J. Food Sci. Nutr.
Vol. 3, No. 2, p. 133~142 (1998)

Orthokinetic Stability of β-Lactoglobulin-Stabilized Emulsions : Effects of Protein Heat Treatment and Surfactant Addition

Soon-Taek Hong

Dept. of Food Science, Howon University, Kusan 573-718, Korea

Abstract

Effects of protein heat treatment and surfactant addition on the orthokinetic stability of β-lactoglobulin-stabilized emulsions have been investigated under turbulent flow conditions. In studies on protein-stabilized emulsions, samples which had been subjected to heat treatment (i.e., the protein solution or the emulsion) have been found to be more prone to orthokinetic coalescence than the untreated ones. The emulsions stabilized with protein heated above the denaturation temperature (i.e., 70°C) showed the bigger initial average droplet size, which resulted in an increased orthokinetic coalescence rate. The storage of the protein-stabilized emulsion at high temperature prior to the shearing experiment also made the emulsion less stable in the shear field. Interestingly, the addition of DATEM has been found to produce a substantial increase in orthokinetic stability of the heat-denatured protein-stabilized emulsion system, although Tween 20 is the opposite case.

Key words: orthokinetic stability, β-lactoglobulin-stabilized emulsions, protein heat treatment, surfactant

INTRODUCTION

One of the primary concerns of food manufacturers in the emulsion industry is to be able to control or manipulate emulsion stability. Orthokinetic stability relates to stability of an emulsion in a shear field. A protein-stabilized emulsion that is very stable with respect to coalescence under quiescent conditions may become destabilized when subjected to a strong shear field (e.g., vigorous stirring). Under turbulent flow conditions, it has been reported (1,2) that the destabilization behavior of β-lactoglobulin-stabilized emulsions depends on a number of factors such as the oil volume fraction, the initial average droplet size, and the presence of low concentrations (e.g., $R \leq 1$) of small-molecule surfactant Tween 20. Increasing the volume fraction of dispersed phase results in an increased collision rate, thereby making the droplets more prone to coalescence. Similar results were reported by Husband and Adams (3) for the suspension of carboxylated polymer latice and Lips et al. (4) for a buttermilk protein-stabilized emulsion containing Tween 60. Previous results had shown (5) that a small addition of Tween 20 (i.e., $R \leq 1$) caused the adsorbed layer of protein to be mobile without significant protein displacement from the interface. This interfacial behavior of the surfactant apparently triggers the orthokinetic instability of the emulsion, suggesting the importance of the mechanical strength of the adsorbed layer of protein on the orthokinetic destabilization of the emulsion system. This observation is in line with the results of Dickinson et al. (6) who showed a positive correlation between protein film surface viscosity and quiescent (perikinetic) coalescence stability. In addition, with sodium caseinate-stabilized emulsions, Chen et al. (2) have demonstrated that the presence of calcium (i.e., ≥ 8 mM $CaCl_2$) leads to an order of magnitude increase in the effective orthokinetic coalescence rate as compared with the calcium-free emulsion.

Droplets which do not flocculate cannot coalesce. This means that the most important factors affecting coalescence stability are usually those which also affect droplet flocculation. The mechanism for the stabilization of protein-stabilized emulsion comprises a combination of steric and electrostatic effects. The optimum coalescence stability of a protein-stabilized emulsion may therefore be expected when the interfacial layer is as thick as possible, and as heavily hydrated and charged (7). Accordingly, van Dam et al. (8) found that increasing protein surface concentration led to an increase in orthokinetic stability of emulsions stabilized with either skim milk powder or caseinate, with the former emulsions being the more stable, possibly due to the for-

mation of a more viscoelastic layer at the interface. This observation is consistent with results of Goff and Jordan (9) who investigated the relative effectiveness of several surfactants in destabilizing ice-cream mix. They found that the mix containing the surfactant producing a greater reduction in the interfacial tension exhibited more pronounced destabilization under shear forces. It was suggested that fat globules coated with a lower protein concentration, caused by the presence of surfactant, were more susceptible to coalescence.

The rate of shear is an important factor in orthokinetic destabilization. It governs the distance of approach of the droplets and the number of encounters (10). Oles (11) investigated the aggregation of monodisperse polystyrene spheres in Couette flow. It was found that a high shear-rate led to an increase in the aggregation rate and to a decrease in the stable size of the aggregates.

Increasing the temperature of casein-stabilized emulsion may induce droplet flocculation. Lips et al. (12) have attributed such an effect to a loss in stabilizing power of the adsorbed layer of protein, as measured by the stability factor. They also found that the adsorbed layer thickness of caseinate on latex decreased with increase in temperature. This can result in a loss of steric stabilization, possibly leading to an overall decrease in stability of an emulsion. With buttermilk protein-stabilized emulsions, Lips et al. (4) studied the effect of temperature on orthokinetic destabilization. A transition point with a minimum stability was observed at 35°C. The authors suggested that the progressive transition from ramified coalesced aggregated structures to full coalescence with increasing temperature is primarily responsible for the observed minimum in orthokinetic stability.

Food processing (e.g., emulsion preparation) usually involves several unit operations such as mixing/separation, hydration/drying, and concentration/dilution. These steps are often carried out at relatively high temperatures. Food emulsions usually contain both proteins and small-molecule surfactants, and most food proteins formulated in the emulsions are susceptible to heat denaturation during such processing, although some exceptions (e.g., casein) are observed. Therefore, if heat treatment is applied to an emulsion during or after preparation, one can expect the properties of the emulsion to be changed, mainly due to the different properties of the heat-denatured protein molecules. Despite the likely significant effects of heat treatment on emulsion properties, relatively little attention has been directed in this area, especially in terms of the orthokinetic stability of heat-treated emulsions. The aim of this work is to study the effect of heat treatment on the shear-induced coalescence of emulsion droplets. In addition, effects of surfactants will be further investigated.

MATERIALS AND METHODS

Materials

The bovine β-lactoglobulin (purity>99 wt %), Tween 20 (purity>99 wt %) 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

Instruments

In this investigation, two different types of shearing apparatus were used: the Silverson blender and a 'home-built' concentric cylinder shearing apparatus. The disruption or aggregation of droplets in a shear field depends strongly on the type of flow. For fluids in motion, two different flow patterns may be identified (13) based on the value of the dimensionless Reynolds number (*Re*): (i) Laminar flow (ii) Turbulent flow: For a stirred vessel, the Reynolds number is given by:

$$Re = (ND^2 \rho)/\eta \quad (1)$$

where N is the rotational speed of the agitator, D is the diameter of the impeller, ρ is the density of the fluid and η is the viscosity of the fluid. With a vessel of standardized geometry, the flow will be turbulent if the Reynolds number exceeds *ca.* 10^4. The flow will be 'transitional' for Reynolds numbers between 10 and 10^4, and laminar below $Re \approx 10$ (14).

The mean size of the droplets is determined by the intensity of the turbulent flow, i.e., by the turbulent

energy dissipation rate per mass E_0. The energy dissipation rate averaged over the vessel is approximated by Groeneweg et al. (14)

$$E_{0\,(av)} = (P_0 N^3 D^5) / V \quad (2)$$

where P_0 is the dimensionless power number, which depends on Re (6.3 for $Re>10^4$ for liquid stirred in a baffled vessel and ca. 2 for $10^4<Re<10^5$ for liquid stirred in a unbaffled vessel) (15) and V is the volume of the vessel (assumed to be the same as the volume of emulsion sample). It should be noted that the approximate eqn. (2) is based on a standard geometrical configuration of a vessel which is not the same as in the present study.

The silverson blender: Most of the experiments reported here were carried out using the Silverson blender under conditions of a turbulent flow field. The blender has a blade (33 mm diameter) attached to a variable speed motor. The blade is inserted into a vortex beaker with a curved shape of baffle which contains the sample. The Reynolds number can be estimated from eqn. (1). For a rotation rate of 9,000 r.p.m., the estimated value of Re for $\rho=10^3$ kg m^{-3} and $\mu=10^{-3}$ N m^{-2} s is of the order of 10^5. The energy dissipation rate can be estimated using eqn. (2). For the sample volume of 85 mL, the estimated E_0 is of the order of 10^5 m^2 s^{-3}.

The concentric cylinder shearing apparatus: This shearing apparatus was designed and built in the Procter Department of Food Science at Leeds University, U.K. Fig. 1 shows a schematic diagram of the concentric cylinder shearing apparatus. A motor is used to rotate a stainless steel rotor (Fig. 1) in a stainless steel well which contains the emulsion sample. The well is enclosed in a plastic water jacket linked to a thermostatically controlled water bath via a pump; hence the temperature of the sample can be controlled during the shearing experiment. A tightly fitting lid is used to prevent air incorporation. The speed of the rotor can be adjusted, and is measured by a digital tachometer. Further information on this apparatus has been documented by Williams (10). The rotor used in this investigation is of a particular type (30 mm diameter) designed to enhance the rate of shear-induced coalescence (Fig. 1). In a preliminary test, this rotor was found to be more effective in creating shear-induced coalescence as compared with the conventional concentric cylindrical shaped bob. The type of flow at a given rotation speed can be determined from eqn. (1). For a rotation rate of 3,500 r.p.m., the estimated value of Re for $\rho=10^3$ kg m^{-3} and $\mu=10^{-3}$ N m^{-2} s is of the order of 10^4. The energy dissipation rate can be estimated using eqn. (2). For the sample volume of 90 ml contained in an unbaffled vessel, the estimated E_0 value is of the order of 10^3 m^2 s^{-3}.

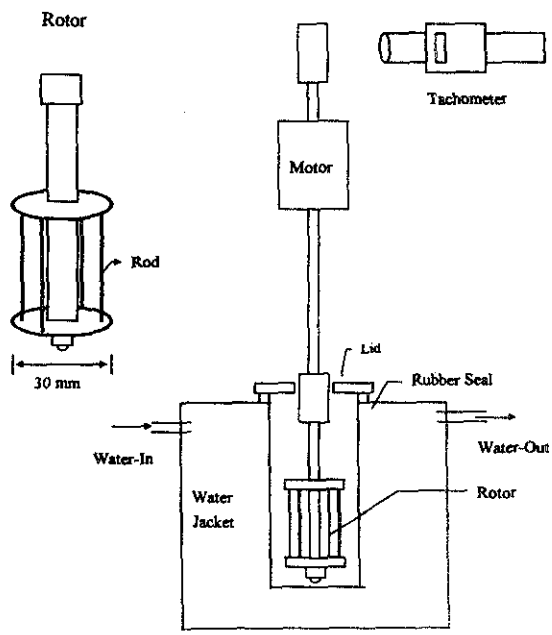

Fig. 1. A schematic diagram of the concentric cylinder shearing apparatus.

Preparation of heat-treated β-lactoglobulin

The native protein solution (0.5 wt % β-lactoglobulin in 20 mM bis-tris buffer, pH 7) prepared at room temperature (~20°C) was placed in a 100 ml flask. This was heated in a water bath at 40, 60, 70, and 80°C for 30 min, and then cooled immediately to room temperature to produce the heat-treated β-lactoglobulin sample.

Emulsion preparation

Native or heat-treated protein-stabilized emulsions (0.4 wt % β-lactoglobulin, 20 wt % *n*-tetradecane, pH 7) were produced by a high-pressure laboratory homogenizer (Shield model S-500) at an operating pressure of 206 bar. The oil and protein solution were poured into a 100 ml cylinder, the inlet tube of the homogenizer placed at the interface between the two phases with the outlet tube inserted into the aqueous phases. As homogenization proceeds the inlet tube is moved up gradually to incorporate all of the oil (this constitutes the preparation of premix). The premix thus obtained was

passed twice through the homogenizer. The droplet size distribution of the freshly made emulsion was then measured using the Malvern Mastersizer S2.01.

Assessment of orthokinetic coalescence

Orthokinetic coalescence was evaluated by monitoring the change in the average volume-surface diameter d_{32} of emulsion samples during shear. An emulsion was poured into the shearing apparatus (Silverson blender or concentric cylinder shearing apparatus), and then sheared continuously (except for sample removal) for a period of time at a constant speed of 9,000±200 r.p.m. (Silverson blender) or 3,500±30 r.p.m. (concentric cylinder shearing apparatus). Small quantities of emulsion (~0.5 ml) were withdrawn from the shearing device at certain intervals for droplet size determination. Samples sheared with the Silverson blender were: (i) the emulsion stabilized with heat-treated protein (EHP) (ii) the native protein-stabilized emulsion which had been stored at 70°C for 2 h (NPEH) and (iii) the native protein-stabilized emulsion (NPE) and the emulsion stabilized with heat-denatured (70°C, 30 min) protein both containing added surfactant (DATEM). For the experiments carried out using the concentric cylinder shearing apparatus, the emulsions stabilized with heat-denatured (70°C, 30 min) protein were sheared at room temperature or 70°C. The concentric cylinder shearing apparatus is used for the purpose of studying the effect of high temperature (i.e., 70°C) on shear-induced coalescence of emulsion droplets, as temperature can be better controlled with this equipment. This apparatus also allows for flow conditions such as the rotation rate and air incorporation etc. to be more tightly controlled. Fig. 2 summarizes experimental procedure for the orthokinetic stability studies.

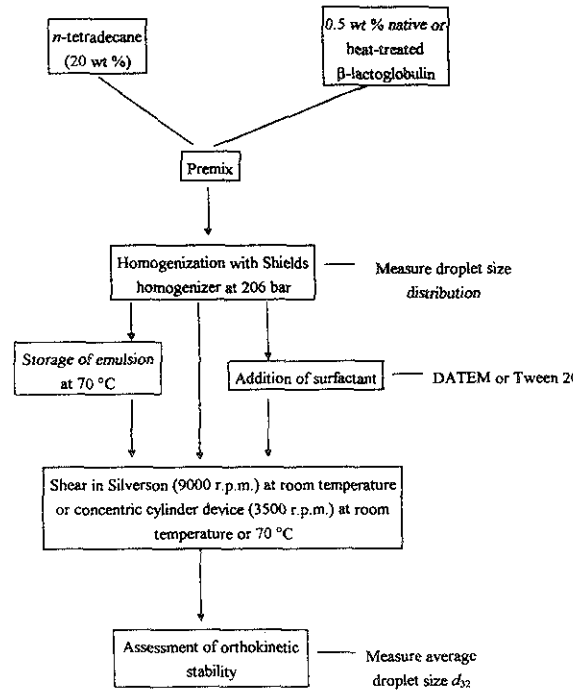

Fig. 2. Summary of experimental procedure for orthokinetic stability studies.

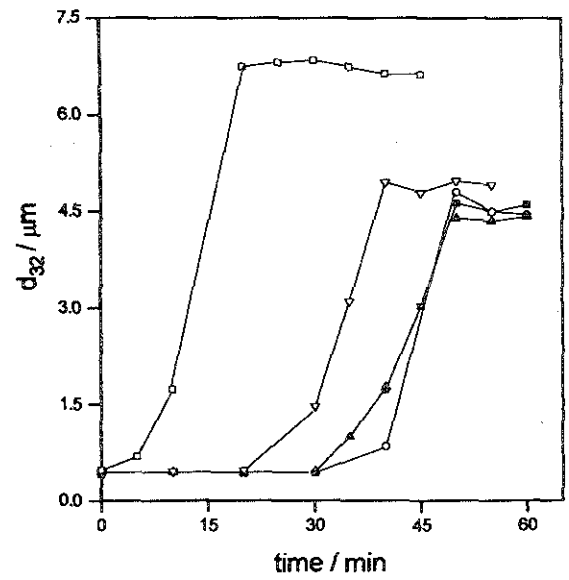

Fig. 3. Influence of heat treatment of β-lactoglobulin on shear-induced coalescence of emulsion droplets (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).
Protein solutions (0.5 wt %) were heated at different temperatures: ▲, room temperature; O, 40°C; ■, 60°C; ▽, 70°C; □, 80°C. The emulsions were sheared with a Silverson blender at room temperature.

RESULTS AND DISCUSSION

Effect of heat treatment

Fig. 3 shows the influence of heat treatment of β-lactoglobulin on the shear-induced coalescence stability of the emulsion (0.4 wt % β-lactoglobulin, 20 wt % *n*-tetradecane, pH 7). The emulsions were prepared with the native protein and with protein heated at different temperatures for 30 min (40, 60, 70 and 80°C), and then sheared at room temperature using the Silverson blender. The initial d_{32} values were found to be 0.40±0.01 μm

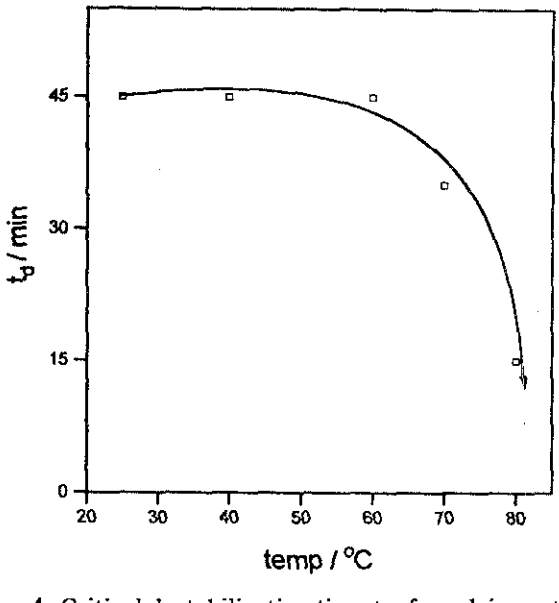

Fig. 4. Critical destabilization time t_d of emulsions stabilized with protein heated at different temperatures (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).

for the native protein and heat-treated protein (40, 60°C) stabilized emulsions, and 0.42±0.01 μm and 0.44±0.01 μm for the emulsions made with protein heated at 70 and 80°C, respectively. In Fig. 3 the volume-surface average droplet diameter (d_{32}) was plotted against shear time. From the same set of results, the dependence on heating temperature of the destabilization time t_d, corresponding to the time at which the average droplet diameter has increased to half of its maximum value (10), is presented in Fig. 4. The general observation to be made is that the average droplet diameter remains approximately constant for a period of time before increasing rapidly to a maximum, after which it levels off. Throughout the investigation, a similar pattern was seen for all the protein-stabilized emulsions destabilized by the flow field. With the native β-lactoglobulin-stabilized emulsion, there was found to be no significant change in d_{32} (±0.05 μm) when sheared for a period of *ca.* 30 min. Thereafter, however, d_{32} begins to increase significantly, followed by a rapid increase reaching a maximum value of 4.6 μm at 50 min. This pronounced divergence arises because the Smoluchowski rate constant for the orthokinetic stability of emulsion systems (2) is proportional to the cube of the diameter of the droplets. In this case, the characteristic destabilization time t_d is estimated to be 45±5 min. Within experimental uncertainty, a similar value of t_d is found for the emulsions made with protein heated at 40 and 60°C. With the emulsions containing protein which had been heated at 70°C or higher, a qualitatively similar trend in the plots of d_{32} is observed, but t_d is found to be substantially shifted to lower values, i.e., t_d=35±5 min for the emulsion made with β-lactoglobulin heated at 70°C, and t_d=15±5 min for the emulsion made with β-lactoglobulin heated at 80°C.

There could be two explanations for these findings. The first relates to the emulsifying properties of heat-denatured protein. It is reasonable to assume that, up to 60°C, the denaturation process of the protein emulsifier β-lactoglobulin is fully reversible with no irreversible aggregation. On the other hand, there is evidence (16) that above 70°C the denaturation behavior begins to change with the formation of irreversible aggregates. The formation of such aggregates is likely to lead to a loss of solubility, and therefore to a lowering of the emulsifying properties (17,18). Higher initial d_{32} of the emulsions made with protein heated at 70 and 80°C (compared to the native or mild heat-treated protein-stabilized emulsions) may be due to the lower emulsifying properties of heat-denatured protein. Based on the Smoluchowski theory of orthokinetic destabilization, therefore, an increased rate of coalescence might be expected for the larger emulsion droplets made with protein heated at 70 and 80°C. Table 1 presents the average droplet diameter d_{32} of destabilized emulsions after an arbitrary shearing time of 60 min, i.e., $t \gg t_d$. It can be seen that heat-denatured protein-stabilized emulsions have a substantially larger limiting droplet size. This is another indication that the emulsifying properties of the protein are impaired by the heat treatment.

Alternatively, changes in electrostatic repulsion could be the explanation. According to the theory of emulsion

Table 1. Average droplet diameter d_{32} of destabilized emulsions after an arbitrary shearing time of 60 min

Temperature(°C)	d_{32}(±0.20 μm)
RT[1)]	4.55
40	4.45
60	4.41
70	4.9
80	6.63

[1)]Room temperature

stability, proteins stabilize emulsions through a combination of steric and electrostatic interactions (19). Electrostatic stabilization is favored by dense monolayers of highly charged globular proteins at low ionic strength, whereas steric stabilization is favored by thick adsorbed layers of flexible proteins under good solvent conditions (20). According to the results of Hong (21), the heat-denatured protein has a lower ζ-potential than that of the native protein, possibly because of the involvement of ionic groups on the protein molecules in forming aggregates during heat treatment. Accordingly, this also leads to a decrease in the ζ-potential of emulsion droplets coated with heat-treated protein. Therefore, decreased orthokinetic stability may be expected in emulsions stabilized by heat-denatured protein, as those emulsion droplets may a show lower stability factor (i.e., a lower energy barrier) arising from less strong electrostatic repulsive forces. However, it appears that the latter effect (i.e., less electrostatic repulsion) on the observed reduced orthokinetic stability may not be significant in this investigation. This is because the difference in ζ-potential between the emulsion droplets coated with the heat-denatured and the native protein is rather small (*ca.* 2 mV).

The influence of heating of the β-lactoglobulin-stabilized emulsion on its shear-induced coalescence stability is shown in Fig. 5. The emulsion made with native protein was left at 70°C for 2 hours (NPEH) before the start of the shearing experiment ($d_{32} \approx 0.40$ μm before heat treatment, and 0.43 μm after heat treatment). The average droplet diameter d_{32} is plotted against shearing time for the heat-treated emulsion (NPEH) and for the emulsion stabilized with heat-treated (70°C, 30 min) protein (EHP) for comparison. Heat treatment, as expected, has a substantial effect on the orthokinetic stability of emulsion system. It was found that the critical destabilization time t_d for the NPEH was shortened to 25±5 min compared to that of the EHP (35±5 min). Upon heating of β-lactoglobulin-stabilized emulsions (50°C, 2 hours), it has been reported (10) that the orthokinetic stability of emulsion depends on pH: at a pH near to the pI of the protein, the heat-treated emulsion becomes much more susceptible to shear-induced coalescence, suggesting that droplet flocculation is mainly responsible for the decrease in the emulsion stability. A similar destabilization mechanism seems to operate here. Heating of the emulsion may cause the adsorbed or bulk protein to aggregate inducing droplet flocculation (22), possibly by cross-linking via disulfide bonds (justified by differences in d_{32} before and after heat treatment). This probably explains the increased rate of orthokinetic destabilization of emulsion. In addition, the much higher limiting d_{32} (where $t > t_d$) found in the heat-treated emulsion (NPEH) is probably indicative of extensive denaturation of protein, and implies that the emulsifying properties of heat-denatured protein have been reduced substantially by heat treatment.

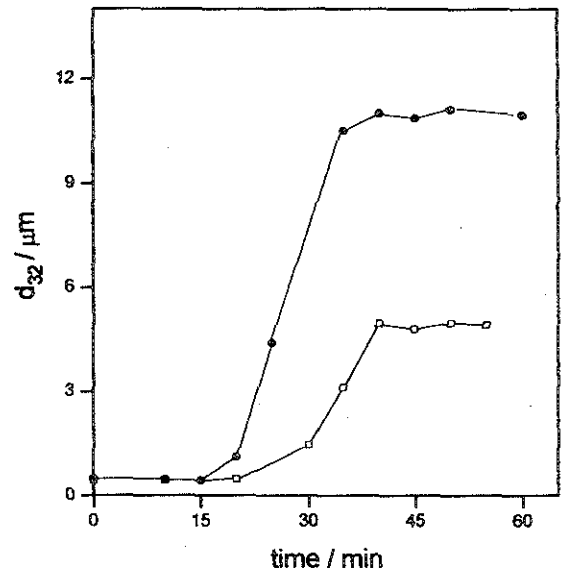

Fig. 5. Influence of heat treatment of β-lactoglobulin-stabilized emulsion on shear-induced coalescence (0.4 wt % protein, 20 wt % oil, pH 7).
The average droplet diameter d_{32} is plotted against shearing time for two different emulsions: ●, heat-treated emulsion (70°C, 2 hours); □, heat-denatured (70°C, 30 min) protein-stabilized emulsion. The emulsion samples were sheared at room temperature using a Silverson blender.

The results discussed so far are for experiments carried out at room temperature. The results discussed below were obtained using the concentric cylinder shearing device with temperature control. Fig. 6 shows the results of shear-induced coalescence experiments on emulsion droplets conducted at two temperatures (20 and 70±1°C). The average droplet diameter d_{32} is plotted against shearing time. The emulsions in Fig. 6 were prepared with heat-denatured protein (70°C, 30 min) to accelerate the destabilization process. With the emulsion sheared at room temperature, it can be seen that there

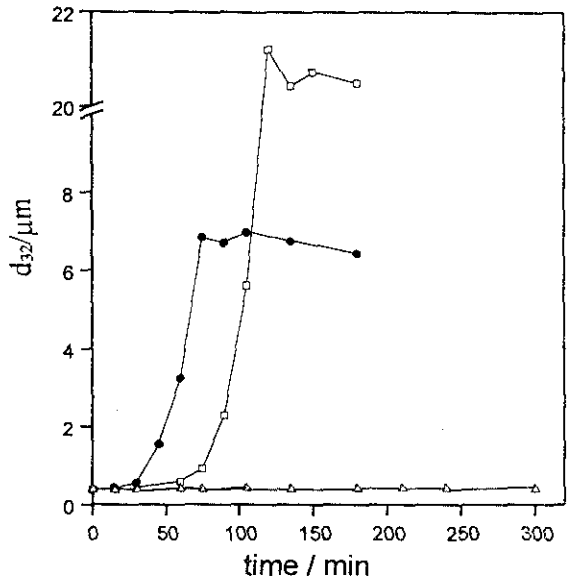

Fig. 6. Influence of shearing temperature on orthokinetic coalescence stability of an emulsion (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).
The average droplet diameter d_{32} is plotted against shearing time for two shearing temperature: □, 70°C, no surfactant; ●, 70°C, with Tween 20 (R=8); △, room temperature, no surfactant. The heat-treated protein-stabilized emulsions were sheared using the concentric cylinder shearing apparatus.

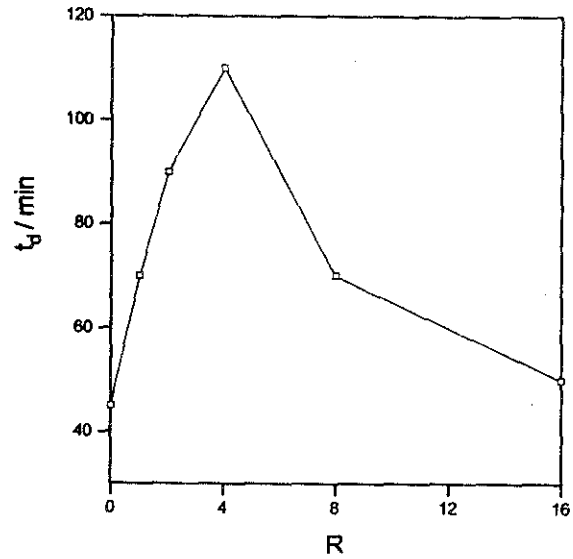

Fig. 7. Influence of DATEM on the critical destabilization time t_d of native β-lactoglobulin-stabilized emulsion (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).
DATEM dissolved at 45°C is added to the fresh emulsion at room temperature. The emulsions containing surfactant were sheared at room temperature using a Silverson blender.

is no significant change in d_{32} throughout the whole period of shearing (i.e., up to 300 min). This contrasts with results found for the same emulsion with the Silverson blender (i.e., $t_d \approx 35$ min). This difference is probably because of the lower Reynolds number and lower energy dissipation rate in this investigation. However, for emulsions sheared at 70°C, poor orthokinetic stability was observed, with the emulsion containing Tween 20 (R=8) being less stable than the one without surfactant; the characteristic destabilization time t_d in the absence of surfactant is estimated to be 110±10 min, as compared with 60±10 min in the presence of surfactant.

For the emulsion sheared at room temperature, the average droplet size remains unchanged with shearing time, reflecting a very large stability factor W produced by the adsorbed protein film (1). However, the stability factor can be decreased at high temperatures (e.g., above the denaturation temperature of adsorbed β-lactoglobulin). It is expected that, at 70°C the adsorbed protein becomes unfolded exposing reactive thiol groups (23). This will in turn lead to the formation of intermolecular disulfide bonds between the protein molecules adsorbed at the same droplet surface and between protein molecules adsorbed at different droplets. The latter process would be expected to lead to the flocculation of emulsion droplets (i.e., cross-linking by disulfide bonds between flocculated droplets) (24). During shearing at 70°C, one would expect the rate of formation of flocculated droplets to be greatly enhanced, since there would be more chances for the droplets in a shear field to come into contact. This may explain the decreased orthokinetic stability of emulsion sheared at 70°C. On the other hand, lower orthokinetic stability observed in emulsion containing surfactant can be attributed to a 'loosened' interfacial film of protein in the presence of Tween 20, which is in agreement with the results of Dickinson et al. (1).

Effect of DATEM

Fig. 7 shows the influence of the anionic surfactant DATEM added after homogenization on the shear-induced coalescence of emulsion droplets. The critical destabilization time t_d (±5 min) is plotted against surfactant/protein molar ratio R in the range 0~16. It can be seen that, while the control emulsion (no surfactant)

exhibits a t_d value of *ca.* 45 min, a small addition of surfactant leads to a substantial increase in t_d (i.e., a substantial increase in the orthokinetic stability) reaching a maximum value of 110 ± 5 min at $R\approx4$. With further addition of surfactant, however, the emulsion becomes more susceptible to shear, i.e., there is a reduced orthokinetic stability.

In order to attempt to explain these findings, it is necessary to refer to the effect of DATEM on the electrophoretic mobility of emulsion droplets (21). It was observed that the net negative charge of emulsion droplets increased with increasing surfactant concentrations due to complexation with interfacial protein. Bee et al. (25) have also shown that DATEM-coated emulsion droplets are negatively charged, due to the carboxyl groups in the molecule. Therefore, one would expect considerably enhanced electrostatic repulsive forces between the DATEM-coated emulsion droplets. This would result in an increase in the orthokinetic stability of emulsion systems, possibly by providing an additional energy barrier to be overcome before droplet coalescence can take place. On the other hand, it appears that the presence of a relatively large amount of surfactant acts as a promoter of the destabilization process.

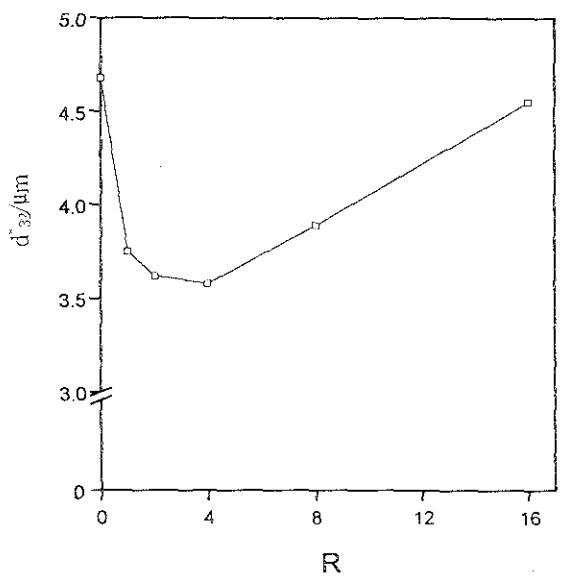

Fig. 8. Influence of surfactant/protein molar ratio R on the limiting average droplet diameter d^*_{32} of a native β-lactoglobulin-stabilized emulsion (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).
The emulsion samples containing surfactant were sheared at room temperature using a Silverson blender.

This is probably caused by the re-solidification of the surfactant during shearing (m.p. of DATEM≈45°C). These re-solidified crystals of surfactant would tend to pierce the thin film between pairs of droplets in a shear field triggering a so-called 'partial coalescence' (22). At higher surfactant concentrations, the emulsion samples are found to be more susceptible to a shear field, suggesting increased rate of the partial coalescence. This presumably simply arises from the increased quantity of re-solidified surfactant crystals.

Fig. 8 shows the limiting average droplet diameter d^*_{32} (±0.2 μm) of destabilized emulsions presented in Fig. 7 after an arbitrary shearing time of 130 min ($t\gg t_d$). One can see that small amount of surfactant addition (i.e., $R\approx1$) produces a large reduction in d^*_{32} (i.e., from 4.6 μm at $R\approx0$ to 3.75 μm at $R\approx1$), followed by a further gradual decrease with further addition of surfactant up to $R\approx4$. At higher surfactant concentrations (i.e., $R\geq8$), however, there is a relatively rapid increase in d^*_{32}. These results are consistent with the stability characteristics of emulsion discussed in Fig. 7. From the critical droplet radius (a_{crit}) dependency on the interfacial tension (26), the reduction in d^*_{32} with R (i.e., up to $R\approx4$) can be attributed to a lowering of interfacial tension in the presence of increasing concentration of DATEM (27). However, at higher surfactant concentrations, this is not true because the re-solidified surfactant is presumably not so effective in lowering of interfacial tension, thereby resulting in larger d^*_{32}.

It is interesting to check if DATEM is also effective in increasing orthokinetic stability of heat-denatured protein-stabilized emulsion. This is illustrated in Fig. 9 by plotting t_d against surfactant/protein molar ratio R. The emulsions were made with heat-denatured protein (70°C, 30 min). As observed earlier (Fig. 8), a small amount of surfactant addition leads to an substantial increase in orthokinetic stability of the emulsion, even though the emulsifying properties of protein in the emulsion might have been impaired by heat treatment. This improvement probably arises from the enhanced electrostatic repulsion between droplets caused by DATEM. It is noteworthy that just a low surfactant addition (i.e., just $R\approx1$) is adequate to compensate for the loss of emulsion stability arising from the heat treatment. In fact, the critical destabilization time t_d for the emulsion containing surfactant ($R\approx1$) is higher than that of the native protein-stabilization emulsion (see arrow). Such

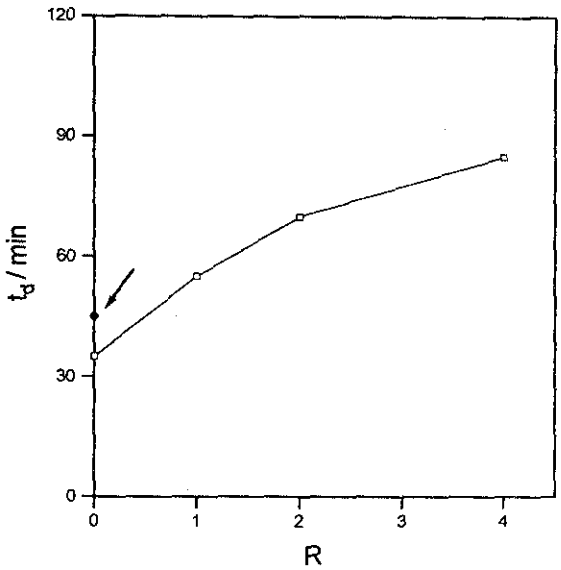

Fig. 9. Influence of DATEM on the critical destabilization time t_d of a heat-denatured β-lactoglobulin-stabilized emulsion (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7).
The emulsion samples were made with heat-denatured protein (70°C, 30 min), and sheared at room temperature using a Silverson blender. The arrow denotes the t_d value for the native protein-stabilized emulsion.

stabilizing properties of DATEM could be potentially useful in the formulation of heat-treated food emulsions.

CONCLUSIONS

In studies on protein-stabilized emulsion, samples which had been subjected to heat treatment (i.e., the protein solution or the emulsion) have been found to be more prone to orthokinetic coalescence than the untreated ones. With the protein solution heated above 70°C, the denaturation behavior begins to change with the formation of irreversible aggregates (16), probably causing a lowering of the emulsifying properties. This resulted in the bigger initial average droplet size in emulsions stabilized with the protein. Based on the Smoluchowski theory of orthokinetic destabilization, this bigger initial d_{32} value is likely to result in an increased coalescence rate. The storage of the protein-stabilized emulsion at high temperature prior to the shearing experiment also makes the emulsion less stable in the shear field. Heating of an emulsion may cause the adsorbed or bulk protein to aggregate forming bridges between droplets. This flocculation leads to increased orthokinetic destabilization of the emulsion. Interestingly, in contrast to Tween 20, the addition of DATEM has been found to produce a substantial increase in orthokinetic stability of the heat-denatured protein-stabilized emulsion system. Such stabilizing properties of DATEM could be potentially useful when food emulsions are prepared with heat-sensitive protein at high temperatures.

REFERENCES

1. Dickinson, E., Owusu, R. K. and Williams, A. : Orthokinetic destabilization of a protein-stabilized emulsion by a water-soluble surfactant. *J. Chem. Soc. Faraday Trans.*, **89**, 865 (1993)
2. Chen, J., Dickinson, E. and Iveson, G. : Interfacial interactions, competitive adsorption and emulsion stability. *Food Structure*, **12**, 135 (1993)
3. Husband, J. C. and Adams, J. M. : Shear-induced aggregation of carboxylated polymer lattices. *Colloid Polym. Sci.*, **270**, 1194 (1992)
4. Lips, A., Westbury, T., Hart, P. M., Evans, I. D. and Campbell, I. J. : On the physics of shear-induced aggregation in concentrated food emulsions. In "*Food colloids and polymers: stability and mechanical properties*" Dickinson, E. and Walstra, P. (eds.), Royal Society of Chemistry, Cambridge, p. 31 (1993)
5. Courthaudon, J.-L., Dickinson, E., Matsumura, Y. and Clark, D. C. : Competitive adsorption of β-lactoglobulin + Tween 20 at the oilwater interface. *Colloids Surf.*, **56**, 293 (1991)
6. Dickinson, E., Murray, B. S. and Stainsby, G. : Coalescence stability of emulsion-sized droplets at a planar oil-water interface and the relationship to protein film surface rheology. *J. Chem. Soc. Faraday Trans.*, **84**, 871 (1988)
7. Graham, D. E. and Phillips, M. C. : The conformation of proteins at interfaces and their role in stabilizing emulsions. In "*Theory and practice of emulsion technology*" Smith, A. L. (ed.), Academic Press, London, p. 75 (1976)
8. van Dam, B., Watts, K., Campbell, I. J. and Lips, A. : On the stability of milk protein-stabilized concentrated oil-in-water food emulsions. In "*Food macromolecules and colloids*" Dickinson, E. and Lorient, D. (eds.), Royal Society of Chemistry, Cambridge, p. 215 (1995)
9. Goff, H. D. and Jordan, W. K. : Action of emulsifiers in promoting fat destabilization during the manufacture of ice cream. *J. Dairy Sci.*, **72**, 18 (1989)
10. Williams, A. : Orthokinetic stability and inferfacial interactions in simple and multiple emulsions. PhD Thesis, University of Leeds (1994)
11. Oles, V. : Shear-induced aggregation and breakup of polystyrene latex particles. *J. Colloid Interface Sci.*, **154**, 351 (1992)
12. Lips, A., Campbell, I. J. and Pelan, E. G. : Aggregation mechanisms in food colloids and the role of biopolymers. In "*Food polymers, gels and colloids*" Dickinson, E. (ed.),

Royal Society of Chemistry, Cambridge, p. 1 (1991)
13. Holland, F. A.: *Fluid flow for chemical engineers.* Edward Arnold, London, p. 1 (1973)
14. Groeneweg, F., van Dieren, F. and Agterof, W. G. M.: Droplet break-up in a stirred water-in-oil in the presence of emulsifiers. *Colloids Surf. A.*, **91**, 207 (1994)
15. Holland, F. A. and Chapman, F. S.: *Liquid mixing and processing in stirred tanks.* Reinhold Publishing Corp., New York, chap. 2 (1968)
16. de Wit, J. N. and Swinkels, G. A. M.: A differential scanning calorimetric study of the thermal denaturation of bovine β-lactoglobulin. *Biochim. Biophys. Acta*, **624**, 40 (1980)
17. Voutsinas, L. P., Cheung, E. and Nakai, S.: Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. *J. Food Sci.*, **48**, 26 (1983)
18. Nakai, S.: Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *J. Agric. Food Chem.*, **31**, 676 (1983)
19. Dickinson, E. and Stainsby, G.: *Colloids in foods.* Applied Science, London (1982)
20. Dickinson, E.: Emulsion stability. In "*Food hydrocolloids: structures, properties and functions*" Nishinari, K. and Doi, E. (eds.), Plenum Press, New York, p. 387 (1994)
21. Hong, S. T.: Influence of surfactants on properties of β-lactoglobulin emulsions and heat-treated emulsion gels. PhD Thesis, University of Leeds (1996)
22. Dickinson, E.: *An Introduction to food colloids.* Oxford University Press, Oxford (1992)
23. Kella, N. K. and Kinsella, J. E.: Structural stability of β-lactoglobulin in the presence of kosmotropic salts. *Int. J. Peptide Protein Res.*, **32**, 396 (1988)
24. McClements, D. J., Monahan, F. J. and Kinsella, J. E.: Disulfide bond formation affects the stability of whey protein isolate emulsions. *J. Food Sci.*, **58**, 1036 (1993)
25. Bee, R. D., Hoogland, J. and Ottewill, R. H.: Food surfactants at triglyceride-water and decane-water interfaces. In "*Food clloids and polymers: stability and mechanical properties*" Dickinson, E. and Walstra, P. (eds.), Royal Society of Chemistry, Cambridge, p. 341 (1993)
26. Walstra, P.: Formation of emulsions. In "*Encyclopedia of emulsion technology, basic theory*" Becker, P. (ed.), Marcel Dekker, New York, Vol. 1, p. 57 (1983)
27. Krog, N.: Function of emulsifiers in food system. *J. Am. Oil Chem. Soc.*, **54**, 124 (1977)

(Received March 4, 1998)