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Physico-chemical interactions of triton in aqueous lysozyme solution

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ABSTRACT

Protein and surfactant often occur together in various formulations of the chemical industry and are capable of modifying surfaces, altering colloid stability and controlling bulk viscosity. Protein – surfactant interactions are expected to have significant structural consequences of solvent medium. In solution, proteins induce formation of surfactant micelles which then bind to the polymer segments, leading to the formation of complexes. These complexes associate to form an insoluble phase which becomes soluble in the presence of excess of amphiphiles producing free micelles. The simultaneous presence of surfactants and proteins is required to achieve ideal dispersion effects in which surfactants provide emulsification capacity, interfacial tension control, whereas polymers impart colloidal stability and special rheological features. Thus, the practical importance of surfactant-(protein)polymer systems has motivated to carry studies of such systems, in which techniques such as viscosity and conductivity measurement, dialysis, fluorescence spectroscopy, NMR and neutron scattering have been used in order to explore the nature and kind of interactions present in these systems. These interactions are, of course characteristics of the protein and surfactant chosen. In the present studies, therefore, density and sound velocity of Triton X-100 (TX-100) in aqueous solutions of Lysozyme, which are sensitive to structural changes, have been measured over a wide temperature range (20-40°C). Molar volume (ϕ_v) has been calculated from density measurements and various acoustical parameters such as apparent adiabatic compressibility (β) and apparent molar compressibility (ϕ_k) have been calculated from the sound velocity data in order to account for the consequences of protein- surfactant interactions. Further information to this effect has been obtained by extending the work to include the viscosity measurements as well. The activation energy parameter has also been calculated using viscosity values.

INTRODUCTION

Protein surfactant interaction, particularly at a molecular level, is an important research area[1] that attracts the interests of researchers and has been extensively studied in aqueous solutions[2-4]. Studies include both oppositely[5-7] and similarly[8,9] charged protein - surfactant and protein - non ionic surfactants systems[10]. However, the understanding of the interactions at molecular level is complicated, since proteins are complex biomacromolecules with unique primary structure expressed in terms of their amino acid sequences. These molecular constituents contribute to a wide variety of interactions with surfactant molecules. Proteins and lipids have long been recognized to interact at interfaces as well as in the bulk solution and can thus affect the solution properties to a considerable extent[11-13]. Surfactants can bind to the protein not only in the monomer form but also in an aggregated state, depending on the surfactant concentration[2,3]. The interactions may result in stabilization or destabilization of the protein structure, depending upon the surfactant concentration and the natural environment of the protein[14,15].

It is known in general that anionic surfactants inter-

act strongly with proteins and form protein–surfactant complexes, which would induce the unfolding of proteins[3], whereas, cationic surfactants show a weaker primary interaction with proteins[3,16]. However, in contrast to these surfactants, non-ionic surfactants bind very weakly to proteins[3]. It is attributed to the low critical micelle concentration (CMC) of some non-ionic surfactants and the absence of the electrostatic interaction between protein and non-ionic surfactant that make micelle formation in the bulk solution a more favourable process than binding to proteins[3,17]. Surfactants, particularly non-ionic types, are often added to prevent and/or minimize protein aggregation during fermentation, purification, freeze-drying[18].

TX-100 ($C_{14}H_{22}O(C_2H_4O)_n$) is a nonionic surfactant which has a hydrophilic polyethylene oxide group and a hydrocarbon lipophilic or hydrophobic group. The hydrocarbon group is a 4-(1, 1, 3, 3-tetramethylbutyl)-phenyl group. TX-100 is widely used in biological works, such as separation of proteins from cell membrane,[19] and mixed with phospholipids it produces effective substrates for studying enzymes of phospholipid metabolism.

In the present study, we have investigated the properties of a two component system comprising lysozyme, a water soluble protein and the non-ionic surfactant, TX-100, both of which are found in many products. The principal aim has been to develop an understanding, at the molecular level, of the relationship between properties of protein/surfactant mixtures in the bulk phase.

EXPERIMENTAL

Ordinary tap water of conductivity range 3–5×10-6 S cm^{-1} at 25 °C was distilled with the help of Harco double distillation unit. The water so obtained has conductance value ~1–4×10^{-7} S cm^{-1} at 25 °C and pH in the range 6.5–7.0. Water of these specifications was used for all experiments.

Lysozyme and TX-100 used in this study was obtained from Merck Chemicals. Both the chemicals were kept in the refrigerator and were used as supplied.

Densities and sound velocities were measured using calibrated pyknometer and Ultrasonic Interferrometer (Model-8, single frequency). Viscosity measurements were carried out with a calibrated Jacketed Ubelholde viscometer. The precision achieved in viscosity measurements was ±0.02%.

Density (d) and sound velocity (v) for surfactant TX-100 in aqueous solutions of 0.25, 0.50, 1.0% w/v lysozyme in the concentration range (0.00043 - 0.16756 mol kg^{-1}) were measured over a wide temperature range (20 - 40 °C) at interval of 5°C.

DISCUSSION

Density and sound velocity measurements

From density and sound velocity values, we have evaluated different parameters like Apparent molar volume (ϕ_v), Apparent molar compressibility (ϕ_k) and Compressibility coefficient (β) using relations[21,22]:

$$\phi_v = 1000 (d_o - d) / (m d d_o) + M / d$$

$$\phi_k = 1000 (\beta - \beta_o) / (m d_o) + \phi_v \beta$$

$$\beta = 1 / (v^2 d)$$

where m is molarity of the solution, M is molecular weight of surfactant. v_o, d_o, β_o and v, d, β are the velocities, densities and compressibilities of pure solvent (aqueous solution of lysozyme) and solution.

ϕ_v values thus obtained are negative over entire temperature range. Although this parameter is expected to contain contributions from hydrophobic and hydrophilic interactions, the negative values of this parameter seem to reflect the predominance due to intermolecular hydrophobic interactions between protein and surfactant with the loss of hydrophobic hydration[23]. The dependence of ϕ_v in aqueous solutions of lysozyme as a function of surfactant concentration and temperature has been shown in Figure 1. A perusal of this figure shows that at low surfactant concentration, ϕ_v values increase sharply up to 0.00089 – 0.00109 mol kg^{-1} of the surfactant concentration. This increase may indicate some interaction taking place between TX-100 and lysozyme which may be due to negative charge present on polyethylene moiety of surfactant with some positive sites[24] of protein. However, another interesting feature observed is the hump in ϕ_v values in the concentration region ~ 0.00109 – 0.01458 mol kg^{-1} which is more predominant at higher lysozyme concentration (0.50% and 1.00% w/v gelatin) showing definitely the presence of hydrophobic manifestations. Hence we expect weak electrostatic as well as hydrophobic interactions up to

this concentration of TX-100. A similar behaviour has also been reported from studies such as foaming and solution properties of protein – non ionic surfactant system e.g. fluorescence studies[25] have shown that in BSA both number of binding molecules (n) and binding con-

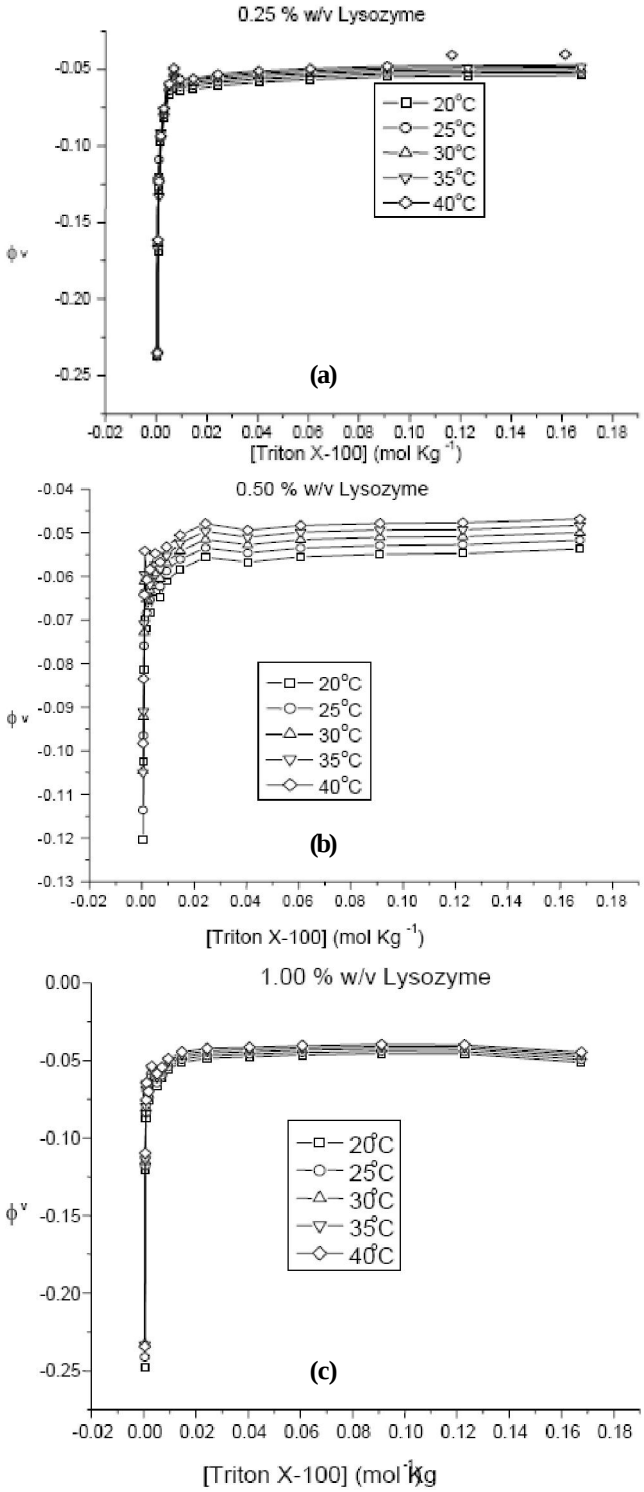


Figure 1 : Apparent molar volume as a function of [Triton X-100] in (a) 0.25 % w/v (b) 0.50 % w/v and (c) 1.0 % w/v lysozyme at different temperatures.

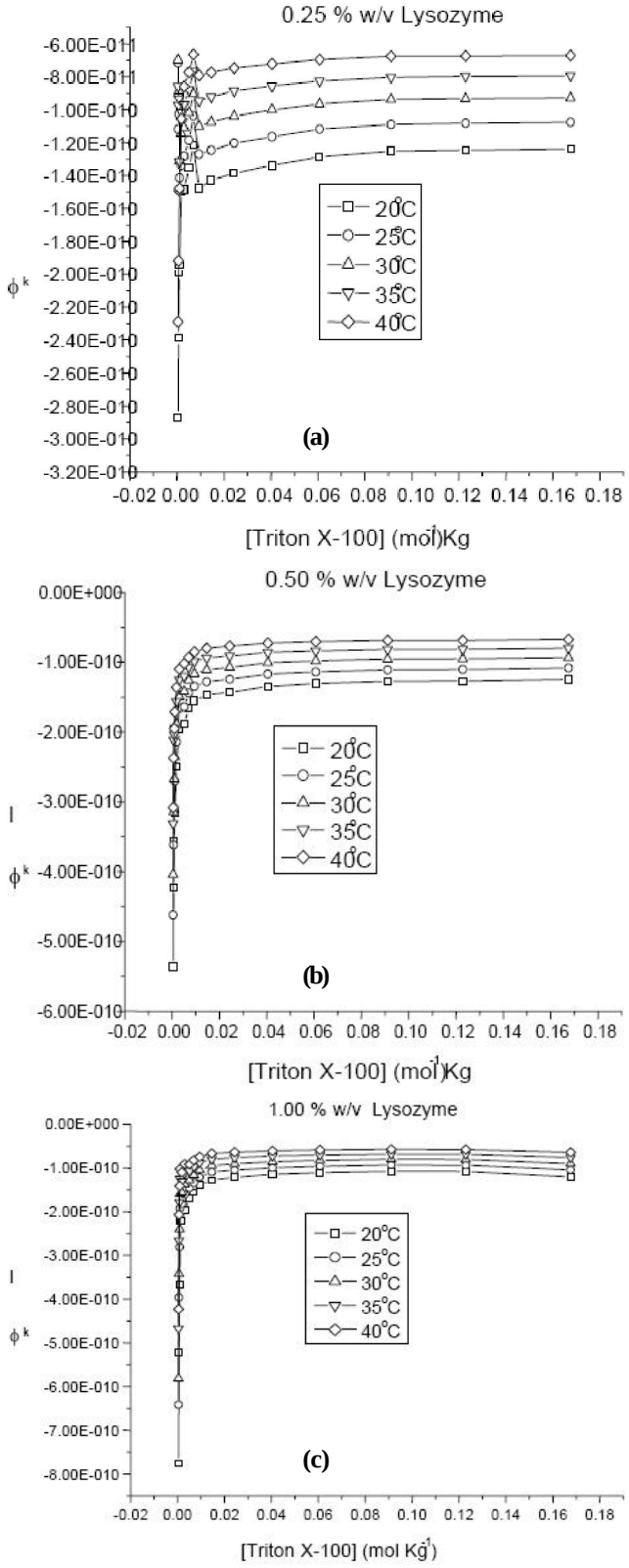


Figure 2 : Apparent molar compressibility as a function of [Triton X-100] in (a) 0.25 % w/v (b) 0.50 % w/v and (c) 1.0 % w/v lysozyme at different temperatures.

stant (K) changes with varying concentration of TX -100. Below CMC of TX -100 both n and K were found to be higher than above CMC values showing binding constant decreased with increased bulk constant of TX -100 concentration in the bulk.

With further increase in TX -100 concentration in aqueous lysozyme solution, ϕ_v values becomes strikingly independent of the surfactant concentration, indicating that surfactant molecules now prefer to undergo micellization rather than interacting with the protein molecules. It was suggested that micelle formation and association[26] with protein represent competitive phenomena. Similar observation has also been reported in BSA- TX -100 system with the help of ITC and DSC studies[27].

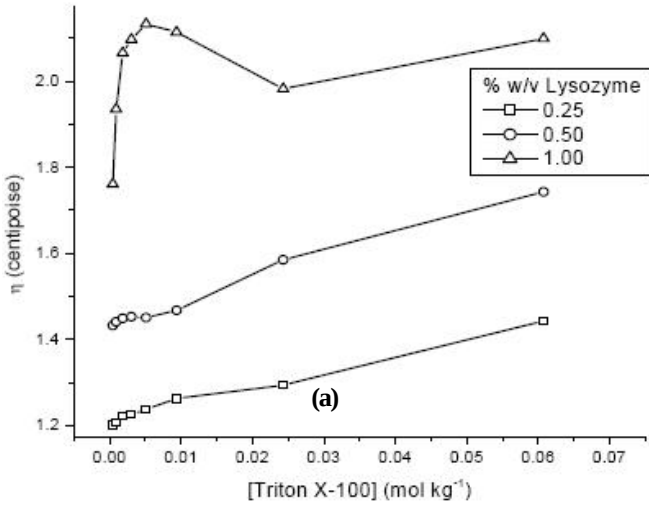
Figure 2 represents the dependence of ϕ_k as a function of concentration and temperature. The results of ϕ_k are also supported by the trends obtained from ϕ_v values. These figures reveal that there exists a reasonably good consistency as regards to the concentration and temperature dependence of ϕ_v and ϕ_k values.

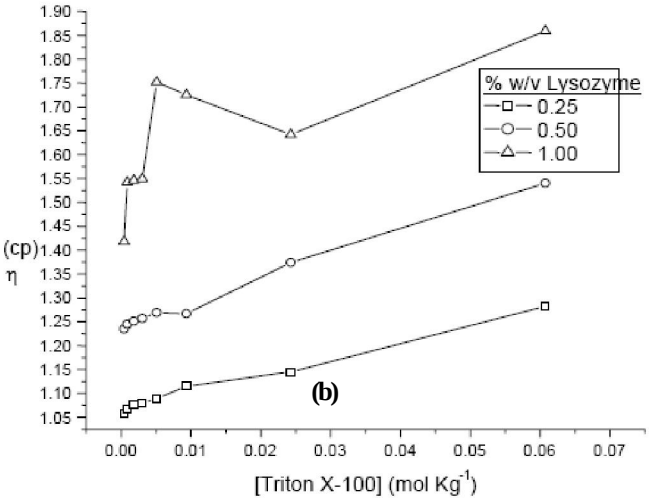
Adiabatic compressibility coefficient (β) values are reported in the TABLE 1. β values show a regular decrease with increase in surfactant concentration, a result similar to as reported earlier[24]. However, it is proposed that the observed changes can be attributed to the intrinsic ionic compressibility and to the structural (hydrophobic hydration) factors. A relatively sharper decrease observed in β values beyond 0.01458 mol kg^{-1} of TX-100 further provides an insight for micellization within the surfactant molecule causing more compactness.

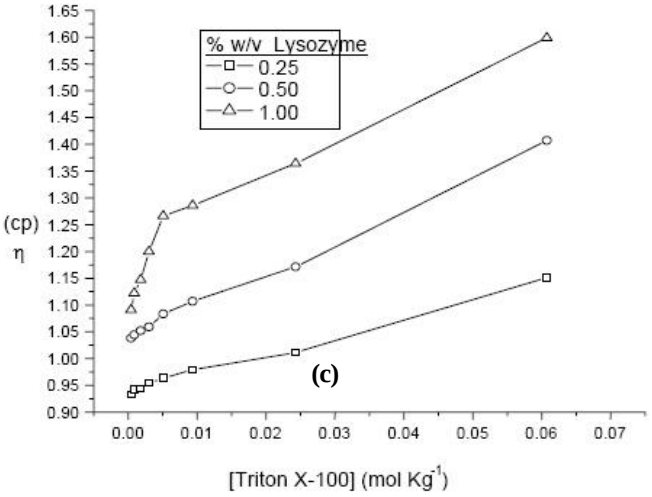
Viscometeric studies

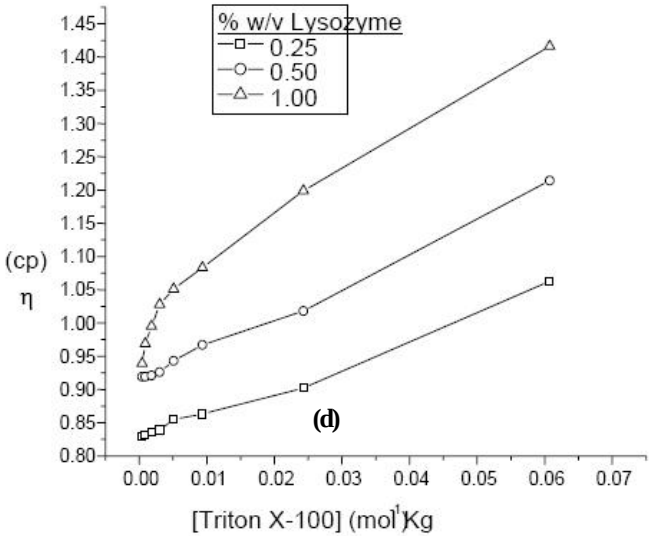
Viscosity studies have also been found to be very effective in order to understand the conformational as well as structural changes of proteins in the presence of surfactants. Therefore, viscometric behaviour of TX-100 in aqueous solutions of lysozyme has been studied at different temperatures. The concentration dependence of viscosity, η of TX-100 has been presented in Figure 3. The η values increases non-linearly at low surfactant concentration followed by a linear behaviour at higher surfactant concentration at all studied temperatures. This is the general behaviour of protein-surfactant system and is attributed to non-cooperative binding and increased intermolecular contacts at lower concentration followed by cooperative binding at higher concentration[28]. Also increase in η values with addition of lysozyme is in proportion to the protein concentration.

The viscosity data has further been analyzed in the light of the activation parameter of the viscous flow pro-









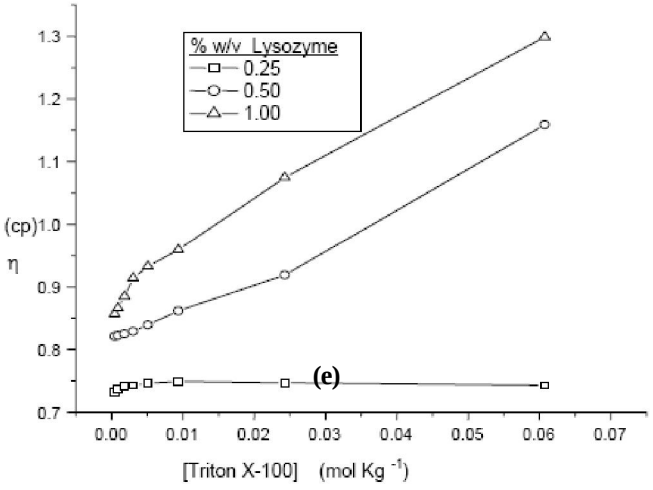


Figure 3 : Viscosity coefficient as a function of [Triton X-100] in 0.25 % w/v, 0.50 % w/v and 1.0 % w/v lysozyme at different temperatures.

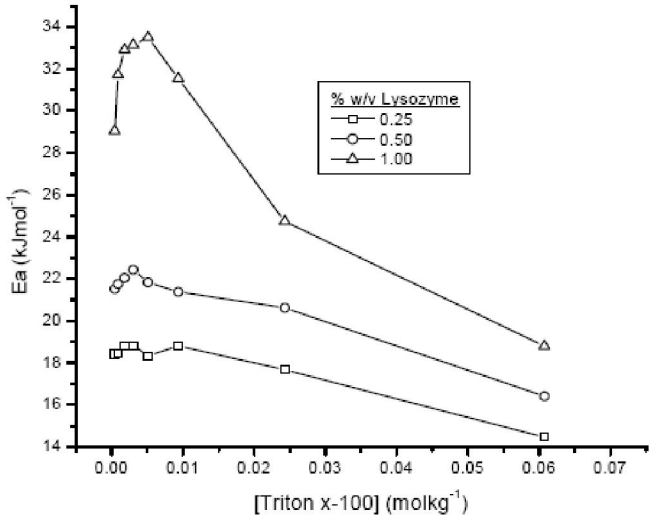


Figure 4 : Activation energy as a function of [Triton X-100] concentration at different concentration of lysozyme.

cess of the protein – surfactant system. The activation energy, E_a has been estimated from the slope = E_a / R of the equation

$$\eta = A \exp (E_a / RT)$$

by plotting log η against 1/T. In all cases, the plots were found to be linear over the entire temperature range studied. A dependence of E_a on surfactant concentration in different aqueous solutions of lysozyme has been presented in Figure 4. The activation energy is found to be linear, except at low surfactant concentration. These observations, no doubt, corroborate the conclusion drawn earlier.

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