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Bridging flocculation in binary protein stabilized emulsions

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Abstract. High volume fraction oil-in-water emulsions (55 vol% *n*-tetradecane) have been prepared under standard homogenization conditions at pH 7 with two different binary protein emulsifier systems, sodium caseinate + gelatin and whey protein + gelatin. Light microscopy has shown that the state of droplet aggregation is sensitively dependent on the quantity and composition of the proteinaceous emulsifier. The behaviour is interpreted in terms of bridging flocculation caused by partial displacement of gelatin from the droplet surface by the more surface-active milk proteins.

Introduction

The structural, rheological and textural properties of food colloids are controlled to a large extent by the state of aggregation of the constituent particles (1). One way in which colloidal particles may become aggregated is by macromolecular bridging. During the preparation of protein-stabilized oil-in-water emulsions, bridging flocculation is possible if the amount of proteinaceous emulsifier available during homogenization is too low to cover fully the newly created interface. Bridging flocculation may occur via protein particles (e.g. casein micelles) as well as by individual macromolecules. The clustering of fat globules encountered during milk homogenization is attributed to the sharing of adsorbed casein micelles between two or more fat globules (2).

Emulsion formation in mixed protein systems is affected by competition between proteins for adsorption at the oil–water interface (3). Recently, it was reported that, for high volume fraction oil-in-water emulsions made with sodium caseinate + gelatin, the texture of the product is very sensitive to the relative proportions of the two proteins in the emulsifier mixture (4). At certain emulsifier concentrations and compositions, corresponding to gelatin-rich mixtures, it was not possible to produce a stable, free-flowing liquid-like emulsion under the emulsification conditions employed (4); what emerged from the homogenizer was a thick cream-like product, presumably highly flocculated, and occasionally accompanied by free oil. The cross-over from liquid-like emulsions to aggregated cream-like emulsions was sufficiently clear-cut to allow the construction of a state stability diagram (4,5) based on subjective assessment, but supported also by quantitative measurements of the time-dependent emulsion shear modulus (4).

In the present paper we report observations made with the light microscope which confirm the sensitivity of the degree of flocculation in emulsions made with caseinate + gelatin to the emulsifier composition. We also report results for the alternative binary system whey protein + gelatin in order to see whether the behaviour is specific to caseinate + gelatin or is a more general phenomenon. Complementary measurements of interfacial tensions for the whey protein + gelatin system have also been made in order to assist in the interpretation of the

behaviour in terms of competitive adsorption between gelatin and the milk proteins at the emulsion droplet interface.

Materials and methods

Spray-dried sodium caseinate (moisture content 1.9 wt%, calcium ion content 7.7 mg/kg) was obtained from the Scottish Milk Marketing Board. Food-grade gelatin (pI 4.8), derived from the alkaline treatment of bovine bones, was supplied by P.Leiner and Sons Ltd (UK). Four different samples of whey protein were used: samples A, B and C were obtained from Milk Products (New Zealand) Ltd, and sample D was supplied by DMV Campina bv (Veghel, The Netherlands). Table I gives whey protein sample reference numbers and analytical compositions as determined by the suppliers. AnalaR grade *n*-tetradecane was obtained from Sigma Chemicals, and the AnalaR grade buffer salts from BDH Chemicals.

Concentrated oil-in-water emulsions were prepared using the 'jet' homogenizer described previously (4,5). The advantage of this type of homogenizer is its ability produce on a small scale high volume fraction emulsions having a highly reproducible droplet-size distribution, but without the need for any premixing with its associated problems of air incorporation. Emulsions were prepared at 45°C and 110 bar operating pressure, with a constant oil volume fraction (55 vol% *n*-tetradecane) and variable amounts of protein in phosphate buffer (pH 7.0). Ten minutes after formation, emulsions were assessed according to their visual appearance and pourability, and each sample was categorized as being liquid-like, cream-like or paste-like (4). Relative viscosities of emulsion samples at 20°C were determined with a Rheomat 30 viscometer (Contraves Industrial Products, Middlesex), with the samples confined between concentric cylinders at a shear-rate of 10 s^{-1}.

Evidence for bridging flocculation was sought by examining emulsions under the light microscope using the Nomarski differential interference contrast technique (6) based on the principle of two mutually perpendicular plane-polarized light waves. This technique allowed us to distinguish readily between *n*-tetradecane oil droplets (high refractive index), which appear in the photomicrographs in relief, and air bubbles (low refractive index), which appear 'flat'. Prior to microscopic analysis, emulsions were diluted 1:10 in phosphate buffer and thoroughly stirred. Some samples were stained with toluidine blue to enhance contrast. As an approximate indication of the degree of flocculation,

Table I. Composition of whey protein samples (wt%)

Component	Sample A (WP4034)	Sample B (WP4035)	Sample C (ProtoB)	Sample D (BV19)
Protein	90.2	90.7	83.8	78
Moisture	6.2	7.8	4.0	5
Fat	0	0	0.91	7
Lactose	0	0	8.2	4
Ash	1.6	2.2	4.75	2.6

the samples were given values of an aggregation number N_A on a scale from $N_A = 0$ (no aggregation) to $N_A = 7$ (extremely aggregated) (7).

Interfacial tension measurements were made at the planar *n*-tetradecane–water interface using the Wilhelmy plate method (8). Measurements were made at 25°C over a period of 24 h for pure and mixed protein solutions (usually 10^{-3} wt%) in phosphate buffer (pH 7.0, ionic strength 0.05 mol/dm^3).

Results and discussion

The subjective assessment of the caseinate + gelatin emulsions into liquid-like, cream-like or paste-like products, depending on emulsifier concentration and composition, was found to conform closely with that of the earlier work of Castle *et al.* (4). Figure 1 shows some points on the state diagram, with emphasis on the region of gelatin-rich compositions ($w_g \sim 0.8$) and total protein emulsifier concentrations in the range $1.0 \leqslant c_p/\text{wt\%} \leqslant 1.5$. In this region of the state diagram there is a peninsula of cream-like emulsion points in a 'sea' of liquid-like ones.

The extent of flocculation at six points on the caseinate + gelatin state diagram can be seen from the photomicrographs in Figure 2. Parts (a), (b) and (c) of Figure 2 refer to three emulsions of the same total protein content (1 wt%) but different composition. The gelatin-rich emulsion (a) with $w_g = 0.9$ is liquid-like and unaggregated ($N_A = 0$). At the slightly lower gelatin fraction of $w_g = 0.75$, the cream-like emulsion in (b) is seen to be highly aggregated ($N_A = 6$) and also rather more polydispersed than emulsion (a). On further

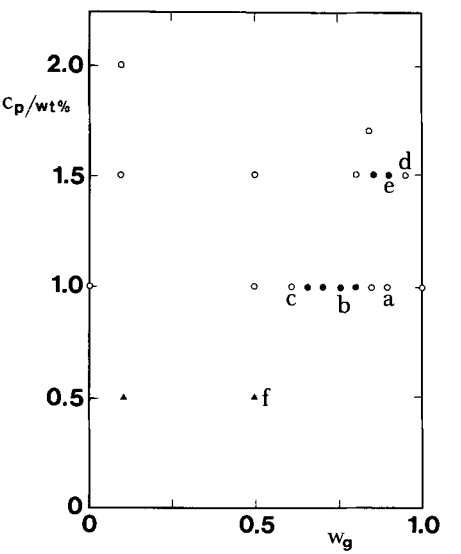

Fig. 1. State stability diagram for caseinate + gelatin emulsions (55 vol% *n*-tetradecane) made by jet homogenization. Total protein content, c_p, is plotted against gelatin weight fraction, w_g: ○, liquid-like; ●, cream-like; ▲, paste-like. Points (a–f) refer to the pictures in Figure 2.

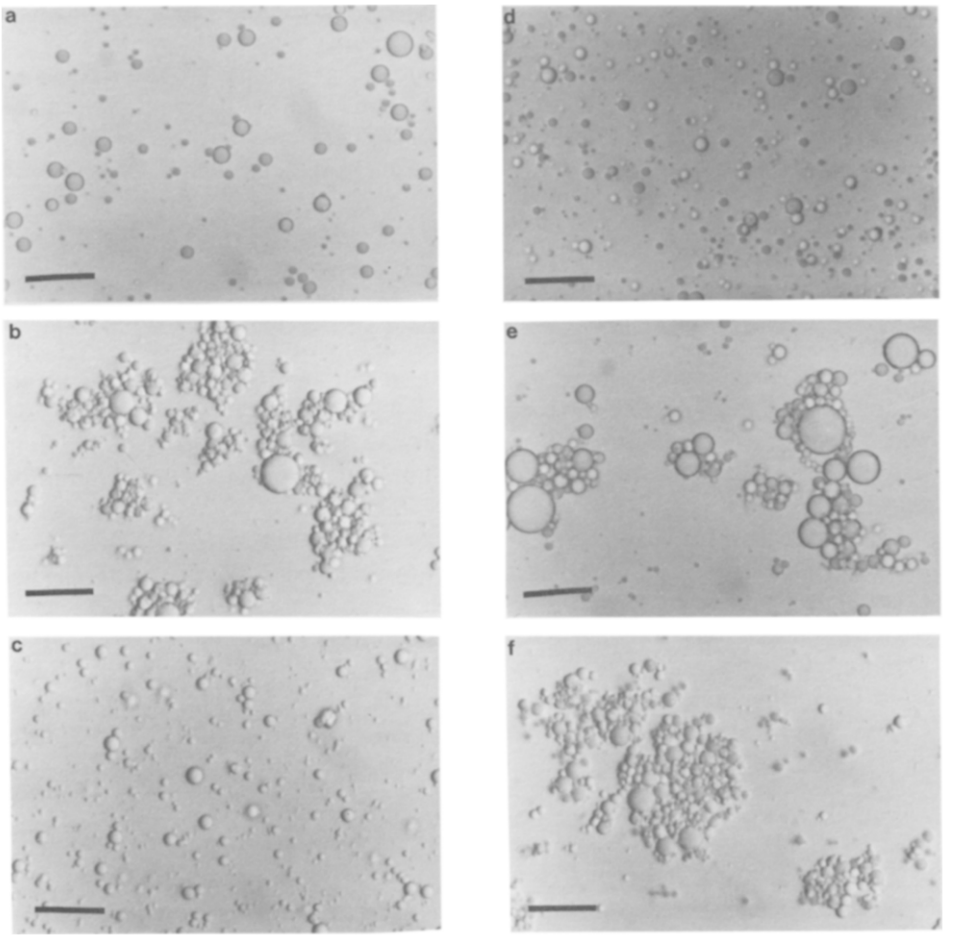

Fig. 2. Photomicrographs of caseinate + gelatin emulsions: (**a**) c_p = 1.0 wt%, w_g = 0.9; (**b**) c_p = 1.0 wt%, w_g = 0.75; (**c**) c_p = 1.0 wt%, w_g = 0.6; (**d**) c_p = 1.5 wt%, w_g = 0.95; (**e**) c_p = 1.5 wt%, w_g = 0.9; (**f**) c_p = 0.5 wt%, w_g = 0.5. Bars correspond to 20 μm.

lowering the gelatin fraction to $w_g = 0.6$, the resulting emulsion (c) is now liquid-like again, and only slightly aggregated ($N_A = 2$). A rather similar sequence of changes occurs on lowering the gelatin fraction at $c_p = 1.5$ wt%, though the range of cream-like states is distinctly narrower. Figure 2(d) refers to a liquid-like emulsion with $w_g = 0.95$ which is essentially unaggregated ($N_A = 1$). An increase of just 5% in the caseinate fraction to $w_g = 0.9$, however, produces a cream-like emulsion (e) which is coarser, more polydisperse and highly aggregated ($N_A = 6$). When the total protein content is low, the resulting emulsions are referred to as being paste-like because they do not flow on pouring. Figure 2(f) refers to such an emulsion with $c_p = 0.5$ wt% and $w_g = 0.5$; it is extremely aggregated ($N_A = 7$).

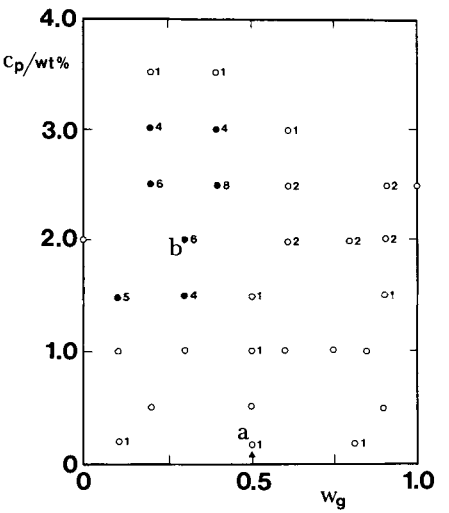

Fig. 3. State stability diagram for whey protein (sample A) + gelatin emulsions (55 vol% *n*-tetradecane) made by jet homogenization. Total protein content c_p is plotted against gelatin weight fraction w_g: ○, liquid-like; ●, cream-like; ▲, paste-like. Points (a) and (b) refer to the pictures in Figure 4. Numbers next to points denote emulsion relative viscosities.

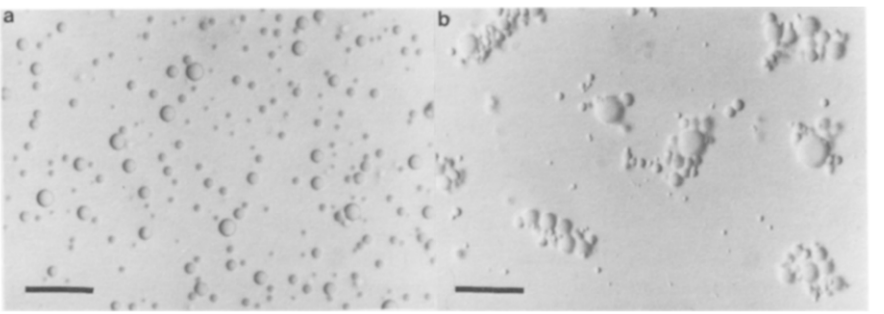

Fig. 4. Photomicrographs of whey protein (sample A) + gelatin emulsions: **(a)** c_p = 0.2 wt%, w_g = 0.5; **(b)** c_p = 2.0 wt%, w_g = 0.3. Bars correspond to 20 μm.

The state diagram for the whey protein A + gelatin system is shown in Figure 3. As with Figure 1, there is a distinct region of the diagram which is associated with cream-like emulsions, but, unlike in Figure 1, the region occurs at low gelatin fractions ($w_g < 0.5$). Other differences from the caseinate + gelatin case are (i) the cream-like region is bounded on all sides by liquid-like states; (ii) cream-like states occur at much higher total protein contents (up to c_p = 3.0 wt%); and (iii) paste-like states appear only at very low total protein contents (c_p = 0.1 wt%). Figure 4 shows two contrasting photomicrographs. Figure 4(a) refers to an emulsion of low total protein content (c_p = 0.2 wt%) made with equal amounts of whey protein (sample A) and gelatin (w_g = 0.5); the emulsion is stable, liquid-like and only very slightly aggregated (N_A = 1). Figure 4(b)

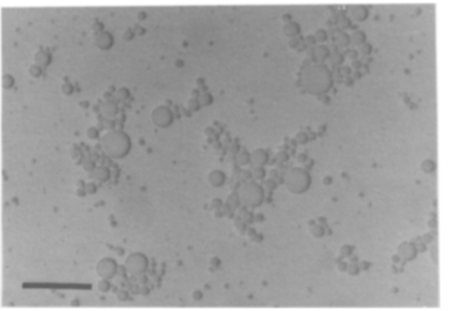

Fig. 5. Photomicrograph of caseinate + gelatin emulsion (c_p = 1.0 wt%, w_g = 0.75) after being subjected to extensive vigorous shearing (cf. Figure 2b). Bar corresponds to 20 μm.

shows a typical cream-like sample (c_p = 2.0 wt%, w_g = 0.3) with a high degree of aggregation (N_A = 6). There is a good general correlation between the extent of droplet aggregation observed under the microscope and the emulsion viscosity. The numbers beside some of the state points in Figure 3 denote relative viscosity values rounded to the nearest integer.

While the extent of flocculation was not significantly affected by mild stirring, it was found that flocs were disrupted by vigorous shear flow conditions. Figure 5 shows a photomicrograph of the caseinate + gelatin emulsion system with c_p = 1.0 wt% and w_g = 0.75 after continuous shearing in Couette flow at 400 s^{-1} for 30 min. Comparison with Figure 2(b), which shows the same sample before shearing, indicates that the floc structure is more open after shearing, although the degree of aggregation (N_A = 6) is more or less the same.

For the whey protein + gelatin emulsions, it appears that transition from flocculated to unflocculated states is sensitive to the nature of the whey protein sample. The results in Figure 3 refer to emulsions made with whey protein sample A. With none of the other samples (B, C or D) was the behaviour remotely similar. Except at very low protein concentrations (c_p = 0.1 wt%), liquid-like emulsions were always obtained with sample B irrespective of total protein content or composition. Samples C and D, on the other hand, always produced paste-like products in the presence or absence of gelatin. It is probably significant that, of the four whey protein samples examined, the most surface-active is sample A, as measured by the value of the steady-state tension of a 10^{-3} wt% solution at the *n*-tetradecane–water interface (see Table II). The reason for the paste-like emulsion produced with samples C and D is probably connected with their poor solubility compared with the other two samples; this may be associated with the high contents of ash (sample C) and fat (sample D). The reason why sample B does not produce aggregated cream-like emulsions is not clear from the analysis in Table I. Possibly it is not sufficiently surface-active to displace bridging gelatin molecules from the interface at the crucial point during emulsification (see below).

Table II compares interfacial tensions of whey protein + gelatin (10^{-3} wt% total protein) with values for the individual components under the same

Table II. Interfacial tension γ (± 0.2 mN/m) at the *n*-tetradecane–water interface (pH 7.0, ionic strength 0.05 M) after 24 h at 25°C (total bulk protein content = 10^{-3} wt% unless stated otherwise)

Protein	γ (mN/m)
Whey sample A	18.6
Whey sample B	24.4
Whey sample C	26.0
Whey sample D	26.4
Whey sample A (10^{-4} wt%)	27.1
Gelatin	34.4
Whey sample A + gelatin ($w_g = 0.5$)	23.1
Whey sample A + gelatin ($w_g = 0.9$)	27.6
No protein	48.0

conditions (pH 7, ionic strength 0.05 M, 25°C). We note that the tension for the 1:1 mixture ($w_g = 0.5$) is much lower than for gelatin alone, but still significantly above that for whey protein (sample A) alone. The tension for the 1:9 mixture (10^{-3} wt%, $w_g = 0.9$) is close to that for 10^{-4} wt% whey protein but substantially higher than that for 10^{-3} wt%. These results contrast strongly with corresponding data for caseinate + gelatin reported previously (9), where it was found that the tension for a 1:9 mixture (10^{-3} wt%, $w_g = 0.9$) becomes essentially the same as that for pure caseinate (10^{-3} wt%, $w_g = 0.0$) after 15–20 h of adsorption. We can infer from these tension experiments that casein has the capacity to predominate over gelatin at the oil–water interface to a considerably greater extent than has whey protein. This is consistent with earlier measurements (9) of competitive adsorption in mixed protein emulsion systems, where it was shown that β-lactoglobulin (the major component of whey), when added to a gelatin-stabilized emulsion after homogenization, is less effective than casein in displacing gelatin from the emulsion droplet interface.

On the basis of the evidence presented here, we infer that bridging flocculation by gelatin is a possible cause of droplet aggregation in the cream-like emulsions, and that the range of protein concentration and composition over which bridging flocculation occurs is related to the competitive adsorption between the gelatin and the milk protein during or very shortly after homogenization. The more surface-active milk protein is presumably adsorbed preferentially, but not in sufficient quantity to prevent gelatin also from being adsorbed and bridging across to other partly covered droplet surfaces during emulsification. The effect requires that the total amount of protein be limited; otherwise gelatin would be completely displaced by the excess of milk protein and then there would be no gelatin bridging between droplets. The fact that the effect occurs at higher w_g values for caseinate (Figure 1) than for whey protein sample A (Figure 3) may be because the former is able to produce the required degree of displacement at a lower protein content than the latter. The reason why the cream-like region in Figure 3 is bounded at low c_p by the liquid-like region (which extends down to $c_p = 0.1$ wt%) is not clear at this stage.

Recent measurements of the electrophoretic mobility of emulsion droplets stabilized by a mixture of proteins would seem to suggest (10) that milk proteins

displace gelatin from the primary oil–water interface into secondary layers and not directly into the bulk phase. That is, gelatin will readily coat the surface of a milk protein-stabilized emulsion droplet in the manner of a protective lyophilic colloid (1). So, in addition to direct bridging of gelatin molecules between two emulsion droplet oil–water interfaces, we may also have flocculation through indirect bridging of shared secondary layers of gelatin on different emulsion droplets. However, such aggregates formed by indirect secondary-layer bridging might be expected to be rather weakly flocculated, unlike those produced in this study, which were rather strong. Additional independent evidence for gelatin adsorbed in secondary layers comes from surface viscosity experiments at the oil–water interface in which a second protein (e.g. casein or whey protein) is introduced into the sub-phase below a previously adsorbed film of gelatin, or vice versa. The experiments performed so far (11,12) would seem to suggest that gelatin, having been initially displaced from the interface by the more surface-active milk protein, becomes subsequently incorporated into a mixed interfacial film via casein–gelatin (or whey protein–gelatin) interactions. Complementary interfacial pressure measurements suggest (12) that the primary layer is essentially pure casein, and so, by inference, the gelatin is assumed to be in a secondary layer.

A possible alternative interpretation of the flocculation behaviour observed here would be bridging of emulsion droplets, not by gelatin, but rather by caseinate (or whey protein). According to this explanation, the droplet surface coverage by caseinate at high w_g values is incomplete; even so, because of its superior ability to adsorb, caseinate displaces some gelatin from the interface, and connects gelatin-covered droplets to one another. At this stage, we certainly cannot rule out bridging by milk protein, especially as clustering of fat globules during milk homogenization is attributed to casein bridging (2), albeit by casein micelles and not the individual casein molecules or small-scale aggregates of sodium caseinate. We have looked for bridging flocculation in emulsions made with caseinate + whey protein, but have not found anything at all resembling that in caseinate + gelatin or whey protein + gelatin. Studies of other mixed food emulsifier systems are currently under consideration in an attempt to throw further light on the mechanism involved.

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