CHAPTER 6

VISOCOMETRIC STUDIES OF CATIONIC SURFACTANTS WITH SOLUBLE OVALBUMIN

INTRODUCTION

Surfactant have been widely studied due to its significance in both applied and fundamental process: detergency, catalysis, flotation, lubrication, colloid stabilization, foaming, emulsification, protein denaturatin, tension moderation in membranes, membrane permeation and drug delivery (1). Surfactant also used to synthesis of nano and mesomaterials using their capability to form self organized aggregate structure (2). Therefore, the determination of the packing ordering, and its relation to the properties of the surfactant aggregates is of fundamental importance (3, 4). The field of polymer surfactant system applications have been considerably extended by many type of polymer (5).

Polymer-surfactant interactions (6-17) have been extensively investigated by researchers due to their manifold applications in the field of food and pharmaceutical industry, and in analytical biochemistry. Surfactant solutions have a general tendency to (18, 19) solubilize a certain amount of additives that correlated with their structural organization and mutual interactions. The interaction of protein with mostly cationic and anionic surfactants ingredients are the particular interact because they are used to co-operatively in formulated complexes. The interaction of coagulant protein with surfactant sodium dodecyl sulphate (SDS) and sodium-dodecyl benzene sulphonate (SDBS) were also investigated by capillary viscometry (20).

The mechanism for protein-surfactant interactions are polyelectrolyte absorption (21) hydrophobic (22). Different type of physico-chemical techniques, like surface tension, conductivity,
viscosity etc. have been used to investigate the interaction between cationic and anionic surfactants with protein (23-29). These interactions in aqueous media give rise to the formation of association structures, thereby modifying the solution and interfacial properties (30, 31). Hydrophobic and electrostatic interaction are two main driving forces for the association between surfactants and proteins. This protein has many experimental applications. Interaction of proteins with surfactants is of great importance in the field of industrial (cosmetics, paints, foods), biological and pharmaceutical sciences (32).

Singh and Coworkers (33) have reported the interaction of surfactant with gliadin by dialysis equilibrium method. The micellization behaviour of anionic and cationic surfactants in the presence of charged (HSA and RNA) and neutral polyvinyl pyrrolidone (PVP) polymer has been studied by conductance measurements (34). Singh et al. have also studied the interaction of transfusion gelatin molecules with cetyl prodinium chloride (35). The viscometric study on gliadin with sodium dodecyl sulphate and cetyl pyridinium Bromide were made (36). Singh and Coworkers have studied the effect on conductance and pH value of synthetic polymer PVP in presence and absence on ionic surfactants (37) and viscometric studies on the interaction of anionic surfactant with α-amylase (38). Alif et al. (39) have investigated the effect of surfactant on hydrilla verticillates by several methods. Sharma studies the effect of bovine milk cascin on surfactant using viscometric method (40) and Dharp and Coworkers (41) have also studied the interaction of surfactant with lipid and protein. In this chapter of the thesis the effect of soluble ovalbumin with cationic surfactants viz., cetyl pyridinium chloride (CPC) and Dodecyl pyridinium chloride (DPC) have been studied using viscometric method.

**EXPERIMENTAL**

**Reagents**: Soluble ovalbumin was prepared from ovalbumin (Sigma
by the method of Smith and Back (42) as described by Arora and Singh (43). The strength of clear solution of protein was determined by evaporating its known volume in an air oven at 105°-110°C. Stock solution of CPC and DPC were prepared in double distilled water. Solutions of hydrochloric acid and potassium chloride (BDH) were used for pH adjustment. Carbonate free KOH was prepared by a standard method. The buffer solutions used were prepared from Reagent Grade Chemicals.

**pH-measurements**: These measurements were carried out as described in Chapter 2.

**Viscosity Measurements**: The viscosity measurements were made with the help of an Ostwald Viscometer of relatively long capillary (flow time for water was 65 sec.) at a temperature of 25±1°C, in a water bath. The kinetic energy correction was found to be negligible. The viscometric stand was arranged in such a way that the viscometer was always situated in the same position in the bath. Protein and surfactant stock solutions were centrifuged at 16000 rpm for 60 minutes to remove particulate matter. The densities of the solvent and solutions were determined with the help of a pyknometer. The viscosity values were calculated by the following relation:

\[
\eta_{rel} = \frac{\eta}{\eta_b} \times \frac{t \times d}{t_0 \times d_0}
\]

where \(\eta_{rel}\) is the relative viscosity, \(t\) and \(d\) are the flow time and density of the solution, while \(t_0\) and \(d_0\) are the time and density of the solvent (water).

**Procedure**:

The following sets of solutions were arranged for viscosity measurements.

(i) A fixed amount of protein (5 mg/ml) was taken in two different pyrex glass tubes. Varying amounts of hydrochloric acid (0.1 M)
or caustic potash (0.1 M) were added, the total volume made 15 ml by adding required amount of distilled water and pH as well as viscosity was recorded.

(ii) A fixed amount of protein (5 mg/ml) and surfactant, CPC/DPC (each 0.002 M) was taken in different pyrex glass tube and varying amount of HCl or KOH were added, the pH and viscosity was determined.

(iii) A fixed amount of protein (5 mg/ml), varying amounts of CPC/DPC were added and the total volume was made upto 15.0 ml. The viscosity of the solutions were determined.

(iv) Different amount of protein in presence of CPC/DPC (each stock 5 g/ml) were mixed in different boiling test tubes and the total volume in each case was adjusted to 15.0 ml (continuous variation) so that mixtures having different protein-surfactant ratio were obtained. Viscosity measurements were made below isoelectric point of soluble ovalbumin.

(v) To a fixed amount of protein (2 mg/ml) varying amounts of CPC/DPC were added and total volume was made upto 15.0 ml. The viscosity of this set was measured at pH-value 7.0, 8.0, 9.0 and 10.0, respectively.

(vi) Varying amounts of protein (2 mg to 48 mg/ml) were taken along with the same amounts of CPC/DPC and the pH was adjusted to 3.0 and 5.80, respectively. The viscosity of this set was measured and the data analysed to get intrinsic viscosity of the polymer at infinite dilution with the help of reduced viscosity.

RESULTS AND DISCUSSION

Viscosity is one of the important tools to study the conformational and rheological changes in protein during their interactions with ionic surfactants. The initial increase in viscosity corresponds to binding of surfactant monomers to the protein chain. Further increase in viscosity
with increase in concentration of surfactant might be due to the cross-linking of the several aggregates of micelles formed by free surfactant monomer in the solution (15, 44). The measured relative viscosity is converted to reduced viscosity \( \eta_{\text{red}} \) by using the following expression.

The following relation applies for dilute solution of surfactant and protein or their mixture:

\[
\eta_{\text{sp}} = \eta_{\text{red}} - 1 = \frac{\eta}{\eta_0} - 1
\]

where \( \eta_{\text{sp}} \) is specific viscosity, \( \eta \) is the viscosity of surfactant or protein or mixture, \( \eta_0 \) is the viscosity of pure solvent.

Reduced viscosity \( \eta_{\text{red}} \) = \( \frac{\eta_{\text{sp}}}{C} \)

where \( C \) is the concentration of surfactant in g/ml. Thus reduced viscosity of solution of surfactant in absence and presence of protein were calculated by the above expression.

The reduced viscosity \( \frac{\eta_{\text{sp}}}{C} \) of soluble ovalbumin solution in the absence or presence of cationic tenside are shown in Figure 1, as a function of pH. The reduced viscosity curve has a minimum at the isoelectric point (IEP) and a maximum on either side of this pH. At this pH the macromolecule of soluble ovalbumin is probably in the contracted form owing to the attractive forces between balanced charges i.e. existence of the zwitter ion in protein structure, while on both sides of isoelectric point, the macromolecule has a net overall charge, which may cause the molecule to extend by repulsion. However, in more acidic or basic solutions, the repulsion forces will be again reduced, owing to the increase in concentration of free ions (H\(^+\) ions from HCl and OH\(^-\) ions from KOH). The constance of reduced viscosity between pH 7.00 to 10.00 resonably explains the stability of protein conformation as also reported in case of soluble ovalbumin. An abrupt rise in viscosity above pH 11.0 indicates extensive unfolding of protein

(152)
chain in the presence of ionic surfactant (44).

The reduced viscosity vs. pH curves in the presence of 0.002 molar CPC/DPC show minima at the IEP, but the values of viscosity number are higher than protein alone, which may be due to combination of tenside cation with neutral protein molecule to give it a net overall charge and consequently cause the molecule to extend itself. The shift of IEP towards higher pH can be explained in terms of the reaction of the surfactant cations with anionic groups of protein. Owing to the binding of surfacant cations to the anionic carboxyls, the total negative charge on protein molecule decreases. This explains the shift of IEP towards more alkaline side. The lesser viscosity on both sides of the IEP in the presence of cationic surfactants show the contraction of protein owing to formation of salt like complexes. In higher pH range surfactant caused unfolding of protein as is indicated from the nature of viscosity pH-curves. It can also be noted that CPC caused much more structural change the DPC. These results are in line as reported by Singh et al. (35) in the binding of cationic surfactants to transfusion gelatin.

The effect of CPC/DPC at the isoelectric pH is quite interesting. Figure 2 shows the variation of the relative viscosity as a function of surfactant concentration at the isoelectric point. It is apparent from the plots that, as the concentration of two surfactant increases, the relative viscosity of mixed solutions increase, attains maximum and then decreases.

This suggests that the combination of surfactant to neutral protein increases the net charge causing an extension in the molecule. The decrease in viscosity after maximum in viscosity surfactant concentration curve can be due to the screening effect of counter ions. At still higher surfactant concentration the viscosity becomes constant. This would represent a state where the screening effect of counter
ions is maximum.

The viscosity variations of protein-surfactant systems (continuous variations) are plotted in Figure 3. The plots exhibit maxima at surfactant-protein ratio of 1:2 which indicates the formation of complex of one to two composition. However, the higher viscosity of CPC-protein than DPC-protein mixtures supports to the higher binding capacity of CPC (C₁₆) as compared to DPC (C₁₃). The relative position of the two curves again go to show that CPC has more protein unfolding power than DPC.

Figures 4 and 5 show the change of relative viscosity obtained upon the addition of CPC/DPC within certain ranges of the surfactant concentrations, either before precipitation or after redissolution. It is seen that relative viscosity values are greatly reduced as a small amount of surfactant is added until precipitation takes place and that after resolubilization of the precipitate initially produced, the mixed solutions exhibited nearly Newtonian behaviour while it was non-Newtonian before precipitation. The mechanism of the phenomenon may be considered as follows. The more or less expanded protein molecule at the alkaline side of the isoelectric point may be changed into the folded hydrophobic state by the continuous combination of surfactant cations and consequently decreases until precipitation takes place. On addition of more surfactant, however, a second adsorption layer would be formed by van der Waals attraction forces between carbon chains (−CH₂ groups), which makes the molecule more hydrophobic and therefore the precipitate solubilized again. The mixed solution thus formed behave similarly to the solution at the IEP without the surfactant. It is interesting to note that the relative viscosity of the mixed solutions, directly before precipitate formation or directly after redissolution of the precipitate, is somewhat less than that of the solution at its isoelectric point. This suggests that soluble ovalbumin in such mixtures may be in a more highly folded state than the
molecules at the IEP, CPC reduces the relative viscosity more than DPC. It may be lesser value of CMC of CPC than that of DPC.

The precipitation zone in Figures 4 and 5 is indicated by means of vacant space. It can be noted that the amount of surfactant needed to bring about complete precipitation increases as the pH of the mixed solution increases as we move away from the IEP e.g. at pH 7.00 the effective concentration is 0.028 mole while at pH 10.00, it is approximately 0.056 mole.

This indicates that the protein/surfactant ratio rises the net negative charge on protein increases (45). Thus the binding process appears to follow a one to one stoichiometry so far as the number of negatively charged groups (carboxyls and phosphates) on OAS molecule are concerned (43). The observed differences between the viscosity concentration behaviour at higher and lower pH can be ascribed to the “desolvation” phenomenon of the protein molecule in the presence of the added tenside. The exposed protein/particles then reacts with the surfactant cations to form a protein-surfactant (P-S) complex. The increases in relative viscosity at higher surfactant concentrations must be correlated with the solvation phenomenon. Kryut et al. (46) reported similar type of viscosity vs. cencentration curves in the polyphenol-gelatin system.

The effect of respective cationic surfactant on protein conformation is explained by means of intrinsic viscosity i.e. limiting value of viscosity number (reduced viscosity). This quantity is also a measure of the effective hydrodynamic volume per gram of the macromolecule. The limiting viscosity number (η) of soluble ovalbumin in the presence of varying amounts of cationic surfactant were calculated by plotting the viscosity number against protein concentrations (mg/ml) and then extrapolating the curves to zero protein concentration (Figures 6 to 8).
\[ \eta_{sp}/C = \lim_{C \to 0} \]

These values of limiting viscosity number are complied in the following Tables 1 to 2.

It is observed that the viscosity numbers (reduced viscosity) increase as the quantity of the respective cationic surfactant increase. This rise the visocisty number may be correlated with the conformational changes produced in soluble ovalbumin molecule by the added cationic surfactant. It could also be noticed that hexadecyl pyridinium chloride \((C_{16})\) produced a greater increase in viscosity numbers, than did the dodecyl pyridinium chloride \((C_{12})\). Thus showing higher reactivity of the former. These conclusions are in line with those reported earlier in case of transfusion gelatin molecule (35).

The higher limiting viscosity numbers \([\eta]\) below iso-electric point of soluble ovalbumin \((p\text{H} 3.00)\) could not be due to electroviscous effect as both the protein and surfactant possessed same charged (cationic). However, such an anomalous behaviour could be explained on the basis of protein swelling, presumably due to the electrostatic repulsion in between a large number of positively charged groups \((43)\). The swelling of a protein in the acid range due to the repulsion of similarly charged locii may cause opening up of the soluble ovalbumin molecule and the surfactant cations can penetrate into the swollen regions. The relatively lower viscosity at \(p\text{H} 5.80\) as compared to \(p\text{H} 3.00\) could be explained by the fact that in the vicinity of IEP the soluble ovalbumin exists as a compact molecule, and the additional surfactant caused less unfolding due to the availability of balanced charges on the protein surface. Most probably the surfactant breaks the salt bridges between anionic and cationic sites and becomes attached there \((47)\). The stepwise rising values of limiting viscosity number with increasing quantity of surfactant may be due to the solubilization and unfolding of the biopolymer. This nature depends on chain length, mode of distribution of polar sites, flexibility of polypeptide chains, tightness of packing
Table 1: Intrinsic viscosity [η], (mg/ml) for CPC-OAS system at pH 3.00 and 5.80 at 25°C temperature (OAS Conc. = 5mg/ml).

<table>
<thead>
<tr>
<th>Conc. of CPC x 10^3 (mg/ml)</th>
<th>Value of intrinsic viscosity [η]</th>
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<tbody>
<tr>
<td></td>
<td>At pH 3.00</td>
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<tr>
<td>0.0</td>
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<td>2.0</td>
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<td>8.0</td>
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<td>57.5</td>
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<tr>
<td>26.0</td>
<td>72.0</td>
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<tr>
<td>30.0</td>
<td>75.0</td>
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</tbody>
</table>

Table 2: Intrinsic viscosity [η], (mg/ml) for DPC-protein system at pH 3.00 and 5.80 at 25°C temperature (OAS Conc. = 5mg/ml).

<table>
<thead>
<tr>
<th>Conc. of DPC x 10^3 (mg/ml)</th>
<th>Value of intrinsic viscosity [η]</th>
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<tr>
<td></td>
<td>At pH 3.00</td>
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<tr>
<td>0.0</td>
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and on the number of cross links i.e. sulphide links. The progressive rise in flow property is due to a corresponding elongation of the macromolecule and a consequent increase in the dissymmetry of the polypeptide units.

From the flow behaviour of soluble ovalbumin-cationic surfactant mixtures, a probable mechanism for surfactant binding and subsequent opening may be provided. The combination of surfactant with protein involves two possible modes of linkages. One of these is chiefly electrostatic involving ionic bonds, while the other appears to be nonelectrostatic involving forces which usually bind surfactant ions into micelles. In the beginning when a little quantity of surfactant is added the ionic linkages are established, but in the presence of larger amounts of surfactant, the nonelectrostatic types of forces are more probable. This is also evident from the values of Huggin’s constant (slope of reduced viscosity vs. concentration curves) which goes on increasing continuously showing a large amount of electrostatic interaction to be present when the concentration of surfactants are low, whereas the phenomenon of uncurling seems to go towards completion with increasing concentration.

The view of Lundgren (48) that the secondary association of extra surfactant is in the form of a loose combination due to a polar attraction with that which is already electrostatically bound finds support from the present investigations. At higher surfactant concentrations, if the binding is due to a polar attraction, there should be less of particle interaction and the increase in the values of Huggin’s should not be as much as is observed in the initial stages of surfactant addition. The presence of hydroxyl groups (alcoholic and phenolic) on protein surface may also cause the formation of hydrogen bonds with the added surfactant. It may therefore be concluded that surfactant-protein interaction involved ionic, hydrophobic and hydrogen-bonding in forming the complexes depending upon the pH and concentration of the respective surfactant.
REFERENCES


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