Interfacial Properties of Milk Protein-Stabilized Emulsions As Influenced by Protein Concentration†

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Interfacial area, protein load, and interfacial energetics were determined on emulsions produced from sodium caseinate (CAS) and whey protein isolate (WPI). Protein concentration in the aqueous phase varied from 0.125 to 1%. At low concentrations, whey protein emulsion showed larger interfacial area (smaller oil droplets) than casein emulsions. However, for concentrations higher than 0.5% the reverse trend was observed. Protein load increased with protein concentration, but leveled off at a concentration of 0.5% for whey protein emulsions. From contact angle measurements on hydrated layers, the interfacial interaction energy between oil droplets was calculated. As protein concentration increased, the short-range polar (AB) contribution to interaction energy became repulsive while the long-range nonpolar (LW) contribution remained attractive. The degree of orientation of the hydration layer was calculated from the AB contribution to oil droplet surface energy. Increasing protein concentration reduced water molecule orientation at the surface of oil droplets. The hydration layer of casein-stabilized oil droplets showed a lower degree of orientation than the hydration layer of whey protein-stabilized oil droplets. Results are discussed in relation to emulsion stability.

INTRODUCTION

In food emulsions, proteins are widely used to stabilize the interface between the oil and the aqueous phase. During emulsion formation, mass transport of proteins to newly formed interface takes place mainly by turbulence-controlled convection (Dickinson et al., 1989; Walstra and Oortwijn, 1982). Reduction of interfacial tension is the driving force for protein adsorption. Once adsorbed, protein molecules spread out and undergo rearrangement to form a stabilizing film (Waniska and Kinsella, 1985). Further adsorption to an existing film depends on protein ability (mainly hydrophobicity and flexibility) to penetrate the film and compress already adsorbed proteins (Kinsella, 1984; Shimazu et al., 1981). Rapid coverage of newly formed droplets is responsible for the formation of finely dispersed emulsions.

The stability of protein-stabilized emulsions is governed by the nature of the film surrounding the oil droplets. Interaction forces between droplets could induce the formation of aggregates and eventually the coalescence of oil droplets. Among the forces responsible for emulsion stability/instability, electrostatic, steric, and bridging interactions received much attention (Halling, 1981). Comparatively, forces originating from interfacial interactions were not investigated. Calculations of interfacial interactions rely on measurement of contact angles made by liquid drops placed on the surface to be studied. Contact angle values are related to the surface energy parameters through the Young equation (Zizman, 1964). Development of this approach for hydrated biological materials allowed measurement of the short-range polar (AB) contribution as well as the long-range nonpolar (LW) contribution to the surface energy (van Oss et al., 1986a). The polar contribution can be further characterized into its electron donor and electron acceptor components (van Oss et al., 1986b).

Measurement of surface energy parameters on hydrated biological materials was used to improve understanding of various phenomena, such as protein solubility (van Oss et al., 1986b), polymer separation methods (van Oss et al., 1978), adhesion of bacteria (Mafu et al., 1991; Busscher et al., 1984), phagocytosis (Absolum et al., 1982), biocompatibility (Kaelble and Moacanin, 1977), milk deposit formation in heat exchangers (Britten et al., 1988), and milk protein interactions (Britten et al., 1989). It was the purpose of this work to use the surface energy approach, in conjunction with surface area and protein load determination, to better understand the formation and stability of milk protein-stabilized emulsions.

MATERIALS AND METHODS

Emulsion Formation. Sodium caseinate (90.6% protein) (Champlain Ltd., Tara, ON) and whey protein isolate (89.3% protein) (Le Sueur Isolates, Le Sueur, MN) were suspended in sodium phosphate buffer (0.005 M, pH 7.0) to protein concentrations of 0.125, 0.25, 0.5, and 1.0%. Protein suspensions were dialyzed for 16 h against the same buffer to equilibrate soluble minerals. Emulsions containing 30% commercial soya oil (Crisco Ltd., Toronto, ON) were produced with a single-stage mini-lab homogenizer (Type 8.30 H, Rannie, Albertslund, Denmark) operating at pressures of 20 MPa for the first pass and 3 MPa for the second. The homogenization temperature was kept at 40 ± 2 °C, and emulsions were cooled to room temperature immediately after formation.

Interfacial Area of the Emulsion. Interfacial area (IA) of the emulsions was calculated from the turbidity of diluted emulsions (Pearce and Kinsella, 1978). Emulsions were diluted in sodium phosphate buffer (0.005 M, pH 7.0) containing 0.5% sodium dodecyl sulfate to a final oil volume fraction of 6×10^{-5}. Optical density was measured at 500 nm with a Beckman DU-7 spectrophotometer (Beckman Instruments, Palo Alto, CA). Calculations were performed according to the method of Cameron et al. (1991)

$$IA = 2T \quad (1)$$

where T is the turbidity ($T = 2.303OD_{500} \times$ dilution/0.01-m light path). IA corresponds to the surface area per unit volume of the emulsion. Interfacial area unit is m^{-1}, but in the present study, results were expressed as m^2/mL of emulsion.

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† FRDC Contribution 284.

0021-8561/93/1441-1187$04.00/0 Published 1993 by the American Chemical Society

Table I. Surface Tension Components of Water, DMSO, and α-Bromonaphthalene (in mJ m^{-2})

	γ^{TOT}	γ^{LW}	γ^+	γ^-	ref
water	72.8	21.8	25.5	25.3	van Oss (1989)
DMSO	44.0	44.0	0	30.0	van Oss (1987a)
α-bromonaphthalene	44.4	43.6	0.4	0.4	van Oss (1987a)

Protein Load. Protein load was calculated from protein depletion in the serum phase after emulsion formation. Serum phase was separated from the emulsion by centrifugation (25000*g* for 60 min). Protein was determined in the aqueous phase before and after emulsion formation using a modified biuret reaction (BCA protein assay reagent, Pearce, Rockford, IL). Calibration curves were prepared with sodium caseinate and whey protein isolates previously analyzed according to the Kjeldahl method. Protein load results were expressed as mg/m^2. For that purpose, protein concentration depletion in the aqueous phase was divided by the interfacial area of the emulsion (IA).

Preparation of Samples for Contact Angle Measurements. Hydrated layers of oil droplets were obtained by dead-end vacuum filtration of a 10-mL emulsion sample through a cellulose membrane of 47-mm diameter and 0.22-μm pore size (Millipore Corp., Bedford, MA). Prior to filtration, the emulsion was washed to remove nonadsorbed proteins from the aqueous phase. A 2-mL emulsion sample was diluted to 10 mL in phosphate buffer (0.005 M, pH 7.0) and centrifuged (3000*g*, 30 min). The cream layer was collected and suspended in phosphate buffer to a volume of 10 mL. This procedure was repeated twice. After washing, protein concentration in the aqueous phase was lower than 0.001%. Filtration of a 10-mL sample of washed emulsion was stopped after 9 mL had passed through the membrane. This precaution was taken to avoid drying of the oil droplet layer.

Contact Angle Measurements. The filtration membrane supporting the hydrated layer of oil droplets was carefully placed over two thicknesses of fully wet Whatman No. 1 filter paper to slow down water evaporation. Microsyringes provided with square-cut Teflon tips were used to place drops of deionized water, α-bromonaphthalene, or dimethyl sulfoxide (DMSO) (Aldrich Chemical Co. Inc., Milwaukee, WI) on the surface of oil droplet layers. The contact angle was measured within 5 s using a goniometer in conjunction with a 100× telescope (Gaertner Scientific Corp., Chicago, IL). Contact angles of new drops deposited on fresh sites of the material were measured at intervals of 5 min. Contact angles were plotted as a function of time to determine the plateau values for the three liquids used. These plateau values were identified as the thermodynamically significant contact angles (van Oss et al., 1975) and were used for further calculations.

Surface Energy Calculations. The contact angle of a liquid drop is related to the surface energy parameters of both the liquid and the surface through the Young extended equation (van Oss and Good, 1988):

$$1 + \cos\theta = (2/\gamma_L^{TOT})[(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + (\gamma_S^+\gamma_L^-)^{1/2} + (\gamma_S^-\gamma_L^+)^{1/2}] \quad (2)$$

where θ is the advancing contact angle and γ the surface energy. Subscript L refers to the liquid and subscript S to the surface. Superscripts LW, +, and − refer, respectively, to the Lifshitz–van der Waals, electron acceptor, and electron donor contributions to the surface energy, while TOT refers to the total surface energy. The liquids used in this study were characterized with respect to their surface energy components (van Oss, 1989; van Oss et al., 1987a), and values are listed in Table I. Thus, experimentally measured contact angle and the energy parameters of the liquid can be substituted in eq 2 to produce an equation with three unknowns which correspond to the surface energy parameters of the oil droplets. Using the three liquids, three equations with three unknowns were produced and solved with the procedure IML from the statistical analyses system (SAS Institute, 1989). The short-range polar contribution to surface energy (γ^{AB}) was calculated from the γ^+ and γ^- components according to the method of van Oss and Good (1988):

$$\gamma_S^{AB} = 2(\gamma_S^+\gamma_S^-)^{1/2} \quad (3)$$

Statistical Analysis. The emulsions were prepared in triplicate according to a completely randomized design. Analysis of variance was used to determine if the factors (protein source and concentration) and their interaction had a significant effect on measured properties (SAS Institute, 1989). Statistical analyses were performed at $\alpha = 0.05$. Contact angles and surface energy parameters were compared through multiple comparisons of least-squares means at controlled α level of 0.05.

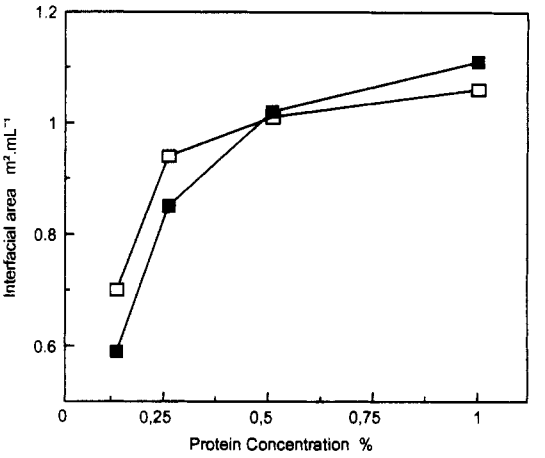

Figure 1. Effect of protein concentration on interfacial area of milk protein-stabilized emulsions. (■) Casein-stabilized emulsions; (□) whey protein-stabilized emulsions.

RESULTS AND DISCUSSION

Emulsion Formation. During emulsification, oil droplets are fractionated and proteins adsorb to maintain the integrity of the newly formed interface. Uncovered oil droplets are unstable and undergo coalescence within the homogenization valve (Pearce and Kinsella, 1978). The interfacial area of the emulsion indicates the ability of proteins to rapidly form a stabilizing film which prevents coalescence. The total interfacial area of the emulsion was measured as a function of protein concentration (Figure 1). Increasing protein concentration reduced the time required to form the stabilizing film and resulted in increased surface area (or smaller oil droplets) ($p = 0.0001$). It appeared that the total interfacial area eventually leveled off with increasing protein concentration. Maximum interfacial area mainly depends on the energy density of the homogenization process (Haque and Kinsella, 1989; Tornberg and Lundh, 1978). The effect of concentration on interfacial area of the emulsion depended on the protein source ($p = 0.0001$). At low protein concentration (0.125 and 0.25%) emulsions from whey proteins showed larger interfacial area (producing smaller oil droplets) than emulsions from caseins. In low-concentration conditions, rate of transport to the interface is critical to ensure rapid membrane formation (Walstra and Oortwijn, 1982). Whey proteins are structured as globular complexes which promote mass transport onto newly formed interface during the homogenization process. Tornberg (1978) suggested that in low-concentration conditions caseinates migrated to the interface via the casein monomers, which can explain the slow rate of transport. As concentration increased, the difference between protein fractions decreased, and for concentrations higher than 0.5%, caseins stabilized a larger interface than whey proteins. At higher protein concentration, newly formed interface is rapidly covered by a thin film and protein transport is no longer a key factor. However, further adsorption of protein molecules is required to produce a stable membrane resisting coalescence. The ability of protein molecules to adsorb and spread out at an interface already covered by a protein film is limited by the electric charge and the

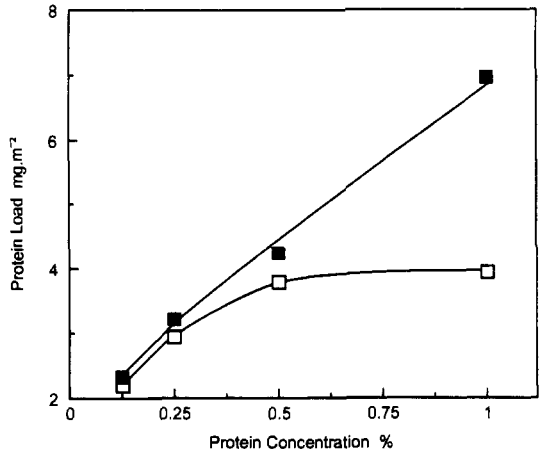

Figure 2. Effect of protein concentration on the protein load of milk protein-stabilized emulsions. (■) Casein-stabilized emulsions; (□) whey protein-stabilized emulsions.

surface pressure of the existing film (MacRitchie and Alexander, 1963a,b). It was shown that caseins, due to their strong amphiphilic nature and flexible structure, had better interfacial activity (Graham and Phillips, 1979), which explains the larger interfacial area stabilized by caseins at higher protein concentration.

The amount of protein adsorbed at the oil–water interface was estimated by protein load determination (Figure 2). As protein concentration increases, protein load (or membrane thickness) increased (p = 0.0001). However, the two protein fractions under study behaved differently (p = 0.0021). The protein load of casein-stabilized emulsions increased almost linearly with concentration (within the range tested). Strong interfacial activity of caseins allows penetration and spreading out at a crowded interface (Kinsella, 1984). The protein load of whey protein-stabilized emulsions leveled off as protein concentration in the aqueous phase reached about 0.5%. The lower interfacial activity prevented the formation of thick protein films, and the interface was considered to be saturated with respect to whey proteins. The dispersity of proteins also influences the emulsion protein load (Walstra and Oortwijn, 1982). Larger particles adsorb to interfaces with limited spreading, leading to higher surface concentration. As casein concentration increased, formation of larger polymers was promoted (Pepper, 1972), which could explain the higher protein load.

Interfacial Interactions. Advancing contact angles were measured with three solvents on hydrated layers of oil droplets isolated from milk protein-stabilized emulsions. With these values a set of three equations (eq 2) was solved to determine the surface energy parameters of oil droplets as a function of protein concentration in the aqueous phase (Table II). The nonpolar long-range (LW) contribution to the surface energy of oil droplets rapidly decreased with protein concentration and leveled off at a concentration of 0.125% for casein-stabilized emulsions and 0.25% for whey protein-stabilized emulsions. Both electron acceptor (γ^+) and electron donor (γ^-) components of the short-range polar contribution increased with protein concentration. The γ^+ component was significantly higher for casein-stabilized droplets than whey protein-stabilized droplets at any concentration tested. For both protein sources, the γ^- component rapidly leveled off at a value around 60 mJ m^{-2}.

From the surface energy parameters, the polar short-range (ΔG^{AB}) and nonpolar long-range (ΔG^{LW}) contributions to interfacial interaction energy between oil droplets were calculated as derived from van Oss et al. (1986b)

$$\Delta G^{AB} = -4[(\gamma_1^+\gamma_1^-)^{1/2} + (\gamma_3^+\gamma_3^-)^{1/2} - (\gamma_1^+\gamma_3^-)^{1/2} - (\gamma_3^+\gamma_1^-)^{1/2}]^2 \quad (4)$$

$$\Delta G^{LW} = -2[(\gamma_1^{LW})^{1/2} - (\gamma_3^{LW})^{1/2}]^2 \quad (5)$$

where subscript 1 refers to the surface energetics of oil droplets (Table I) and subscript 3 refers to the surface energetics of water (Table I).

The polar short-range (AB) contribution to the interfacial interaction energy increased from a strong negative value (attraction) to a strong positive value (repulsion) as protein concentration increased (p = 0.0001) (Figure 3a). For casein-stabilized oil droplets, the change from AB attraction to AB repulsion required very low protein concentration. At 0.125% concentration, the AB interaction energy already showed its maximum positive value (40 mJ m^{-2}), while for whey protein-stabilized oil droplets, the maximum positive value occurred at 0.25% protein concentration. It was shown that despite a slightly lower protein load (Figure 2), whey proteins stabilized a larger interfacial area than caseins in the low-concentration region (Figure 1). In these conditions, whey proteins formed a thinner film than caseins around oil droplets. Extensive spreading of whey protein molecules resulted in low density of polar residues at the surface of oil droplets, which could explain the lower AB interaction energy of whey protein-stabilized droplets. For concentrations higher than 0.25%, the AB interaction energies of oil droplets stabilized by both protein sources were similar.

The long-range nonpolar (LW) contribution to the interfacial interaction energy also increased with protein concentration (p = 0.0001) (Figure 3b). The LW interaction energy was low in magnitude and remained negative (attraction) within the concentration range tested. These results suggest that as protein concentration increases, the protein film surrounding oil droplets contributes to emulsion stability through polar short-range (AB) repulsion forces between oil droplets. However, nonpolar long-range (LW) attraction forces promote the association of oil droplets and impair emulsion stability. It was noted that for concentrations higher than 0.25% both protein sources showed similar interaction energy contributions. Then the interfacial interaction energy cannot explain the stability difference observed between casein- and whey protein-stabilized emulsions (Britten and Giroux, 1991).

Hydration Layer. Water molecules, in the bulk, are in random orientation. However, water molecules in close contact with the interface (hydration layer) are oriented according to surface characteristics. The structure of water molecules in contact with hydrophobic surfaces is well documented (Dickinson and Stainsby, 1982). The degree of orientation of water molecules at an interface is estimated by the percentage decrease in γ^{AB} of the hydrated surface, relative to γ^{AB} of bulk water (γ^{AB}_{water} = 51 mJ m^{-2}) (van Oss and Good, 1988). The degree of orientation of water molecules in the hydration layer surrounding oil droplets was calculated from γ^{AB} values of Table II. Results are presented on Figure 4. In low protein concentration conditions, water was highly ordered at the oil droplet surface. However, as protein concentration increased, the degree of orientation decreased (p = 0.0001). A very low concentration of casein brought the degree of orientation of water molecules down to about 60%. For whey protein-stabilized droplets, a higher protein concentration was required. A high degree of orientation is associated with the monopolarity of the surface (large γ^- compared to γ^+) (van Oss et al., 1987b). The hydrogen atoms of water molecules are bound to the

Table II. Contact Angles (Plateau Values) of Selected Liquids and Corresponding Surface Energy Components of Fat Droplets from Protein-Stabilized Emulsions[a]

source	concn, %	contact angles, deg: water	DMSO	α-bromo-naphthalene	γ^{LW}, mJ m^{-2}	γ^{+}, mJ m^{-2}	γ^{-}, mJ m^{-2}	γ^{AB}, mJ m^{-2}
CAS	0	19.3$^{\alpha}$	85.0$^{\alpha}$	9.7$^{a\alpha}$	40.2$^{\alpha}$	0.09$^{\alpha}$	2.8$^{\alpha}$	1.0$^{\alpha}$
	0.125	23.7$^{abc\beta}$	10.0^{a}	9.7^{a}	30.5^{a}	1.66^{a}	62.6^{a}	20.4^{a}
	0.250	22.7$^{c\gamma}$	9.0^{a}	0^{b}	30.8$^{a\beta}$	1.74^{ab}	62.1^{ab}	20.8^{ab}
	0.500	25.7$^{bd\delta}$	10.7^{ab}	0^{b}	29.9$^{a\gamma}$	1.98^{bc}	61.2$^{ab\beta}$	22.0^{b}
	1.000	26.0$^{ad\epsilon}$	13.0$^{b\beta}$	0^{b}	29.9$^{a\delta}$	1.99^{c}	60.2$^{b\gamma}$	21.9^{b}
WPI	0	19.3$^{\alpha}$	85.0$^{\alpha}$	9.7$^{c\alpha}$	40.2$^{\alpha}$	0.09$^{d\alpha}$	2.8$^{\alpha}$	1.0$^{c\alpha}$
	0.125	25.7$^{d\beta}$	62.3	37.0	34.6	0.01^{d}	25.1	1.0^{c}
	0.250	24.7$^{d\gamma}$	14.7^{c}	21.7	30.3$^{b\beta}$	1.17	64.1	17.3
	0.500	24.0$^{d\delta}$	14.3^{c}	11.7^{c}	30.6$^{b\gamma}$	1.58^{e}	61.2$^{c\beta}$	19.7^{d}
	1.000	25.0$^{d\epsilon}$	15.0$^{c\beta}$	10.0^{c}	30.2$^{b\delta}$	1.74^{e}	60.4$^{c\gamma}$	20.5^{d}

[a] Results from the same protein source sharing the same letter are not significantly different; results from the same protein concentration sharing the same Greek symbol are not significantly different (α = 0.05).

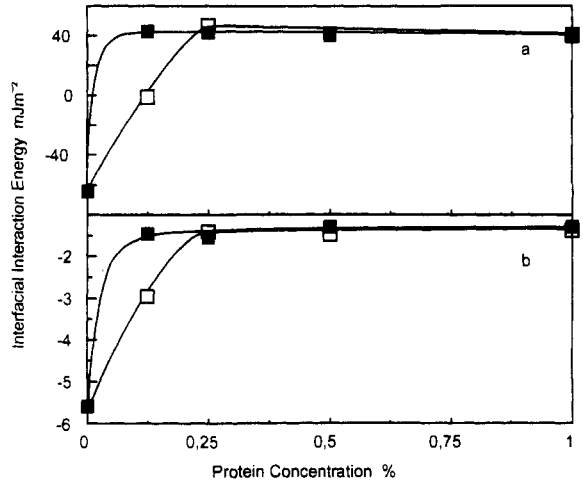

Figure 3. Effect of protein concentration on the interfacial interaction energy of oil droplets in milk protein-stabilized emulsions. (a) Short-range, polar (AB) contribution to interfacial interaction energy; (b) long-range, nonpolar (LW) contribution to interfacial interaction energy; (■) casein-stabilized emulsions; (□) whey protein-stabilized emulsions.

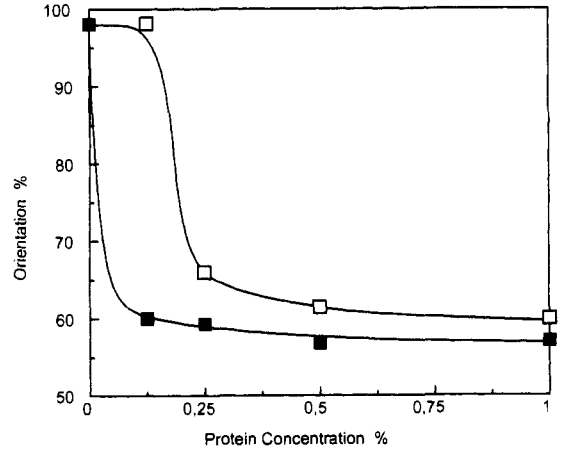

Figure 4. Effect of protein concentration on the degree of orientation of water molecules in the hydration layer of oil droplets in milk protein-stabilized emulsions. (■) Casein-stabilized emulsions; (□) whey protein-stabilized emulsions.

protein residues covering the oil droplets, while the oxygens are exposed to the solvent. As observed in Table II, a significant electon acceptor (γ^{+}) contribution to the surface energy was detectable only when protein concentration reached 0.25% and remained under 2 mJ m^{-2} compared to a γ^{-} component of 60 mJ m^{-2}. At any concentration tested, the degree of orientation of water molecules was higher on whey protein-stabilized oil droplets than on casein-stabilized ones (p = 0.0001). The orientation of water molecules in the hydration layer governs the entropy and affects the free energy of the emulsion. Stronger orientation results in a more negative entropy value, which contributes to the reduced stability of whey protein emulsions as compared to casein emulsions. In previous studies, we clearly showed that whey protein-stabilized emulsions were less stable to coalescence than casein-stabilized emulsions (Britten and Giroux, 1991). The balance between the electron donor and electron acceptor components of the surface energy of droplet membrane is responsible for the orientation of the hydration layer and apparently plays a significant role in emulsion stability. The asymmetry of γ^{-} and γ^{+} components (Table II) was responsible for strong orientation of the hydration layer, which remained over 50% even at the highest protein concentration tested. Selection of amphiphilic molecules with a balanced γ^{-}/γ^{+} ratio to prepare food emulsions is likely to improve the stability by reducing the entropy of the system. These ingredients might, however, reduce the short-range polar (AB) repulsive forces. Work is in progress to isolate and evaluate emulsifying properties of such molecules.

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Received for review December 28, 1992. Revised manuscript received March 29, 1993. Accepted May 10, 1993.