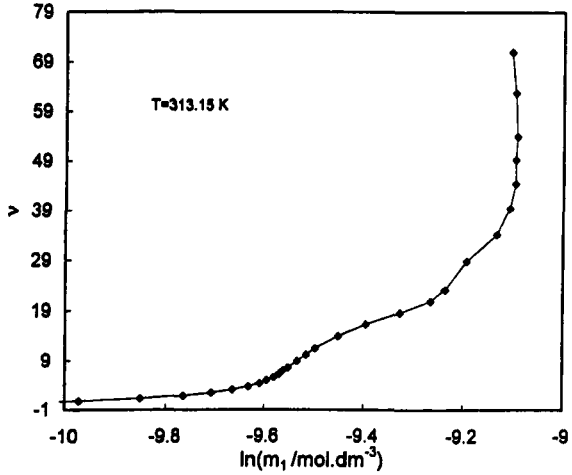

FIGURE 2 The binding isotherm of CTAB/lysozyme interaction. Concentration of buffer solution and lysozyme are constant and equal to 0.05 mol · dm $^{-3}$ and 0.05 w/V%, respectively.

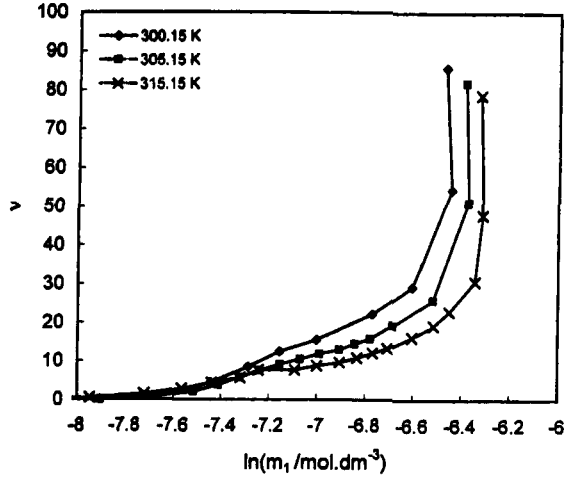

FIGURE 3 The binding isotherm of DTAB/lysozyme interaction at various temperatures.

concentration at different temperatures (300.15, 305.15 and 315.15 K) (Tab. I). The increase in temperature shifts the binding isotherm to a lower free concentration of surfactant. The calculation of the apparent

TABLE I Monomer concentration of surfactant, m_l with the ratio of bound amount per number of mole of lysozyme, ν for DTAB/lysozyme at three different temperatures

$T=300.15\,K$		$T=305.15\,K$		$T=315.15\,K$	
$ln\ m_l$ $(mol\cdot dm^{-3})$	ν	$ln\ m_l$ $(mol\cdot dm^{-3})$	ν	$ln\ m_l$ $(mol\cdot dm^{-3})$	ν
−7.90	0.09	−7.90	0.08	−7.95	0.54
−7.68	1.00	−7.67	0.81	−7.72	1.49
−7.55	2.64	−7.52	2.01	−7.56	2.84
−7.44	4.36	−7.41	3.82	−7.44	4.31
−7.28	8.47	−7.32	5.74	−7.32	5.66
−7.15	12.54	−7.24	7.51	−7.24	7.60
−7.00	15.58	−7.15	9.11	−7.09	7.64
−6.77	22.21	−7.06	10.54	−7.00	8.76
−6.60	29.32	−6.99	11.88	−6.91	9.63
−6.44	54.33	−6.90	13.03	−6.83	10.85
−6.47	85.75	−6.84	14.51	−6.77	12.14
		−6.78	15.84	−6.71	13.40
		−6.68	19.29	−6.60	16.24
		−6.52	25.93	−6.51	19.25
		−6.37	51.03	−6.45	22.92
		−6.39	81.89	−6.34	30.65
				−6.31	47.91
				−6.32	78.74

binding constant, K_{app}, can be applied to the entire binding isotherm. This is based on the Wyman binding potential concept [15]. The binding potential, Π is calculated from the area under the binding isotherm according to the equation:

$$\Pi = RT \int_{\nu=0}^{\nu} \nu_i d \ln m_l \tag{1}$$

and is related to an apparent binding constant, K_{app}, as follows:

$$\Pi = RT \ln(1 + K_{app} m_l^{\nu}) \tag{2}$$

ν_i is calculated using the following equation:

$$\nu_i = \frac{C_t - m_l}{C_P} \tag{3}$$

C_t, C_P and m_l are the total surfactant concentration, lysozyme concentration and free surfactant concentration, respectively.

Values of K_{app} were determined by application of Eqs. (1) and (2) were used to determine values of the Gibbs energy (ΔG_{app}) and the

Gibbs energy of binding per surfactant ion (ΔG_ν):

$$\Delta G_\nu = \frac{\Delta G_{app}}{\nu_i} = -\frac{RT}{\nu_i} \ln K_{app} \tag{4}$$

Figure 4 shows Gibbs energy of binding of interaction of lysozyme with DTAB. Initially there is a competition between the binding of surfactant to lysozyme, and exothermic process, and unfolding of the lysozyme chain, an endothermic process.

According to Figure 4, the process of binding involves two regions. In the first region ΔG_ν decreases as binding values are increased; therefore there are more sites available on the lysozyme to fill these sites. On the other hand the lysozyme chains are unfolded until the balance between the number of free and bound sites shifts in favour of lysozyme-bound surfactant and reaches the minimum value of ΔG_ν. We believe that when the surfactant concentration exceeds this critical value there are steric factors and electrostatic repulsion between head groups of the surfactant. Thus in the second region ΔG_ν increases. This Gibbs energy of binding, ΔG_ν for DTAB at various ν_i and temperatures (300.15, 305.15 and 310.15 K) are tabulated in Table II.

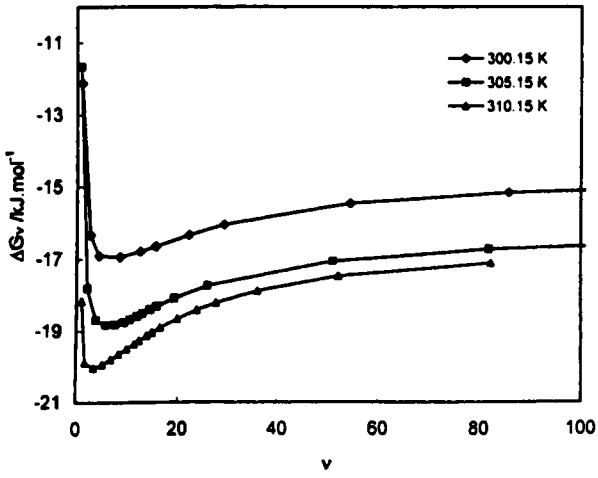

FIGURE 4 Plot of Gibbs energy of binding *versus* ν for interaction between DTAB and lysozyme at various temperatures. Concentration of buffer solution and lysozyme are constant and equal to 0.05 mol·dm^{-3} and 0.05 w/V%, respectively.

TABLE II Gibbs energy of DTAB/lysozyme interaction at various ν and temperatures. Concentration of buffer solution and lysozyme are constant and equal to $0.05\,mol \cdot dm^{-3}$ and 0.05 w/V%, respectively

$T=300.15\,K$		$T=305.15\,K$		$T=315.15\,K$	
ν	ΔG_ν ($kJ\,mol^{-1}$)	ν	ΔG_ν ($kJ\,mol^{-1}$)	ν	ΔG_ν ($kJ\,mol^{-1}$)
1.01	−12.11	0.81	−11.66	1.01	−18.15
2.64	−16.32	2.09	−17.80	1.71	−19.88
4.37	−16.90	3.82	−18.68	3.39	−20.03
8.47	−16.93	5.74	−18.83	5.10	−19.93
12.55	−16.78	7.51	−18.81	6.79	−19.79
15.59	−16.64	9.11	−18.75	8.35	−19.64
22.22	−16.33	10.54	−18.67	9.93	−19.49
29.33	−16.05	11.88	−18.58	11.43	−19.36
54.33	−16.47	13.03	−18.50	12.43	−19.26
85.75	−15.17	14.50	−18.39	13.94	−19.13
		15.84	−18.30	14.94	−19.03
		19.29	−18.07	16.48	−18.90
		25.93	−17.73	19.88	−18.64
		51.03	−17.05	23.76	−18.40
		81.89	−16.72	27.70	−18.20
				35.91	−17.88
				52.03	−17.47
				82.27	−17.11

The process of micellization of ionic surfactant represents a balance between several forces favouring and resisting aggregation. One of the main forces resisting aggregation is the crowding together of the ionic head groups at the surface of the micelle. The effect of a synthetic water-solube lysozyme with various hydrophobic segments has been investigated. As the concentration of the backing electolyte increases, the critical concentration at which the surfactant first binds to the lysozyme decreases. This is analogous to the effect of electrolyte on the micellization of the pure surfactant. For a given head group, the strength of the interaction has been found to be enhanced by increasing the hydrophobic chain length of the surfactant.

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