CHAPTER 3

The effects of different solution additives on the self-association of a "super-simplified" protein model

3.1. Introduction:

The results described in chapter 2 implicate that the arginine’s effect on protein self-association may have come from its effect on protein side chain. In contrast, a traditional osmolyte (like sucrose) effects only on backbone and does not influence the sidechains. In this chapter, we have aimed to validate this hypothesis using a number of surfactants. We assume that the self-association of surfactants can be considered as a ‘super-simplified’ model of protein self-association.

Self-association of biological molecules has been studied extensively because of their relevance in a number of biological questions. For example, self-association of proteins may lead to formation of amyloids which has been implicated in several human diseases. Additionally self-association of therapeutic proteins could lead to immunogeneity and other undesirable complications in their manufacturing and formulation development. Self-association of surfactants is considered important as they offer a suitable model system for biological membranes (1-5). Encapsulation by surfactants has been used as an efficient method of drug delivery (6). Self-association of surfactants leads to formation of micelles and other aggregated forms which may play crucial roles in their interaction with proteins or other biomolecules (7-11).

We have been studying protein self-association using fluorescence correlation spectroscopy (FCS) and other biophysical methods. Fluorescence correlation spectroscopy (FCS) is an important single molecular technique to study diffusional and conformational properties of labeled molecules (12-20). In a FCS experiment, the sample is kept in a small observation volume under thermodynamic equilibrium. Fluorescence fluctuations, which occur due to the molecular diffusion in and out of the observation volume, are analyzed by measuring the correlation functions providing a measure of the hydrodynamic radius ($r_H$). FCS can be used in combination with other fluorescence techniques to study chemical kinetics and dynamics in the
μsec time scale (13, 21, 22). Using FCS we have shown recently that arginine, an amino acid, can be used to inhibit protein self-associations (23). We have shown further by measuring μsec conformational dynamics using FCS that arginine inhibits formation of mis-folded intermediate states in the unfolding transition of proteins (18). Our data and other results available in the literature suggest that the ability of arginine to suppress protein self-association may arise from its interaction to the side-chains of the native and native like states of proteins (24-27). This property would be in contrast with other protein stabilizers and osmolites which interact only with the unfolded state of proteins without affecting their native states (18).

To get further insight into this, we have carried out FCS measurements to study the effect of different stabilizers including arginine on the self-associations of two surfactants with same chain length and opposite charge. We assume that the self-association of the surfactants could be modeled using an extended state (the monomer) and a self-associated state (micelles) without using any contribution from the native or native like states. We show further that the use of high concentration of urea or glycerol affects the process of self-association presumably by affecting the interaction of water with the surfactant molecules. We observe that self-association is favorable at low pH and in the presence of salt. While sucrose inhibits self-association, the behavior of arginine depends on the nature of surfactants. Arginine favors self-association of SDS, a negatively charged surfactant. However with DTAB, a surfactant of identical chain length but opposite charge, arginine inhibits micellization.

3.2. Materials and Methods:

Sample and reagents: Teramethyl rhodamine-5- maleimide (TMR) was obtained from Molecular probes (Eugene, OR). SDS, DTAB, Urea, and NaCl were purchased from Sigma- Aldrich in the highest available grade.

FCS experiments and FCS analysis have been carried out as discussed in chapter2.

The correlation function data was further analyzed by the maximum entropy method (MEM) recently applied to FCS using the MEMFCS algorithm (28). In this method, the multicomponent correlation function can be represented by n, the number of noninteracting fluorescent species, each of which can have a diffusion time of 0.001 to 500 ms. MEMFCS
minimizes the parameter $\chi^2$ and also maximizes the entropic quantity $S = \sum p_l \ln p_l$
(Where $p_l = \alpha_l \sum \alpha_j$) to obtain an optimized fit (23,28).

3.3. Results and Discussion:

Two common surfactants, namely, sodium dodecyl sulfonate (SDS) and dodecyl trimethyl ammonium bromide (DTAB), are chosen for the present study. We chose SDS because its self-association properties have been extensively studied. As a model ionic surfactant, SDS is also widely used to study the folding of different proteins (29,30). SDS has been shown to stabilize partially folded intermediate states whose characterization by NMR and other spectroscopic techniques provides useful information about the thermodynamics and kinetics of protein folding (31). SDS has been shown to refold proteins under strongly denaturing conditions and even in the presence of 10M urea (31). Although the exact mechanism of SDS-induced refolding is still not known, the surfactants have been found to interact with proteins using ionic and hydrophobic interactions. Although significant refolding of the secondary structure of cytc has been observed with SDS, a charged surfactant, the extent of refolding is much less with lauryl maltoside, a neutral surfactant with an identical chain length (31). We monitor the diffusion of a fluorescent dye molecule, tetramethylrhodamine-5-maleimide (TMR), in the presence of different concentrations of surfactants. It has been assumed that the interaction of TMR with the surfactant monomer can be differentiated from its interaction with surfactant self-associated molecules by the hydrodynamic radii ($r_H$) measurements. Rhodamine fluorophores have been shown to bind SDS (32).

3.3.a. Analysis of correlation plots obtained with the FCS experiments using TMR in the presence of different concentration of SDS:

Correlation functions obtained by FCS experiments in the absence and presence of different concentration of SDS have been shown in figure 3.1. To obtain the diffusion time ($\tau_D$) values the correlation functions have been fit to equation 2.1 using a single-component system. The goodness of the fit has been verified by the randomness of the residual distributions. Hydrodynamic radii ($r_H$) of the surfactant molecules have been calculated from equation 2.3 and 2.4 from the values of $\tau_D$. Fitting of the FCS data using two diffusing components (the first
component with a diffusion time of $\tau_{D1}$ and amplitude of $a_1$ and the second component with a diffusion time of $\tau_{D2}$ and an amplitude of 1-$a_1$ does not improve the fit (figure 3.1). The correlation function fits and the residual distributions obtained for both models are supplied in figure 3.1. The correlation function data have been further analyzed using MEMFCS techniques (Figure 3.2b). MEMFCS provides a bias-free solution to complement the conventional method of analyzing the correlation function data. MEM analyses also show the presence of only one diffusion component in the presence of different concentration of SDS (Figure 3.2b). In the absence of SDS, the value of $\tau_D$ has been observed to be 31 µs, which increases to 53 and 110 µs in the presence of 3 and 10 mM SDS, respectively. MEM fitting shows, in the absence of SDS, of the data shows the maximum at 25 µs, and in presence of 3 and 10 mM SDS shifts the maxima to higher values at 43 and 93 µs, respectively (Figure 3.2b). Diffusion times value obtained from MEMFCS techniques are very much similar that we obtained from equation 2.1.
Figure 3.1: correlation functions obtained by FCS experiments with TMR molecule in the presence of
0mM (a,b), 3mM (c,d) and 10mM (e,f) SDS. The residual distributions of the fits are shown at the bottom of the figures. The use of one diffusional component has been found appropriate to fit the autocorrelation function data obtained with the free dye (a, c, e). Addition of an extra exponential component does not improve the fittings (b, d, f).
Figure 3.2: (a) The normalized correlation functions obtained with TMR in the absence and presence of 3mM and 10mM SDS. The data were fit to Equation 2.1 using a model containing one diffusing component. (b) Profiles of the MEM analyses of the correlation functions data in the absence and presence of 3mM and 10mM SDS. (c) Dependence of $r_H$ and count per particle with SDS concentrations. The left axis represents the $r_H$ data while the right axis shows the data for the count per particle. FCS experiments are carried out at room temperature using 20 mM sodium phosphate buffer at pH 7.4.
Figure 3.2c shows the variation of $r_H$ and the counts per particle with SDS concentration. Both observables follow sigmoidal profiles. The values of $r_H$ increase with SDS concentration as self-association increases the size of the molecules. Because the self-association of monomers leading to large particles decreases the number of monomers (and hence the number of particles) for a particular count rate, the counts per particle increase with self-association as observed in the present data (Figure 3.2c). Interestingly, the increase in $r_H$ begins at 2.5 mM SDS, which is less than the first critical micellar concentration (cmc$_1$) of SDS under this solution condition (8 mM) (33). There could be several reasons behind this early onset of the increase in $r_H$ with SDS concentration. First, there may be adduct formation between TMR and negatively charged SDS monomers occurring below the cmc as reported earlier (33). Second, the surfactant monomers may self-associate to form small surfactant aggregates at less than the reported value of the cmc as shown by Zettl. et al (34). To obtain further insight into this, we have carried out FCS experiments with DTAB using TMR. Interestingly, the increase in $r_H$ with DTAB concentration starts at 12 mM (see below), which is similar to the reported cmc of DTAB (35). The present FCS experiments hence indicate a concentration of self-association (Csa) where Csa is similar to the cmc of DTAB but is significantly less than the cmc of SDS.

3.3.b. Effect of urea concentration on SDS self-association:

Figure 3.3a shows MEM profiles of TMR at 0, 3, and 10 mM SDS in the absence and presence of different concentrations of urea at pH 7.4. In the absence of urea, MEM profiles are well separated, with maxima at 25, 43, and 93 ps for 0, 3, and 10 mM SDS, respectively (Figure 3.3a, bottom panel). In the presence of 3 M urea, their separation decreases significantly and profiles overlap with each other (Figure 3.3a, middle panel). As a result, the maxima of MEM profiles do not show any major SDS concentration variations (27, 27, and 49 μs in the presence of 0, 3, and 10 mM, respectively). In the presence of 5 M urea, their separation decreases even further, resulting in significant overlaps between MEM profiles (Figure 3.3a, top panel). As discussed earlier, the values of $\tau_D$ (obtained from conventional or MEM analysis) have been used to determine $r_H$. Figure 3.3b shows the variation of $r_H$ with SDS concentration in the presence of different concentrations of urea. In the absence of urea (filled squares), $r_H$ increases drastically as the self-association of the surfactant monomers begins. The presence of urea (open squares for
3M and filled circles for 5M urea) hinders self-association, resulting in an increase in the concentration at which self-association begins (Figure 3.3b).

Figure 3.3: Urea dependence of self-association of SDS: (a) MEM profiles obtained with 0 mM, 3mM and 10 mM SDS in the absence (bottom panel) and presence of 3M and 5M urea. (b) SDS concentration dependence of $r_H$ in the absence and presence of 3M and 5M urea.

3.3.c. Effect of pH on SDS self-association:

Figure 3.4a shows MEM profiles of 0, 3, and 10 mM SDS at pH 2 and 11. MEM profiles at pH 7.4 are shown as a comparison. MEM profiles are similar at pH 7.4 and 11. At low pH, however,
the profile for 3 mM SDS is significantly shifted. Also, at pH 2, MEM profiles for 3 and 10 mM SDS are superimposable. Figure 3.4b shows the variation of $r_H$ with SDS concentration at pH 2 and 11; the data at pH 7.4 is shown as a comparison. Compared to that at pH 7.4, self-association at pH 2 occurs at a very low SDS concentration. The transition of self-association is considerably sharper at pH 2 with a high degree of cooperativity. The concentration of self-association is slightly higher at pH 11 than at pH 7.5. We have studied the effect of urea on the self-association of SDS at pH 2 and 11. Figure 3.5 shows the variation of $r_H$ with SDS concentration at pH 2 and 11 in the presence of different urea concentrations. As observed at pH 7.4, the use of urea at pH 2 and 11 increases the concentration of SDS needed to initiate self-association.

![Figure 3.4](image)

Figure 3.4: pH dependence of self-association of SDS: (a) MEM profiles of 0 mM, 3 mM and 10 mM SDS at pH 7.4, pH 2 and pH 11. (b) SDS concentration dependence of $r_H$ at pH 7.4, pH 2 and pH 11.
3.3.d. Effect of different solution additives on SDS self-association:

We have carried out FCS experiments with TMR in the presence of different solution additives (e.g., glycerol, sucrose, and arginine) to study their effects on the self-association of SDS. MEM profiles with different additive solutions in the absence and presence of 3 and 10 mM SDS are shown in Figure 3.6. MEM profiles with sucrose show less dispersion between 0 and 3 M SDS. With glycerol, MEM profiles do not show any dispersion and the data at 0, 3, and 10 mM SDS overlap. With arginine, MEM profiles show a large shift between 0 and 3 mM SDS. No change in MEM profiles is observed between 3 and 10 mM SDS. To gain better insight into the effects of different solution additives, $r_\text{H}$ values are plotted with SDS concentrations in the presence of different concentrations of each additive (Figure 3.7).

The data at Figure 3.7a suggest that self-association occurs at higher SDS concentrations in the presence of sucrose. With glycerol, the process of self-association is slow and gradual (Figure 3.7b), which is supported by the MEM profile data in Figure 3.6. The presence of glycerol leads to the complete absence of dispersion in the MEM profiles at different concentrations of SDS (Figure 3.6). The addition of 500 mM arginine, in contrast, results in an

![Figure 3.5: Effect of urea on the self-association of SDS at (a) pH 2 and at (b) pH 11 monitored by $r_\text{H}$. The presence of urea inhibits formation of aggregates at both the pH2 and pH11.](image)
earlier onset of the formation of self-associated species (Figure 3.7c), which is also shown in the MEM profiles (Figure 3.6).

Figure 3.6: Effect of solution additives (Arginine, Glycerol and Sucrose) on MEM profiles in the absence and presence of 3 mM and 10 mM SDS. Experiments were performed in 20 mM phosphate buffer at pH 7.4.
Figure 3.7: The variation of $I_h$ with SDS concentrations in the presence of different additives: (a) the effect of sucrose; (b) the effect of glycerol; (c) the effect of arginine and Sodium chloride. The concentration of each additive is mentioned in their respective Figures.
The midpoint of the transition is calculated for each of the solution additives using a sigmoidal fit and plotted with the molar concentration of the additive (Figure 3.8). The lines drawn through the data in Figure 3.8 are generated using third-order polynomial fits. These lines should be used for representation purposes only and to understand the trend. It is evident from Figure 3.8 that, in the case of glycerol and urea, the midpoint of the self-association increases with the concentration of the additives, indicating that they inhibit the self-association of SDS. In the case of NaCl and arginine, however, the trend is reversed and these two additives facilitate the self-association of SDS. According to the data presented here, the propensity of SDS self-association follows the order arginine > NaCl > pH 2 > phosphate buffer, pH 7.4 > sucrose > pH 11 > urea > glycerol.

3.3.e. Effect of solution additives on the self-association of DTAB:

FCS experiments with TMR in the presence of DTAB, a positive surfactant with the same chain length as SDS, yield a concentration of self-association (Csa) of 12 mM, which is similar to the reported cmc (35). We have carried out FCS experiments in the presence of different solution additives in order to understand the effects of these additives on the self-association of DTAB.
Figure 3.9 shows $r_H$ values of different concentrations of DTAB in the presence of 5 M urea, 500 mM sucrose, 20% glycerol, and 500 mM arginine. Representative MEM profiles of different concentrations of SDS in the presence of additives are shown in Figure 3.10. As observed before in the case of SDS, the presence of urea, sucrose, and glycerol increases the Csa of DTAB molecules. Similarly, NaCl decreases the Csa, suggesting that the presence of salt favors self-association. Interestingly, the effect of arginine has been found to be exactly opposite to its behavior toward SDS self-association. Although arginine favors the self-association of DTAB, the presence of it inhibits that of DTAB (Figure 3.9).

![Image of Figure 3.9: FCS experiments on the self-association of DTAB. The values of $r_H$ are plotted with the concentration of DTAB in the presence of different solution additives. The name of each additive is mentioned in their respective Figure.](image-url)
Figure 3.10: MEM profiles of the FCS experiments carried out with different concentrations of DTAB in the presence solution additives: The concentrations of the surfactant and additives are shown in the figure. In the absence of any solution additives (in phosphate buffer at pH 7.4), MEM profiles shift to the right with the increase in DTAB concentration due to self-association. In the presence of urea and glycerol, MEM profiles are superimposable indicating significant increase in the concentrations of self-association. The presence of arginine results in gradual shift of the MEM profiles although it is considerably slower than that in its absence (in phosphate buffer at pH 7.4).

The self-association of surfactants has been studied in great detail using multiple techniques including conductivity, light scattering, and rheology measurements (36-38). Steady-state and time-resolved fluorescence studies using suitable fluorophores have also been performed to explore the solution properties of surfactants and their association behaviours (39-42). Although the use of single-molecule techniques including FCS is becoming popular in the study of different aspects of nucleic acid and protein chemistry, their use in the field of surfactants has been relatively uncommon (34,43,44).

FCS data are generally analyzed by using models of a number of discrete diffusing components. Because the individual components and their amplitudes can influence each other, particularly in the case of multicomponent systems, this method can be quite confusing and potentially inadequate for analyzing heterogeneous systems. We have used MEM, which has recently been developed by Sengupta et al. and used extensively to study protein aggregation and
other systems (28,45). MEM provides a bias-free method of fitting FCS data using the continuous distribution of a large number of diffusing components.

The FCS study presented in this article shows an increase in $r_H$ that begins at around 2.5 mM SDS. This concentration is significantly less than the reported cmc value of SDS in water (8 mM). This apparent discrepancy may arise because FCS is sensitive enough to detect the formation of relatively small particles in solution such as the formation of adducts between SDS and TMR. Assuming $r_H$ of TMR (molecular weight 481) to be approximately 7.5 Å, the calculated $r_H$ of a TMR-SDS (TMR bound SDS monomer) molecule can be calculated to be 8.9 Å, which is remarkably similar to the value of $r_H$ observed (8.8 Å) in the present study at 1 mM SDS. Likewise, the formation of small complexes of three and six molecules of TMR-bound SDS would yield $r_H$ values of approximately 13 and 16 Å, and identical values are observed at 3 and 3.5 mM SDS. Short complexes in such a size range would be difficult to detect by techniques traditionally used to measure the cmc values of a surfactant molecule. Experiments are underway in our laboratory to detect these small adducts using high-resolution transmission electron microscopy. The ability of FCS to detect and monitor these early events makes it an important technique. It would be very exciting if the single-molecule resolution of FCS could be used to monitor the lag phase of protein self-association. Although the presence of conformationally altered intermediates would add to the complexity in the initial stage of the protein aggregation pathway, FCS has been shown to monitor subtle changes in the conformational orientation of a number of proteins (13,14,18,23).

Although FCS offers high sensitivity and single-molecule resolution for studying surfactant micellization, there are several important issues that need careful consideration. Because micelles are dynamic, it is expected that the diffusion coefficient measured by FCS may depend on the relative binding affinity of the used fluorophore (here TMR) to the surfactant micelles compared to that of the surfactant monomer. Because this relative binding affinity would almost certainly change under different solution conditions (e.g., at low pH or in the presence of glycerol or urea), the value of Csa obtained by FCS may be influenced significantly. This effect can be overcome using a fluorophore that is covalently attached to a monomer molecule. To determine if the binding affinity between TMR and the surfactants has any
significant influence on the measured Csa values, we have performed separate dynamic light
scattering measurements with SDS at pH 7.4 in the presence of several solution additives. The
values of the count rate at different SDS concentrations (representative plots are shown in Figure
3.11) are used to calculate the cmc of SDS. Although the cmc values determined by the count rate
data have been found to be different from Csa, their relative trend has been found to be similar to
that observed by the FCS data. Second, MEM assumes the diffusing components to be
noninteracting, but this assumption may not hold in the case of highly interacting micellar
systems. It is important to analyze the FCS data obtained with a heterogeneous and dynamic
system using different methods and models to rule out any possible fitting artifacts. Finally, care
should be taken to exclude the contributions of the refractive index and viscosity, which not only
could lead to an artificial increase in the diffusion time but the refractive index mismatch could
potentially lead to significant focal volume aberrations.

![Figure 3.11](image1.png)

**Figure 3.11:** Count rates obtained by the dynamic light scattering (DLS) experiments are plotted with
SDS concentrations, which provide a separate method of measuring the cmc of SDS in the presence of
different solution additives. In phosphate buffer at pH 7.5, the count rate measurements provide a cmc of
SDS is about 8mM. In the presence of arginine the value of cmc decreases while the addition of glycerol
as a solution additive increases the cmc of SDS.
A comparison between the protein and surfactant self-association behavior could be interesting. Protein self-association has been studied in detail because of its relevance in several human diseases and also because of its critical role in the formulation development of biotechnologically derived drugs or "biologics" (47-50). Research in our laboratory emphasizes the role of the stabilizers or excipients such as arginine to influence the conformational properties of the native, nativelike, and unfolded states of a protein (18, 23). The self-association of a surfactant may be considered to be a "supersimplified" model of the self-association of a protein excluding the contribution of the native or nativelike states. The self-association of a protein is an equilibrium composed of at least three interacting units: the native state, the unfolded states, and the self-associated states. The presence of native or unfolded-like intermediate states with altered conformational properties also adds to the complexity. The exclusion of the side chains in a surfactant system (and conformational heterogeneity) excludes the presence of the native state, resulting in a two-state equilibrium between the unfolded state and the self-associated state. Although research in protein folding had traditionally focused on the native state, there is growing recent interest to study the unfolded state of a protein (51-57). Another significant difference between these two kinds of self-association comes from the degree of reversibility. Surfactant self-association has been found to be reversible in the presence of all of the additives studied. (Figure 3.12 shows one representative example in phosphate buffer at pH 7.4.) The reversibility of protein self-association, however, depends on the nature of the proteins, the solution conditions, and the nature of the additives present in the solution (unpublished data).
Figure 3.12: The reversibility of SDS self-association/dissociation is measured by FCS experiments and the values of $r_H$ are plotted with the SDS concentrations. For the dissociation measurements, FCS experiments are performed using a SDS micelle at 15mM SDS as the initial concentration; which is subsequently diluted using phosphate buffer at pH 7.5. Surfactant self-association/dissociation has been found to be completely reversible in the presence of every additive studied.

The self-association of an ionic surfactant (such as SDS and DTAB used in this study) can be governed by two opposing physicochemical interactions. The hydrophobicity of the surfactants would favor their association, but the headgroup charges would repel each other. The use of low pH is expected to neutralize the negative charge of the SDS molecules, leading to an overall enhancement of the favorable hydrophobic interaction that is consistent with the present results at pH 2. An apparent similarity occurs in the case of proteins because many proteins have been shown to exhibit an enhanced aggregation propensity at low pH because of the enhanced hydrophobic interactions. A high pH, such as pH 11 used in this study, should have no effect on SDS self-association because the overall charge and hydrophobicity of SDS molecules remain identical to these values at pH 7.4, which is also observed in this study. The observed effect of salt in increasing the affinity of the self-association of SDS and DTAB can be explained by the
screening of the charges of the surfactant headgroups, resulting in more favorable hydrophobic interactions.

The effect of urea, glycerol, and other small additive molecules on the self-association of ionic and nonionic surfactants has been studied (58). The addition of urea to increase the cmc of surfactants has been reported earlier, and it has been proposed that urea weakens the hydrophobic interactions in aqueous solution (59). Two different mechanisms have been regularly used in the literature to explain the effect of urea on surfactant self-association. The indirect mechanism considers urea to be a "water breaker", and the direct mechanism suggests the replacement of water molecules from the hydration shell as induced by urea (59,60). A third mechanism has also been proposed that suggests that urea increases the water polarity, increasing the solvation of the polar groups (59,64). It has been shown recently that the use of a direct or indirect mechanism to understand urea action is inadequate in general terms; instead the actual mechanism depends on a particular surfactant system, depending on how the solvation occurs (59). For a protein system, it is generally believed that urea interacts with the side chains and backbone of a protein more tightly than water or another hydrophobic molecule, leading to its ability to dissolve the core hydrophobic region (61). Urea has been commonly used to break protein aggregates and to purify protein molecules from inclusion bodies. Cosolvents such as glycerols have been shown to have complex effects on self-association that cannot be explained by simple salting-in or salting-out effects (58). Glycerol is known for its effect to stabilize the native state of a globular protein. It is expected to act like an Osmolite, which interacts with the unfolded state of a protein and excludes itself from the surface of the protein. The use of glycerol and other small organic molecules, however, is shown to decrease the cohesive forces in water, which results in an increase in the solubility of surfactants (62). The end result would be an increase in the concentration at which self-association begins as observed in the case of glycerol used in this study. The use of ethanol, isopropanol, and glycerol as additives has been shown to disfavor micellization and decreases in the aggregation number (62). We have chosen arginine for this study for several reasons. Although arginine has been widely used as an efficient agent to inhibit protein aggregation (63), little or no information is available on its effect on the self-association of a surfactant. Although the structure of arginine is similar to that of guanidine, a molecule commonly used to denature proteins, arginine is commonly used as a protein stabilizer
and as an inhibitor of protein self-association. It has been suggested that arginine may have dual
effects: a protein-stabilizing osmolyte-like effect and a destabilizing denaturant-like effect (23,
63). Its ability to interact with the protein side chains, particularly with the aromatic amino acids,
has been proposed to be responsible for its behavior in inhibiting protein self-association (63).
The present results suggest that in the case of a “supersimplified” model that does not have the
presence of sidechain contributions; the effect of arginine depends solely on the charge of the
surfactant systems. Arginine favors the self-association of negatively charged SDS, but it play an
inhibitory role in DTAB self-association. DTAB is positively charged, and hence it may offer
repulsive interactions with the positive guanidine group present in arginine.

3.4. Conclusions:

Self-association of a surfactant has been considered a ‘super-simplified’ model of the self-
association of a protein excluding the contribution of their side chains. Self-association of the
surfactants could be a useful model using an extended state (the monomer) and a self-associated
state (micelles) without using any contribution from the native or native like states. In addition,
the availability of different surfactants with various charge and head groups allows selective
tweaking of the ionic and hydrophobic properties of this ‘super-simplified’ model. In this
chapter, we have carried out a detailed study of the self-association of two surfactant systems
namely SDS and DTAB. These two surfactants are chosen because of their same chain length
and opposite charge.

Fluorescence correlation spectroscopy is used to monitor the self-association of SDS and DTAB
monomers at single-molecule resolution. Tetramethylrhodamine-5-maleimide (TMR) has been
chosen as a probe because rhodamine dyes have been shown to bind surfactant micelles.
Correlation functions obtained by FCS experiments have been fit using conventional discrete
diffusional component analysis as well as the more recent maximum entropy method (MEM).
Hydrodynamic radii calculated from the diffusion time values increase with surfactant
concentration as the monomers self-associate. Effects of several solution additives on the self-
association property of the surfactants have been studied. Urea and glycerol inhibit self-
association, and arginine shows a dual nature. Arginine has a duel character, it favors self-
association of SDS, a negatively charged surfactant, however, with DTAB, a surfactant of identical chain length but opposite charge, arginine inhibits the micellization.
3.5. **References:**


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