J. Food Sci. Nutr.
Vol. 3, No. 2, p. 143~151 (1998)

# Electrophoretic Mobility to Monitor Protein-Surfactant Interactions

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Abstract

Protein-surfactant interactions have been investigated by measuring ζ-potential of β-lactoglobulin-coated emulsion droplets and β-lactoglobulin in solution in the presence of surfactant, with particular emphasis on the effect of protein heat treatment (70°C, 30 min). When ionic surfactant (SDS or DATEM) is added to the protein solution, the ζ-potential of the mixture is found to increase with increasing surfactant concentration, indicating surfactant binding to the protein molecules. For heat-denatured protein, it has been observed that the ζ-potential tends to be lower than that of the native protein. The effect of surfactant on emulsions is rather complicated. With SDS, small amounts of surfactant addition induce a sharp increase in zeta potential arising from the specific interaction of surfactant with protein. With further surfactant addition, there is a gradual reduction in the ζ-potential, presumably caused by the displacement of adsorbed protein (and protein-surfactant complex) from the emulsion droplet surface by the excess of SDS molecules. At even higher surfactant concentrations, the measured zeta potential appears to increase slightly, possibly due to the formation of a surfactant micellar structure at the oil droplet surface. This behaviour contrasts with the results of the corresponding systems containing the anionic emulsifier DATEM, in which the ζ-potential of the system is found to increase continuously with $R$, particularly at very low surfactant concentration. Overall, such behaviour is consistent with a combination of complexation and competitive displacement between surfactant and protein occurring at the oil-water interface. In addition, it has also been found that above the CMC, there is a time-dependent increase in the negative ζ-potential of emulsion droplets in solutions of SDS, possibly due to the solublization of oil droplets into surfactant micelles in the aqueous bulk phase.

**Key words**: protein-surfactant interactions, electrophoretic mobility, ζ-potential, β-lactoglobulin, protein heat treatment, SDS, DATEM

## INTRODUCTION

Most food emulsions are in part stabilized electrostatically according to DLVO theory arising from adsorbed charged molecules, such as proteins, small-molecule surfactants, etc. As well as competing for space at the oil-water interface, the two kinds of surface-active species will tend to interact in the bulk phase and at the interface, possibly leading to the formation of a distinct complex (1,2). Interactions between proteins or polymers and low-molecular-weight surfactants have been extensively studied over the past several decades (3-6). The major thermodynamic driving force for interaction is the hydrophobic effect, i.e., the association of the alkyl chain of the surfactant molecules with the hydrophobic regions of dissolved polymer molecules. At the same time, the head group of ionic surfactants may also be involved in attractive interactions with oppositely charged groups along the polymer chain (1).

The electrophoretic mobility of a protein-coated particle depends on the distribution of electrical charge in the macromolecular layer at the surface of shear (7). The technique may be used to probe competitive adsorption between proteins and between protein and surfactant by monitoring the change in emulsion droplet mobility. According to Dickinson et al. (8), the mobility of α-lactalbumin emulsion droplets is reduced by the addition of β-casein towards that for β-casein droplets. On the other hand, no such effect was observed for β-casein addition to β-lactoglobulin emulsion droplets. This behavior is consistent with other experiments carried out on the same systems, implying either the displacement of previously adsorbed protein (i.e., α-lactalbumin by β-casein) or the absence of protein displacement (i.e., β-lactoglobulin by β-casein). It has been established that the addition of water-soluble

surfactant to a protein-stabilized emulsion leads to reduced protein surface concentration to an extent which depends on the surfactant concentration (9). Since protein-coated emulsion droplets carry a substantial charge, the interfacial protein displacement may result in a change in the surface charge density of the droplets i.e., in the ζ-potential. Recently, several workers have reported a change in calculated ζ-potential (or measured electrophoretic mobility) of the emulsion droplet as interfacial protein is displaced by the small-molecule surfactant, i.e., in systems containing β-casein+Tween 20 (10,11).

The ζ-potential also appears to be affected by the native protein-protein or protein-surfactant interfacial interactions. According to Chen and Dickinson (12), the addition of cationic gelatin to a β-lactoglobulin- stabilized emulsion causes the ζ-potential of the emulsion droplets to increase as a consequence of interaction between the two species. This increase in ζ-potential reaches a maximum at gelatin concentration of *ca.* 0.3 wt %, corresponding to the point of charge neutralization. On the other hand, the results obtained for SLES 2EO addition to a gelatin-stabilized emulsion are rather more complicated. It was found that the zeta potential decreases or increases depending on the surfactant concentration, suggesting the protein-surfactant interfacial interactions or the displacement of interfacial gelatin.

In the current study, protein-surfactant interactions will be further explored by measuring the electrophoretic mobility (or calculated ζ-potential) of protein-coated emulsion droplets and protein in solution in the presence of various surfactants-well studied anionic SDS and water-dispersible anionic DATEM (food-grade). A comparison will be made between the native and the heat-denatured protein (70°C, 30 min). In addition, for some experiments, time-dependent development of ζ-potential will be determined for protein-coated emulsion droplets in the presence of surfactant. Hopefully, the results obtained here can perhaps be related in some way to the interfacial and rheological properties of protein-stabilized emulsion or emulsion gels containing the surfactant.

## MATERIALS AND METHODS

### Materials

The bovine β-lactoglobulin (purity>99 wt %), sodium dodecyl sulphate (SDS), N-ethylmaleimide(NEM), and *n*-tetradecane (purity>99.9 wt %) were obtained from Sigma Chemicals. Commercial-grade DATEM (17% esterified tartaric acid; major fatty acid-palmitic and stearic) was donated by Danisco Ingredients (Brabrand, Denmark). Buffer solutions were prepared with analytical grade reagents and double-distilled water.

### Methods

#### Emulsion preparation

The native protein was dissolved in bis-tris buffer (20 mM, pH 7) at room temperature and this was placed in a 100 ml flask in a water bath at 70°C for 30 min, then cooled immediately to room temperature to produce the heat-treated sample. The emulsion (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris, pH 7) was prepared with both the native and heat-treated protein using the jet homogenizer (13)

#### The zetasizer

Laser Doppler electrophoresis was carried out using the Zetasizer 4 (Malvern Instrument, Malvern) fitted with capillary cell ZET 5104. The capillary cell in the instrument was cleaned periodically and reassembled in the correct position. Before taking measurements, the laser beams were carefully aligned so that they crossed exactly at one of the two stationary layers. This enabled the electro-osmosis flow to be cancelled out. For this alignment, a AZ55 electrophoresis standard latex suspension (which comprises a carbozylated polystyrene latex) was used to make the laser beams visible. The standard latex suspension was prepared by diluting the AZ55 dispersion with 0.020 M phosphate buffer at pH 7. The mean value of the zeta potential of these latex particles should be *ca.* $-55\pm2$ mV at 25°C under conditions of proper alignment.

#### Determination of electrophoretic mobility

Electrophoretic mobility measurements were carried out on emulsions or protein solutions (0.4 wt % β-lactoglobulin, 20 mM bis-tris, pH 7) at room temperature using a Malvern Zetasizer 4, fitted with the ZET5104 sample cell. The mobility of samples was measured at a certain count rate (*ca.* 1500 kcps) which was achieved by diluting the emulsion samples (no dilution for the protein solution) extensively with buffer solution containing the required amounts of surfactant (dilution extent≈1 : 6000). The diluted emulsion or the protein

solution (*ca.* 10 ml) was then injected through the sample cell in each experiment, and the quoted result, expressed as zeta potential $\zeta$ is the average of three independent measurements. The measured $\zeta$-potential of diluted emulsion samples containing surfactant was found to change with time. Unless, otherwise stated, the measurement was carried out 10 min after sample preparation, i.e., after dilution of emulsion.

## RESULTS AND DISCUSSION

### β-Lactoglobulin+SDS systems

The interactions between protein and the anionic surfactant SDS have been studied by monitoring the change in calculated zeta potential upon addition of surfactant. The binding of the anionic surfactant to β-lactoglobulin at pH 7 would be expected to lead to an increase in the net charge on the protein-surfactant complex. This is illustrated in Fig. 1 which shows a plot of the zeta potential of the protein (surfactant complex as a function of surfactant concentration for native or heat-denatured β-lactoglobulin (70°C, 30 min) solutions (0.4 wt % protein, 20 mM bis-tris buffer, pH 7). Pure β-lactoglobulin molecules (in the absence of added surfactant) have a zeta potential of $\zeta \approx -26.5$ mV ( $\zeta = -26.7 \pm 0.5$ mV for native β-lactoglobulin, $\zeta = -26.1 \pm 0.5$ mV for heat-treated β-lactoglobulin). For both samples, the addition of surfactant produces an increase in the effective net charge on protein suggesting the formation of β-lactoglobulin/SDS complex. Qualitatively similar results were found in systems containing β-lactoglobulin+SLES 2EO (12). These observations also in part support the results of the interfacial shear viscosity experiments that, due to the slow breaking down of protein (surfactant complex under shear, shear-thinning behaviour was observed with the interfacial film containing β-lactoglobulin+SDS, and measurements of rheology of protein and protein-stabilized emulsion gels obtained with the same system that the gel strength was found to depend on interactions between protein and surfactant (14). What is noteworthy here is that, with increasing surfactant concentration, the heat-denatured protein exhibits a lower increase in magnitude of the negative zeta potential compared to that of the native protein; the difference was enlarged at higher surfactant concentrations, possibly due to more pronounced surfactant binding to native β-lactoglobulin. Less surfactant binding of heat-denatured protein is consistent with the results of Oakes (15). The explanation may lie in the formation of protein aggregates during heat treatment, which leads to a reduction in the hydrophobicity of protein (16,17). This means that heat-denatured protein has less binding sites (hydrophobic sites) available for SDS interaction, and therefore a lower zeta potential.

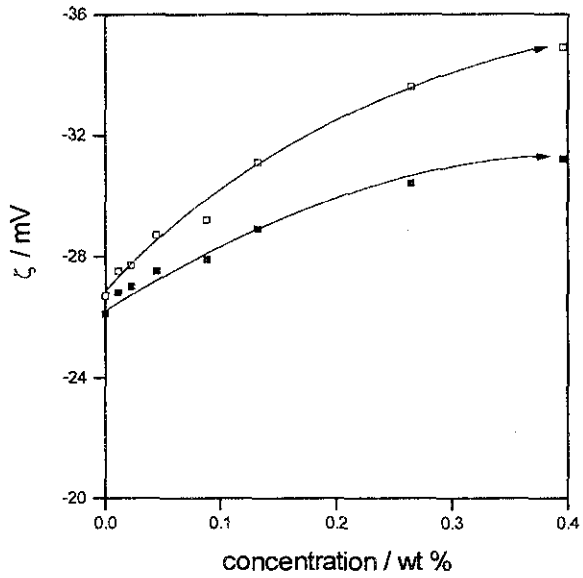


**Fig. 1. Influence of SDS on the zeta potential of β-lactoglobulin solutions (0.4 wt % protein, 20 mM bis-tris buffer, pH 7).**
Zeta potential $\zeta$ is plotted against surfactant concentration for the two protein solutions: □, native protein; ■, heat-denatured protein (70°C, 30 min).

Likewise, with the protein-stabilized emulsion, the net negative charge at the protein-coated droplet surface might be expected to increase in magnitude as a consequence of surfactant binding. This is illustrated in Fig. 2 by the plot of the zeta potential of the emulsion droplets ($d_{32} \approx 0.55$ μm) against surfactant concentration (β-lactoglobulin emulsion: 1.6 wt % protein, 38 wt % oil, 20 mM bis-tris buffer, pH 7). The electrophoretic mobility was measured 10 minutes after sample preparation. Oil droplets coated solely with adsorbed β-lactoglobulin have a zeta potential of $\zeta = -58.4 \pm 1.0$ mV. A relatively small addition of SDS induces a sharp increase in zeta potential, which reaches a maximum (negative) value of $\zeta \approx -70$ mV at a surfactant concentration of *ca.* 0.06 wt % ($2.08 \times 10^{-3}$ M), slightly below the CMC of SDS i.e., $2.9 \times 10^{-3}$ M in buffer (18). Further surfactant addition leads to a gradual reduction in $\zeta$-potential. Then, at even

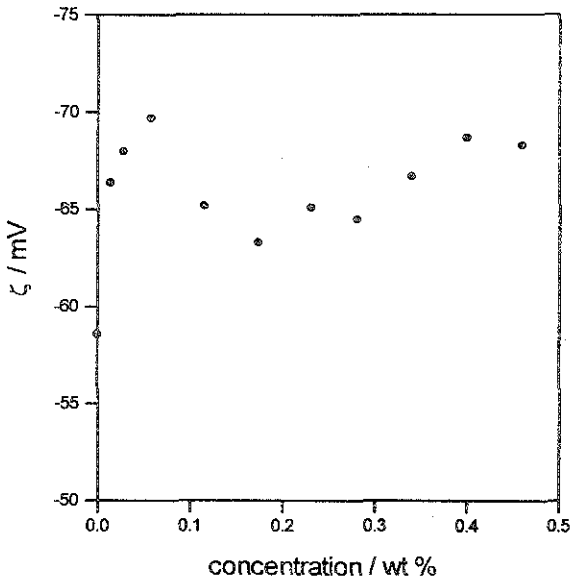


Fig. 2. Zeta potential $\zeta$ of protein-stabilized emulsion droplets (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7) in solutions of different SDS concentration.

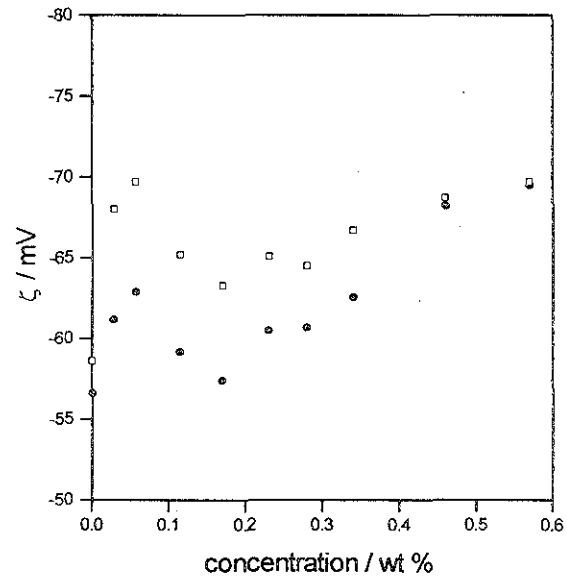


Fig. 3. Effect of heat denaturation on zeta potential $\zeta$ of heat-denatured protein-stabilized emulsion droplets (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7). Zeta potential $\zeta$ is plotted against surfactant concentration: □, native protein; ●, heat-denatured protein (70°C, 30 min).

higher surfactant concentrations, the calculated zeta potential appears to increase slightly again.

At low surfactant concentrations (i.e., up to 0.06 wt %), the observed increase in zeta potential is undoubtedly the result of surfactant binding. An increase in surfactant concentration, however, leads to the displacement of interfacial protein or protein-surfactant complex from the interface, as reflected in the gradual reduction in zeta potential. At the end of the decrease, it could be assumed that the oil droplets are solely coated with surfactant layer. At this point onwards, further surfactant adsorption onto the oil droplet could be possible, forming a surfactant micellar structure at the oil droplet surface (19-21). This may explain the slight increase in zeta potential observed at higher surfactant concentrations. It is also noteworthy that SDS-coated oil droplets exhibit a substantial zeta potential value (higher than that of pure β-lactoglobulin-coated oil droplets) at the surfactant concentration of 0.17 wt % ( $\zeta \approx -63$ mV), at which value presumably all the interfacial protein had been displaced. According to Hunter (1981), near the bulk CMC, densely packed and vertically oriented ionic surfactant layers (called hemi-micelles) are formed on a hydrophobic surface, eventually leading to the formation of surfactant micelles with further increase in surfactant concentration. Therefore, it could be presumed that at a surfactant concentration of 0.17 wt % (well beyond the CMC), surfactant micelles or hemi-micelles are formed at the oil droplet surface, leading to a high zeta potential.

Fig. 3 shows the zeta potential $\zeta$ of heat-treated protein-stabilized emulsion droplets ($d_{32} \approx 0.47$ μm) as a function of surfactant concentration, together with results for the native protein for comparison. The emulsion (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7) was prepared with heat-denatured protein (70°C, 30 min). Oil droplets coated solely with heat-denatured β-lactoglobulin have a zeta potential of $\zeta = -56.4 \pm 1.0$ mV, which is slightly lower in (negative) magnitude than those of native β-lactoglobulin-coated droplets. The general trend of changes in zeta potential with added surfactant is qualitatively similar to that of the native protein-stabilized emulsion droplets, but the absolute values are found to be lower at all surfactant concentrations, with the difference becoming less with increasing surfactant concentration. For instance, at a surfactant concentration of 0.06 wt %, where a maximum zeta potential is observed as a result of surfactant binding, the emulsion droplets have a zeta potential of $\zeta \approx -63$ mV (compared with $\zeta \approx -70$ mV for the native protein-stabilized emulsion droplets at the same surfactant concentration).

At low surfactant concentration (i.e., up to 0.06 wt %), the lower zeta potential is consistent with the view that less surfactant binds to heat-denatured protein, as indicated by the data in Fig. 1. Also, the smaller droplet size (i.e., $d_{32} \approx 0.47$ μm as compared with $d_{32} \approx 0.55$ μm for the native protein-stabilized emulsion) probably leads to a lower protein surface concentration, thereby possibly resulting in a lower zeta potential. Heat-denatured protein-stabilized emulsions have a higher specific surface area compared to the native protein-stabilized emulsions (arising from the smaller droplet size). Therefore, a higher concentration of surfactant is required to achieve the same level of surface coverage (i.e., zeta potential) as in the native protein-stabilized emulsion. This results in the observed lower zeta potential of heat-treated emulsion droplets at surfactant concentrations greater than *ca.* 0.2 wt %. Despite the initial difference, however, the zeta potential for the two systems reaches a similar level at higher surfactant concentrations (e.g., ~0.5 wt %), due to saturation of the surfactant molecules at the oil droplet surface.

Ageing the β-lactoglobulin emulsion also produces less surfactant binding, as indicated by the decrease in zeta potential with storage time (Fig. 4). Two emulsion samples were aged for certain period of time; one of the emulsions contains 20 mM NEM. At a certain storage time, an aliquot of the emulsion sample was diluted with buffer (for the determination of zeta potential) containing 0.06 wt % SDS where a maximum zeta potential is observed (Fig. 2). It has been reported previously for aged emulsions (22-24) that adsorbed β-lactoglobulin is partially polymerized via the formation of disulphide bonds. As observed earlier (Fig. 1 and Fig. 3), polymerized protein molecule has less binding capacity to surfactant. This explanation is confirmed in experiments involving addition of thiol blocking agent NEM to the fresh emulsion to stop the polymerization process. Within experimental uncertainty, no changes in zeta potential for storage time is observed for the NEM-containing emulsion sample, implying that surfactant binding without polymerization is hardly affected by ageing. Therefore, the observed decrease in the zeta potential of pure protein-stabilized emulsion droplets in Fig. 4 can be reasonably attributed to the formation of polymerized β-lactoglobulin molecules at the oil-water interface.

Note that the emulsion samples prepared for the electrophoretic mobility measurements were extremely

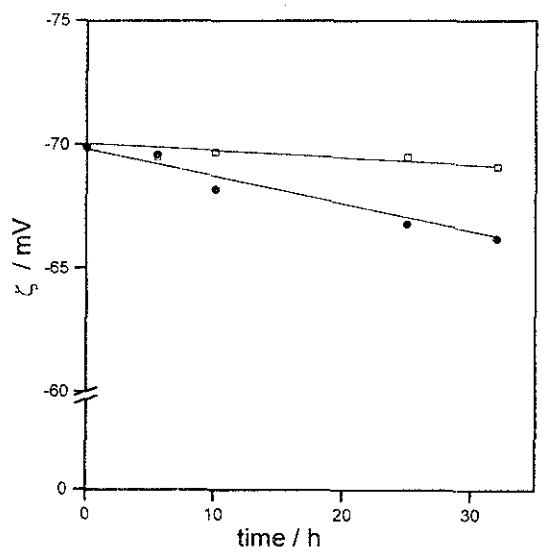


**Fig. 4. Effect of storage of protein-stabilized emulsion on surfactant binding (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7).**
Two emulsion samples were aged for 30 hours with: □, 20 mM NEM; ●, control (no NEM). Zeta potential ζ is plotted against storage time for the emulsion droplets (made from the aged emulsion) in solution containing 0.06 wt % SDS.

dilute. Hence, in the mobility experiments, the actual values of surfactant/protein molar ratio $R$ in Fig. 2 are in the hundreds or higher. This means that there were adequate surfactant molecules available in the bulk aqueous phase. In order to investigate the effect of these free surfactant molecules or micelles on the zeta potential, the diluted emulsion samples containing various concentrations of added SDS were left for 30 hours at room temperature before measurements were carried out. Fig. 5 shows the results of storage on the measured zeta potential of the emulsion droplets. The calculated zeta potential is plotted against surfactant concentration, together with results taken from Fig. 2 for comparison. All values for the stored emulsion samples are higher than those of the 10-minutes-old sample; a more pronounced difference is observed at higher surfactant concentrations. At low surfactant concentrations (i.e., up to 0.06 wt %), there is a rapid increase in zeta potential, followed by a gradual increase with increasing surfactant concentration. With further addition of surfactant, i.e., above 0.3 wt %, a plateau emerges, which is much higher than found with the 10-minutes-old sample at the corresponding surfactant concentration.

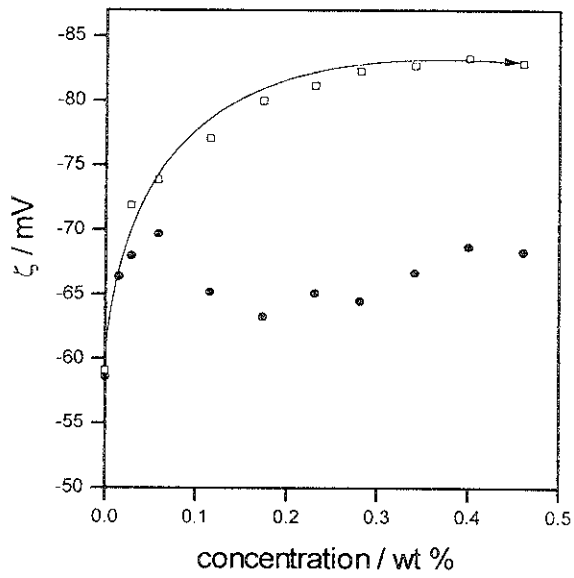


**Fig. 5. Effect of sample storage on the measured zeta potential of protein-stabilized emulsion droplets (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7) dispersed in surfactant solution.**
Zeta potential ζ is plotted against SDS concentration for the diluted emulsion samples stored for a certain period of time: □, 30 h; ●, 10 min.

This time-dependent increase in zeta potential is unexpected. The results could be better understood by considering the behaviour of a simple system containing oil droplets+anionic surfactant. Such an assumption could be justifiable since the protein content in the diluted emulsion samples is extremely low relative to the amount of added surfactant, and from the fact that the time-dependent increase was found to be more substantial after all the interfacial protein had presumably been displaced. An explanation for these findings involves the slow solubilization of emulsion droplets into surfactant micelles, which in the aqueous phase can solubilize hydrophobic molecules into their hydrophobic interiors (25). This process is time-dependent, leading to changes in the concentration, size distribution, and composition of emulsion droplets (26-28). What could be envisaged in the diluted emulsion sample during storage is that, beyond the CMC, the oil droplets surrounded with a surfactant monolayer are solubilized into surfactant micelles in the aqueous bulk phase. Most probable mechanisms for this process are: (i) the surfactant micelles collide with the oil-water interface, incorporate some oil, and then move back into the aqueous phase; or (ii) thermal fluctuations at the oil-water interface induce collective desorption of oil and surfactant molecules in the form of surfactant micelles (26). As a result of the solubilization, the droplet concentration is decreased. The mean size of the droplets, however, tends to either decrease or increase, depending on the predominance of solubilization or Ostwald ripening. The decrease in the concentration of emulsion droplets probably in turn results in an increase in free surfactant molecules released from solubilized oil droplets and/or micelles saturated with oil molecules. These surfactant molecules would then be available for further adsorption as micelles onto the remaining oil droplet surface. This could cause the measured zeta potential to increase substantially. In addition, the observed plateau value at high surfactant concentrations probably indicates the saturation adsorption of surfactant molecules at the oil droplet surface. On the other hand, in the absence of surfactant micelles, the time-dependent increase in zeta potential could be due to further adsorption of surfactant to the oil droplet surface and/or further complexation between protein and surfactant at the oil-water interface. This would explain the increase in zeta potential observed in low surfactant concentrations, i.e., up to 0.06 wt %.

The time-dependent increase in zeta potential appears to be more pronounced at the beginning of the storage period. Fig. 6 shows the time-dependent zeta potential of emulsion droplets as a function of storage time for systems containing various concentration of added surfactant. The rate-limiting step in the solubilization process is most likely to be the transport of oil molecules across the oil-water interface and into the micelles, rather than the number of collisions between micelles and the dispersed oil phase (26,27). This means that, at the beginning of storage, pure free surfactant micelles are abundant in the aqueous bulk phase, leading to a high rate of solubilization; but with time, micelles saturated with solubilized oil would predominate, and this would then result in a slow down of the solubilization process. Hence, based on the earlier discussion, the increased rate of solubilization can be expected to lead to the rapid increase in zeta potential observed at the beginning of storage.

β-Lactoglobulin+DATEM systems

The commercial food emulsifier DATEM is widely used in the bakery industry because of its binding ability to gluten. In the previous study (29), the interfacial

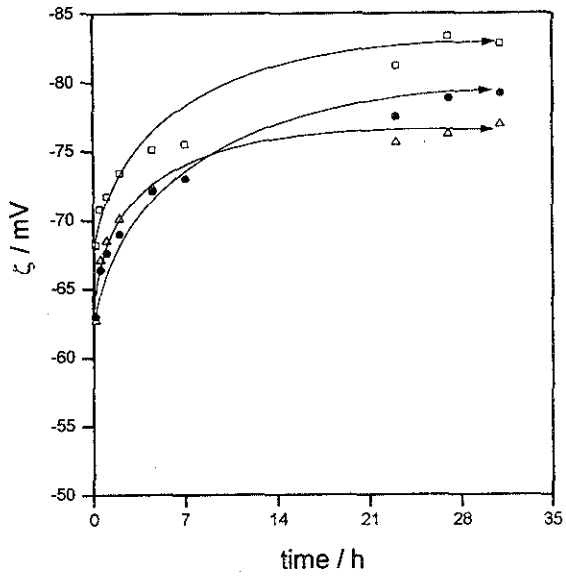


**Fig. 6. Time-dependent zeta potential ζ of protein-stabilized emulsion droplets (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7) in solutions of different SDS concentration: △, 0.11 wt %; ●, 0.17 wt %; □, 0.46 wt %.**

The diluted emulsion samples containing surfactant were stored at room temperature, and small amounts of sample were withdrawn for measurement during storage.

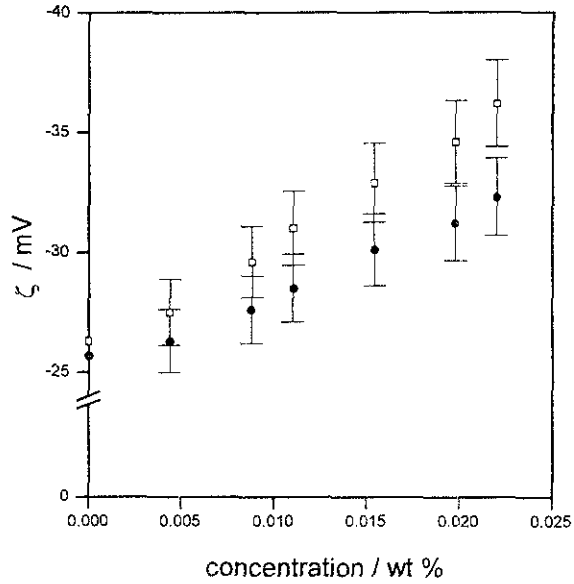


**Fig. 7. Influence of DATEM on the zeta potential of β-lactoglobulin solutions (0.4 wt % protein, 20 mM bis-tris buffer, pH 7).**

Zeta potential ζ is plotted against surfactant concentration for the two protein solutions: □, native protein; ●, heat-denatured protein (70°C, 30 min). Error range was calculated from three independent sets of experiments.

interaction of DATEM with protein has been discussed. In this work, this is further investigated by measuring its effect on the zeta potential.

First, we consider the effect of DATEM on the zeta potential ζ of a native or heat-denatured β-lactoglobulin solution (0.4 wt % protein, 20 mM bis-tris buffer, pH 7) measured at room temperature as shown in Fig. 7. The DATEM solution (1 wt %) was dissolved by heating at 50°C prior to mixing with the protein solution, and the mixture was further heated to ensure complete solubilization. Nevertheless, the measured values were found to have relatively large error range (±*ca.* 5%) compared to the protein+SDS system (±*ca.* 2%), possibly arising from the re-crystallization of surfactant (m.p. of DATEM≈45°C) after sample preparation. Hence, experiments with DATEM were carried out at very low surfactant concentration range to reduce such a possible artefact. As shown in Fig. 7, the addition of DATEM is simply to increase the magnitude (negative) of the zeta potential of protein solution, indicating the formation of a protein/surfactant complex, but less surfactant binding was observed with heat-denatured protein, similar to what was found with the protein+SDS system (Fig. 1). Similarly, the addition of DATEM to the diluted emulsion samples also produces an increase in the

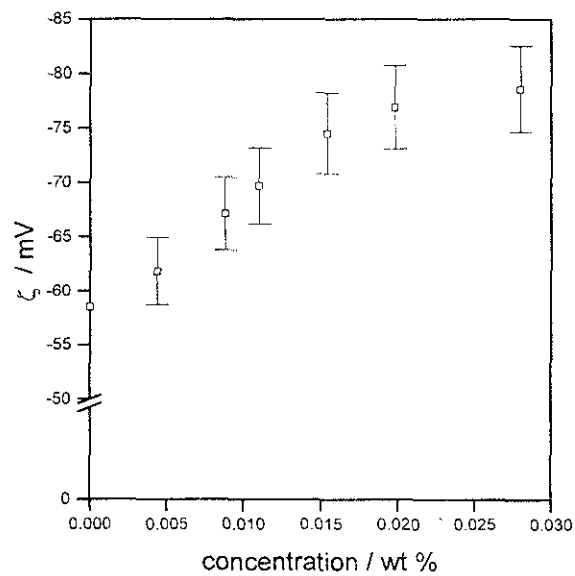


**Fig. 8. Zeta potential ζ of protein-stabilized emulsion droplets (1.6 wt % β-lactoglobulin, 38 wt % *n*-tetradecane, 20 mM bis-tris buffer, pH 7) in solutions of different DATEM concentrations.**

calculated zeta potential (Fig. 8). At very low surfactant concentrations (i.e., up to 0.015 wt%), the zeta potential was found to increase linearly with surfactant concentration. Further surfactant addition seems to cause the rate of increase to reduce slightly.

Although DATEM is used extensively in food industry, little is known about the molecular interactions involved between protein and DATEM. Due to the free carboxylic group in the molecule, the surfactant can possibly be bound to protein by ionic interactions (30); hydrophobic interactions between the fatty acid moiety of the surfactant and apolar part of the protein is also likely. It is noteworthy, both in Figs. 7 and 8, that the extent of increase in zeta potential is greater than that for the protein+SDS system (Fig. 1 and 2) at the corresponding surfactant concentrations. This probably suggests a higher extent of interaction of the surfactant with protein. This behaviour seems to manifest itself in protein gels and protein-stabilized emulsion gels containing DATEM, leading to a pronounced reinforcing effect (31).

## CONCLUSIONS

We have shown that the $\zeta$-potential of protein-coated emulsion droplets and protein in solution in the presence of surfactant is greatly dependent on both surfactant concentration and whether the protein is heat-denatured. When ionic surfactant (SDS or DATEM) is added to the protein solution, the $\zeta$-potential of the mixture is found to increase with increasing surfactant concentration. For heat-denatured protein, it has been observed that the $\zeta$-potential tends to be lower than that of the native protein. Results on emulsions are quite different. With SDS, small amounts of surfactant addition induce a sharp increase in zeta potential arising from the specific interaction of surfactant with protein. With further surfactant addition, there is a gradual reduction in the $\zeta$-potential. At even higher surfactant concentrations, the measured zeta potential appears to increase slightly. This behaviour contrasts with the results of the corresponding systems containing the anionic emulsifier DATEM, in which the $\zeta$-potential of the system is found to increase continuously with $R$.

We speculate here that, for protein-coated emulsion droplets (in systems containing SDS at low $R$ or DATEM) and protein in solution, the increased (negative) zeta potential with increasing surfactant concentration is attributable to surfactant binding to the protein molecules. With protein-coated emulsion droplets, on the other hand, the displacement of adsorbed protein (and protein-surfactant complex) from the emulsion droplet surface by surfactant may account for the observed decrease in the zeta potential and the formation of a surfactant micellar structure at the oil droplet surface may be the reason for the slight increase in the zeta potential of systems containing SDS at high $R$. Taken as a whole, it is concluded that such behaviour is generally consistent with a combination of complexation and competitive displacement between surfactant and protein occurring both at the oil-water interface and the bulk aqueous phase.

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(Received March 4, 1998)