

# Protein Displacement from the Emulsion Droplet Surface by Oil-Soluble and Water-Soluble Surfactants

Eric Dickinson\* and Sumio Tanai

Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, United Kingdom

Competitive adsorption of  $\beta$ -casein with a combination of oil-soluble and water-soluble surfactants has been investigated in emulsions (20 wt % oil, 0.4 wt % protein, pH 7) made with soya oil and with *n*-tetradecane at 20 °C. The oil-soluble surfactant present during emulsification is  $C_{12}E_2$  (diethyl glycol dodecyl ether), glycerol monostearate (GMS), or glycerol monopalmitate (GMP). The water-soluble surfactant added after emulsification is  $C_{12}E_8$  (octaethylene glycol dodecyl ether) or Tween 20 (polyoxyethylene sorbitan monolaurate). The oil-soluble surfactant in the dispersed phase is found to reduce substantially the protein surface concentration in the presence of water-soluble surfactant. The monoglycerides GMS and GMP are particularly effective in reducing the amount of  $C_{12}E_8$  or Tween 20 required for complete protein displacement from the soya oil-water interface. On the other hand,  $C_{12}E_2$  does not affect the  $C_{12}E_8$  bulk concentration required for complete protein displacement from the hydrocarbon oil-water interface. Lowering the temperature from 20 to 5–10 °C, either before or after addition of water-soluble surfactant, leads to substantially increased protein displacement, especially in emulsions containing GMS.

In food emulsions such as ice cream or mayonnaise, there are two classes of adsorbing species that have a strong tendency to accumulate at the oil-water interface: proteins (usually derived from milk or eggs) and small-molecule surfactants (polar lipids and their derivatives). The composition of the stabilizing layer depends on the competitive adsorption of the proteins and surfactants (de Feijter et al., 1987; Dickinson and Woskett, 1989; Dickinson, 1991) and is important for understanding the physicochemical factors affecting the formation, stability, and rheology of food colloids (Darling and Birkett, 1987; Dickinson, 1989; Bergenstahl and Claesson, 1990).

Recent studies in our laboratory (Courthaudon et al., 1991a-c) on competitive adsorption in model emulsion systems stabilized by pure milk proteins have shown that the addition of water-soluble nonionic surfactant after emulsification leads to complete removal of protein from the droplet surface at high surfactant/protein ratios. It has also been demonstrated (Courthaudon et al., 1991c,d) that the presence of oil-soluble nonionic or zwitterionic surfactant during emulsification leads to a reduction in the protein surface coverage of the resulting emulsion. As most food emulsion products contain both water-soluble and oil-soluble surfactants, it is a logical step to attempt to quantify the competitive displacement of protein in model systems containing both types of species.

A commercially important application of competitive adsorption of milk proteins and emulsifiers is the weakening of protein-fat binding in whippable dairy emulsions such as ice-cream mix (Berger, 1976). It has been demonstrated recently (Barfod et al., 1991) that when ice-cream mix containing added glycerol monostearate (GMS) is cooled from 20 to 5 °C, there is a significant reduction in the protein load at the emulsion droplet surface. This change in surface composition on cooling is accompanied by a substantial increase in emulsion whippability (Barfod et al., 1991) and a substantial reduction in the tension at the oil-water interface (Krog, 1991).

This paper reports on the competitive displacement of the milk protein  $\beta$ -casein from the oil-water interface by

a combination of water-soluble surfactant (added after homogenization) and oil-soluble surfactant (present during homogenization). The water-soluble surfactants are research grade  $C_{12}E_8$  (octaethylene glycol *n*-dodecyl ether) and food grade Tween 20 (polyoxyethylene (20) sorbitan monolaurate). The oil-soluble ones are  $C_{12}E_2$  (diethylene glycol *n*-dodecyl ether), glycerol monostearate (GMS), and glycerol monopalmitate (GMP). To avoid problems of crystallization, emulsions are made with an oil phase that remains liquid over the temperature range (0–20 °C) investigated. As a bridge between real food colloids and previous work on model systems in our laboratory, comparisons are made between nonfood surfactants and food grade emulsifiers and between hydrocarbon oil and triglyceride oil droplets.

## MATERIALS AND METHODS

**Materials.** The surfactant  $C_{12}E_8$  (>99 wt %) was obtained from Nikko Chemicals (Tokyo);  $C_{12}E_8$  has a critical micelle concentration (cmc) of  $1.09 \times 10^{-4}$  M and an estimated hydrophile-lipophile balance (HLB) of 13.1. The Tween 20 was obtained from Sigma Chemical Co. (St. Louis, MO) with a quoted fatty acid content of 50% lauric acid and the balance primarily myristic, palmitic, and stearic acid; Tween 20 has a cmc of  $4 \times 10^{-5}$  M and an estimated HLB of 16.7. Two samples of  $C_{12}E_2$  (>99 wt %) were used, one from Nikko Chemicals and the other from Fluka Chemicals (Glossop, U.K.). High-purity samples (>99 wt %) of glycerol monostearate and glycerol monopalmitate were obtained from Fluka Chemicals and Grindsted Products (Brand, Denmark), respectively.

Two different samples of freeze-dried  $\beta$ -casein were used. One was prepared from whole milk as described previously (Dickinson et al., 1988), and the other was purchased from Sigma as a lyophilized salt-free powder. Analysis of both samples by fast protein liquid chromatography (FPLC) on a Mono-Q ion-exchange column gave a single sharp peak indicating the absence of any other casein impurities.

Two different samples of soya oil were used. The first was a high-purity triglyceride sample from Karlshamns LipidTeknik (Stockholm, Sweden); it was shown by high-pressure liquid chromatography (Courthaudon et al., 1991d) to be entirely free of monoglycerides, diglycerides, and free fatty acids and was used without further purification. The second sample was obtained from Sigma; it was made free of surface-active contaminants by

\* To whom correspondence should be addressed.

**Table I. Average Droplet Diameter  $d_{32}$  of Emulsions (0.4 wt %  $\beta$ -Casein, 20 wt % Oil, pH 7) Made with Different Oil Phases Containing Various Concentrations of Oil-Soluble Surfactants (Values Refer to Weight Percent in Whole Emulsion)**

oil phase	surfactant	concn, wt %	$d_{32}$ , <sup>a</sup> $\mu\text{m}$
<i>n</i> -tetradecane			0.98
<i>n</i> -tetradecane	$\text{C}_{12}\text{E}_2$	0.1	0.66
<i>n</i> -tetradecane	$\text{C}_{12}\text{E}_2$	0.5	0.41
soya oil <sup>b</sup>			0.92
soya oil	$\text{C}_{12}\text{E}_2$	0.1	0.80
soya oil	$\text{C}_{12}\text{E}_2$	0.5	0.72
soya oil	GMS	0.05	0.83
soya oil	GMS	0.2	0.82
soya oil	GMS	0.5	0.78
soya oil	GMP	0.2	0.81

<sup>a</sup> Estimated precision  $\pm 0.02 \mu\text{m}$ . <sup>b</sup> Emulsions made with samples of soya oil from Karlshamns LipidTeknik (used as received) and Sigma (purified as described under Materials) gave identical droplet-size distributions (and protein displacement behavior) within the experimental uncertainty of the measurements.

twice passing through a Florisil column (50 g of oil through 4 g of 100-mesh Florisil at each pass) as described by Gaonkar (1989). AnalaR grade *n*-tetradecane (>99 wt %) was purchased from Sigma. Buffer salts were of AnalaR grade from BDH Chemicals (Poole, U.K.). The water was double-distilled.

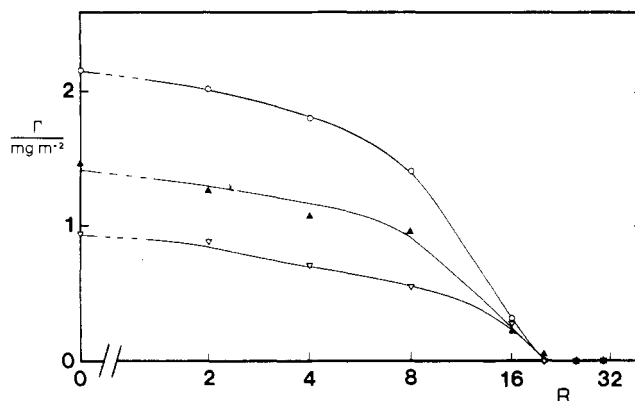
**Emulsion Preparation.** An aqueous solution of 0.5 wt %  $\beta$ -casein was prepared in 20 mM Bis-Tris propane buffer (pH 7.0). Appropriate amounts of oil-soluble surfactant ( $\text{C}_{12}\text{E}_2$ , GMS, or GMP) were dissolved in *n*-tetradecane or soya oil. The oil and aqueous phases (20:80 by weight) were mixed at 20 °C for 30 s, and the coarse pre-mix was then homogenized at 20 °C using a small-scale single-stage valve homogenizer (Dickinson et al., 1987) operating at a pressure of 250 bar. A Malvern Mastersizer S2.01 was used to determine the droplet-size distribution, from which was derived the volume-surface average diameter  $d_{32}$  and the specific surface area (area per unit mass of emulsion). Over the time scale of the displacement experiments (see below), the emulsion samples ( $d_{32} < 1 \mu\text{m}$ ) showed no change in droplet-size distribution.

**Displacement Experiments.** A freshly made emulsion (20 wt % oil, 0.4 wt %  $\beta$ -casein) was divided into several aliquots, and to each was added at 20 °C a suitable amount of water-soluble surfactant ( $\text{C}_{12}\text{E}_8$  or Tween 20) to produce a set of samples with water-soluble surfactant/protein molar ratio in the range 0–50. It was confirmed that, as noted previously (Courthaudon et al., 1991b,c), there is no change in  $d_{32}$  following addition of  $\text{C}_{12}\text{E}_8$  or Tween 20. After storage at 20 °C for 2 h, each aliquot was centrifuged at  $1.5 \times 10^4 g$  for 15 min to separate the emulsion droplets from the aqueous serum phase. The  $\beta$ -casein concentration in the serum was determined as described previously (Dickinson et al., 1988) using a Pharmacia FPLC Mono-Q ion-exchange chromatography column with a linear NaCl gradient (0–0.5 M). The protein surface coverage was inferred from the known surface area of the emulsion and the amount of  $\beta$ -casein present in the aqueous phase after centrifugation.

With some of the soya oil-in-water emulsions containing 0 or 0.2 wt % GMS, competitive displacement behavior was studied at temperatures below 20 °C. After addition of  $\text{C}_{12}\text{E}_8$  at 20 °C, the aliquot was cooled to 10, 5, or 0 °C; it was then left for 2 or 24 h prior to centrifugation at the low temperature followed by protein analysis as described above. In an alternative procedure, the  $\text{C}_{12}\text{E}_8$  was added after the samples were cooled to the low temperature; samples were cooled to 15, 10, 5, or 0 °C, then  $\text{C}_{12}\text{E}_8$  was added, and the samples were stored for 2, 3, 4, and 5 h, respectively.

## RESULTS AND DISCUSSION

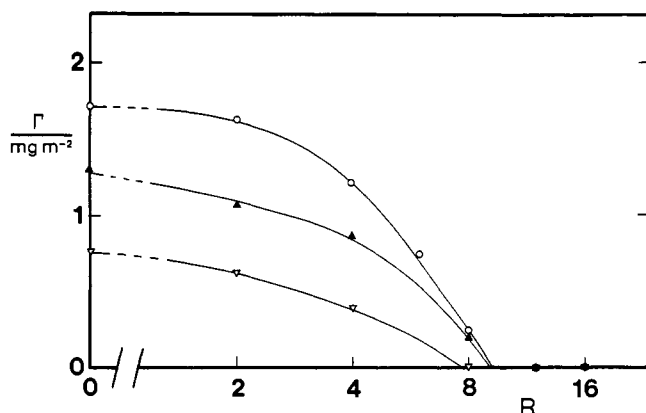
The average droplet diameter  $d_{32}$  of each freshly made emulsion is recorded in Table I. The presence of oil-soluble surfactant ( $\text{C}_{12}\text{E}_2$ , GMS, or GMP) during homogenization leads to a reduction in emulsion droplet size as noted previously (Dickinson et al., 1989a; Courthaudon et al.,



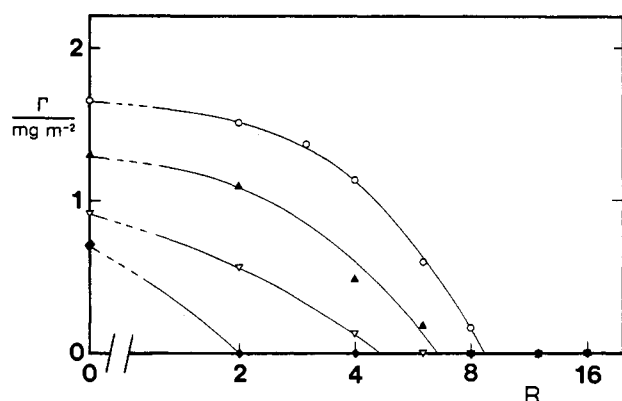
**Figure 1.** Effect of  $\text{C}_{12}\text{E}_8$  added after homogenization at 20 °C on the protein surface coverage in *n*-tetradecane-in-water emulsions (0.4 wt %  $\beta$ -casein, 20 wt % oil, pH 7) containing various amounts of  $\text{C}_{12}\text{E}_2$  dissolved in the *n*-tetradecane. The surface concentration  $\Gamma$  is plotted against the  $\text{C}_{12}\text{E}_8/\beta$ -casein molar ratio  $R$ : (O) no  $\text{C}_{12}\text{E}_2$  present; ( $\blacktriangle$ ) 0.1 wt %  $\text{C}_{12}\text{E}_2$ ; ( $\nabla$ ) 0.5 wt %  $\text{C}_{12}\text{E}_2$  (concentrations refer to weight percent  $\text{C}_{12}\text{E}_2$  in whole emulsion).

1991c). The value of  $d_{32}$  appears to be more affected by oil-soluble surfactant when the oil phase is *n*-tetradecane than when it is soya oil. Reductions in droplet size also occur when water-soluble surfactants are present during emulsification (Courthaudon et al., 1991c), but here we restrict attention to systems in which  $\text{C}_{12}\text{E}_8$  or Tween 20 is added after emulsion formation to investigate the competitive displacement of protein by water-soluble surfactant under conditions of total constant surface area. (Changes in protein surface coverage due to surfactant addition prior to homogenization arise from changes in specific surface area as well as from direct surfactant/protein competition at the interface.)

The competitive displacement behavior for the system *n*-tetradecane/ $\text{C}_{12}\text{E}_2/\text{C}_{12}\text{E}_8$  at 20 °C is shown in Figure 1. The protein surface concentration  $\Gamma$  is given as a function of the  $\text{C}_{12}\text{E}_8/\beta$ -casein molar ratio  $R$  for emulsions containing 0, 0.1, and 0.5 wt %  $\text{C}_{12}\text{E}_2$ . The data for the emulsions with no  $\text{C}_{12}\text{E}_2$  present provide confirmation of results published previously (Courthaudon et al., 1991c);  $\beta$ -casein is completely removed from the interface at  $R \approx 20$ . With  $\text{C}_{12}\text{E}_2$  dissolved in the *n*-tetradecane, the surface coverage  $\Gamma(R)$  is reduced for  $0 \leq R < 20$ , although the amount of  $\text{C}_{12}\text{E}_8$  required for complete protein displacement remains approximately the same ( $R \approx 20$ ), even in the presence of 0.5 wt %  $\text{C}_{12}\text{E}_2$  (i.e., at a  $\text{C}_{12}\text{E}_2/\beta$ -casein molar ratio exceeding 100:1). From these results, we can infer that the presence of the oil-soluble polyoxyethylene surfactant reduces the concentration of disordered protein that can be accommodated in an adsorbed layer at the oil-water interface, but it appears that the adsorption strength of those protein molecules that are adsorbed is roughly the same as that in the absence of oil-soluble surfactant since it takes the same amount of water-soluble surfactant to remove protein completely from the interface. In the language of the statistical theory of Cohen Stuart et al. (1984), we say that the critical displacer concentration is not significantly affected by the chemical nature of the adsorbent phase. Possibly what happens in molecular terms is that, as the bulk  $\text{C}_{12}\text{E}_8$  concentration becomes reasonably large (say  $R \geq 10$ ), adsorbing  $\text{C}_{12}\text{E}_8$  molecules displace  $\text{C}_{12}\text{E}_2$  molecules into the bulk oil phase as well as protein segments into the aqueous phase. In this way, as the  $\text{C}_{12}\text{E}_8$  concentration approaches the critical displacer concentration ( $R \rightarrow 20$ ), the chemical composition of the interfacial region becomes independent of the  $\text{C}_{12}\text{E}_2$  concentration because it has effectively itself been dis-



**Figure 2.** Effect of  $C_{12}E_8$  added after homogenization at 20 °C on the protein surface coverage in soya oil-in-water emulsions (0.4 wt %  $\beta$ -casein, 20 wt % oil, pH 7) containing various amounts of  $C_{12}E_2$  dissolved in the soya oil. The surface concentration  $\Gamma$  is plotted against the  $C_{12}E_8/\beta$ -casein molar ratio  $R$ : (O) no  $C_{12}E_2$  present; ( $\blacktriangle$ ) 0.1 wt %  $C_{12}E_2$ ; ( $\nabla$ ) 0.5 wt %  $C_{12}E_2$ .



**Figure 3.** Effect of  $C_{12}E_8$  added after homogenization at 20 °C on the protein surface coverage in soya oil-in-water emulsions (0.4 wt %  $\beta$ -casein, 20 wt % oil, pH 7) containing various amounts of GMS dissolved in the soya oil. The surface concentration  $\Gamma$  is plotted against the  $C_{12}E_8/\beta$ -casein molar ratio  $R$ : (O) no GMS present; ( $\blacktriangle$ ) 0.05 wt % GMS; ( $\nabla$ ) 0.2 wt % GMS; ( $\blacklozenge$ ) 0.5 wt % GMS.

placed from the surface by  $C_{12}E_8$ . So, at  $R = 16$ , the amount of protein adsorbed is essentially the same for 0.5 wt %  $C_{12}E_2$  as it is for pure  $n$ -tetradecane.

Figure 2 shows the same data as Figure 1 except with soya oil replacing  $n$ -tetradecane as the dispersed phase. As already noted previously (Courthaudon et al., 1991d), the protein surface coverages at the triglyceride-water interface are consistently lower than those at the hydrocarbon-water interface. We see evidence in Figure 2 for a weaker binding energy of  $\beta$ -casein for soya oil droplets than for  $n$ -tetradecane droplets. Complete protein displacement is achieved at  $R \geq 10$  in the absence of  $C_{12}E_2$  and at a slightly lower value ( $R \geq 8$ ) for  $C_{12}E_2$  present at 0.5 wt %. It appears that the protein is more readily displaced by polyoxyethylene surfactants from the triglyceride-water interface. The molecular explanation could be that the hydrophobic residues of the  $\beta$ -casein molecule are less strongly solvated by the more polar triglyceride "solvent" than by the hydrocarbon. Hence, it requires a lower  $C_{12}E_8$  concentration to reduce the interfacial free energy to the value necessary for complete protein displacement (Dickinson et al., 1990).

Protein displacement data for the system soya oil/GMS/ $C_{12}E_8$  are presented in Figure 3. We can see from a comparison of Figures 2 and 3 that GMS is considerably more effective in facilitating the displacement of protein

**Table II.** Effect of Tween 20 Added after Homogenization at Surfactant/Protein Molar Ratio  $R$  on Protein Surface Concentration  $\Gamma$  in Emulsions (0.4 wt %  $\beta$ -Casein, 20 wt % Soya Oil, pH 7, 20 °C) Containing Various Amounts of  $C_{12}E_2$  or GMS in the Oil Phase (Concentrations Refer to Weight Percent in Whole Emulsion)

$R^a$	$\Gamma, ^b \text{ mg m}^{-2}$				
	no $C_{12}E_2$ or GMS	0.1 wt % $C_{12}E_2$	0.5 wt % $C_{12}E_2$	0.05 wt % GMS	0.2 wt % GMS
0	2.06	1.44	0.90	1.28	0.95
2	1.88	1.09	0.66	1.15	0.80
4	1.60	0.76	0	0.83	0.26
6				0.39	0
8	1.20	0.37	0	0	0
12		0	0	0	0
16	1.06	0		0	0
30	0.44				
40	0				

<sup>a</sup> Estimated experimental error <5%. <sup>b</sup> Estimated maximum experimental error  $\pm 0.08 \text{ mg m}^{-2}$ .

from the interface by  $C_{12}E_8$  than is  $C_{12}E_2$ . At a GMS concentration of 0.5 wt % (i.e., a GMS/ $\beta$ -casein molar ratio of 90:1), the protein surface concentration is  $\Gamma = 0.7 \text{ mg m}^{-2}$  at the soya oil-water interface in the absence of water-soluble surfactant, but  $\Gamma$  is reduced to zero at a very low  $C_{12}E_8$  concentration ( $R \geq 2$ ). Analogous experiments with glycerol monopalmitate (GMP) have shown that GMP is even more effective than GMS in facilitating protein displacement. For soya oil-in-water emulsions containing 0.2 wt % GMP, the protein surface coverages are  $\Gamma = 0.57, 0.36,$  and  $0.0$  for  $R = 0, 2,$  and  $4$ , respectively, as compared with  $\Gamma = 0.92, 0.56,$  and  $0.13$  for the corresponding emulsions containing 0.2 wt % GMS in the presence of the same amounts of added  $C_{12}E_8$ .

Commercial grade glycerol monostearate (a mixture of mono- and diglycerides) is widely used as an "emulsifier" in the formulation of food colloids. In simulated cream liqueurs, it has been demonstrated (Dickinson et al., 1989b) that commercial grade GMS (or commercial grade sodium stearyl lactylate) displaces a significant proportion of the adsorbed casein from the surface of the emulsion droplets, although even at 1 wt % emulsifier there is still enough protein coverage to provide effective steric stabilization against droplet coalescence. It is clear that the present study with pure  $\beta$ -casein displaced by purified monoglycerides is qualitatively consistent with the displacement behavior which occurs in the simulated cream liqueur, which contains a mixture of milk proteins and low molecular weight surfactants, as well as various other components (alcohol, sucrose, etc.). Protein displacement in the simulated cream liqueur does not lead, however, to any loss of emulsion stability. In fact, the presence of commercial grade GMS leads to a substantial improvement in stability with respect to creaming as well as a longer shelf life on storage at elevated temperatures (Dickinson et al., 1989b). The reason for this improved stability is not fully understood, but it may involve an interaction between the protein and a liquid crystalline state of the emulsifier.

We now turn to results obtained with the food grade emulsifier Tween 20. The data are recorded in Table II. We can see that the qualitative trends of protein displacement behavior are the same for the Tween 20 as for the  $C_{12}E_8$ , but there are some quantitative differences related to the chemical nature of the water-soluble surfactant. In the absence of GMS or  $C_{12}E_2$ , the molar ratio of surfactant to protein required for complete displacement is roughly twice that for  $C_{12}E_8$ ; this difference is greatly reduced, however, when oil-soluble surfactant is present.

**Table III. Effect of Temperature on Protein Displacement by  $C_{12}E_8$  in Emulsions (0.4 wt %  $\beta$ -Casein, 20 wt % Soya Oil, pH 7) in the Absence of Oil-Soluble Surfactant<sup>a</sup>**

R	$\Gamma$ , mg m <sup>-2</sup>			
	20 °C	10 °C	5 °C	0 °C
0	1.66 (1.62)	1.20 (1.15)	1.48 (1.45)	1.68 (1.73)
2	1.51 (1.54)	0.98 (1.00)	1.25 (1.28)	1.58 (1.57)
4	1.23 (1.10)	0.40 (0.38)	0.77 (0.74)	1.23 (1.20)
8	0.18 (0.15)	0 (0)	0 (0.06)	0.19 (0.15)
12	0 (0)	0 (0)	0 (0)	0 (0)

<sup>a</sup> Protein surface concentration  $\Gamma$  is given for systems analyzed after 2 h of storage at the reduced temperature following additions of  $C_{12}E_8$  to freshly made emulsions at 20 °C. The numbers in parentheses record  $\Gamma$  values after 24 h of storage.

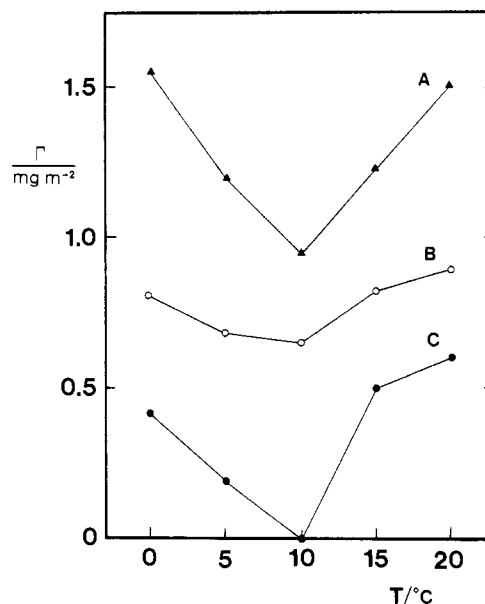
**Table IV. Effect of Temperature on Protein Displacement<sup>a</sup>**

R	$\Gamma$ , mg m <sup>-2</sup>			
	20 °C	10 °C	5 °C	0 °C
0	0.82 (0.84)	0.78 (0.74)	0.81 (0.78)	0.88 (0.82)
2	0.56 (0.50)	0 (0)	0.22 (0.18)	0.44 (0.44)
4	0.13 (0.10)	0 (0)	0 (0)	0.13 (0.12)
6	0 (0)	0 (0)	0 (0)	0 (0)

<sup>a</sup> Conditions same as in Table III except that emulsion oil phase contains 1 wt % GMS (i.e., 0.2 wt % GMS in whole emulsion).

It is noted that GMS dissolved in the soya oil is particularly effective in reducing the amount of Tween 20 required for complete removal of protein from the emulsion droplet surface. In fact, for the emulsions containing 0.2 wt % GMS, the value of  $R \approx 6$  for the critical displacer concentration at 20 °C is similar for both Tween 20 (Table II) and  $C_{12}E_8$  (Figure 3). Both Tween 20 and  $C_{12}E_8$  are water-soluble nonionic surfactants, and we therefore do not expect them to interact strongly with  $\beta$ -casein in aqueous solution, although there may be some weak cooperative binding of surfactant to protein at surfactant concentrations approaching the cmc (Dickinson and Woskett, 1989). However, it is clear from theoretical considerations (de Feijter et al., 1987) that the primary thermodynamic driving force for protein displacement is not the attractive interaction between protein and surfactant in bulk solution but rather the preference of the surfactant for adsorption sites at the oil-water interface. The situation with water-soluble anionic surfactants (e.g., sodium dodecyl sulfate), which bind strongly to proteins at low surfactant concentrations, is more complex (Dickinson et al., 1990).

All of the results reported so far were obtained in experiments carried out at ambient temperature (20 °C). We now consider the effect of a reduction in the temperature on the displacement behavior. Tables III and IV give protein surface coverages in soya oil-in-water emulsions containing 0 and 0.2 wt % GMS, respectively. For a given concentration of  $C_{12}E_8$  added after homogenization, the measured protein surface concentration at 10 °C is significantly lower than that at 20 °C, but it increases again to a value close to that at 20 °C when the temperature is reduced to 0 °C. It seems that equilibrium is reached after a reasonably short period of time: surface coverages determined after storage times of 2 and 24 h are more or less the same within the estimated experimental uncertainty (5–10%). The ability of  $C_{12}E_8$  to displace  $\beta$ -casein from the oil-water interface in the presence of GMS is especially marked in the temperature range 5–10 °C. These results are consistent with recent findings (Barfod et al., 1991; Krog, 1991) of a strong displacement of protein from the interface during the phase transition of adsorbed monoglyceride on cooling from 15 to 5 °C. Such observations may be relevant to the making of whippable dairy



**Figure 4.** Effect of temperature and the presence of GMS in the oil phase on protein displacement in emulsions (0.4 wt %  $\beta$ -casein, 20 wt % soya oil, pH 7) with  $C_{12}E_8$  added at a  $C_{12}E_8/\beta$ -casein molar ratio  $R$  after cooling from 20 to 15 (2 h), 10 (3 h), 5 (4 h), and 0 °C (5 h). The surface concentration  $\Gamma$  is plotted against temperature  $T$ : (A) no GMS present,  $R = 2$ ; (B) 0.2 wt % GMS,  $R = 1$ ; (C) 0.2 wt % GMS,  $R = 2$ .

emulsions such as ice-cream mix (Berger, 1976). The function of the added emulsifier in such products is supposedly to promote the controlled destabilization of the emulsion to induce fat globule agglomeration for stabilizing the air cells of the resulting frozen foam. When monoglycerides are used for this purpose, it is necessary prior to freezing to let the emulsion age for several hours at, say, 5 °C to make a satisfactory ice cream.

In this study there appears to be an optimum temperature of around 10 °C corresponding to maximum protein displacement. This is indicated by the results plotted in Figure 4 for the experiments in which emulsions were first cooled to the lower temperature (15, 10, 5, or 0 °C) prior to addition of the water-soluble surfactant ( $C_{12}E_8$ ). The minimum in  $\Gamma$  around 10 °C is also evident, however, in the absence of GMS, and so the temperature effect may be due as much to changes in adsorption and aggregation behavior of the  $\beta$ -casein with temperature as to changes in the surface state of the monoglyceride. Possibly, even, the temperature effect is predominantly a function of the protein adsorption behavior at the triglyceride-water interface, with the role of the monoglyceride being simply to amplify the extent of the protein displacement. In the presence of 0.2 wt % GMS at 10 °C, the protein is completely removed from the interface when  $C_{12}E_8$  is added at a concentration corresponding to  $R = 2$ .

## CONCLUSIONS

The main general conclusion of this work is that the presence of a mixture of water-soluble and oil-soluble surfactants in an oil-in-water emulsion leads to much greater displacement of adsorbed milk protein than with either surfactant present alone. Qualitatively the same behavior is found with hydrocarbon and triglyceride oils and with pure synthetic and food grade surfactants.

Complete removal of protein from the emulsion droplet surface can only be achieved by addition of a water-soluble surfactant. The influence of the oil-soluble surfactant on the amount of water-soluble surfactant required for

complete displacement appears to depend on the nature of the surfactants and the oil phase. The critical displacer concentration of  $C_{12}E_8$  is unaffected by  $C_{12}E_2$  dissolved in *n*-tetradecane but is greatly reduced by GMS dissolved in purified soya oil.

The influence of GMS on the protein surface coverage in emulsions with added water-soluble surfactant is strongly affected by temperature, reaching a maximum effect in the temperature range 5–10 °C. This experimental study of competitive adsorption in model systems containing known amounts of pure surfactants may provide the basis for understanding the role of commercial mono-glyceride emulsifiers in food colloids such as ice cream and cream liqueurs.

One additional complication in real dairy colloids is the partially crystalline nature of the milk fat over the temperature range investigated in this study. It would be interesting to extend these experiments to emulsions made from high melting point fats to explore the influence of triglyceride crystallization on the competitive adsorption behavior.

#### LITERATURE CITED

- Barfod, N. M.; Krog, N.; Larsen, G.; Buchheim, W. Effects of Emulsifiers on Protein/Fat Interaction in Ice-Cream Mix during Ageing. 1. Quantitative Analyses. *Fat Sci. Technol.* **1991**, *93*, 24–29.
- Bergenstahl, B. A.; Claesson, P. M. Surface Forces in Emulsions. In *Food Emulsions*, 2nd ed.; Larsson, K., Friberg, S. E., Eds.; Dekker: New York, 1990; pp 41–96.
- Berger, K. G. Ice-Cream. In *Food Emulsions*, 1st ed.; Friberg, S., Ed.; Dekker: New York, 1976; pp 141–213.
- Cohen Stuart, M. A.; Fleer, G. J.; Scheutjens, J. M. H. M. Displacement of Polymers. 1. Theory. Segmental Adsorption Energy from Polymer Desorption in Binary Solvents. *J. Colloid Interface Sci.* **1984**, *97*, 515–525.
- Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Clark, D. C. Competitive Adsorption of  $\beta$ -Lactoglobulin + Tween 20 at the Oil-Water Interface. *Colloids Surf.* **1991a**, *56*, 293–300.
- Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Williams, A. Influence of Emulsifier on the Competitive Adsorption of Whey Proteins in Emulsions. *Food Struct.* **1991b**, *10*, 109–115.
- Courthaudon, J.-L.; Dickinson, E.; Dagleish, D. G. Competitive Adsorption of  $\beta$ -Casein and Nonionic Surfactants in Oil-in-Water Emulsions. *J. Colloid Interface Sci.* **1991c**, *145*, 390–395.
- Courthaudon, J.-L.; Dickinson, E.; Christie, W. W. Competitive Adsorption of Lecithin and  $\beta$ -Casein in Oil-in-Water Emulsions. *J. Agric. Food Chem.* **1991d**, *39*, 1365–1368.
- Darling, D. F.; Birkett, R. J. Food Colloids in Practice. In *Food Emulsions and Foams*; Dickinson, E., Ed.; Royal Society of Chemistry: London, 1987; pp 1–29.
- de Feijter, J. A.; Benjamins, J.; Tamboer, M. Adsorption Displacement of Proteins by Surfactants in Oil-in-Water Emulsions. *Colloids Surf.* **1987**, *27*, 243–266.
- Dickinson, E. Food Colloids—An Overview. *Colloids Surf.* **1989**, *42*, 191–204.
- Dickinson, E. Competitive Adsorption and Protein/Surfactant Interactions in Oil-in-Water Emulsions. In *Microemulsions and Emulsions in Foods*; El Nokaly, M., Cornell, D. G., Eds.; ACS Symposium Series 448; American Chemical Society: Washington, DC, 1991; pp 114–129.
- Dickinson, E.; Woskett, C. M. Competitive Adsorption between Proteins and Small-Molecule Surfactants in Food Emulsions. In *Food Colloids*; Bee, R. D., Richmond, P., Mingins, J., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1989; pp 74–96.
- Dickinson, E.; Murray, A.; Murray, B. S.; Stainsby, G. Properties of Adsorbed Layers in Emulsions containing a Mixture of Caseinate and Gelatin. In *Food Emulsions and Foams*; Dickinson, E., Ed.; Royal Society of Chemistry: London, 1987; pp 86–99.
- Dickinson, E.; Rolfe, S. E.; Dagleish, D. G. Competitive Adsorption of  $\alpha_1$ -Casein and  $\beta$ -Casein in Oil-in-Water Emulsions. *Food Hydrocolloids* **1988**, *2*, 397–405.
- Dickinson, E.; Mauffret, A.; Rolfe, S. E.; Woskett, C. M. Adsorption at Interfaces in Dairy Systems. *J. Soc. Dairy Technol.* **1989a**, *42*, 18–22.
- Dickinson, E.; Narhan, S. K.; Stainsby, G. Stability of Cream Liqueurs containing Low-Molecular-Weight Surfactants. *J. Food Sci.* **1989b**, *54*, 77–81.
- Dickinson, E.; Euston, S. R.; Woskett, C. M. Competitive Adsorption of Food Macromolecules and Surfactants at the Oil-Water Interface. *Prog. Colloid Polym. Sci.* **1990**, *82*, 65–75.
- Gaonkar, A. G. Interfacial Tensions of Vegetable Oil/Water Systems: Effect of Oil Purification. *J. Am. Oil Chem. Soc.* **1989**, *66*, 1090–1092.
- Krog, N. Thermodynamics of Interfacial Films in Food Emulsions. In *Microemulsions and Emulsions in Foods*; El Nokaly, M., Cornell, D. G., Eds.; ACS Symposium Series 448; American Chemical Society: Washington, DC, 1991; pp 138–145.

Received for review August 29, 1991. Accepted November 4, 1991.

Registry No. GMP, 26657-96-5; GMS, 31566-31-1;  $C_{12}E_8$ , 3055-98-9;  $C_{12}E_2$ , 3055-93-4.