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# Thermodynamics and micellization of cetyltrimethyl ammonium bromide in the presence of lysozyme

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ABSTRACT

To study the structural changes in lysozyme (cationic protein) on the addition of N-cetyl-N,N,N-trimethyl ammonium bromide (CTAB) and to understand the mechanism underlying aggregation of resulting protein–surfactant complex, surface tension, density and sound velocity measurements of lysozyme–CTAB solutions were carried out over a temperature range of 25–40 °C. The critical micelle concentration (cmc) of CTAB has been calculated from surface tension measurements and is found to increase with increase in lysozyme concentration as well as with temperature. Surface tension data have been further used to calculate the interfacial parameters; maximum surface excess concentration ($\Gamma_{max}$), minimum area per molecule ($A_{min}$), standard free energy of adsorption ($\Delta G^{o}_{ad}$), surface pressure at cmc ($\Pi_{cmc}$) and standard free energy of transfer ($\Delta G^{o}_{tr}$), that have direct bearing on the consequences of such interactions at the molecular level. The negative values of standard Gibbs energy changes indicate spontaneity of micellization. Apparent molar volume ($\phi_v$) and apparent molar adiabatic compressibility ($\phi_\kappa$) have also been calculated using density and velocity of sound. These parameters have been examined as a function of surfactant concentration, lysozyme concentration and temperature to understand the consequences of interactions between these two components. A good qualitative correlation is found to exist with regard to the surfactant–lysozyme interaction obtained from the experimental data from different techniques.



## 1. Introduction

The surfactants find their wide spread applications as emulsifier, detergents and foaming agents [1–4] owing to their ability to lower the interfacial tension. The amphiphilic nature of surfactants is responsible for their tendency to concentrate at the interfaces and to aggregate in solutions into various supramolecular structures such as micelles and bilayers. Ionic surfactants and proteins both have charged groups and hydrophobic portions [5] and as a result the interaction between surfactants and proteins is relatively complex, involving different types of intermolecular forces. The ionic head groups of surfactants may bind to oppositely charged group on the protein surface by electrostatic forces, whereas non-polar tail groups of surfactants may bind to non-polar sites on the protein surface through hydrophobic forces [6]. These interactions, however are highly dependent on the nature of proteins as well as surfactant.

Chicken egg white lysozyme (molecular weight 14.6 kDa) is a small globular protein, consists of 129 amino acids, containing 18 cationic and 12 anionic residues, stabilized by four disulfide bonds. The importance of lysozyme relies on its extensive use as a model system to understand the underlying principles of protein structure, function, dynamics and folding, through theoretical and experimental studies [7,8]. High natural abundance is also one of the reasons for choosing lysozyme as a model protein for studying protein–CTAB interaction. Another important aspect of lysozyme is its ability to carry drug [9]. According to the X-ray crystal structure, lysozyme possesses a relatively rigid structure [10]. It contains six tryptophan (Trp) residues, three of them are located in the substrate binding site, two are located in the core hydrophobic region and one is separated from all other residues. Trp62 and Trp108 are the most dominant fluorophores [11].

In contrast to anionic surfactants, the literature on complexation of cationic surfactants and water-soluble proteins is limited [12–14]. Lysozyme has a tendency to bind with cetyltrimethylammonium bromide because of hydrophobic effect in spite of electrostatic repulsion [15]. So the aim of the present work is to investigate the effect of concentration of lysozyme on micellization of CTAB, which is a cationic surfactant. For this purpose, several samples covering a wide range of concentrations of protein and surfactant have been prepared. The physicochemical properties of these samples have been evaluated by using surface tension, density and ultrasonic velocity measurements. The conformational and rheological changes in lysozyme have also been investigated.

## 2. Experimental

### 2.1. Materials

Ordinary tap water was distilled with the help of Millipore (Elix) distillation unit, which was further distilled in the presence of alkaline

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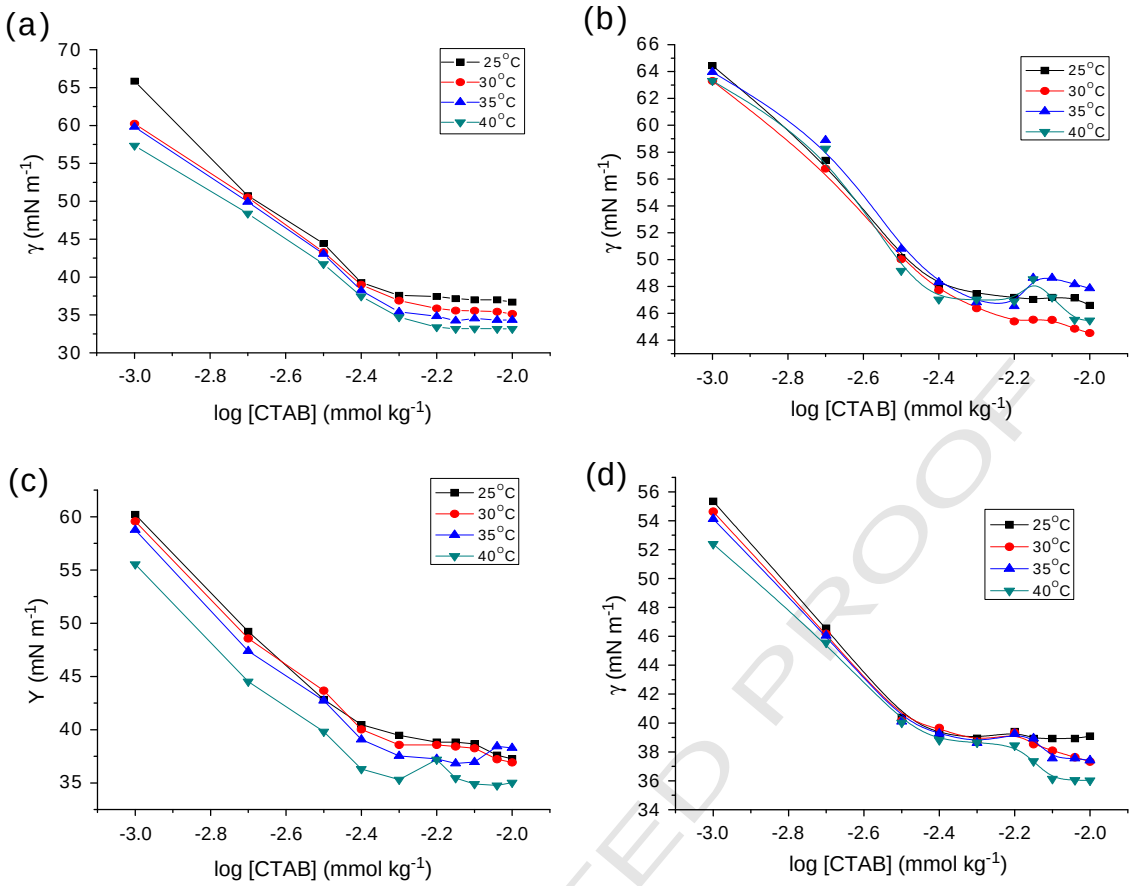


**Fig. 1.** γ versus log[CTAB] in aqueous solution containing (a) 0.0, (b) 0.05, (c) 0.1 and (d) 0.4% w/v of lysozyme.

potassium permanganate through a (750 mm) long vertical fractionating column. The water so obtained has specific conductance 2–3μS $cm^{-1}$ at 298 K and pH in the range of 6.5–7. Water of these specifications was used for all experiments as well as to calibrate survismeter and density and sound velocity analyzer-5000 (DSA-5000). Hen egg-white lysozyme from Sigma Chemical Co. was used as received. AR grade cetyltrimethyl ammonium bromide (CTAB) of purity > 99% was also obtained from S.D. Fine Chem. Ltd. A pure sample of CTAB was obtained by several recrystallizations from ethanol [16].

### 2.2. Equipment and experimental procedures

All the measurements were carried out in a high precision water thermostat supplied by Narang Scientific Works, New Delhi.

**Table 1**
Interfacial parameters of CTAB in 0, 0.05, 0.1 and 0.4% w/v lysozyme at different temperatures.

| Temperature (°C) | cmc (mM) | $\Pi_{cmc}$ (mM $m^{-1}$) | $A_{min}$ ($nm^2 \times 10^2$/molecule) | $\Gamma_{max} \times 10^{10}$ (mol/$cm^2$) | $\Delta G^o_m$ (kJ $mol^{-1}$) | $\Delta G^o_{ad}$ (kJ $mol^{-1}$) | $\Delta G^o_{tr}$ (kJ $mol^{-1}$) |
|---|---|---|---|---|---|---|---|
| *0% w/v lysozyme* | | | | | | | |
| 25 | 3.46 | 32.37 | 46.38 | 3.58 | −7.56 | −16.60 | −12.10 |
| 30 | 3.67 | 32.33 | 60.16 | 2.76 | −7.97 | −19.68 | −11.55 |
| 35 | 4.28 | 33.78 | 62.65 | 2.65 | −8.82 | −21.57 | −11.37 |
| 40 | 4.67 | 36.36 | 63.61 | 2.61 | −9.35 | −23.28 | −11.36 |
| *0.05% w/v lysozyme* | | | | | | | |
| 25 | 4.02 | 27.34 | 73.14 | 2.27 | −8.25 | −20.29 | −10.72 |
| 30 | 4.89 | 25.53 | 76.16 | 2.18 | −9.24 | −20.95 | −10.47 |
| 35 | 5.21 | 24.77 | 75.13 | 2.21 | −9.65 | −20.86 | −10.57 |
| 40 | 5.62 | 25.88 | 79.06 | 2.1 | −10.11 | −22.43 | −10.48 |
| *0.1% w/v lysozyme* | | | | | | | |
| 25 | 4.78 | 32.25 | 60.16 | 2.76 | −8.99 | −20.67 | −10.92 |
| 30 | 4.97 | 31.25 | 62.89 | 2.64 | −9.3 | −21.14 | −10.93 |
| 35 | 5.26 | 28.93 | 64.35 | 2.58 | −9.69 | −20.90 | −10.95 |
| 40 | 5.69 | 27.02 | 65.62 | 2.53 | −10.15 | −20.83 | −10.94 |
| *0.4% w/v lysozyme* | | | | | | | |
| 25 | 4.16 | 31.15 | 68.32 | 2.43 | −8.41 | −21.23 | −10.84 |
| 30 | 4.78 | 30.1 | 73.79 | 2.25 | −9.14 | −22.52 | −10.59 |
| 35 | 4.91 | 29.65 | 76.16 | 2.18 | −9.41 | −23.01 | −10.64 |
| 40 | 5.01 | 27.61 | 85.14 | 1.95 | −9.64 | −23.80 | −10.49 |