

Orthokinetic Flocculation of Caseinate-Stabilized Emulsions: Influence of Calcium Concentration, Shear Rate, and Protein Content

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Calcium-induced flocculation of caseinate-stabilized soybean oil-in-water emulsions in conditions of Couette flow was studied. A concentrated emulsion (20% oil, 0.5–2.0% sodium caseinate in 20 mM imidazole, pH 7) was diluted 20 times in buffer containing concentrations of CaCl₂ between 9 and 17 mM and sheared at rates between 335 and 1340 s⁻¹. The average particle size (d_{43}), measured by integrated light scattering, increased in a sigmoidal manner with shearing time. An increased shear rate resulted in an increased flocculation rate, because of the increased number of collisions between particles, but a decreased value of the maximum d_{43} , because higher shear rates increasingly disrupted the flocs. The flocculation rate was increased by increasing the calcium concentration, indicating an increased collision efficiency. The orthokinetic stability of the emulsions was increased with increased protein content, and it is postulated that the increased surface coverage and hydrodynamic thickness of the adsorbed protein layer increased steric repulsion between droplets, so that higher calcium concentrations were necessary to induce sufficient conformational change of the proteins to allow flocculation. At high caseinate concentrations, calcium may also induce precipitation of unadsorbed caseins from the serum to the oil–water interface, thereby increasing steric repulsion and hence increasing orthokinetic stability.

Keywords: *Orthokinetic flocculation; caseinate-stabilized emulsions; calcium-induced flocculation; shear*

INTRODUCTION

Oil-in-water emulsions stabilized by adsorbed proteins may be caused to aggregate because of changes in their environment, which can be chemical (e.g., pH, ionic strength, presence of specific ions) or physical (e.g., heating, shearing). Emulsions stabilized by sodium caseinate flocculate when the calcium ion activity in solution is high enough. Calcium has the ability to reduce electrostatic repulsion between emulsion droplets, because the binding of positively charged calcium ions to the negatively charged phosphoserine residues on both α_{s1} - and β -caseins decreases the charge density of the droplet surface, and unbound calcium ions decrease the thickness of the electrical double layer of the droplets. Because of their binding to the phosphoserine, the calcium ions also induce conformational changes in the adsorbed proteins, which will have the effect of decreasing steric repulsion. Finally, calcium may form bridges between adsorbed casein molecules on different droplets (Dickinson and Stainsby, 1982; Dickinson et al., 1992; Brooksbank et al., 1993; Agboola and Dalgleish, 1995; Dalgleish, 1997).

The kinetics of flocculation of emulsions differ between quiescent and shearing conditions. Generally, the rate of flocculation of emulsions is higher when they are sheared, because the frequency of particle collision is

increased. Flocculation of emulsion droplets under quiescent conditions is initiated by Brownian motion and can generally be described with Smoluchowski's theory for perikinetic flocculation (Overbeek, 1952; Darling, 1987). According to this theory the rate of reduction in particle concentration N of monodisperse spherical particles is given by

$$dN/dt = \alpha\beta N^2 \quad (1)$$

where α is the collision efficiency factor, β the collision frequency, and N the concentration of particles. The collision frequency is given by

$$\beta = 4Dd = \frac{1}{3}kT\eta^{-1} \quad (2)$$

where D is the diffusion coefficient, d the particle diameter, k Boltzmann's constant, T the temperature, and η the solvent viscosity. The perikinetic flocculation rate is dependent on the number of particles but is independent of the particle size.

When the emulsion is subjected to a shear force, collisions are induced by Brownian motion, but also by differences in the applied velocities of the emulsion droplets, and hence the frequency of particle collision is increased. Under shearing conditions the collision frequency, according to Smoluchowski's theory for orthokinetic flocculation (Overbeek, 1952; Darling, 1987) is given by

$$\beta = \frac{2}{3}\dot{\gamma}d^3 \quad (3)$$

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where $\dot{\gamma}$ is the shear rate. Thus, the rate of orthokinetic flocculation is strongly dependent on the particle size.

In addition to inducing flocculation of the droplets, shear flow also disrupts larger flocs (as long as the flocculated droplets do not coalesce), thereby limiting the maximal particle size (Mühle, 1993). The average particle size as a function of shearing time shows a sigmoidal shape (Chen et al., 1993; Lips et al., 1993; Dickinson and Williams, 1994; Schokker and Dalgleish, 1998). Initially, the rate of aggregation is small: in this lag phase droplets and small flocs aggregate. Subsequently, the rate of aggregation increases strongly because of the effect of size (eq 3), because the mechanism of aggregation of caseinate-stabilized emulsions in the presence of calcium ions is flocculation [i.e., fractal dimensionality (FD) < 3] rather than coalescence (FD = 3) (Dickinson et al., 1992; Agboola and Dalgleish, 1995), so that the effective particle size is relatively large because of solvent inclusion (Lips et al., 1993). Finally, the growth of particle size levels off because an equilibrium between orthokinetic flocculation and breakup is established. The particle size distribution progresses during the course of shearing from monomodal at low average particle size, to bimodal, to monomodal at a higher average size (Chen et al., 1993; Lips et al., 1993; Dickinson and Williams, 1994; Schokker and Dalgleish, 1998).

This paper presents a study of the calcium-induced orthokinetic flocculation of sodium caseinate-stabilized oil-in-water emulsions. The objective of this study was to determine the influence of the calcium content, shear rate, and protein concentration on the orthokinetic stability of the emulsions.

MATERIALS AND METHODS

Materials. Spray-dried sodium caseinate (NaCas) (Alanate 180) was supplied by the New Zealand Dairy Board, Wellington, New Zealand. The calcium content of the sodium caseinate was 0.1% of the dry matter. Soybean oil, imidazole, CaCl₂, and NaCl were purchased from Sigma Chemical Co. (St. Louis, MO) and were used without further purification.

Emulsion Preparation. Oil-in-water emulsions were prepared using 20% v/v soybean oil and sodium caseinate solutions in 20 mM imidazole (pH 7.0), which contained 0.5, 1.0, 1.5, or 2.0% w/w of protein in the aqueous phase. Before homogenization, the sodium caseinate solutions were filtered through a 0.22 μm membrane (Millipore Corp., Bedford, MA). Pre-emulsions were prepared using a Polytron rotor-stator mixer (Brinkman Instruments, Westbury, NY). Emulsions were prepared using a Microfluidizer 110S (Microfluidics Corp., Newton, MA), operated at an air pressure of 0.2 MPa, corresponding to 28 MPa of liquid pressure, and the mixture was passed through the interaction chamber 10 times. The 0.5, 1.0, 1.5, and 2.0% caseinate-stabilized emulsions had particle sizes (d_{43}/d_{32}) of 0.53/0.42, 0.44/0.36, 0.43/0.35, and 0.45/0.36 μm , respectively.

Shear. Before the shear experiments, the (concentrated) emulsions were diluted by a factor of 20 into a buffer of 20 mM imidazole (pH 7.0) containing various amounts of CaCl₂, between 9 and 17 mM in the aqueous phase. The diluted emulsions (volume fractions $\Phi = 0.01$) were sheared in a Couette shear flow apparatus, as described previously (Schokker and Dalgleish, 1998), at speeds between 100 and 400 rpm, corresponding with shear rates ($\dot{\gamma}$) of 335–1340 s⁻¹. The first sample for particle size analysis was taken immediately after the shear flow apparatus had been filled, just before the shearing started; other samples were taken after shear stress had been applied for a desired period of time. These samples were immediately diluted 1:1 in 20 mM imidazole containing 5 mM CaCl₂ and 50 mM NaCl (pH 7.0), to prevent further flocculation (Schokker and Dalgleish, 1998).

Particle Analysis. The particle size distribution and average sizes of the particles (d_{32} and d_{43}) were measured by integrated light scattering using a Mastersizer X (Malvern Instruments Inc., Southboro, MA). The presentation factor was 0303 (i.e., relative refractive index and absorption of 1.414 and 0.001, respectively). Samples were presented to the Mastersizer in an MSX1 Small Volume Presentation Unit containing 20 mM imidazole, 5 mM CaCl₂, and 50 mM NaCl (pH 7.0) and mixed at minimum speed and just long enough to obtain thorough mixing, to prevent the breakage of flocs by the shear in the mixing unit (Schokker and Dalgleish, 1998).

RESULTS AND DISCUSSION

Effect of Calcium Concentration. Concentrated emulsion ($\Phi = 0.2$, [NaCas] = 0.5%) was diluted 20-fold in 20 mM imidazole buffer (pH 7.0) containing 9–12 mM CaCl₂ and sheared ($\dot{\gamma} = 670 \text{ s}^{-1}$) for various times to induce flocculation. [We did not check our sheared samples microscopically for coalescence, but assumed that only flocculation had taken place, in accordance with previous studies in our laboratory (Agboola and Dalgleish, 1995; Agboola, 1996); however, Dickinson and Davies (1999) found coalescence in extensively sheared caseinate emulsions. Even if coalescence had taken place, it would not alter the conclusions, because it would almost certainly be preceded by flocculation.] With increasing calcium concentrations the rate of flocculation increased, resulting in a decreased time of onset of the exponential flocculation and an increased exponential flocculation rate (Figure 1A), as found by Dickinson and Davies (1999). At intermediate calcium concentrations (11 mM) the average particle size as a function of shearing time showed a sigmoidal curve. However, when the calcium concentration was high (12 mM), flocculation was already exponential before the first sample was drawn. At low calcium concentrations (9 and 10 mM) flocculation was slow and not all stages in the development of particle size distribution were reached within 2 h, which was the maximum experimental time, whereas at even lower calcium concentrations the shearing time was not long enough to give any observable increase in particle size.

At a given shear rate the collision frequency of emulsion droplets is constant, so the flocculation rate must be a measure of the collision efficiency. Under the experimental conditions at which the emulsion was produced ($\Phi = 0.2$; [NaCas] = 0.5%), most of the casein molecules are adsorbed on the surfaces of the oil droplets (Fang and Dalgleish, 1993), and the collision efficiency is determined by properties of the interfacial protein layer. Evidently, in the presence of 9 mM CaCl₂, electrostatic and steric repulsion between droplets, caused by the protein layer, was still too high to allow the droplets to flocculate (i.e., collision efficiency was negligibly small), whereas at higher calcium concentrations repulsive forces were reduced enough to allow flocculation, and the collision efficiency increased with increasing calcium concentration.

At neutral pH the caseinate-stabilized emulsion droplets are negatively charged, mainly due to the presence of phosphoserine residues and, to a lesser extent, carboxylic acid residues on the casein molecules (Swaisgood, 1992). Addition of calcium ions decreases these charges as result of binding of calcium ions to specific sites and by acting as a counterion to negatively charged residues (Agboola and Dalgleish, 1995). This causes the ζ -potential of the droplets to decrease, but even the addition of a considerable excess of calcium ions does

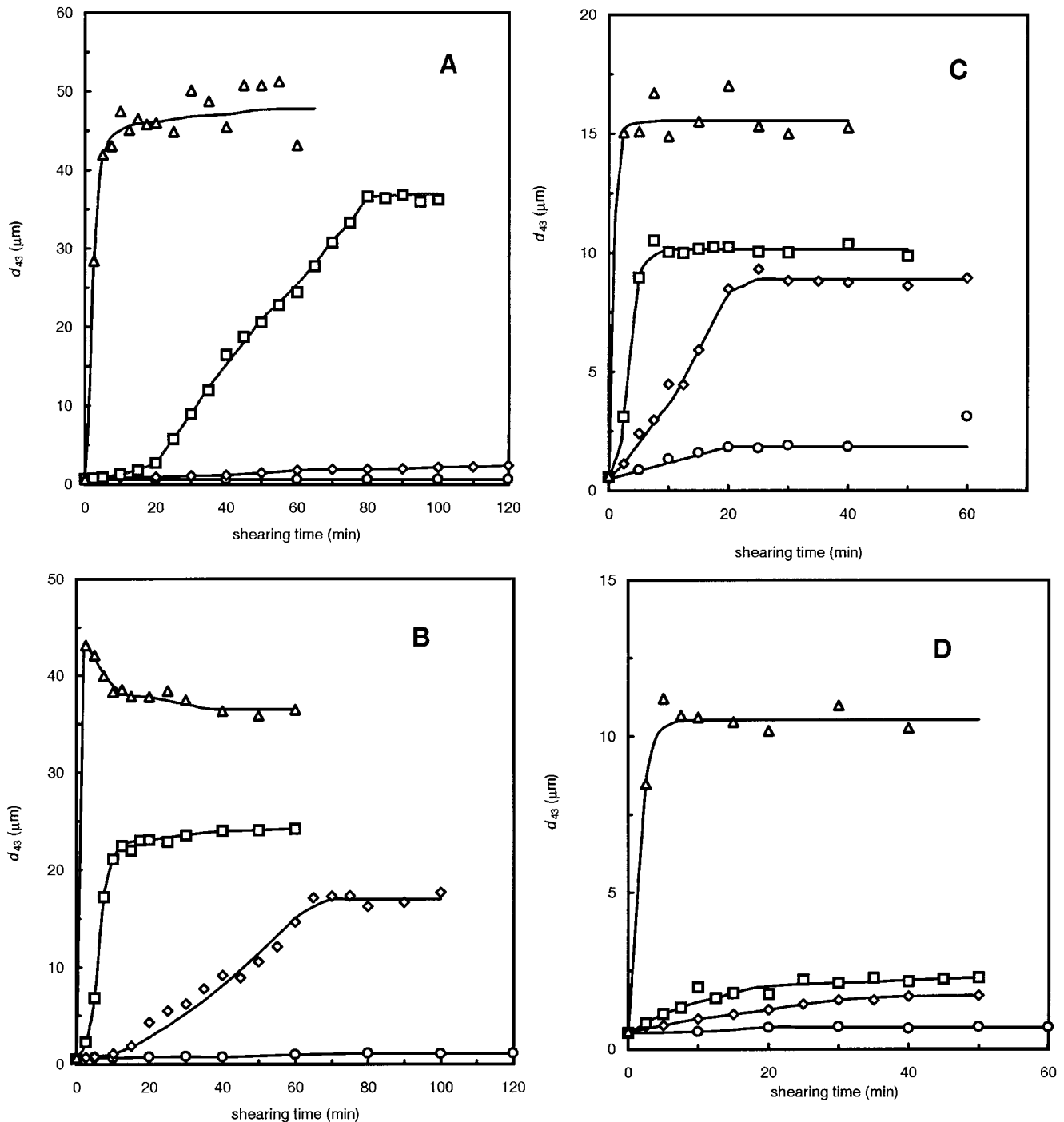


Figure 1. Effect of calcium and caseinate concentrations on orthokinetic aggregation of caseinate-stabilized emulsions ($\Phi = 0.01$; $\dot{\gamma} = 670 \text{ s}^{-1}$): (A) $[\text{NaCas}] = 0.025\%$, (O) $[\text{CaCl}_2] = 9 \text{ mM}$, (\diamond) 10 mM , (\square) 11 mM , (Δ) 12 mM ; (B) $[\text{NaCas}] = 0.05\%$, (O) $[\text{CaCl}_2] = 10 \text{ mM}$, (\diamond) 11 mM , (\square) 12 mM , (Δ) 13 mM ; (C) $[\text{NaCas}] = 0.075\%$, (O) $[\text{CaCl}_2] = 12 \text{ mM}$, (\diamond) 13 mM , (\square) 14 mM , (Δ) 15 mM ; (D) $[\text{NaCas}] = 0.10\%$, (O) $[\text{CaCl}_2] = 14 \text{ mM}$, (\diamond) 15 mM , (\square) 16 mM , (Δ) 17 mM .

not completely neutralize the charges (Dalgleish, 1984; Agboola, 1996). The critical calcium concentration necessary to induce flocculation is considerably above the concentration at which the ζ -potential is found to remain constant, which suggests that the last stage of stabilization against flocculation is through steric repulsion rather than through electrostatic repulsion. This would confirm findings by Dickinson et al. (1998), who found that the stability of caseinate-stabilized emulsions toward NaCl was not affected by ionic strength or pH. The fact that CaCl_2 could destabilize the emulsion and NaCl could not suggests that the final destabilization of caseinate-stabilized emulsions against calcium-

induced flocculation is caused by calcium ions inducing conformational changes of the adsorbed casein molecules at the interface, resulting in thinning of the protein interlayer and thereby decreasing steric repulsion. Brooksbank et al. (1993) have suggested a similar calcium-induced destabilization mechanism for polystyrene latices coated with β -casein.

The maximal average particle size of the flocs (d_{max}) was dependent on the calcium concentration: for a given shear force, increasing calcium concentrations resulted in an increased d_{max} (Figure 1A), as was reported by Dickinson and Davies (1999). The value of d_{max} is determined by the resultant of interdroplet attractive

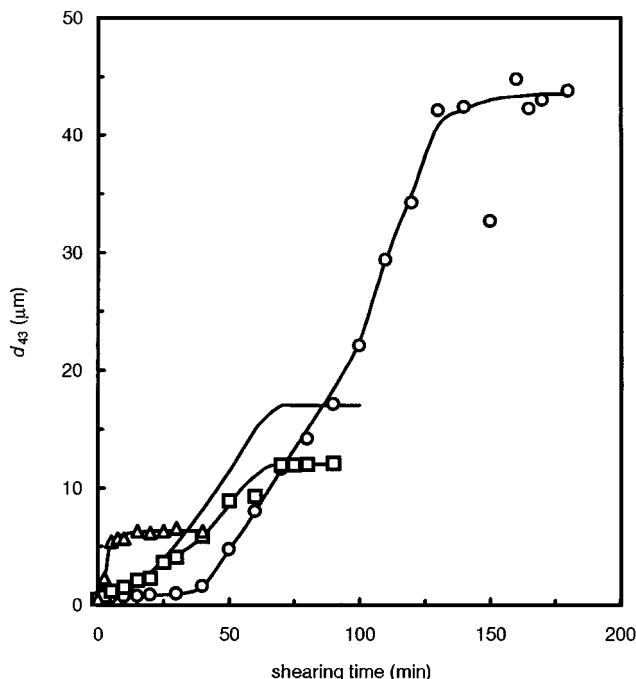


Figure 2. Effect of shear rate on orthokinetic aggregation of caseinate-stabilized emulsion ($\Phi = 0.01$, $[\text{NaCas}] = 0.05\%$; $[\text{CaCl}_2] = 11 \text{ mM}$): (○) $\dot{\gamma} = 335 \text{ s}^{-1}$; (◇) 670 s^{-1} ; (□) 1005 s^{-1} ; (△) 1340 s^{-1} .

forces, causing association of droplets and flocs, and disruptive hydrodynamic forces, which break up the larger flocs. Because the shear flow was the only disruptive force, d_{max} at constant shear flow should be a measure of the strength of the flocs and, therefore, of the adhesive forces between the emulsion droplets in the aggregate (Mühle, 1993). The increase of d_{max} with increasing calcium concentration may be explained because interdroplet repulsive forces are diminished and because of increased possibilities for the formation of calcium bridges.

Effect of Shear Rate. To investigate the effect of shear rate on the orthokinetic flocculation, concentrated caseinate-stabilized emulsion ($\Phi = 0.2$, $[\text{NaCas}] = 1.0\%$) was diluted 20 times in 20 mM imidazole buffer (pH 7.0) containing 11 mM CaCl_2 and was sheared at 335, 670, 1005, and 1340 s^{-1} for various times. Results are shown in Figure 2. The d_{43} of emulsions increased with time during the course of shearing, showing a sigmoidal shape, but at high shear rate ($\dot{\gamma} = 1340 \text{ s}^{-1}$) flocculation was already exponential before the first sample was drawn. An increased shear rate resulted in an earlier onset of exponential particle size growth and a slight increase of rate of flocculation in the exponential phase. The flocculation rate is often found to be slightly less than proportional to the shear rate: increasing the shear rate increases the number of collisions between particles (eq 3) but reduces the collision efficiency (Gregory, 1989).

As expected, increasing the shear rate resulted in a smaller maximum average particle size of the flocs. Floc breakage in laminar flow is caused by erosion of small flocs or primary droplets from the larger flocs. Larger flocs are more prone to break down due to the higher forces (moment); for smaller flocs the rate of floc breakage is negligible. Consequently, the flocs grow only to a certain limiting size, beyond which breakage to smaller units occurs. The maximum stable size of flocs was found to be inversely proportional to the shear rate

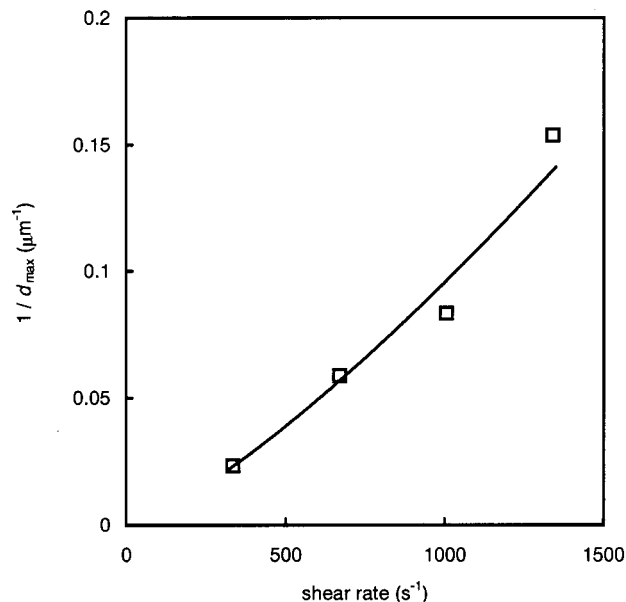


Figure 3. Effect of shear rate on maximal floc size in caseinate-stabilized emulsion ($\Phi = 0.01$, $[\text{NaCas}] = 0.05\%$; $[\text{CaCl}_2] = 11 \text{ mM}$).

(Figure 3): higher shear forces also broke up smaller aggregates (Gregory, 1989; Mühle, 1993).

Effect of Protein Concentration. To investigate the effect of the protein content of the emulsion on the orthokinetic stability, concentrated emulsions ($\Phi = 0.2$) were prepared with 0.5, 1.0, 1.5, or 2.0% sodium caseinate. These emulsions were diluted 20-fold in 20 mM imidazole buffer (pH 7.0) containing 9–17 mM CaCl_2 and were sheared ($\dot{\gamma} = 670 \text{ s}^{-1}$) for various times (Figure 1). At all caseinate contents the rate of flocculation and the maximum floc size increased with increasing calcium concentrations, as described above. The calcium concentration needed to induce orthokinetic flocculation increased with increasing caseinate concentration. For a given calcium concentration, d_{max} decreased with increasing caseinate concentration (Figure 4).

The higher concentration of calcium required to induce orthokinetic flocculation of emulsions with higher protein content cannot be explained directly by increased binding of calcium by caseins, because the total concentration of caseins present in the diluted sheared emulsion is very small (0.025–0.1%). From the calcium binding isotherms of the two major caseins in sodium caseinate, α_{s1} -casein (Dalgleish and Parker, 1980) and β -casein (Parker and Dalgleish, 1981), it can be estimated that an increase of only 0.05 mM CaCl_2 would be sufficient to maintain the level of calcium bound to the protein.

The amount of protein used to stabilize an emulsion affects several parameters of the emulsion droplets, such as average particle size, surface coverage (Γ), and the conformation of the proteins at the interface. It is not expected that, at a given oil content, orthokinetic flocculation is strongly affected by the initial average particle size because the number and diameter of the droplets cancel and also because in our case the diameters of all the emulsions are rather similar. However, the properties of the interfacial protein determine the extent of electrostatic and steric repulsion between the emulsion droplets and, hence, the orthokinetic stability of the emulsion. We speculate that the increased

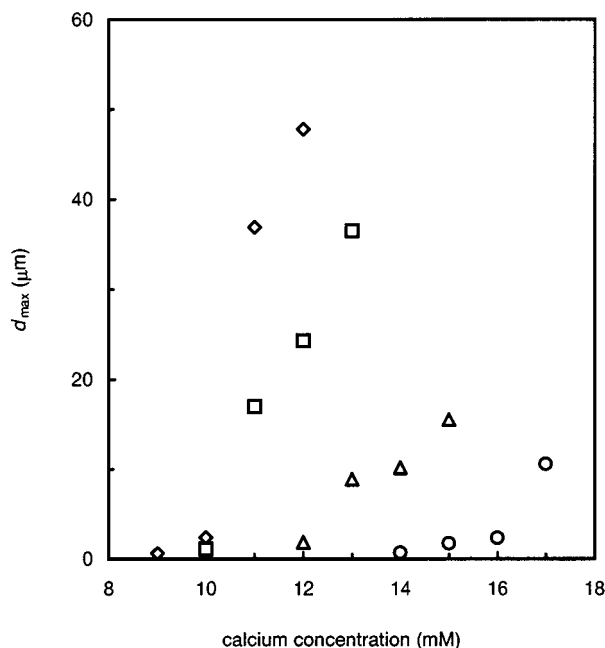


Figure 4. Effect of calcium and caseinate concentrations on maximal floc size in caseinate-stabilized emulsion ($\Phi = 0.01$; $\dot{\gamma} = 670 \text{ s}^{-1}$): (\diamond) [NaCas] = 0.025%; (\square) 0.05%; (\triangle) 0.075%; (\circ) 0.1%.

amount of calcium needed to induce flocculation has its origin in the increased electrostatic and steric repulsion of droplets in emulsions with higher casein concentrations.

At relatively low caseinate concentrations (0.3–1.5% in the concentrated emulsion) the surface coverage of the emulsion droplets increases with increasing protein concentrations. The adsorption isotherm typically shows a minimal $\Gamma = 1 \text{ mg/m}^2$, below which the emulsion is not stable, and levels off at $\Gamma = 3 \text{ mg/m}^2$. Above $\sim 1.5\%$ sodium caseinate the surface coverage remains constant (Fang and Dalgleish, 1993). Because binding of calcium cannot completely neutralize the negative charges of the casein molecules (Dalgleish, 1984), the electrostatic repulsion between emulsion droplets is greater at higher surface coverage, and more calcium ions would be necessary to shield the charges enough to allow flocculation.

The amount of protein used to stabilize an emulsion also affects the conformation of the adsorbed caseins at the oil–water interface. For the lowest adsorption the interfacial protein layer is $\sim 5 \text{ nm}$ thick, which represents a maximal spreading of the casein molecules on the surface. At maximal adsorption the adsorbed protein layer is considerably thicker, namely 10 nm , because segments of the casein molecules protrude further into the solution (Fang and Dalgleish, 1993). A thicker interfacial layer increases steric repulsion between emulsion droplets, and more calcium would be required to induce the same extent of conformational change of the adsorbed casein molecules (i.e., thinning of the protein interlayer) to decrease the steric repulsion enough to allow flocculation.

The orthokinetic stability of the emulsion stabilized with 2% caseinate (in the concentrated emulsion) was considerably higher than that of the 1.5% caseinate-stabilized emulsion. Because at caseinate concentrations $> 1.0\text{--}1.5\%$ the surface coverage and hydrodynamic thickness of the adsorbed protein layer are fairly constant (Fang and Dalgleish, 1993), this increased

stability cannot be explained by increased electrostatic and steric repulsion between the droplets. This of course assumes that the adsorbed casein is not rapidly desorbed when the emulsion is diluted; because proteins are generally difficult to desorb, we believe this to be true. Possibly, the increased orthokinetic stability is due to the amount of protein in the serum: in emulsions stabilized with low caseinate concentrations all of the casein present is adsorbed on the oil droplet interface, whereas at higher caseinate contents a considerable proportion of nonadsorbed casein is in solution (Fang and Dalgleish, 1993). We speculate that, if the calcium concentration gets high enough, casein molecules precipitate from the serum phase onto the droplet interface, as was found by Dickinson and Davies (1999), thereby increasing the electrostatic and steric repulsion between emulsion droplets. The casein molecules from the serum may aggregate by calcium bridging to casein molecules on the interface, thereby forming a multilayer, or precipitation of casein molecules may lead to a more compact packing of the proteins in the adsorbed monolayer, as was found in salt stability (NaCl) studies of casein-stabilized emulsions (Casanova and Dickinson, 1998).

The maximal average floc size, hence the floc strength, was also very dependent on the caseinate concentration, reflecting the extent of electrostatic and steric repulsion between droplets at different protein concentrations. The large d_{max} for emulsions stabilized with 0.5 and 1.0% caseinate may be due to coalescence. It may also partially be ascribed to bridging flocculation: the surface coverage in these emulsions has not yet reached its maximal value (Fang and Dalgleish, 1993), and (after flocculation) casein molecules of one droplet may adsorb to another droplet, rendering the separation of the linked droplets more difficult. Furthermore, d_{max} appeared to be inversely dependent on the amount of free casein in the emulsion, possibly because the adhesive forces between droplets, where casein molecules have precipitated from the serum phase onto their interface, are decreased.

CONCLUSIONS

In this paper we have demonstrated that the calcium-induced orthokinetic destabilization of caseinate-stabilized emulsions is influenced by an intricate interplay of the calcium and protein contents. The influence of calcium ions on the flocculation stability is much more complex than a simple ionic strength effect, which decreases the electrostatic repulsion by decreasing the thickness of the electrical double layer of the emulsion droplets. Because calcium ions bind to the phosphoserine groups of the caseins, they reduce the charge of the droplets, and consequently the electrostatic repulsion between droplets, much more effectively than NaCl (Swaisgood, 1992). Because the critical calcium concentration necessary to induce orthokinetic flocculation is considerably above the concentration at which the ζ -potential remains constant, the last stage of stabilization of caseinate-stabilized emulsions must be through steric repulsion rather than electrostatic repulsion. Presumably the final step in the destabilization is caused by calcium ions inducing conformational changes of the adsorbed casein molecules on the interface, resulting in thinning of the protein interlayer, which would decrease steric repulsion between the droplets sufficiently to allow them to flocculate. Increased protein

contents increase the orthokinetic stability because they increase the surface coverage and hydrodynamic thickness of the adsorbed protein layer, giving an increased steric repulsion, and higher calcium concentrations are necessary to induce sufficient change in conformation of the adsorbed proteins to allow flocculation. At high caseinate concentrations, at which not all casein is adsorbed at the interface, calcium may induce precipitation of unadsorbed caseins from the serum to the oil-water interface, thereby increasing steric repulsion and hence increasing orthokinetic stability.

More research will be necessary to establish the effect of calcium and protein on the orthokinetic stability of caseinate emulsions. For a better understanding, it would be valuable to have a better insight into the condition of the protein interlayer as a function of protein and calcium contents (i.e., surface coverage, hydrodynamic thickness of the protein layer, ζ -potential), and study of caseinate emulsions may have to be combined with research on well-defined systems, such as β - and α_{s1} -casein emulsions or emulsions stabilized by well-defined binary systems of pure caseins.

LITERATURE CITED

- Agboola, S. O. Studies on the stability of oil-in-water emulsions formed using milk proteins. Ph.D. Thesis, University of Guelph, 1996.
- Agboola, S. O.; Dalglish, D. G. Calcium-induced destabilization of oil-in-water emulsions stabilized by caseinate or by β -lactoglobulin. *J. Food Sci.* **1995**, *60*, 399–404.
- Brooksbank, D. V.; Davidson, C. M.; Horne, D. S.; Leaver, J. Influence of electrostatic interaction on β -casein layers adsorbed on polystyrene latices. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 3419–3425.
- Casanova, H.; Dickinson, E. Influence of protein interfacial composition on salt stability of mixed casein emulsions. *J. Agric. Food Chem.* **1998**, *46*, 72–76.
- Chen, J.; Dickinson, E.; Iveson, G. Interfacial interactions, competitive adsorption and emulsion stability. *Food Struct.* **1993**, *12*, 135–146.
- Dalglish, D. G. Measurements of electrophoretic mobilities and zeta-potentials of particles from milk using laser Doppler electrophoresis. *J. Dairy Res.* **1984**, *51*, 425–438.
- Dalglish, D. G. Adsorption of protein and the stability of emulsions. *Trends Food Sci. Technol.* **1997**, *8*, 1–6.
- Dalglish, D. G.; Parker, T. G. Binding of calcium ions to bovine α_{s1} -casein and precipitability of the protein-calcium ion complexes. *J. Dairy Res.* **1980**, *47*, 113–122.
- Darling, D. F. Kinetic aspects of food emulsion behaviour. In *Food Structure and Behaviour*; Blanshard, J. M. V., Lillford, P., Eds.; Academic Press: London, U.K., 1987; pp 107–147.
- Dickinson, E.; Davies, E. Influence of ionic calcium on stability of sodium caseinate emulsions. *Colloids Surf. B* **1999**, *12*, 203–212.
- Dickinson, E.; Stainsby, G. *Colloids in Foods*; Applied Science: London, U.K., 1982.
- Dickinson, E.; Williams, A. Orthokinetic coalescence of protein-stabilized emulsion. *Colloids Surf. A* **1994**, *88*, 317–326.
- Dickinson, E.; Hunt, J. A.; Horne, D. S. Calcium induced flocculation of emulsions containing adsorbed β -casein or phosvitin. *Food Hydrocolloids* **1992**, *6*, 359–370.
- Dickinson, E.; Semenova, M. G.; Antipova, A. S. Salt stability of casein emulsions. *Food Hydrocolloids* **1998**, *12*, 227–235.
- Fang, Y.; Dalglish, D. G. Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. *J. Colloid Interface Sci.* **1993**, *156*, 329–334.
- Gregory, J. Fundamentals of flocculation. *Crit. Rev. Environ. Control* **1989**, *19*, 185–230.
- Lips, A.; Westbury, T.; Hart, P. M.; Evans, I. D.; 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., Walstra, P., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1993; pp 31–44.
- Mühle, K. Floc stability in laminar and turbulent flow. In *Coagulation and Flocculation: Theory and Applications*; Dobiáš, B., Ed.; Dekker: New York, 1993; pp 355–390.
- Overbeek, J. Th. G. Kinetics of flocculation. In *Colloid Science Volume 1: Irreversible Systems*; Kruyt, H. R., Ed.; Elsevier: Amsterdam, The Netherlands, 1952; pp 278–301.
- Parker, T. G.; Dalglish, D. G. Binding of calcium ions to bovine β -casein. *J. Dairy Res.* **1981**, *48*, 71–76.
- Schokker, E. P.; Dalglish, D. G. The shear-induced destabilization of oil-in-water emulsions using caseinate as emulsifier. *Colloids Surf. A* **1998**, *145*, 61–69.
- Swaisgood, H. E. Chemistry of caseins. In *Advanced Dairy Chemistry, Vol. 1, Proteins*; Fox, P. F., Ed.; Elsevier Applied Science: London, U.K., 1992; pp 63–110.

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