Short communication

Air-water interface interaction of anionic, cationic, and non-ionic surfactants with a coagulant protein extracted from *Moringa oleifera* seeds studied using surface tension probe

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Abstract

The interaction of coagulating protein extracted from Moringa oleifera seeds with the anionic surfactant sodium bis (2-ethyl-1-hexylsulfosuccinate (AOT), the cationic surfactant hexadecylpyridinium chloride (HDPC) and non-ionic surfactant Triton X-100 (TX-100) were investigated by surface tension measurement. The protein extract interacts strongly with anionic surfactant AOT suggesting that the former is cationic under conditions studied. HDPC exhibits mild interaction with protein extract whereas the interaction with Triton X-100, if any, could not be detected by this technique.

Keywords: coagulant protein, critical aggregation concentration, critical micelle concentration, protein-surfactant interaction, surface activity, surface tension

Introduction

Coagulation-flocculation followed by sedimentation and disinfection is used worldwide in the water treatment industry. Aluminium salts, iron salts and synthetic polymers are the most common used coagulants in water treatment worldwide. The cost and environmental side effects of these compounds are their two main disadvantages (Martyn et al., 1989; Broin et al., 2002). For instance, several serious drawbacks of using aluminium salts have been pointed out, particularly concerning health problems related to residual aluminium in treated waters, such as Alzheimer's disease (Martyn et al., 1989; Fatoki and Ogunfowokan, 2002). Therefore, it is desirable to develop other cost-effective and more environmentally acceptable coagulants/flocculants. There has been increased interest in the use of organic derived plant materials, such as *Moringa oleifera* (MO) seeds (Jahn and Dirar, 1979, Grabow et al., 1985; Jahn, 1986; 1988; Olsen, 1987; Muyibi and Evison, 1995a;b; Ndabigengesere et al., 1995; Ndabigengesere and Narasiah, 1998; Folkard, 1999; Sajidu et al., 2006).

MO is a tropical plant belonging to the family Moringaceae. The plant grows quickly even on soils having relatively low humidity. It is known to be non-toxic to humans or animals. MO seeds extracts have been shown to have large effects on turbidity removal (92 to 99% reduction) (Jahn, 1986; Muyibi and Evison, 1995a;b). Seed extracts have also been reported to dramatically decrease clay and bacteria contents in raw water (Grabow et al., 1985; Madsen et al., 1987; Muyibi and Evison, 1995). Moreover, it has been suggested that crushed MO seeds could be as efficient as aluminium salts for coagulation of raw water impurities. Water treated with MO seed extract produces less sludge volume compared to alum (Ndabigengesere and Narasiah, 1998). An additional benefit of using coagulants derived from MO is that a number of useful products may be extracted from the seed. In particular, edible and other useful oils may be extracted before the coagulant is fractionated. Residual solids may be used as animal feed and fertiliser, while the shell of the seed may be activated and used as an adsorbent. The coagulant is thus obtained at extremely low or zero net cost.

The main concern in using MO seeds for water treatment is the significant increase in organic load (Ndabigengesere and Narasiah, 1998; Okuda et al., 2001a;b). Jahn (1988) reported that water treated with crude MO seed extracts should not be stored for more than 24 h. The crude extract is therefore not generally suitable for large water supply systems where the hydraulic residence time is very high. Two approaches may allow the use of MO seeds in such systems. Adsorption can be used to remove the organic load from the crude seed extracts. Alternatively, the active coagulating component may be extracted from the seed and used in pure or semi-pure form, thus reducing the total amount of organic material added to the treatment process. In this paper, we have chosen the latter approach.

The extraction and purification of the active component from MO seeds has been described previously by a number of authors (Ndabigengesere and Narasiah, 1998; Broin et al., 2002; Ghebremichael et al., 2005), although there have been conflicting reports as to the nature and properties of the protein compounds extracted. However, several of the reports have described the main water-extractable component as proteinaceous. It was described as a water-soluble protein with a net positive charge (Ghebremichael et al., 2005), as dimeric cationic proteins with molecular mass of 12-14 kDa and isoelectric point (pI) between 10 and 11 (Ndabigengesere et al., 1995). Others reported a molecular mass of 6.5 kDa and pI greater than 10 (Gassenschmidt et al., 1994). Furthermore, the mechanism of coagulation by *Moringa* seeds is not well understood and different authors have attributed it to existence of proteins and non-proteins flocculating agents (Gassenschmidt et al., 1994; Ndabigengesere et al., 1995; Okuda et al. (2001a,b). The varying reports on the nature,

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properties and mechanism of the active component from MO seeds in water treatment thus necessitated further study.

In addition, in general, proteins themselves behave as surfactants and their surface activity is of interest to industry for a wide spectrum of applications (Magdassi, 1996; Izmailova and Yampolskaya, 1998; Wieranga, 2005). Because of their surface-active properties, proteins, for instance, contribute to the formation and improve the physical stability of foam based food products. The data regarding the surfactant behaviour of the coagulating protein extracted from MO seeds is scarce. It is also the surfactant behaviour of proteins that prompted the investigation on interfacial properties of coagulating protein extracted from MO seeds.

Currently, there is increased interest in the search for materials which are biodegradable, non-toxic and derived from renewable resources (Piispanen, 2002). Proteins are an interesting starting material for the development of surfactants because various proteins possess inherent surface active properties (Magdassi, 1996; Magdassi and Kamyshny, 1996). Investigation of seeds protein has been motivated by the need to produce plant protein products with an acceptable functionality, such as solubility, viscosity, emulsifying and foaming properties (Hall, 1996; Schwenke, 1998). Moreover, proteins used in interfacial studies are mainly food proteins because of their natural widespread availability in high concentrations in food systems or raw materials, and are also commercially available due to their easy of isolation and purification (Schwenke, 1998).

The surface and interfacial phenomena involving surfactants and proteins, and their mixtures, are widespread in nature and technology (Izmailova and Yampolskaya, 1998). The protein-surfactant interactions have been studied in the field of chemistry because they can shed light on the functional properties (Vasilescu et al., 1999; Sun et al., 2005). Protein-surfactant mixtures have applications in the food industry, biochemistry, and in pharmaceutical industry (Jones, 1996; Vasilescu et al., 1999; Sun et al., 2005). In research methods, surfactants are widely used in the purification and characterisation of proteins such as in the technique of polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) for the analysis and estimation of molecular masses of proteins (Jones, 1996; Tofani et al., 2004).

Protein-surfactant interactions in aqueous media have been investigated at low protein concentration using surface tension technique for systems such as SDS-lysozyme (Green et al., 2000), SDS-myoglobin (Tofani et al., 2004) and SDS-gelatine (Jones, 1996; Wüstneck and Krägel, 1998). Surface tension technique has also been used in investigating surfactant properties of proteins such as bovine serum albumin (BSA), lysozyme and other proteins from less conventional sources (Cooper et al., 2005). In this paper, we report the interaction between the coagulating protein extracted from MO seeds and surfactants (anionic, cationic and non-ionic) in aqueous media using surface tension measurements. To the best of our knowledge, only surface tension data of the coagulant protein and SDS-coagulant protein system has so far been reported (Maikokera and Kwaambwa, 2007).

Materials and methods

Extraction and purification

The MO seeds and the seed cake were obtained from Mahalapye, Botswana. Seeds were stored at room temperature whereas the seed cake was stored in a cold room at 4°C. The seeds were shelled manually just before the extraction, and the kernel was grounded to a powder using mortar and pestle. The extraction and purification of protein powder was done using the method of Ndabigengesere and Narasiah (1998), which was found to give molecular weight of coagulant protein of about 13 kDa (Ndabigengesere et al., 1995).

Oil was removed by mixing the seeds powder in 40 to 60 °C petroleum ether (37%, w/v) for 30 min on an orbital shaker (Stuart Scientific). The solids were separated by filtration. The water soluble protein was then extracted by mixing the solids in water (200 mℓ) for 30 min and filtered through Whatman paper No. 3.

The protein was precipitated from aqueous filtrate by addition of solid ammonium sulphate, $[(NH_4)_2SO_4]$, to the aqueous filtrate until a saturation of 100% was reached. The precipitated protein was separated by filtration, re-suspended in water and then filtered to remove any insoluble materials. The protein solution was dialysed through cellulose membrane (Sigma Aldrich) with a molecular weight cut-off of 12 to 14 kDa. The active protein remained inside of a dialysis tube. The inside solution was then poured on a carboxymethylcellulose (CM) column equilibrated with water to retain the active protein and step elution was carried out using 1M sodium chloride solution. The eluted solution was dialysed again through the 12 to 14 kDa membrane. The inside solution was freeze dried (Edwards Freeze Dryer) and a white protein powder was obtained. The powder was kept at room temperature.

Surface tension measurements

Surface tension (γ) of protein solutions in the presence and absence surfactant was measured with a Krüss K9 digital tensiometer (Hamburg, Germany) based on the Du Nouy platinum ring detachment method and was calibrated using deionised water. The platinum ring was cleaned before each measurement 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± 2°C). The readings were taken in triplicate for each individual solution to check repeatability and the surface tension values were within an error less than or equal to ±3 $mN \cdot m^{-1}$.

Surfactant solutions in the presence of protein were used to study the protein-surfactant interaction. The surfactants used were anionic surfactant sodium bis(2-ethyl-1-hexylsulfosuccinate (AOT), the cationic surfactant hexadecylpyridinium chloride (HDPC) and non-ionic surfactant Triton X-100 (TX-100), and all were purchased from Sigma-Aldrich and used without further purification. The adsorption at the air-water interface from binary solutions of coagulant 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 (Holmberg, 2001).

Results and discussion

The studies of surface tension could provide evidence of interaction between proteins and surfactants. When strong interactions between proteins and surfactants exist, the surface tension

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curve of protein-surfactant mixtures would deviate from that of surfactants. Figures 1 to 3 show the surface tension curves of systems of AOT, HDPC and TX-100 in the presence and absence of protein. The surface tensions of pure surfactant solutions are also shown for comparison. No surface tension minima were found for all surfactants in the absence of protein, which implied that no surface-active impurities detectable by surface tension measurements were present. The *cmc* was taken as the concentration corresponding to the break in the plots representing the variation of the surface tension, γ, with the surfactant concentration and ln (surfactant concentration). This is based on the Gibbs adsorption equation isotherm (Evans and Wennerström, 1994). The *cmc* values in water estimated from the surface tension plots for AOT, HDPC and TX-100 are 2.48 mM, 0.98 mM, and 0.26 mM, respectively. 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. For instance, the *cmc* values in water at 25°C reported in Holmberg (2001) of the AOT, HDPC and TX-100 are 2.6 mM, 1.1 mM and 0.22 mM, respectively, showing good agreement with the values with our results.

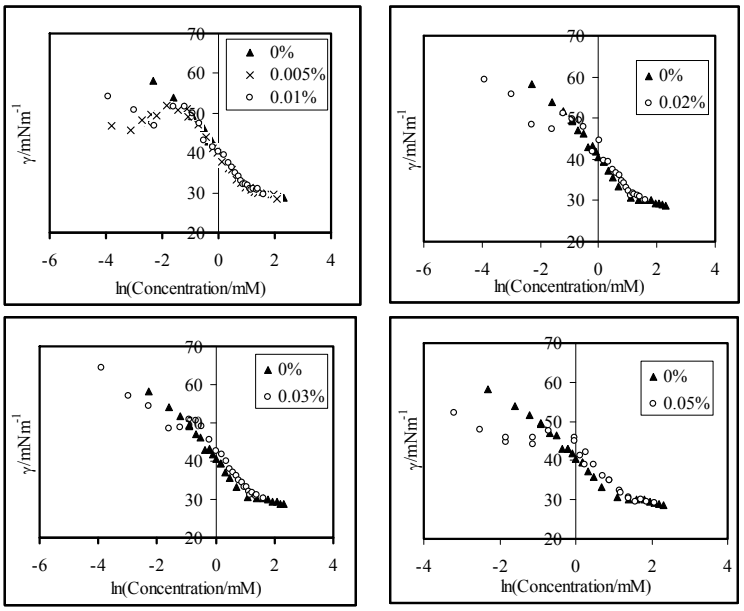

Figure 1
Surface tension of AOT in the presence and absence of protein at room temperature

The surface tension profile for the mixed AOT/protein system shows a number of characteristic features which are absent in the case of HDPC/protein and TX-100/protein systems. The first notable feature is the lowering of surface tension over the low AOT concentration regime in the AOT/protein system as shown in Fig. 1. The addition of protein lowers the surface tension of AOT solutions suggesting clearly the formation of a more surface-active complex arising from the binding of AOT to protein. The decline in surface tension is more pronounced for AOT/protein system followed by HDPC/protein system shown in Fig. 2. Figure 3 shows that the presence of protein has a negligible effect, if any, on the surface tension of the TX-100 solutions. The γ vs. ln (concentration) plot of TX-100-protein systems coincides with that of TX-100 solutions alone, which suggests that in the solution of TX-100-protein system, no interaction of surfactants with protein can be detected by surface tension measurements at protein concentrations used. Therefore, the interactions of non-ionic surfactant TX-100 with protein are similar to those of the surfactant alone in the case of surface activity.

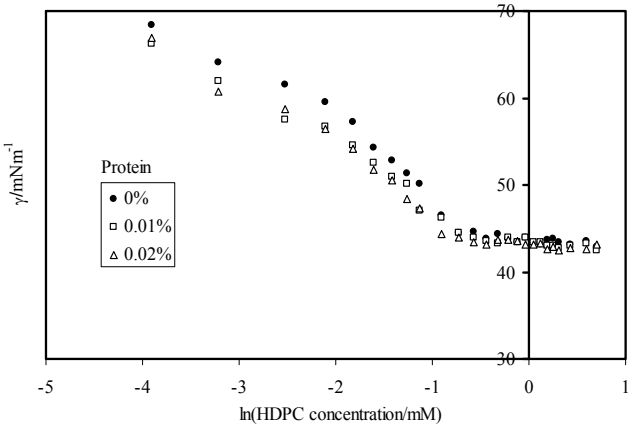

Figure 2
Surface tension of HDPC in the presence and absence of protein at room temperature

It is useful to note that because protein molecules usually contain positive and negative charges, added ionic surfactants readily form complexes with protein, while in comparison, non-ionic surfactants interact rather weakly with protein. Furthermore, when comparing anionic and cationic surfactants, studies have shown that the anionic surfactant interacts more strongly than the cationic surfactant. It has been stated earlier that the protein has an isoelectric point around pH 11. Thus, at pH 7, the coagulant protein is positively charged and this could explain the weaker interaction observed for HDPC/protein system than AOT/protein system.

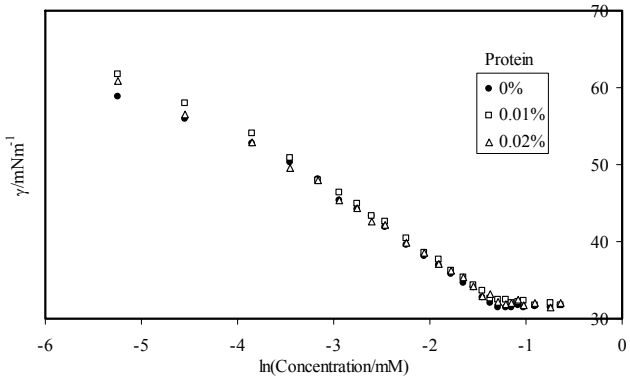

Figure 3
Surface tension of TX-100 in the presence and absence of protein at room temperature

This so-called synergistic effect of anionic/protein system is well documented in literature and is ascribed to specific binding via the strong electrostatic attraction and cooperative association of the surfactant to the protein via hydrophobic affinity (Mata et al., 2006). Electrostatic interactions will occur as the head-group of the surfactant binds to the positive charges on the surface of the protein, while the surfactant's hydrophobic chains interact with adjacent hydrophobic regions of the protein. This is followed by conformational changes of the protein to expose its hydrophobic core, leading to non-specific cooperative association of the surfactant.

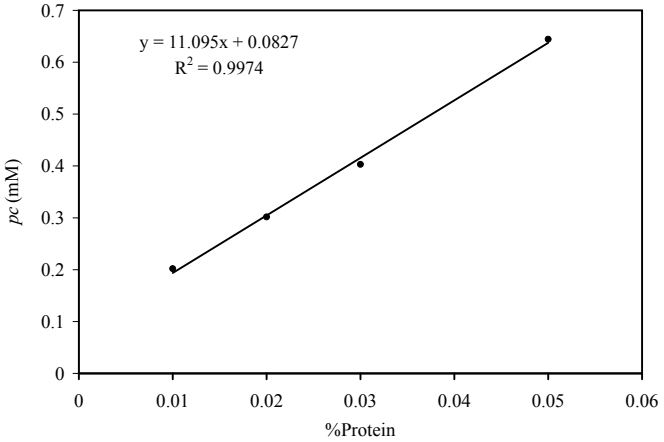

Figure 4
Observed critical aggregation concentration of protein-AOT systems as a function of protein concentration

The effect can be observed at very low surfactant concentrations, where the surfactant alone does not adsorb at the air-water interface. In this region of the surfactant concentration, the surfactant molecules appear to be non-cooperatively associated to the polymer chains.

It is known in general that anionic surfactants interact strongly with proteins (oppositely charged polyelectrolytes) and form protein-surfactant complexes, which would induce the unfolding of proteins (Ananthapadmanabhan, 1993). Cationic surfactants exhibit a lower tendency to interact with proteins (Ananthapadmanabhan, 1993). In contrast to anionic and cationic surfactants, non-ionic surfactants bind weakly to proteins (Ananthapadmanabhan, 1993) and this is attributed to the low *cmc* of some non-ionic surfactants and the absence of the electrostatic interaction between protein and non-ionic surfactant that make micelle formation in the bulk solution a more favourable process than binding to proteins (Ananthapadmanabhan, 1993; Xiao et al., 2005).

For all AOT-protein systems except 0.005% protein/surfactant system, it was observed that there was visible precipitation at a certain surfactant concentration and this was taken to be the surfactant precipitation concentration (*pc*) for a given protein/surfactant system. As stated earlier, the coagulant protein is known to be positively charged in water (isoelectric point of the protein is between 10 and 11) (Ndabigengesere et al., 1995) and so it can form a precipitate with oppositely charged AOT due to the formation of neutral complexes of coagulant protein-anionic surfactant. Green et al. (2000) observed similar precipitation phenomenon for sodium dodecyl sulphate (SDS)-lysozyme system. In Table 1, the observed *pc* values, which increase with increase in protein concentration, are shown. A plot of the observed *pc* vs. % protein is linear as shown in Fig. 4. The complexation of the protein with AOT that leads to the formation of insoluble precipitates is ascribed to charge neutralisation between the protein and surfactant ions.

The data exhibit a slight increase in surface tension at intermediate concentrations, and these are the same solutions in which precipitation was observed. In other words, the *pc* values roughly correspond to the AOT concentrations at which the maxima in surface tensions isotherms in the intermediate AOT concentration range occur (see Fig. 1 and Table 1). Figure 1 shows that before the surface tension rises to maximum, it remains flat for some concentration range, over which addition of AOT did not cause any measurable change in surface tension. This break point is similar to that observed in other polymer/surfactant systems bearing opposite charges. This break point is commonly referred to as the critical aggregation concentration (*cac*) and values AOT/protein system obtained from data plotted in Fig. 1 are given in Table 1. It is important to bear in mind that *cac* implies that below this point there is no interaction between the surfactant and polymer and we believe such assignment to be misleading for the systems bearing opposite charges, because neutralisation between the negatively charged AOT and positively charged fragments of protein will occur, even at very low AOT concentration. Despite the almost constant surface tension over this region, the composition of the complexes at the surface and in the region is expected to vary with addition of AOT and the rising in surface tension indicates an increased solubility of the surface aggregates (Green et al., 2000). This would mean that the surface concentration of the complex has been reduced. At higher AOT concentrations, the precipitate re-dissolved and the surface tension starts to drop. The subsequent decline in surface suggests the adsorption of free AOT monomers.

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

Protein concentration (%)	*cac*(mM)	*pc* (mM)	Surfactant concentration at γ maximum (mM)
0.005	0.0224	-----	0.161
0.01	0.101	0.202	0.202
0.02	0.101	0.302	0.301
0.03	0.202	0.403	0.404
0.05	0.162	0.644	0.485

The other interesting feature of the protein/ionic surfactant systems is the occurrence of the *cmc* as compared with the *cmc* of the pure surfactants. The protein seems to decrease the *cmc* of HDPC for protein concentrations used whereas it seems to increase that of AOT. Generally, in surfactant/polymer systems, the *cmc* in the mixed system is found to be greater than that of pure surfactant, due to binding and association of surfactant onto polymer. The trend observed here is the opposite of what Green et al. (2000) obtained for lysozyme/SDS system and explained it by suggesting that this indicates that the aggregation of the surfactant was likely to be facilitated by the association of the polypeptide fragments. A useful piece of supporting information to this assumption was that the surface tension above the *cmc* in the mixed system was lower than that of the pure surfactant suggesting that above *cmc* the system contains both surfactant-rich and surfactant-lean aggregates and that there was no formation of pure surfactant aggregates. In our case, surface tension above the *cmc* in the polymer/surfactant mixed system is the same as that of the pure surfactant.

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Conclusions and future work

In this paper the concentration dependence of air-water interfacial properties of coagulating protein-surfactant systems in aqueous media by surface tension measurements is reported. The data showed that the order of interaction of the extracted protein with surfactants studied was as follows: AOT > HDPC > Triton X-100. In fact, the surface tension-surfactant concentration plot for protein-non-ionic surfactant system coincided with that of the surfactant alone suggesting that the interactions, if any, could not be detected by surface tension probe under experimental conditions used. The results also do suggest that the protein and AOT are oppositely charged under experimental conditions used and hence the electrostatic interactions exist. The coagulant protein-AOT interaction binding scheme exhibit the behaviour of weakly interacting polymer-surfactant systems.

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