

Surface Activity Interactions in Aqueous Solution of Anionic Surfactants with a Water Treatment Protein from *Moringa oleifera* Seeds

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Abstract

The interaction of anionic surfactants sodium dodecylbenzenesulphonate (SDBS) and sodium bis (2-ethyl-1-hexylsulfosuccinate) (AOT) with the water treatment protein extracted from *Moringa oleifera* seeds has been investigated by surface tension measurements. The results were compared with previously reported studies using surface tension and zeta potential measurements of the same protein with an anionic surfactant sodium dodecylsulphate. There is a strong electrostatic interaction between the protein and the surfactants characterized by a local maximum at intermediate surfactant concentration. This surfactant concentration depends on the protein concentration and corresponds to the surfactant concentration at the point of charge reversal (zeta potential = 0 mV). The number of surfactant molecules required to neutralize the positive charges on the protein was found to be 8 per protein molecule.

Keywords

Anionic, Critical Micelle Concentration, Surface Tension, Surfactant, *Moringa oleifera*, Zeta Potential

1. Introduction

It is well known that the local environment determined by parameters such as pH, salt concentration and the

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presence of surfactant can alter significantly the behaviour of biological macromolecules. The interaction between surfactants and proteins is an active field of research in colloid science because very often in practice the proteins and surfactants are present in the same systems such as industrial, biological, pharmaceutical, and cosmetic systems. Protein-surfactant interaction is important in understanding stability and structural changes in proteins. They may lead to the formation of protein-surfactant complexes which can be soluble or insoluble in aqueous medium [1] [2]. Due to the existence of nonpolar and ionic amino acid side chains in protein molecules, the formation of these complexes is driven by electrostatic interactions between the charged head groups of the surfactant and the oppositely charged units of the protein and also by hydrophobic interactions [3]-[5]. Interaction of globular proteins, especially bovine serum albumin (BSA) with surfactants, in particular sodium dodecylsulphate, SDS, has been extensively studied aiming to understand how surfactant binding affects the protein structure and function [3] [4]. Several spectroscopic and physicochemical techniques have been used to investigate the assembled protein-surfactant complexes [6] [7]. There are several studies that have widened the range of the researches of the interactions of the protein with surfactants, but not enough to understand the role of the hydrophobic and electrostatic interactions between protein and surfactant.

The physicochemical characterisation of the water treatment cationic *Moringa oleifera* (MO) seed protein is important in order to understand the nature of the protein and its interaction with other components particularly in water treatment. This study is an extension of the previous work of the interaction of the water treatment MO seeds protein with an anionic surfactant sodium dodecylsulphate (SDS) studied by surface tension and ultrasonic velocity measurements [8]. The anionic surfactants used in this study of using surface tension were sodium dodecylbenzenesulphonate (SDBS) and sodium bis(2-ethyl-1-hexylsulfosuccinate) (AOT) and their chemical structures are given in **Figure 1**. The surfactants differ in their hydrocarbon chemical chains to SDS (also shown in **Figure 1** for comparison purposes).

The protein from seeds of MO shows some surface activities, *i.e.* it's effective in reducing surface tension of water from 72 mN/m to about 30 - 40 mN/m [9]. The proteins can be identified with some critical concentrations at which the surface tension begins to drop (proteins weakly surface active at low concentrations). The onset of surface activity in the concentration range studied of the MO seeds proteins compared to three common proteins, bovine serum albumin (BSA), lysozyme and frog foam protein, is of the order frog foam > BSA > MO > lysozyme.

Surface tension detected the adsorption of the protein at the air-water interface and indicated that the protein is weakly surface active at low concentrations, requiring substantially higher solution concentration to achieve maximum reduction in surface tension. The poor adsorption of the protein at low concentrations may be attributed to electrostatic repulsion among the charged adsorbed molecules due to high charge density in the protein molecule. The proteins from *Moringa* seeds are reported to be densely charged cationic molecules in water solution. This is in line with the literature report that proteins exhibit greater surface activity near the isoelectric point, because of minimization of electrostatic repulsion among the identically charged adsorbed molecules. In addition, a soluble protein has hydrophilic parts located on the outside of the molecule and the hydrophobic regions are buried resulting in low affinity for the air-water interface. The low surface activity of the protein agrees with high solubility in water observed for the protein.

The weak surface activity is important in water treatment because if very surface active, then one would require high concentrations before getting the protein molecules in the bulky to start the water purification process. It is, however, important to point out that surface activity isn't enough in determining whether or not the protein has water pollutant removal properties but it is merely one of its peculiar properties that play some role in water treatment.

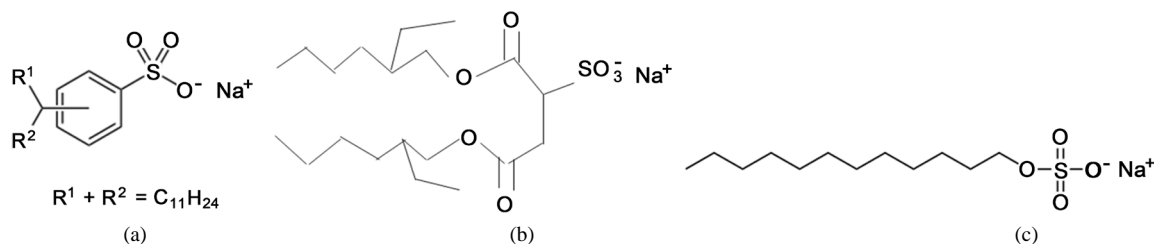


Figure 1. Chemical structures of the anionic surfactants: (a)—sodium dodecylbenzenesulphonate (SDBS); (b)—sodium bis(2-ethyl-1-hexylsulfosuccinate) (AOT); (c)—sodium dodecylsulphate (SDS).

2. Materials and Methods

2.1. Materials

The extraction and purification of protein powder was done using the method of Ndabigengesere and Narasiah [10] [11], and the experimental details are as described previously by Kwaambwa and Maikokera [12]. The anionic surfactants used were sodium dodecylbenzenesulphonate (SDBS) and sodium bis(2-ethyl-1-hexylsulfosuccinate (AOT), and both were purchased from Sigma-Aldrich and used without further purification. Ultrapure water (UHQ, Elga, Wycombe, UK) was used to prepare the mixtures.

2.2. Surface Tension Measurements

Surface tension was measured with a Kruss (Hamburg, Germany) K9 digital tensiometer using the Du Nouy ring method, calibrated using ultrapure water. The platinum ring was cleaned between each reading by flaming (Bunsen burner) to remove any residual deposits. The surface tension measurements were made immediately after pouring the protein/surfactant solution in the sample trough and were done at room temperature ($23^{\circ}\text{C} \pm 2^{\circ}\text{C}$). The readings were taken in triplicate for each individual solution to check reproducibility and the surface tension values were within an error less than or equal to $\pm 3 \text{ mN}\cdot\text{m}^{-1}$.

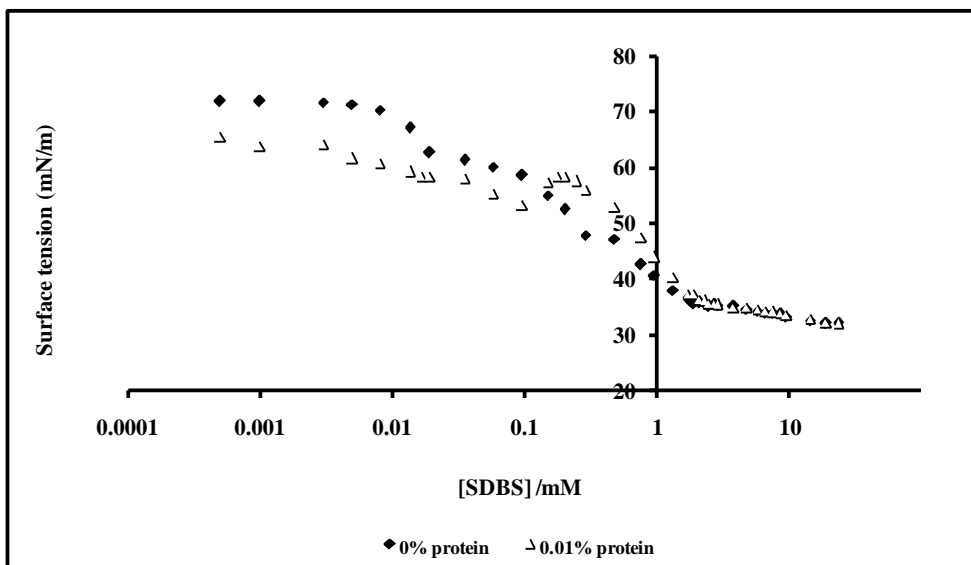
Surfactant solutions in the presence and absence of protein were used to study the protein-surfactant interaction. The freeze-dried protein was dissolved in UHQ water. The adsorption at the air-water interface from binary solutions of protein extracted from MO seeds and surfactant was followed by surface tension measurements by keeping the protein concentration (% w/v) constant and varying the surfactant concentration up to concentrations higher than the critical micelle concentration (CMC). The CMC values in water for most commercial surfactants are available in the literature, and the results from individual laboratories may vary slightly depending upon the method and conditions of the laboratory. The surface tension measurements were measured at room temperature immediately after sample preparation

3. Results and Discussion

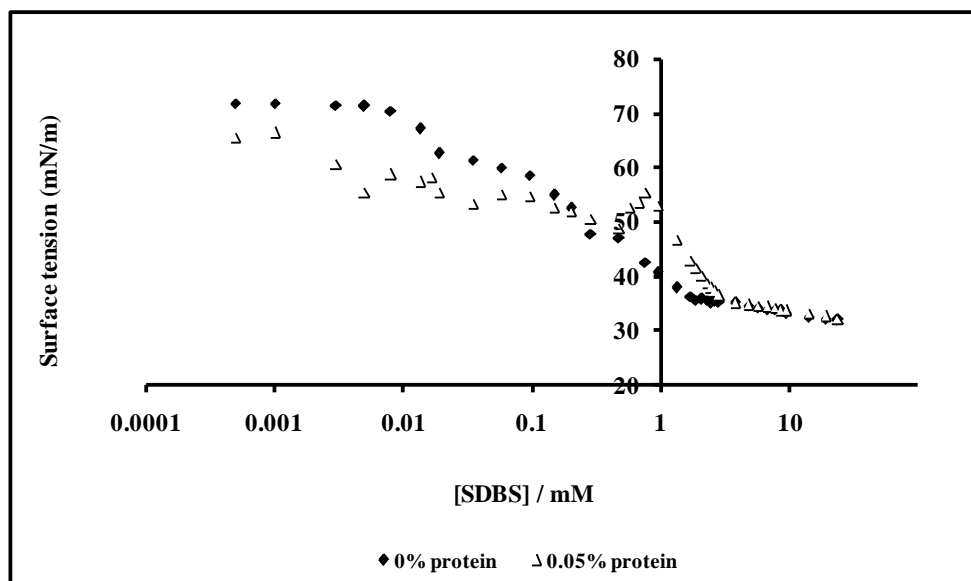
Figure 2 shows the plots representing the variation of the surface tension, γ , with the SDBS concentration, *i.e.* \ln (surfactant concentration). This is based on the Gibbs adsorption equation isotherm [13]. Similar plots were obtained for the protein/AOT systems but are not shown here. The CMC was taken as the concentration corresponding to the break in the plots representing the variation of the surface tension, γ , with \ln (surfactant concentration) for surfactant solutions without protein. The CMC values in water estimated from the surface tension plots for SDBS and AOT were 2.11 mM and 2.48 mM, respectively. The CMC values for SDBS and AOT obtained are in good agreement with the literature values of 2.14 mM and 2.6 mM, respectively, in water at 25°C as reported in Holmberg [14]. **Figure 2** also shows that SDBS has the ability to reduce the surface tension of water to as low as approximately $32 \text{ mN}\cdot\text{m}^{-1}$.

The surface tension data for the mixed solutions of SDBS with different coagulant protein concentration (% w/v) is also shown in **Figure 2**. At low SDBS concentration, the surface tension of the mixed system is lower than that of pure surfactant, which indicates formation of more surface-active complexes arising from the binding of SDBS to the protein. The data exhibit an increase in surface tension at intermediate SDBS concentrations, with a local maximum occurring at 0.2 mM and 0.768 mM for 0.01% and 0.05%, respectively. It is interesting that the maximum in surface tension profile for 0.05% protein occurs at approximately the same SDBS concentration of 0.768 mM as the maximum observed in the solution viscosity [15].

For all protein/surfactant systems, it was observed that there was a certain anionic surfactant concentration at which precipitation was visible as shown in **Figure 3** for protein/AOT system as an example. This surfactant concentration was taken to be the surfactant precipitation concentration (pc) for a given protein/surfactant system and it coincides with the surfactant concentration at the surface tension maximum mentioned above. As stated earlier, the MO protein is known to be positively charged in water (isoelectric point of the protein is between 10 and 11) [16] and so it can form a precipitate with oppositely charged anionic surfactant due to the formation of neutral complexes of protein-anionic surfactant. In **Table 1**, the observed pc values, which increase with increase in protein concentration, are given for protein/AOT system. Green *et al.* [17] observed similar precipitation phenomenon for SDS/lysozyme system. The complexation of the protein with AOT that leads to the formation of insoluble precipitates is ascribed to charge neutralization between the protein and surfactant ions.



(a)



(b)

Figure 2. The effect of coagulant protein on the surface tension of SDBS in water. Panels (a) and (b) are 0.01% and 0.05% protein respectively. The protein/SDBS interaction was studied by means of the Du Nouy ring method.



Figure 3. Typical visual effect of increasing AOT surfactant concentration on MO seeds protein solution (0.05% w/v).

Table 1. Summary of the observed and calculated parameters for the coagulant protein/AOT system.

Protein concentration (%w/v)	Protein concentration (mM)	p_c (mM)
0.01	0.0137	0.202
0.02	0.0274	0.302
0.03	0.0411	0.403
0.05	0.0684	0.644

In summary, it is here stated that the data exhibit a slight increase in surface tension at intermediate concentrations for all three anionic surfactant/protein systems, and these are the same solutions in which precipitation was observed. In other words, the p_c values correspond to the anionic surfactant concentrations at which the maxima in surface tensions isotherms in the intermediate surfactant concentration range occur.

The surface tension profiles systems protein/SDBS and protein/AOT show similar trends to those observed for SDS/protein system. The protein interacts strongly with anionic surfactants such as SDS, AOT and SDBS and might have specific binding sites for such surfactants. It is important to point out that any differences in the surface tension profiles can be attributed to structural differences in the anionic surfactants. For instance, the two anionic surfactants SDBS and SDS only differ in their head groups (SDBS has a sulfonate while SDS has a sulphate head group). **Figure 4(a)** shows a comparison of surface tension profiles for the two surfactants SDBS and SDS in the presence of 0.01% protein. The difference in local maximum positions in the surface tension profiles for the two surfactants in presence of same protein concentration (0.01% protein) may suggest head group effect in the interaction of the two surfactants with the protein. It can be seen that the maximum occurs at lower surfactant concentration for SDBS (maximum at 0.2 mM) than for SDS with a maximum at 0.768 mM.

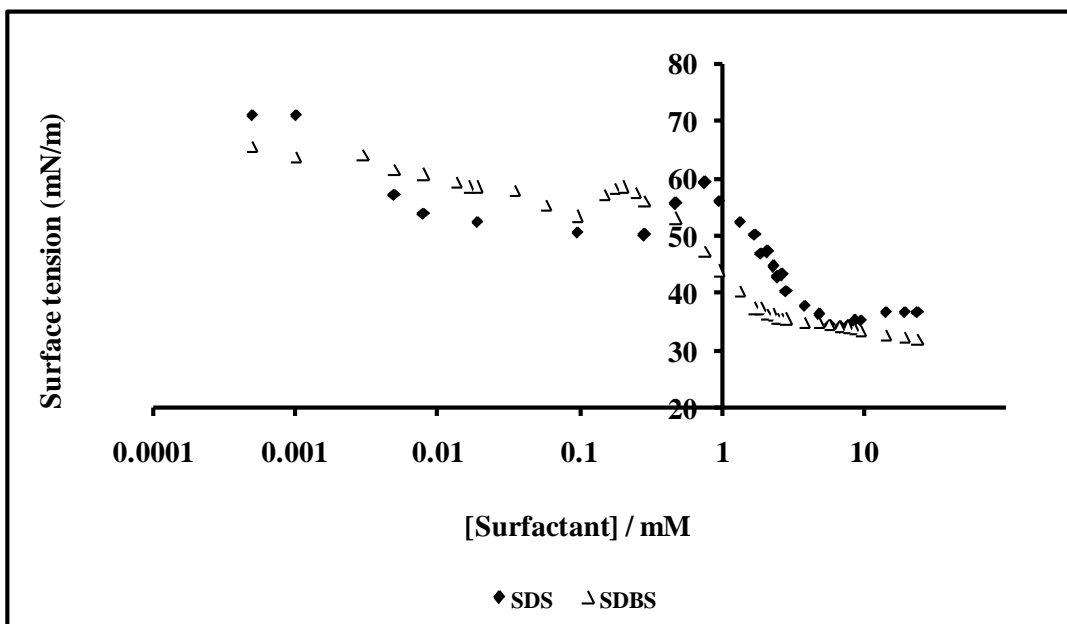
Note that in **Figure 4(b)**, the surface tension data is re-plotted against reduced surfactant concentration (*i.e.* c/CMC) so that the protein-surfactant interactions in different surfactant systems could be compared relative to CMC. In other words, re-scaling of the concentration in this manner should account for the effect of micellisation of the different types of surfactants and magnitudes of the surface tension data which reflects protein-surfactant interactions. The local maximum for the two curves now coincide suggesting that the difference in local maximum positions in **Figure 4(a)** may be due to micellisation of the surfactants (CMC is affected by surfactant head group). These studies provide further support of specific adsorption of surfactants to the protein. The surface tension at similar low surfactant concentrations initially decreased. When the concentration is increased beyond the point at which the interface is fully occupied, further surfactant molecules are dispersed in the bulk solution, either alone or associated with the protein.

Previously Kwaambwa and Rennie [18] studied the effect of SDS concentration on the zeta potential (ζ) of the MO protein in aqueous solution. The zeta potential was used to identify the points of charge reversal in the solutions. The concentration for neutralization increased with protein concentration. The surfactant concentration observed at the local maximum in surface tension corresponds to anionic surfactant concentration at the cross-over point of the zeta potential ($\zeta = 0$ mV).

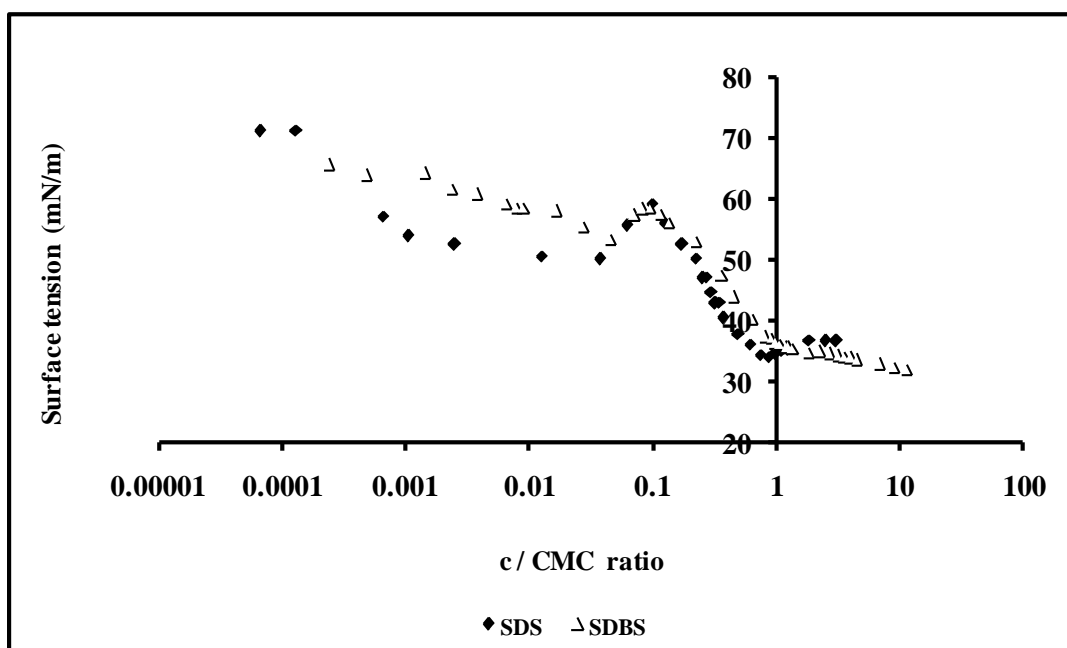
Using the determined amino acid composition [19] and the Protein calculator v3.3 [20], the molecular weight of the protein was estimated to be 7.307 kDa [19]. The % protein from both surface tension measurements and zeta potential could then be converted to a concentration in mM. **Figure 5** shows the plot of surfactant concentration (mM) versus protein concentration for the surface tension and zeta potential data. The data results in a single linear plot independent of the surfactant. The number of surfactant molecules associated with each protein molecule was found to be $\sim 8 \pm 1$ molecules per protein molecule from the slope of linear plot. The value agrees with amino acid sequencing results by Gassenschmidt *et al.* [21] and Broin *et al.* [22], who reported that the protein extracted from MO seeds has 8 negatively charged amino acid residues. The MO protein interacts with surfactants in the order anionic > cationic > zwitterionic/non-ionic [8] [18] [23].

4. Conclusion

The surfactants SDBS and AOT interact strongly with cationic protein extracted from MO seeds as evidenced from surface tension measurements. There is a reasonable agreement in the CMC values determined by surface



(a)



(b)

Figure 4. The effect of 0.01% coagulant protein on the surface tensions of SDS and SDBS in water. (a) Surface tension plotted versus surfactant concentration, c ; (b) Surface tension plotted versus the c/CMC ratio.

tensions (2.11 mM and 2.48 mM for SDBS and AOT, respectively) with the values reported in literature. The interaction behaviour of the protein with these surfactants is similar to that observed with SDS. There is an increase in the surface tension of the intermediate surfactant range which corresponds to the precipitation concentration (pc) and the concentration at which zeta potential is zero (point of charge reversal of the protein from positive to negative). From the points of charge reversal and surface tension maxima, it was estimated that 8 ± 1 anions are required to neutralize the positive charges of each protein molecule. This clearly demonstrates that negative surfactant headgroup is responsible in the protein-surfactant concentration.

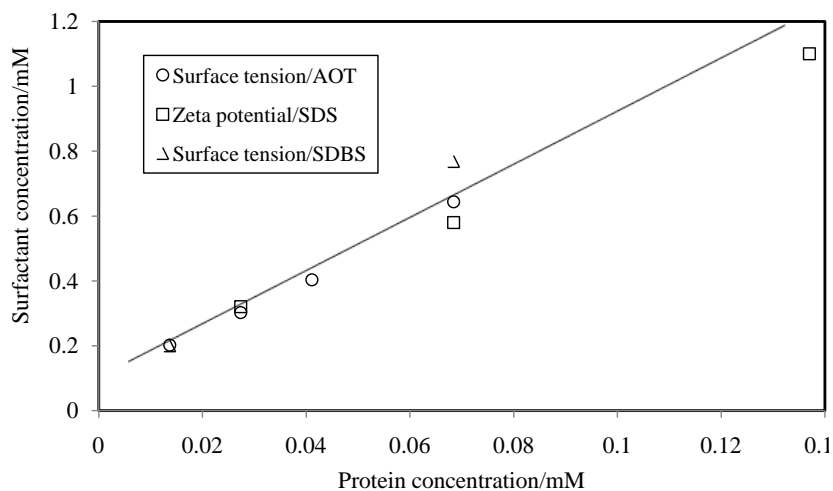


Figure 5. Surfactant concentration at $\zeta = 0$ mV (cross over point) and surface tension maximum as a function of protein concentration. The linear equation fitted to these point is $[\text{Surfactant}] = 8.0 [\text{protein}] + 0.11$.

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