CHAPTER VII
PROTEIN – SURFACTANT INTERACTIONS – A STUDY OF INTRINSIC AND EXTRINSIC FLUORESCENCE

7.1. Perspective of the Work

This study probes the interaction of common surfactants with the protein BSA. Serum Albumins are transport proteins found in blood plasma\(^1\,^2\). They can bind a wide variety of ligands i.e. bilirubin, fatty acids, hematin and drugs to name a few. Thus the main function of serum albumin is to transport metabolites in blood. BSA is one protein that is commonly used for research purposes due to its stability, water solubility and versatile binding capacity\(^1\).

The study of specific and nonspecific interactions of proteins with surfactants is fundamental both from the viewpoint of understanding and application. Surfactants are used to extract proteins from cell membranes. Surfactant–protein interactions are comparable to some extent to lipid – protein interactions in the membranes of living cells\(^3\,^4\) and can account for the transport of metabolites in body fluids\(^1\,^3\). Technical applications of surfactant – protein interactions include drug delivery, cosmetic preparation and often detergent action\(^5\). One important application is in the SDS-PAGE electrophoresis technique to determine the polypeptide composition of proteins\(^6\). This study was undertaken against this backdrop of literature on protein – surfactant interactions. Surfactant binding to proteins have been probed in the past mainly by dialysis method and titration calorimetry\(^7\,^9\). Only a few workers have employed fluorescence methods to monitor protein – surfactant interactions\(^10,^11\). But most of the earlier work involved anionic surfactants. To our knowledge, there are very few reports where interaction of a protein with cationic and nonionic surfactants have been studied in detail by spectroscopic methods.
In this work protein – surfactant interactions have been studied mainly by the fluorescence method. The fluorescence probe method has emerged as an important tool for bio-membrane research. Its advantages are its high degree of sensitivity and low degree of membrane perturbation. The fluorescence probe ANS (1-anilino-8-naphthalene sulfonate) is most usually used in bio-membrane research\textsuperscript{12-19}. Here, ANS has been used as an extrinsic fluorophore (since it binds noncovalently to many proteins), to study how surfactant binding to the protein can alter the probe binding sites. The intrinsic tryptophan (Trp) fluorescence of BSA has also been monitored.

It is well known that ANS exhibits a large fluorescence enhancement on binding to proteins either in the native or denatured form\textsuperscript{10,12-19}. The fluorescence enhancement can be explained on the basis of an ICT excited-state\textsuperscript{12,13} some workers have explained ANS fluorescence using the polarity-dependent TICT concept\textsuperscript{17-19}. In polar media, the main non-radiative pathway in the excited state of ANS is Twisted Intramolecular Charge transfer (TICT). When ANS molecules bind to the nonpolar interior of proteins, TICT is hindered and thus fluorescence enhancement is observed. The protein used in this work is BSA (Bovine Serum Albumin). The binding of ANS to BSA in presence of surfactants is explored fluorimetrically so as to detect structural alterations in the protein induced by surfactants. Both ionic and nonionic surfactants have been used. The fluorescence studies have been complemented by circular dichroism studies.

7.2 Results and Discussions:

7.2.1. Aggregation of Surfactants in Presence of BSA:

Before venturing into a detailed study of surfactant-protein interactions, it was worthwhile to note how the micellisation process of the surfactants was affected by the protein, BSA. In presence of 30 \( \mu \text{M} \) protein, fluorescence of 20 \( \mu \text{M} \) ANS decreases with increasing
concentration of surfactant. This maybe due to competitive binding of ANS and surfactant to the protein surface. It was found that in presence of protein, the break point in $I_f$ versus [surf] plot occurs at concentrations very different from the respective CMC's of the three surfactants\textsuperscript{11,20}. The values are 1.5 mM, 0.9 mM and 0.4 mM for SDS (Fig. 7.1), CTAB and TX-100 respectively. Now, surfactants are known to form aggregates smaller than micelles in presence of polymers. The threshold concentration required for aggregate formation is called the critical aggregation concentration (CAC), which for many surfactants is lower than the CMC\textsuperscript{9a}. Since, [BSA] concentration was fixed initially at 30 $\mu$M, only one CAC i.e. CAC\textsubscript{1} is obtained. Other workers have obtained a second CAC i.e. CAC\textsubscript{2} at very high protein concentrations\textsuperscript{9a}.

![Graph](image)

**Fig. 7.1:** SDS-induced decrease in fluorescence of ANS bound to BSA. [BSA] = 30$\mu$M, [ANS] = 20$\mu$M.

It is interesting to note that the CAC\textsubscript{1} value is lower than the CMC for the anionic surfactant, SDS. For an anionic surfactant, the binding to protein occurs initially to the cationic lysyl, histidyl and arginyl side chains. This specific binding is favoured electrostatically and thus CAC\textsubscript{1} is much lower than normal CMC of SDS. For the
cationic surfactant CTAB, initially the electrostatic interaction is repulsive. At a higher CTAB concentration, hydrophobic interaction predominates and binding occurs. For the nonionic surfactant TX-100, the only driving force for binding is hydrophobic in nature. Thus for CTAB and TX-100, CAC\textsubscript{1} values are not lower than the CMC values. For SDS, binding of SDS molecules to adjacent cationic sites being favoured, protein surfactant aggregates are formed at low concentrations. The CAC\textsubscript{1} values obtained for SDS and CTAB in BSA are close to the values obtained in presence of other proteins i.e. gelatin and lysozyme\textsuperscript{9a}.

7.2.2. Surfactant Binding to BSA – Binding Isotherms and Scatchard Analysis:

The nature of the protein – surfactant interaction can be well understood in terms of the binding isotherms. For this, the intrinsic tryptophan (Trp) fluorescence (\(\lambda_{\text{ex}} = 295\) nm) has been monitored. Fig. 7.2 shows the binding isotherms for BSA-surfactant interaction. To plot the binding curves, the surfactant concentration was gradually increased keeping concentration of BSA constant at 30 \(\mu\)M and change in Trp fluorescence was monitored. The fraction (\(\alpha\)) of a BSA molecule bound by surfactant was plotted versus the total surfactant (bound + free) concentration. In the absence of surfactant, \(\alpha = 0\) and under saturation binding conditions \(\alpha = 1\).

For the SDS – BSA system, \(\alpha\) increases from 0 – 0.2 up to 2 mM SDS. Beyond this concentration, \(\alpha\) was almost constant up to 10 mM SDS. Above this concentration, there is a sharp rise in \(\alpha\) until it attains the value 1.0 at ~ 24 mM SDS (Fig. 7.2a). This indicates that there is rapid, initial specific binding of the anionic SDS molecules to cationic amino acid residues on BSA (Scheme 7.1). Competitive binding between SDS and ANS expels the latter to the aqueous phase, thus ANS fluorescence decreases. Turro et al\textsuperscript{21} reported that TNS, a polarity probe similar to ANS has little or no affinity for SDS micelles.
In the concentration range, 2-10 mM SDS, there is a plateau-like region in the binding curve of SDS. This may correspond to aggregation of surfactant molecules. Beyond 10 mM SDS, there is a sharp rise in the binding curve. In this region, massive cooperative binding of the surfactant begins to occur on the protein leading to its uncoiling (Scheme 7.1). Uncoiling leads to exposure of many hydrophobic binding patches on the protein which were previously buried inside the hydrophobic interior of the native form. Thus more binding occurs leading to large cooperative effects.

![Graph](image)

Fig. 7.2a : Binding isotherm for SDS binding to BSA, [BSA] = 30 μM

For the CTAB – BSA system, (Fig. 7.2b), $\alpha$ increases to 0.1 at ~2.5 mM CTAB and then $\alpha$ increases to the saturation value of 1 upto 8 mM CTAB. Beyond this limit, $\alpha$ remains constant with increasing [CTAB]. For binding of CTAB to BSA unlike SDS, no initial plateau is observed. For SDS binding to BSA, the plateau in the binding curve between 2-10 mM SDS corresponds to surfactant aggregation on the protein surface. For explaining CTAB binding to BSA, the structure of BSA needs to be briefly discussed. BSA molecule has a net negative charge at pH = 7. BSA contains two Tryptophans (Trp) at residues...
212 and 134 of which Trp at 212 is buried in the hydrophobic interior and Trp at 134 is more solvent exposed\cite{1}. The structure of the molecule resembles a prolate ellipsoid with a linear array of 3 domains. Binding of CTAB to BSA is driven mainly by hydrophobic interaction and to a lesser extent by electrostatic attraction between a cation and an anion. Saturation of binding sites by CTAB binding is achieved at a much lower concentration compared to SDS. This is because, SDS binds to BSA in a highly cooperative manner exposing many previously hidden sites. For CTAB, degree of cooperativity is less.

Scheme 7.1 : Model for surfactant-protein interaction.
For the TX - BSA system (Fig. 7.2c), $\alpha$ increases to the saturation value of 1 at 8 mM TX. After this concentration, $\alpha$ remains almost constant up to 25 mM TX-100. TX-100 being nonionic, its binding to BSA is driven only by hydrophobic forces. Another point to
be noted is that for TX-BSA, the saturation value i.e. \( \alpha = 1 \) is reached at a much lower surfactant concentration (8 mM) than for SDS-BSA i.e. 24 mM. This is because, for TX-100, once the hydrophobic patches on BSA have been covered, no more binding occurs. But for SDS, initial electrostatic binding causes massive uncoiling of BSA exposing many more binding sites for SDS binding.

The decrease of ANS fluorescence has been found to be maximum in presence of SDS i.e. 95% decrease compared to 80% for CTAB and 40% for TX-100 at 25 mM surfactant. One reason for the large decrease of ANS fluorescence induced by SDS is that, both the ligands being anionic, there is mutual repulsion between them. Thus SDS dislodges more ANS molecules to the bulk aqueous phase decreasing their fluorescence drastically. Thus ANS fluorescence is a good reporter of surfactant binding to protein.

A better understanding of the strength of ligand binding to a macromolecule can be reached from a quantitative analysis of the experimental data. One such method involves the application of the Scatchard analysis\(^2\) to fluorescence data. The Scatchard equation is

\[
\frac{r}{c} = \frac{K}{n + K}\frac{1}{r} \quad \text{[7.1]}
\]

Where \( K \) = binding constant

\( n \) = number of binding sites on the macromolecule

\( c \) = free ligand concentration

\( r \) = moles of ligand bound per mole of macromolecule,

The Scatchard plot is a plot of \( r / c \) versus \( r \). Here two regions of surfactant concentrations have been used – one low i.e. 0.1 mM and one high i.e. 10 mM. If binding is non-cooperative, then the Scatchard plot is linear. In this study, the Scatchard plots for binding of all the three surfactants to BSA are nonlinear. Thus the process of surfactant binding is cooperative i.e. binding of 1 molecule on the protein influences the binding of subsequent molecules. The Scatchard plots reveal that binding of all three surfactants to BSA shows negative cooperativity Fig. 7.3(i)-(iii). The linear portion of the curve gives the value of \( K \) i.e. the binding constant. The linear portion when
extrapolated, intersects the r axis. This point of intersection yields the value of n i.e. the number of binding sites. For all the three surfactants, the curve can be best represented as an intersection of two straight lines indicating that there are two classes of binding sites on the protein for the surfactants. The n and K for each type of site have been determined (Table 7.1).

Fig. 7.3(i): Scatchard plot for SDS binding to BSA. [SDS] < 1mM

Fig. 7.3(ii): Scatchard plot for CTAB binding to BSA. [CTAB] < 1mM
When surfactant concentration is maintained at 0.1 mM, for one type of binding site, n ≈ 5 for all the three surfactants with binding constant $K_1$ of the order of $10^5 - 10^6$ M$^{-1}$. The Scatchard plots indicate a second type of site exhibiting weaker binding with $K_2$ values of the order $10^4$ M$^{-1}$. The number of binding sites ($n_2$) for this second set varies for the three surfactants with SDS showing the highest $n_2$ i.e. 13, followed by TX-100, $n_2 = 12$ and CTAB has the lowest $n_2$ value i.e. 7 (Table 7.1). $\Delta G$'s for binding have been indicated in Table 7.1.

Table 7.1: Values of n and k for surfactant binding to BSA obtained from Scatchard analysis:

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Concentration (mM)</th>
<th>$n_1$, $n_2$</th>
<th>$K_1$, $K_2$ (M$^{-1}$)</th>
<th>$\Delta G$ (KJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>0.10, 10.00</td>
<td>5, 13, 980</td>
<td>$4.8 \times 10^5$, $1.6 \times 10^4$ $1.3 \times 10^3$</td>
<td>-32.4, -24.0, -17.7</td>
</tr>
<tr>
<td>TX-100</td>
<td>0.10, 10.00</td>
<td>5, 12, 580</td>
<td>$3.5 \times 10^5$, $3.4 \times 10^4$ $3.8 \times 10^2$</td>
<td>-31.6, -25.8, 14.7</td>
</tr>
<tr>
<td>CTAB</td>
<td>0.10, 10.00</td>
<td>5, 7, 822</td>
<td>$10.5 \times 10^5$, $1.3 \times 10^4$ $3.6 \times 10^2$</td>
<td>-34.3, -23.4, -14.6</td>
</tr>
</tbody>
</table>

($n_1$, $n_2$ – two classes of binding sites)
Enthalpies of binding to some proteins per mole of SDS (ΔH) have been measured by other workers using microcalorimetry. ΔH values at pH=7 indicate that binding of SDS to many proteins is exothermic. This combined with our results (i.e. negative ΔG) indicates that ΔS for SDS increases on binding to the protein. This is characteristic of a substantial hydrophobic contribution to the binding process arising from the disordering of water molecules surrounding the surfactant. Thus surfactant binding to proteins involves two stages – 1) initial binding to ionic sites on the protein driven by ionic interaction of surfactant head groups with ionic sites on the proteins, 2) binding of alkyl chains of the surfactant to hydrophobic patches close to the ionic sites in the protein. For nonionic surfactants, the main driving force is hydrophobic interaction with nonpolar patches on the proteins. Confirmation to this statement comes from the observation that chemical modification of ionic sites yields lower n values and reducing the alkyl chain of surfactants weakens binding.

Thus the Scatchard analysis reveals that binding of all the three surfactants to BSA involves two types of sites. Within the region of high affinity binding (i.e. high K value), the number of available binding sites is fixed i.e. 5. The binding constant (K) for binding to these sites is highest for CTAB and lowest for TX-100 (Table 7.1). Now the BSA molecule is found to have a net negative charge of −18 at pH = 7. Thus initial binding of surfactants to protein is driven by the ionic interaction between the surfactant head group and the protein as well as hydrophobic interaction. CTAB head group being positively charged will participate in an attractive interaction with the net negative charge on BSA, whereas for SDS, this interaction will be repulsive. Moreover, the binding isotherms indicate that at 0.1 mM surfactant, co-operativity in binding is in the nascent stage. Hence the higher K1 value for CTAB. K1 for TX-100 binding, however, represents purely hydrophobic interaction with nonpolar patches on the protein. But the number of binding sites (n) for all three surfactants is the same. Thus the results obtained here corroborate
previous findings with SDS i.e. strong fatty acid - like high affinity binding to BSA\textsuperscript{21,24}. We have found a second type of binding with $K_2$ values of the order of $10^4$ and $n_2$ ranging from 7 – 13. This result and the findings for CTAB and TX-100 are new. $n_2$ values follow the order SDS > TX > CTAB. Thus in this zone of surfactant binding, greater number of SDS molecules are bound compared to CTAB. Thus this binding may originate from specific cationic sites on BSA as also hydrophobic interaction with neighbouring hydrophobic patches. This second binding with larger $n$ values must involve other preformed sites.

At high surfactant concentrations i.e. 10 mM, Scatchard plots indicate different values for $n$ and $K$. $K_1$ values are of the order of $10^3$ – $10^2$ M\textsuperscript{-1} with $n$ varying from 580 – 980. The high $n$ values in this surfactant concentration region indicate that here there is massive nonspecific cooperative binding to the protein (Scheme 7.1). This cooperative binding is accompanied by an unfolding of the protein. Although, the disulfide bridges prevent the peptide chain from unfolding completely, a substantial amount of unfolding can occur. Large amounts of bound ionic surfactant can break both the intrachain hydrophobic bonding as well as provide an electrostatic repulsion favouring an extended structure. In this region of surfactant concentration, co-operative effects are observed from the binding curve. Here, SDS exhibits the strongest binding effect with $K \sim 10^3$ M\textsuperscript{-1} (Table 7.1). The number of binding sites too are largest for SDS. Massive protein uncoiling leads to large scale co-operativity. Thus Scatchard data in this region reflects the ability of the surfactants to cause protein unfolding. It is to be noted that at the working pH i.e. 7, BSA bears a net negative charge and thus there can be no net or overall coulombic contribution to binding energy of SDS. Nevertheless, binding of anions occurs to high affinity sites. This may derive a coulombic contribution from binding near local positive charges.

Hydrodynamic and optical properties of the BSA – SDS complex indicate a major conformational change\textsuperscript{9b} from the native form. The
protein in a protein – SDS complex is not globular but rather an exerted polypeptide chain. We have shown that, even for cationic and nonionic surfactants, slight conformational modifications of the protein can occur. The largely negative $\Delta G$ values (Table 7.1) indicate the spontaneity of the binding process. It also seen that binding at $[\text{surfactant}] \approx 0.1$ mM is thermodynamically more favoured than binding at $[\text{surfactant}] \approx 10$ mM. The Gibbs energies of surfactant binding at saturation are comparable to the Gibbs energies of micelle formation$^{25}$. Both micellisation and protein - surfactant binding are cooperative processes. However, the protein – surfactant complexes are more stable than micelles, since the protein presents a complementary hydrophobic surface on which the surfactant can bind. Several models have been proposed for protein – SDS complexes$^{8,15,21}$. The most popular model for the SDS-BSA complex is the “pearl necklace” model in which the flexible denatured polypeptide chain of the protein has small spherical micelles clustered around it$^8$. The location of SDS molecules is such that the sulphate head group forms a salt bridge with cationic amino acid residues and the hydrocarbon tails make hydrophobic contact with the tertiary structure.

### 7.2.3. ANS binding to protein – Extrinsic Fluorescence of the Dye Label

To monitor the protein – surfactant binding process fluorimetrically, not only has the intrinsic tryptophan fluorescence been followed but also the fluorescence of an external label i.e. ANS has been studied. The binding characteristics of ANS to BSA have been studied both in the absence and the presence of surfactants. The Scatchard plots have been obtained using data from studies of ANS fluorescence. The Scatchard analysis for ANS binding to BSA in absence of surfactants indicates three binding sites with $K = 23 \times 10^5$ M$^{-1}$ (Table 7.2). In presence of surfactants, the binding of ANS to BSA
becomes weaker as indicated by the lower K values (Table 7.2). This implies competitive binding of ANS and surfactants to similar sites on the protein. Some nonpolar fatty acids have also been shown to quench the fluorescence of albumin-bound ANS\textsuperscript{26,27}. This is also borne out by the fact that the number of ANS binding sites is reduced from 3 to 1 (for SDS) and 2 (for TX -100 and CTAB) (Table 7.2). At lower concentrations of surfactant i.e. (0.1 mM), Scatchard plots for ANS – protein binding show negative cooperativity. At higher surfactant concentrations (10 mM), positive cooperativity is seen in presence of ionic surfactant CTAB and SDS (Fig. 7.4a). This is because at 10 mM surfactant concentration, substantial protein unfolding occurs, thus exposing more binding sites for ANS.

Table 7.2 : Values of n and k for ANS binding to BSA in presence of surfactants, obtained from Scatchard analysis :

<table>
<thead>
<tr>
<th>[Surfactant]</th>
<th>n</th>
<th>K $\text{M}^{-1}$</th>
<th>$\Delta G$ KJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No surfactant</td>
<td>3.0</td>
<td>23.1x10\textsuperscript{5}</td>
<td>-36.2</td>
</tr>
<tr>
<td>2. 0.1 mM SDS</td>
<td>1.0</td>
<td>18.8x10\textsuperscript{5}</td>
<td>-35.7</td>
</tr>
<tr>
<td>3. 10 mM SDS</td>
<td>-</td>
<td>6.0x10\textsuperscript{5}</td>
<td>-33.0</td>
</tr>
<tr>
<td>4. 0.1 mM TX-100</td>
<td>2.0</td>
<td>8.3x10\textsuperscript{5}</td>
<td>-33.7</td>
</tr>
<tr>
<td>5. 10 mM TX-100</td>
<td>1.5</td>
<td>5.8x10\textsuperscript{5}</td>
<td>-32.8</td>
</tr>
<tr>
<td>6. 0.1 mM CTAB</td>
<td>2.0</td>
<td>8.4x10\textsuperscript{5}</td>
<td>-33.7</td>
</tr>
<tr>
<td>7. 10 mM CTAB</td>
<td>-</td>
<td>8.0x10\textsuperscript{5}</td>
<td>-33.6</td>
</tr>
<tr>
<td>8. 1 M Urea</td>
<td>2.0</td>
<td>25.0x10\textsuperscript{5}</td>
<td>-36.4</td>
</tr>
</tbody>
</table>

The nature of the three ANS binding sites on BSA have been a subject of debate for a long time. ANS is believed to be tightly bound to binding sites located not very deep inside the protein interior. This is evidenced by the large rotational relaxation times of protein-bound ANS\textsuperscript{28}. However, it is difficult to precisely define whether the ANS binding sites in the proteins is wholly hydrophilic or hydrophobic. Previously the hypothesis of hydrophobic binding sites were favoured, based on the work with apomyoglobin, done by Stryer\textsuperscript{29}. It was found that ANS-bound to apomyoglobin was displaced after the addition of hemin, which is known to bind to a highly nonpolar site\textsuperscript{30}.
Fig. 7.4a: Positive co-operative effects seen in ANS binding to BSA at high SDS concentration \( \approx 10 \text{ mM} \)

Fig. 7.4b: Positive co-operative effects seen in ANS binding to BSA at high CTAB concentration \( \approx 10 \text{ mM} \)

However recent findings by titration calorimetry indicate that ANS is dominantly bound to cationic groups of proteins through ion pair formation between the cationic amino acid residues and the sulfonate anion\(^{15a}\). Our findings are completely in agreement with this result. Our results show that the maximum decrease in fluorescence
of protein-bound ANS occurs in presence of SDS. Now SDS is known to initially bind to cationic amino acid sites of BSA. Thus there is a competitive binding between ANS and SDS for attacking the cationic sites of BSA. In this competition, SDS emerges as the winner as its binding is also facilitated by hydrophobic interaction. Moreover SDS concentration is about 1000 times greater than ANS concentration. There is another possibility that some free SDS micelles are also present. However, ANS being anionic is not attracted towards anionic micelles. Thus a net decrease in ANS fluorescence is observed.

If the previous hypothesis of purely hydrophobic ANS-binding protein sites was true, then the nonpolar TX-100 would cause larger decrease in ANS fluorescence than SDS and CTAB. However, this is not so in reality as borne out by our experiments. X-ray study of ANS binding to the protein chymotrypsin shows that ANS is bound in a polar region of the protein surface, near the charged amino acid residues and may become accessible to water on slight alteration of protein structure. Studies on the related protein, HSA (Human Serum Albumin) indicate that the protein comprises three domains each of which has two subdomains. The principal ligand binding sites are in one sub-domain, IIIA. The three binding sites for ANS and surfactants may be in this sub-domain for BSA too.

The changes induced in protein-bound ANS fluorescence by surfactants have been compared to the effects of the well known denaturant, urea. In order to compare the two, we have used low urea concentrations i.e. upto 1M. It is seen that in presence of 1M urea, the binding constant for ANS binding to BSA is almost the same i.e. $23 \times 10^5 \text{ M}^{-1}$ (in absence of urea) and $25 \times 10^5 \text{ M}^{-1}$ (in 1M urea) (Table 7.2). The number of binding sites was the same. Thus, it was seen that surfactant-induced denaturation of protein is more effective than urea as a lower concentration of the former is required. Even at [urea] = 1M which is 100 times larger than the concentration of surfactants used, there is no change in binding constant (K) of protein-bound ANS and the number of binding sites (n). The decrease in ANS fluorescence in
urea was nominal i.e. 25% as compared to the 40 – 95 % decrease observed with surfactants. Thus the mechanisms of urea-induced and surfactant-induced denaturation are different. The former plays an important role in altering solvation while surfactants weaken the hydrophobic and electrostatic interactions in the tertiary protein structure. Only at higher concentrations i.e. 6 – 8 M, urea is effective in denaturing proteins. Urea is also known to exhibit two effects . At low concentration < 1M urea induces ligand binding to protein$^{31,32}$. At concentrations > 1M, urea decreases binding. Since, our urea concentrations were ≤ 1M, we have not observed substantial urea – induced decrease in ANS binding to BSA.

7.2.4. Accessibility of Intrinsic Tryptophan and Bound–ANS in BSA – Effect of Acrylamide

To determine the accessibility of tryptophan residues, fluorescence quenching studies were undertaken with the well-known quencher, acrylamide. Acrylamide was used as it is a nonperturbing quencher which usually does not bind to proteins. Fluorescence quenching in this case proceeds mainly via physical contact between the quenchers and fluorophores and hence is directly dependent on the extent to which the fluorophore can be approached. Thus the extent of accessibility of Trp residues as well as ligand binding sites in BSA can be probed by quenching studies. Stern-Volmer plots for acrylamide quenching were obtained by using the Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + K_{SV} [Q] \quad \cdots \cdots \cdots \cdots [7.2]$$

where $F_0 = $ fluorescence intensity of fluorophore in absence of quencher.

$F = $ fluorescence intensity of fluorophore in presence of quencher.

$K_{SV} = $ Stern-Volmer constant

$[Q] = $ quencher concentration
The Stern-Volmer plots for quenching of tryptophan fluorescence by acrylamide deviate from linearity at quencher concentrations larger than 0.8 M. This indicates that there are two fluorophore populations present and one class of fluorophores is not quite accessible to the quencher. This is because acrylamide cannot penetrate the hydrophobic interior of BSA and only the tryptophan residue i.e. Trp 134 present on the protein surface is quenched. A similar downward curvature of the Stern-Volmer plot is observed for trifluoroacetamide - induced quenching of Lysozyme fluorescence. The linear portion of the data have been fitted yielding the corresponding $K_{SV}$ (Table 7.3). The quenching constant ($k_q$) has been determined using the relation

$$K_{SV} = k_q \cdot \tau_0 \quad [7.3]$$

$k_q$ = quenching constant

$\tau_0$ = lifetime of the fluorophore in absence of quencher.

### Table 7.3: Acrylamide quenching in absence and presence of surfactants:

$[\text{Acrylamide}] = 0$-$2$ M

<table>
<thead>
<tr>
<th>Medium</th>
<th>$K_{SV,\text{Trp}}$ ($M^{-1}$)</th>
<th>$K_{SV,\text{ANS}}$ ($M^{-1}$)</th>
<th>$k_{q,\text{Trp}}$ ($M^{-1} s^{-1}$)</th>
<th>$k_{q,\text{ANS}}$ ($M^{-1} s^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 20 μM ANS + 30 μM BSA + Acrylamide</td>
<td>2.65</td>
<td>0.45</td>
<td>6.2$x 10^8$</td>
<td>0.25$x 10^8$</td>
</tr>
<tr>
<td>2.(a)20 μM ANS + 30 μM BSA + 0.1 mM SDS + Acrylamide (b)20μM ANS + 30 μM BSA +10 mM SDS + Acrylamide</td>
<td>2.31</td>
<td>0.50</td>
<td>4.8$x 10^8$</td>
<td>0.45$x 10^8$</td>
</tr>
<tr>
<td>2.29</td>
<td>0.93</td>
<td>3.9$x 10^8$</td>
<td>1.82$x 10^8$</td>
<td></td>
</tr>
<tr>
<td>3.(a)20 μM ANS + 30 μM BSA +0.1mMTX-100 +Acrylamide (b) 20 μM ANS + 30 μM BSA +10 mM TX-100 + Acrylamide</td>
<td>2.76</td>
<td>0.50</td>
<td>6.3$x 10^8$</td>
<td>0.38$x 10^8$</td>
</tr>
<tr>
<td>0.55</td>
<td>0.68</td>
<td>1.4$x 10^8$</td>
<td>0.59$x 10^8$</td>
<td></td>
</tr>
<tr>
<td>4.(a) 20 μM ANS +30 μM BSA +0.1 mM CTAB+ Acrylamide (b) 20 μM ANS +30 μM BSA +10 mM CTAB+ Acrylamide</td>
<td>2.29</td>
<td>0.45</td>
<td>5.7$x 10^8$</td>
<td>0.33$x 10^8$</td>
</tr>
<tr>
<td>2.79</td>
<td>0.29</td>
<td>7.2$x 10^8$</td>
<td>0.57$x 10^8$</td>
<td></td>
</tr>
<tr>
<td>5. 20 μM ANS + 30 μM BSA +1 M Urea + Acrylamide</td>
<td>2.59</td>
<td>0.50</td>
<td>5.5$x 10^8$</td>
<td>0.28$x 10^8$</td>
</tr>
</tbody>
</table>

The $k_q$ values obtained are of the order of $10^8$ M$^{-1}$ s$^{-1}$. Since the $k_q$ values for diffusion controlled quenching are known to be $\leq 1 \times 10^{10}$
M⁻¹s⁻¹, quenching of Trp fluorescence in this case is probably diffusion controlled. Partial shielding of the fluorophore (Trp) may be responsible for $k_q$ being less than $1 \times 10^{10}$ M⁻¹s⁻¹.

We were interested in studying how surfactant denaturation of proteins can affect the accessibility of Trp residues to quencher. On increasing acrylamide concentration, we have observed a red shift of Trp fluorescence to 348 nm. This is attributed to the quenched residues. Table 7.3 indicates that the $k_q^{\text{Trp}}$ values decrease in presence of the surfactants. The $K_{sv}^{\text{Trp}}$ values are almost unchanged. This may be because surfactant-binding to BSA causes its denaturation. Denaturation generally exposes previously buried Trp residues i.e. residues 212 and acrylamide being a surface quencher, becomes an active quencher. In this case, surfactant-induced protein denaturation is accompanied by micelle formation whereby the exposed Trp residues face a hydrophobic micellar environment. In this situation, the polar quencher acrylamide will not be able to access the Trp residues. Thus $k_q$ values remain unchanged or at most may decrease. But in 1 M urea, $k_q$ values remain unchanged. It is well known that the denaturating effect of urea starts at much higher concentrations i.e. $>6$ M¹³¹,³².

However, the quenching studies with the extrinsic probe ANS shows the opposite effect. Here interestingly, $k_q^{\text{ANS}}$ increases in presence of surfactant. This indicates that surfactant-induced conformational changes of BSA cause exposure of the ANS binding sites to solvent. Thus ANS fluorescence gets easily quenched. Thus ANS binding sites on BSA are different from the Trp sites. But even in this case, 1 M urea does not cause an appreciable increase in $k_q^{\text{ANS}}$. This once again proves that as far as conformational change of the protein is concerned, surfactants are more effective than urea at low concentrations.
7.2.5. Time Resolved Fluorescence Studies

Fluorescence decays for both Trp and ANS emission were determined. The lifetime values have been listed in Table 7.4. The Trp emission excited at 295 nm could be fit to a bi-exponential decay. In BSA, the bi-exponential fit yielded lifetimes of 3.4 and 11.4 ns (Table 7.4a). The shorter lifetime ($\tau_1$) has been assigned to buried Trp i.e Trp-212 while the longer lifetime ($\tau_2$) is due to the solvent exposed residue i.e. Trp-134. Table 7.4a indicates that the dominant contribution to lifetime of Trp comes from Trp-212 (since $a_1 > a_2$). However, in presence of 10 mM SDS (Fig 7.5a) and CTAB, $a_2$ increases i.e indicating that Trp becomes more solvent exposed. In 1M urea too, $\tau$ values remain almost unchanged. Table 7.4b and Fig 7.5b shows the decrease of lifetime of ANS bound to BSA, in presence of surfactants.

### Table 7.4 a: Lifetimes of tryptophan in BSA in presence of additives

<table>
<thead>
<tr>
<th>Medium</th>
<th>$\tau_1$ (ns)</th>
<th>$a_1$</th>
<th>$\tau_2$ (ns)</th>
<th>$a_2$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 30 µM BSA</td>
<td>3.4</td>
<td>0.90</td>
<td>11.4</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>2. 30 µM BSA + 0.1 mM SDS</td>
<td>3.6</td>
<td>0.90</td>
<td>17.2</td>
<td>0.10</td>
<td>1.1</td>
</tr>
<tr>
<td>3. 30 µM BSA + 10 mM SDS</td>
<td>3.3</td>
<td>0.80</td>
<td>13.7</td>
<td>0.20</td>
<td>0.9</td>
</tr>
<tr>
<td>4. 30 µM BSA + 0.1 mM TX-100</td>
<td>3.4</td>
<td>0.90</td>
<td>11.5</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>5. 30 µM BSA + 10 mM TX-100</td>
<td>3.0</td>
<td>0.90</td>
<td>11.5</td>
<td>0.10</td>
<td>1.1</td>
</tr>
<tr>
<td>6. 30 µM BSA + 0.1 mM CTAB</td>
<td>3.7</td>
<td>0.90</td>
<td>14.5</td>
<td>0.10</td>
<td>1.1</td>
</tr>
<tr>
<td>7. 30 µM BSA + 10 mM CTAB</td>
<td>3.0</td>
<td>0.85</td>
<td>16.9</td>
<td>0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>8. 30 µM BSA + 1.0 M Urea</td>
<td>3.4</td>
<td>0.90</td>
<td>13.3</td>
<td>0.10</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### 7.4 b: Lifetimes of ANS bound to BSA, in presence of additives:

<table>
<thead>
<tr>
<th>Medium</th>
<th>$\tau_1$ (ns)</th>
<th>$a_1$</th>
<th>$\tau_2$ (ns)</th>
<th>$a_2$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 20 µM ANS + 30 µM BSA</td>
<td>14.5</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>2. 20 µM ANS + 30 µM BSA + 0.1 mM SDS</td>
<td>2.9</td>
<td>0.2</td>
<td>17.9</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>3. 20 µM ANS + 30 µM BSA + 10 mM SDS</td>
<td>3.1</td>
<td>0.8</td>
<td>13.4</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>4. 20 µM ANS + 30 µM BSA + 0.1 mM TX-100</td>
<td>2.3</td>
<td>0.2</td>
<td>18.9</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>5. 20 µM ANS + 30 µM BSA + 10 mM TX-100</td>
<td>2.5</td>
<td>0.4</td>
<td>17.3</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>6. 20 µM ANS + 30 µM BSA + 0.1 mM CTAB</td>
<td>2.0</td>
<td>0.1</td>
<td>19.8</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>7. 20 µM ANS + 30 µM BSA + 10 mM CTAB</td>
<td>4.4</td>
<td>0.9</td>
<td>10.8</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>8. 20 µM ANS + 30 µM BSA + 1.0 M Urea</td>
<td>1.9</td>
<td>0.1</td>
<td>18.8</td>
<td>0.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>
The ANS lifetimes were also determined by exciting the solutions at 337 nm with $\lambda_{em} = 465$ nm. For ANS in 30 $\mu$M BSA, the decay was exponential with a $\tau$ value 14.5 ns (Table 7.4b). In surfactants, the BSA-bound ANS decay is biexponential. The lower ANS $\tau$ values can be assigned to those ANS molecules bound to sites on BSA that are more exposed to solvent. The larger $\tau$ value resembles the lifetime of ANS in relatively nonpolar media and is due to those ANS molecules bound to hydrophobic sites on BSA. At lower SDS concentration, the dominant contribution is from the second type of bound ANS with long $\tau_2$ i.e. 17 ns (Fig 7.5b). At high concentration of SDS, the shorter component $\tau_1$ (3.1 ns) becomes dominant (Fig 7.5b). This indicates that SDS causes protein unfolding more effectively at higher concentration i.e. 10 mM, thus making bound ANS more exposed to water. For CTAB too, the same trend is seen. But for TX-100 the contribution of the longer component is always dominant even at higher surfactant concentrations only its magnitude i.e. $a_2$ decreases slightly. Thus TX-100 is not as effective in inducing protein unfolding as the other surfactants. 1M urea does not change the $\tau$ value much. This indicates that urea at this concentration i.e. 1M has no effect on the tertiary or secondary structure of BSA.

In the presence of surfactants at low concentration the contribution of $\tau_2$ i.e. longer lifetime component of ANS, $a_2$ is high. At higher concentration of surfactants especially SDS and CTAB, $a_2$ decreases. Thus surfactants at high concentrations cause exposure of the ANS binding sites (on BSA) to solvent. As far as the Trp emission is concerned, a longer lifetime is generally associated with the more solvent exposed tryptophan residue$^{34,35}$. Our observations indicate that presence of ionic surfactants exposes buried tryptophan residues to the solvent, since the value of $a_2$ increases in presence of SDS and CTAB.
Due to the good overlap between the emission spectrum of Trp and the absorption spectra of ANS, nonradiative energy transfer is possible between them. Trp acts as the donor in this case. The theory
of the energy transfer has been well studied\textsuperscript{35}. The energy transfer can be studied by determining certain parameters – energy transfer efficiency (E), Forster distance (R\textsubscript{0}) and rate constant for energy transfer (k\textsubscript{ET}). These have been defined by Förster's theory of energy transfer\textsuperscript{35b}. The energy transfer parameters for the Trp – ANS system have been evaluated in the absence and presence of surfactants and urea. These have been listed in Table. 7.5. The energy transfer efficiency from Trp to ANS is decreased in presence of surfactants while it is almost unaffected by 1M urea. Thus energy transfer studies too indicate that surfactants can bind to BSA and can effectively expose both donor and acceptor to solvent, thus increasing the D-A distance and decreasing the probability of energy transfer.

<table>
<thead>
<tr>
<th>Medium</th>
<th>(\Phi^D)</th>
<th>E</th>
<th>J((\lambda))</th>
<th>R\textsubscript{0}</th>
<th>r</th>
<th>(&lt;\tau^D&gt;)</th>
<th>k\textsubscript{ET}</th>
<th>(&lt;\tau^A&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 36 (\mu)M BSA +20 (\mu)M ANS</td>
<td>0.044</td>
<td>0.58</td>
<td>7.54\times10^{-15}</td>
<td>19.8</td>
<td>18.8</td>
<td>4.3</td>
<td>3.17\times10^{8}</td>
<td>17.8</td>
</tr>
<tr>
<td>2. 36 (\mu)M BSA + 20 (\mu)M ANS + 0.1 mM SDS</td>
<td>0.038</td>
<td>0.32</td>
<td>1.3\times10^{-14}</td>
<td>21.2</td>
<td>24.0</td>
<td>4.8</td>
<td>0.99\times10^{8}</td>
<td>11.01</td>
</tr>
<tr>
<td>3. 36 (\mu)M BSA + 20 (\mu)M ANS + 10 mM SDS</td>
<td>0.026</td>
<td>0.39</td>
<td>8.4\times10^{-15}</td>
<td>18.5</td>
<td>19.9</td>
<td>5.9</td>
<td>1.09\times10^{8}</td>
<td>5.1</td>
</tr>
<tr>
<td>4. 36 (\mu)M BSA + 20 (\mu)M ANS + 0.1 mM TX-100</td>
<td>0.039</td>
<td>0.45</td>
<td>1.04\times10^{-14}</td>
<td>20.4</td>
<td>21.1</td>
<td>4.0</td>
<td>2.04\times10^{8}</td>
<td>13.6</td>
</tr>
<tr>
<td>5. 36 (\mu)M BSA + 20 (\mu)M ANS + 10 mM TX-100</td>
<td>0.037</td>
<td>0.43</td>
<td>1.11\times10^{-14}</td>
<td>20.5</td>
<td>21.5</td>
<td>3.9</td>
<td>2.09\times10^{8}</td>
<td>11.5</td>
</tr>
<tr>
<td>6. 36 (\mu)M BSA + 20 (\mu)M ANS + 0.1 mM CTAB</td>
<td>0.041</td>
<td>0.37</td>
<td>7.9\times10^{-15}</td>
<td>19.7</td>
<td>21.5</td>
<td>4.4</td>
<td>1.34\times10^{8}</td>
<td>13.1</td>
</tr>
<tr>
<td>7. 36 (\mu)M BSA + 20 (\mu)M ANS + 10 mM CTAB</td>
<td>0.017</td>
<td>0.36</td>
<td>9.1\times10^{-15}</td>
<td>17.4</td>
<td>19.2</td>
<td>3.8</td>
<td>1.46\times10^{8}</td>
<td>5.1</td>
</tr>
<tr>
<td>5. 36 (\mu)M BSA + 20 (\mu)M ANS + 1.0 M Urea</td>
<td>0.043</td>
<td>0.56</td>
<td>7.98\times10^{-15}</td>
<td>19.9</td>
<td>19.1</td>
<td>4.7</td>
<td>2.72\times10^{8}</td>
<td>17.6</td>
</tr>
</tbody>
</table>

The Forster distances (R\textsubscript{0}) have been determined using the overlap integrals J(\(\lambda\)) obtained by numerical integration. From the R\textsubscript{0} values, the donor acceptor distance (r) has been determined. r for Trp – ANS is \(\sim 19\ A^0\) which agrees quite well with the literature value.
of 23 Å. In presence of all the three surfactants, r value increases. This is caused by alteration of protein secondary and tertiary structure. However, 1M urea has no effect on tertiary protein structure and thus has no effect on energy transfer. Thus the energy transfer efficiency in various media can be correlated quite well to the donor-acceptor distance. The $k_{\text{ET}}$ values are of the order of $10^8$ sec$^{-1}$, decreasing in the order BSA > (BSA + 1M urea) > (BSA + TX-100) > (BSA + CTAB) > (BSA+SDS). The capabilities of proteins for binding with ANS can be correlated with the decrease in their Trp fluorescence due to energy transfer to ANS.

7.2.7. Circular Dichroism Studies:

To confirm the above conclusions regarding the effect of surfactants and urea on the tertiary and secondary structure of BSA, circular dichroism (CD) studies were undertaken. To examine the protein secondary structure in detail, the spectra were scanned in the wavelength region 200 – 250 nm to probe the backbone amide transitions. The $\alpha$ - helix is characterised by negative peaks of similar magnitude at 222 nm and 208 nm. The standard method$^{19}$ was used to fit the spectral data and thus determine the percentage of $\alpha$ - helicity$^{37}$. These have been listed in Table 7.6. The surfactants do not present any CD signal in the spectral range 200 – 250 nm, thus the observed CD is due to the protein alone. It is seen that the protein in absence of any additives has an $\alpha$ - helix content of 56%. This result is in agreement with literature value of 55% $\alpha$ - helix$^1$. In presence of surfactants, there is change in the helicity indicating a change in the protein secondary structure (Fig 7.6a). A general trend is observed in the effect of all the three surfactants on helicity of BSA. At low surfactant concentrations $\leq 0.1$ mM, there is little change in the helicity but at higher surfactant concentrations, helicity decreases.
(Fig. 7.6a). However, urea at 1 M concentration has no effect on the helicity of BSA. Our findings are in agreement with the studies of Moriyama et al. They found that on addition of [SDS] ≥ 6 mM, helicity decreases. At high surfactant concentration, preferential adsorption on the protein surface leads to a swelling of the protein which exposes the hydrophobic residues. Thus α - helices are broken to give a more open disordered structure. Surfactant addition also has a considerable effect on the percentage of β-sheet and random coil. Thus we conclude surfactants at higher concentrations, disrupt the α - helical network and lead to a more open, random, solvent- exposed protein structure. Here again, we find that TX-100 causes no change in helicity of BSA. The aromatic region of the protein spectrum i.e. 250 – 320 nm, too shows significant alterations in presence of surfactants (Fig 7.6b). The UV-CD spectra of BSA around 250 – 320 nm show significant perturbation in presence of the surfactants. This indicates significant perturbation of the surface tryptophan and tyrosyl residues. Thus the surfactants perturb the structure of the protein surface, mediating the water layer and microenvironment around the superficial aromatic residues. This conclusion is also reached from the fact that presence of surfactants leads to a lower quenching efficiency of acrylamide and a perturbation of the specific ligand ANS. Thus the results obtained from CD studies are in complete agreement with earlier observations from fluorescence studies.
Table 7.6: Circular dichroism studies of the effect of additives on BSA structure:

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of α-helix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 3 μM BSA</td>
<td>56.00</td>
</tr>
<tr>
<td>2. 3 μM BSA + 0.1 mM SDS</td>
<td>54.00</td>
</tr>
<tr>
<td>3. 3 μM BSA + 10 mM SDS</td>
<td>47.00</td>
</tr>
<tr>
<td>4. 3 μM BSA + 0.1 mM TX-100</td>
<td>56.00</td>
</tr>
<tr>
<td>5. 3 μM BSA + 10 mM TX-100</td>
<td>55.00</td>
</tr>
<tr>
<td>6. 3 μM BSA + 0.1 mM CTAB</td>
<td>55.00</td>
</tr>
<tr>
<td>7. 3 μM BSA + 10 mM CTAB</td>
<td>51.00</td>
</tr>
<tr>
<td>8. 3 μM BSA + 1.0 M Urea</td>
<td>56.00</td>
</tr>
</tbody>
</table>

Fig. 7.6a: Circular dichroism spectra of the effect of surfactants on secondary structure of BSA. In the wavelength region 200 – 250 nm
(i) BSA  (ii) BSA + 10 mM SDS
Fig. 7.6b: Circular dichroism spectra of the effect of surfactants on structure of BSA. In the wavelength region 250 - 320 nm (i) BSA (ii) BSA + 10 mM SDS

7.3. Conclusions:

Protein-surfactant interactions have been examined fluorimetrically by following the intrinsic Trp as well as external label (ANS) fluorescence. The steady-state fluorescence results have been corroborated by time resolved studies and circular dichroism spectra. The most important conclusion is that although the overall nature of protein-surfactant interactions is specific-ionic interaction followed by nonspecific hydrophobic interaction, the details of the process depend on the type of surfactant. It is important to note from the above study that surfactant denaturation of proteins occurs at surfactant concentrations (10 mM) much lower than those required for the commonly used denaturants, urea (6-8 M). For the latter, denaturing action depends on their effect on water structure and weakening of hydrophobic interactions. For surfactants, on the other hand, the driving forces are electrostatic interactions with amino acid residues
followed by the thermodynamically favourable hydrophobic interactions (as evidenced by negative binding free energies). At low surfactant concentration, binding to BSA is strong as characterized by high K values. However, CD spectra suggest that protein is not denatured at low surfactant concentration (0.1 mM). But at high surfactant concentration, the Scatchard plots indicate a considerable role of cooperative effects in surfactant binding to protein and there is considerable protein uncoiling leading to quenching of fluorescence of bound ANS and decrease in α-helicity. Among the three types of surfactants, the nonionic surfactant TX-100 shows the minimum interaction with BSA while SDS and CTAB interact strongly. The common model suggested by SANS studies for the protein-surfactant complex is a “pearl necklace” model in which the flexible denatured polypeptide has spherical micellar clusters wrapped around its backbone. Protein-surfactant complexes are generally more stable than surfactant micelles. The presence of an external marker for BSA (ANS) and the effect of surfactant on its fluorescence, is used to characterize the ligand binding sites. For ANS binding to BSA, there are 3 such sites. Surfactants have been found to displace ANS from its binding sites. This indicates that the binding is competitive in nature. Fluorimetric study of such competitive binding can be used to detect and quantify the binding of nutrients, drugs and other physiologically important compounds to albumin.

It has been found that the acrylamide quenching efficiency for Trp decreases in presence of surfactants. Of the two Trp residues in BSA, the one on loop 3 i.e. Trp 134 is more exposed than the one on loop 4 (Trp 212). Thus the quenching studies reflect the effect of acrylamide on Trp in loop 3. In presence of surfactants, although the protein uncoils, the Trp is surrounded by a hydrophobic envelope offered by the surfactant. This hinders the approach of acrylamide. However, with surfactant-induced protein uncoiling, the ANS binding sites become more exposed to solvent and $k_Q^{ANS}$ increases.
The efficiency of RET from Trp to ANS decreases in presence of surfactants, reiterating the fact that surfactants induce protein uncoiling thus increasing the donor (Trp) – acceptor (ANS) distance. However, urea at low concentrations i.e. 1M has no effect on RET.

The circular dichroism studies indicate that large amounts of the absorbed ionic surfactants CTAB and SDS at concentrations \( \geq 10 \) mM cause substantial uncoiling as evidenced by the CD spectra. The surfactants break intrachain hydrophobic bonding and favour an extended structure due to electrostatic repulsion. Thus the ionic surfactants CTAB and SDS can perturb significantly the structure of BSA as evidenced by our studies – steady-state and time-resolved fluorescence, acrylamide quenching and CD studies. The effect of TX-100 on BSA structure is not as marked as that of CTAB and SDS.

7.4. Importance of this Work:

This study is expected to provide an important insight into the interaction of serum proteins with polar and nonpolar molecules in the biological system. The most important implication of this work is that surfactants can cause breakdown of protein-membrane lipid interactions. This effect bears relevance to the isolation of anion and glucose transporters of human erythrocytes and the reconstruction of receptors for insulin and acetylcholine\(^3^8\). The more interesting development in the field of surfactants in recent times is the study of the role of the pulmonary surfactant in maintaining the stability of the alveolae epithelial membrane\(^3^9,4^0\). Further scope of work using such pulmonary surfactants remains to be explored.

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