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Methylcellulose-induced stability changes in protein-based emulsions

Dipak K. Sarker¹, Monique Axelos, Yves Popineau *

*Institut National de la Recherche Agronomique, Centre de Recherche de Nantes, rue de la Géraudière, BP 71627,
44316 Nantes cedex 3, France*

Abstract

The emulsifying and oil-in-water stabilizing properties of methylcellulose (MeC) were investigated in bovine serum albumin (BSA)-based emulsions. The creaming stability, flocculation, surface concentration of BSA and MeC and droplet size were determined. Results obtained showed modifications of creaming rates that were related to MeC concentrations in the continuous and dispersed phases. Viscosity effects on creaming and changes in average droplet size (d_{43}) relating to droplet coverage were identified and delineated. Studies performed on macroscopic oil–water and air–water interfaces were used to identify interfacial structuring and composition. A good agreement was found between droplet surface composition and the resistance to coalescence of emulsion droplets. Emulsions that demonstrated a more rigid-like adsorbed interfacial layer were more stable with respect to coalescence. This study involving model emulsion systems provides a new insight into the stability of industrial preparations containing mixtures of proteins and polysaccharides. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Coalescence; Competitive adsorption; Rheology; Interface; Stability

1. Introduction

Many food, pharmaceutical and agrochemical commodities exist in the form of emulsions, where one immiscible (dispersed) phase is distributed in another (continuous) phase. These can be oil-in-water (O/W), water-in-oil (W/O) or complex multiple emulsions (e.g. W/O/W). The science of multi-component emulsion formation, stabilization and stability is complex [1,2].

Surface active molecules such as proteins and low molecular weight emulsifiers (e.g. stearates, Spans and Tweens) are often used to stabilize the interfacial layer of oil droplets because they adsorb readily at the interface [2,3]. Retarding droplet coalescence (droplet fusion) is a primary concern to manufacturers since in extreme cases this can result in separation of the dispersion into two distinct, oil and water phases.

Proteins and emulsifiers stabilize emulsions against coalescence in different and mutually incompatible ways [4]. Proteins are relatively slow to diffuse to the interface but form a rigid viscoelastic adsorbed interfacial layer [5,6], whereas

* Corresponding author. Tel.: +33-2-4067-5059; fax: +33-2-4067-5025; e-mail: popineau@nantes.inra.fr.

¹ Present address: SmithKline Beecham Pharmaceuticals, Manor Royal, Crawley, West Sussex, RH10 2JQ, England.

emulsifiers in general, rapidly diffuse from the bulk to the interface of the dispersed phase but lack significantly large interfacial viscoelastic properties. Rapid diffusion and viscoelasticity can both retard droplet coalescence. Viscoelastic effects retard coalescence by providing a 'thick' rigid mechanical barrier to droplet fusion [2,5–8]. With emulsifiers rapid diffusion on a molecular scale to and in the plane of the interface (Marangoni effect) and the associated dragging of continuous phase, thickening the region in between the droplets, serves to stabilize the droplets against coalescence [9].

In the case where the two types of surface active agent are mixed, neither mechanism of interfacial stabilization functions well. The interface is dominated by the most surface active molecular species [8,10–12]. This phenomenon is referred to as competitive adsorption [2,10,11] or competitive displacement, depending on whether one surface active component is allowed to adsorb at the interface before another. The latter often refers to the situation when a more surface active component is added after emulsification, thus allowing one component to adsorb initially relatively unhindered. This competition for space at the interface dictates the stabilization mechanism that prevails and thus the magnitude of resistance to coalescence.

In commercial products, viscosity increasing hydrocolloids are often incorporated in emulsion mixes. In O/W emulsions for example, they act as relatively neutral components which have little, if any, surface activity but retard coalescence by reducing the speed of separation of oil droplet-rich and water-rich regions of the dispersion (creaming), which almost always occurs since the density of oil is generally much lower than that of water. This 'stabilizing' effect is most frequently related to an increase in bulk viscosity [13]. Hydrocolloids often, also function by 'loosely' associating with the adsorbed interfacial or bulk protein molecules [14], and since they can have a tendency to associate with water strongly this can provide a mechanical 'cushion' to close approach of droplets [5,13,15] and subsequent droplet coalescence. Conversely, hydrocolloids can also contribute to the destabilisation of emulsions by

promoting flocculation. The two mechanisms involved then are bridging flocculation, when hydrocolloids interact with adsorbed molecules and create a link between two droplets, and depletion flocculation resulting from lower osmotic pressure between approaching droplets than in the surrounding bulk phase, when non-adsorbed macromolecules are excluded from the region between the droplet surfaces [16].

Methylcellulose is a water-soluble hydrophobically-modified cellulose derivative widely used in a large number of industrial applications for its surfactant properties and unusual thermoreversible gelation upon increasing temperature [17–19]. Methylcellulose is also used frequently because of its adhesion properties (printing), moisture retention (film), adsorption properties (stabilization of paint) and thermothickening ability (cosmetics, pharmaceuticals) [20]. Methylcellulose is characterized by the average degree of substitution (DS), which is defined as the average number of hydroxyl groups substituted per anhydroglucose. This parameter masks the fact that commercial methylcelluloses have a heterogeneous substituent distribution along their backbone [21] which facilitates hydrophobic interactions, leading to gelation. The gel obtained by heating is, in fact, associated with phase separation with a lower critical solution temperature (LCST) [22]. Below the LCST the solution is a one-phase system. Above this temperature, the solution becomes cloudy and the gel formation prevents a complete phase separation, leading to a homogeneous white gel. In solution, MeC is a semi-flexible polymer with a persistence length of ~ 5 nm. When the temperature is increased, aggregation of some molecules results in compact domains with a size depending on temperature and concentration [22]. Previous viscosity and equilibrium dialysis measurements [23] as well as conductimetric studies [22] have shown that the molecule can interact strongly with the surfactant sodium dodecyl sulphate to form associated structures.

The multi-component emulsions prepared industrially are often highly complex interacting systems. Emulsion stability is important [24] as this governs product 'shelf-life'. Delineation of the factors involved in the stability/instability of such

products is difficult to evaluate. This understanding is however simplified by using an approach involving model emulsion systems. In our studies, we evaluate the effect of using relatively low to moderate concentrations ($0.01\text{--}10\text{ mg ml}^{-1}$) of methylcellulose (MeC) together with a good emulsifying protein, bovine serum albumin (BSA) in model 30–40% oil-in-water emulsions under mild solvent conditions.

2. Materials and methods

2.1. Preparation of samples

The protein sample, bovine serum albumin (molecular weight 67 kD; BSA; A-9647) was obtained from the Sigma, St. Louis, MO. The purified methylcellulose Methocel A4C was purchased as a salt from Dow, France (no. 0726693-017). Hirrien et al. [25], using viscometric and NMR measurements, found an average molecular weight of 149 kD and an average monomer molecular weight of 186 g. The Methocel was dialyzed (8 kD) at 5°C to remove contaminating particulate matter, sodium chloride and other salts, lyophilized and stored prior to use according to the method of Chevillard and Axelos [22]. All samples were prepared in 0.1 mol dm^{-3} (M) imidazole-HCl buffer using surface chemically pure water (surface tension 72.8 mN m^{-1}) adjusted to pH 7.0. For the purposes of the experiments *n*-hexadecane ($>99\%$ purity, no. 2088789; Merck, Germany) was cleaned over pure water and dried thoroughly. The concentration of BSA solutions was determined spectrophotometrically at 280 nm using an absorbance coefficient of $0.66\text{ mg ml}^{-1}\text{ per cm}$.

All emulsions containing BSA were formed with 5 mg ml^{-1} ($75\text{ }\mu\text{M}$) solutions since this corresponded to the extended linear region of a particle size versus concentration curve. Stock solutions of $\approx 30\text{ mg ml}^{-1}$ ($215\text{ }\mu\text{M}$) MeC were used to formulate mixes.

Emulsion, BSA and MeC assays were performed on solutions that were centrifuged at 15000 rpm for 20 min recovered and re-centrifuged then stored in well-sealed containers at

-80°C , to prevent microbial degradation prior to protein or viscometric assay. All sample concentrations referred to in the text were calculated on the basis of the test solution or aqueous phase of the emulsion formed.

2.2. Measurements on solutions, dispersions and macroscopic interfaces

2.2.1. Surface concentration measurements using OPA reagent

This spectrophotometric method was used to measure the BSA content of emulsion centrifugates and stock solutions. A calibration curve was generated using BSA standard solutions ($r^2 = 0.99$). The chromophore that is quantified is created by the reaction of free α - or ε -amino groups with *o*-phthalaldehyde (OPA) under denaturing conditions [26,27]. The reagent solution used was composed of 50 ml 0.1 M sodium tetraborate buffer pH 9.3, 1.25 ml 20% sodium dodecyl sulphate, 100 mg dimethyl-2-mercaptoethylammonium chloride and 40 mg OPA dissolved in 1 ml methanol. A total of $50\text{ }\mu\text{l}$ of centrifugate/protein standard was added to 1 ml of reagent solution, mixed thoroughly, allowed to stand for exactly 20 min and the absorbance read at 340 nm at 25°C against a reagent solution blank. Assays were performed in triplicate. BSA surface concentration was calculated from the difference in the solution concentration prior to and after emulsification.

2.2.2. Surface tension

Air–water surface tension measurements of 25 ml dilute BSA (0.1 mg ml^{-1}) and MeC solutions were performed using a Krüss digital tensiometer K10T at $20 \pm 1^{\circ}\text{C}$ in a vapour saturated environment using a platinum–iridium Wilhelmy plate after a thermal equilibration of 30 min.

2.2.3. Bulk viscometry

Coaxial cylinder shear viscosity and oscillatory measurements were performed on 2 ml samples of Newtonian solutions, emulsion centrifugates and on emulsions. Measurements were performed on a Contraves Low Shear 40 equipment with 18 mm spindle (radius 6 mm) at shear rates in the range

0.005–1.0 s⁻¹ at 20°C in a vessel of radius 6.5 mm. The viscosity was calculated from the average values in the plateau of measured viscosity versus shear rate. Oscillatory measurements were performed on a Carri-Med CSL 100 controlled stress rheometer, with a 2% deformation and frequency of oscillation of 0.1–0.7 Hz to test for inter-species interaction in the bulk phase. Shear measurements were also used to assay the concentration of methylcellulose adsorbed at the interface of emulsions (using emulsion centrifugates) by reference to the viscosity of the corresponding native solutions. Centrifugation served to distinguish the rheological properties of the droplets from those of the entire emulsion. The measurement is a relative one, as some fractionation of MeC of the continuous phase will have undoubtedly taken place. Smaller molecules are likely to be adsorbed in preference. Nevertheless, the viscosimetry provides a good basis for an analytical tool.

2.2.4. Surface viscometry

The coaxial cylinder-bicone technique was used to measure the rheological properties of the oil/water interface. Measurements were performed on a Carri-Med CSL 100 controlled stress rheometer, with Carri-Med software and 6 cm diameter ($R_1 = 3$ cm) titanium bicone (no. 930951:TA Instruments, England) at 20°C. This adaptation of the standard equipment allowed measurement of interfacial properties. All measurements were performed in a firmly secured 7 cm diameter ($R_2 = 3.5$ cm) thoroughly cleaned glass vessel with 20 ml of test solution and 20 ml of *n*-hexadecane after thermal equilibration of 1 h. The instrument gives results in terms of measured torque T (Nm) and bicone rotation speed ω (rad s⁻¹). These can be converted to shear stress γ (N/m) and shear rate (s⁻¹), respectively [28], thus:

$$(\sigma) = T/2\pi R_1^2 \quad (1)$$

$$(\dot{\gamma}) = \omega[(R_2^2 + R_1^2)/(R_2^2 - R_1^2)] \quad (2)$$

The magnitude of changes in shear rate as a function of shear stress in the resulting flow curves gives a direct indication of the 'rigid-like' properties of the interface. Modelling of the

parameters to yield absolute viscosity values was not performed as the adsorbed interfacial layer was non-Newtonian.

2.2.5. Surface balance

The compressibility and interfacial area occupied by molecules were measured with a KSV 5000 Langmuir trough-type apparatus and controlling software at 20°C. Surface chemically pure water was used as the blank. A total of 200 μ l of a surface active agent (220 μ M) was applied directly on the surface and was allowed to equilibrate for 4 h prior to measurement. Isotherms were generated when the moveable barrier was advanced at a rate of 10 mm min⁻¹. Surface pressure calculated against that of pure water and a clean surface (surface pressure = 0 mN m⁻¹) was measured by a platinum-iridium Wilhelmy plate. All apparatus was cleaned in ethanol and rinsed exhaustively with surface chemically pure water.

2.3. Measurements on emulsions

2.3.1. Emulsification for particle sizing and coalescence studies

The emulsions were first coarsely mixed using a Polytron PT-MR 3000 blender and anti-foam spindle (PT-DA3012/2 TS) at 20 000 rpm for 1 min, creating a coarse emulsion (pre-mix; nominal droplet diameter, d_{43} of 20 μ m). Finer emulsions were prepared in a separate unit at 20°C. The jet-homogenizer apparatus, described in detail by Burgaud et al. [29], with the configuration used consists of two parallel vertical stainless-steel chambers and pistons with diameters of 0.8 and 1.5 cm, respectively. This basic apparatus rests within a compressed air-fuelled piston pressure chamber (LabPlant, England). The 22.5 ml of 'pre-mixed' emulsion (phase volume oil in water, $\phi = 0.318$) was placed in the dry homogenizer unit. A stainless-steel jet disc with a jet hole diameter of 0.55 mm (LPHJ023) and a starting pressure of 3 bar combine to force the pre-mix, under large turbulent and shear forces at a pressure of 135 bar through the jet hole. This created BSA stabilized droplets with a diameter of ≈ 2 μ m. No difference was found in the size and

distribution of droplets using a pre-mix technique or the technique of using separate chambers for the oil (narrow bore) and surfactant solution (wide bore). Consequently, all experiments were performed with the pre-mix technique. The first 3 ml dispatched from the apparatus were always discarded.

2.3.2. Creaming studies

The basis of the technique is the measurement of conductimetric changes between the rod-like electrodes, located at the base of a 12×2.8 cm internal diameter glass tube in the equipment [30], as the oil droplets concentrate in the upper portion of the emulsion. Each measurement uses 25 ml of surfactant and/or protein solution, *A*, and 15 ml purified *n*-hexadecane, *B* ($\phi = 0.375$). The equipment uses the same Polytron apparatus and conditions used to generate coarser pre-mix emulsions. The average phase volume (ϕ_{av}) of emulsion in the top 15 ml can then be calculated:

$$\phi_{av} = 1 - [(A/B) \times (1 - (C_t/C_{sol}))] \quad (3)$$

where the *C* is the conductivity and the subscripts sol and t refer to the buffered test solution before emulsification and emulsion at time *t*, respectively. These conductivity measurements were used as an indication of emulsion stability, because they are directly related to the creaming of the droplets. Since the conductivity of the buffer under the constant temperature conditions of the room ($20 \pm 1^\circ\text{C}$) remained constant, the recorded conductivity after emulsification was a direct measure of the conducting aqueous phase content of the emulsion in the volume corresponding to the height of the electrodes.

2.3.3. Particle sizing

The average droplet size (droplet diameter, d_{43}) was measured by a light scattering technique. This was performed on a Malvern Mastersizer IP apparatus using a software for an independent size fitting model, 45 mm lens (reverse Fourier optics, 0.1–80 μm diameter range), stirrer speed of ≈ 300 rpm. A refractive index of 1.434 was used for the hexadecane dispersed phase (presentation 0507) and water was used as the dispersant.

3. Results and discussion

3.1. Surface activity, bulk viscosity and creaming

The surface tension of BSA and MeC solutions is presented in Fig. 1. The figure shows that MeC was highly surface active. A cross-over in the curves representing MeC/BSA and MeC solutions alone as a function of augmented MeC concentration illustrate a binding of MeC by BSA had taken place and resulted in the formation of a complex. In addition, the duration of the cross-over with concentration indicates most probably a binding of considerable magnitude occurred. Binding of substrates by both BSA [31] and MeC [32] has already been reported. The data do not indicate if it was exclusively a BSA–MeC complex that was adsorbed at the interface over the whole range concentration studied, or if MeC alone had ‘displaced’ much of the adsorbed BSA. The exact composition of the adsorbed material remains unclear. It is however certain that it was not BSA alone.

With the knowledge that MeC can adsorb preferentially at the interface to BSA a series of creaming study experiments were performed to examine emulsion formation and creaming stability (Fig. 2(a) and (b)). Fig. 2(a) shows that at 10 mg ml^{-1} MeC alone, the emulsions formed were extremely slow to ‘cream’. The poor surface stabi-

Fig. 1. Air–water surface tension of solutions of MeC ($\circ$) alone and in the presence of 0.1 mg ml^{-1} BSA ($\bullet$), presented as a function of MeC concentration.

Fig. 2. Effect of MeC concentration on the creaming of *n*-hexadecane/water emulsions ($\phi = 0.375$). The change in droplet average phase volume against time is plotted for emulsions with MeC alone (graph a) and in the presence of 5 mg ml⁻¹ BSA (graph b). The concentrations of MeC incorporated were: 0 (*), 0.01 (+), 0.1 (○), 0.25 (●), 1 (□), 5 (■) and 10 mg ml⁻¹ (◇). The emulsion oil phase volume (ϕ) was 0.375.

lization properties of emulsions formed from MeC at concentrations < 0.1 mg ml⁻¹ was demonstrated by the inability of concentrations inferior to this to form an emulsion which persisted more than a few seconds. This can be explained in terms of a molecule that exhibits poor surface mobility and cohesive rheological properties [1]. In Fig. 2(b), when results are compared with those of the BSA control, they indicate that inclusion of low concentrations (0.01 mg ml⁻¹ MeC) reduced the creaming rate. Our hypothesis is that this is achieved by polymer which

is associated with the BSA already adsorbed; the association of water with the MeC would increase the overall droplet density from that of the oil (~ 0.77 g cm⁻³) to a value closer to that of water (~ 1 g cm⁻³). The net effect would be to reduce buoyancy effects and reduce droplet creaming [33]. Moderate concentrations (0.1–1 mg ml⁻¹ MeC) actually increased the creaming rate when compared to BSA alone. Mechanisms such as bridging flocculation or depletion flocculation might be involved, but our experiments do not allow to discriminate between them. Small differences between the phase volume of curves corresponding to emulsions formed with MeC and BSA/MeC mixtures at 0.1 mg ml⁻¹ MeC indicated that the emulsions in the mixture were still partially stabilized by BSA. The lower phase volume of the mixtures with moderate MeC concentration as opposed to the absence of MeC (or low concentrations) after 240 min was resulting from a viscosity increase leading to a less concentrated creamed phase. At higher concentrations of MeC (5–10 mg ml⁻¹ MeC) creaming was very strongly reduced. The solutions of these samples, prior to emulsification, were considerably more viscous than those of a lesser concentration. Interestingly, the creaming curves presented in Fig. 2(a) and (b) were essentially superimposable for emulsions with 1 and 10 mg ml⁻¹ MeC, indicating the surface and bulk solution properties of MeC were dictating the creaming properties of the emulsion.

Bulk viscosity changes with bulk MeC concentration are presented in Fig. 3. Negligible change was observed in the range of 0 to 0.25 mg ml⁻¹ MeC but between 0.25 and 1 mg ml⁻¹ MeC a small increase was found. However in the range 1–10 mg ml⁻¹ MeC very large increases in solution viscosity were observed. Evidently, the viscosity of BSA/MeC mixture was dictated by the viscosity of the MeC almost exclusively as the viscosity contribution of BSA was essentially negligible. The exception found was at 5–10 mg ml⁻¹ MeC when the viscosity of the mix was slightly higher than that of MeC alone, indicating a possible BSA–MeC interaction (a synergistic interaction was also indicated in surface tension measurements). The magnitude of interaction was assessed by oscillation viscometry (Table 1). A

Fig. 3. Changes in the bulk viscosity as a function of MeC concentration for samples MeC alone ($\circ$), MeC + 5 mg ml⁻¹ BSA ($\bullet$) and centrifugates of emulsions prepared from MeC + 5 mg ml⁻¹ BSA samples ($\square$).

concentration of 10 mg ml⁻¹ MeC was selected to aid elucidation of suspected minor synergistic interactions. This was the most viscous solution studied and was expected to amplify interactions if present. The complex viscosity and complex modulus were lower for MeC alone than for 5 mg ml⁻¹ BSA/10 mg ml⁻¹ MeC solutions. The loss angle (ϕ), which indicates the elastic (fully elastic = 0°) or viscous (fully viscous = 90°) contributions to the complex modulus, showed the viscosity component was significantly lower for the BSA/MeC mixture than for MeC alone. This indicated that the mixture was, not necessarily 'elastic' but less viscous pointing to a 'partial gelation' in the mixture in excess of that found with MeC alone. A similar interaction might be expected (of a different magnitude) at the interface of droplets but only in the domain where relatively large concentrations of BSA persisted.

This is however, by no means certain as the interaction would depend on the form in which the two components were adsorbed and would require rather extensive and favorable interaction and conformations when adsorbed.

The bulk viscosity was also used to assay the quantity of adsorbed interfacial MeC in emulsions by quantifying the MeC in the emulsion centrifugate. The concentration difference between the solution viscosity before and after emulsification was considered to represent the MeC adsorbed at the interface. Reduction in viscosity from those obtained for the BSA/MeC or MeC systems did not occur in the centrifuged samples when droplets had been removed. This method is perhaps only appropriate for relatively crude estimations of concentration, as we assume a linear change in viscosity between points on the calibration curve (Fig. 3), this is not entirely the case but rather represents a close approximation. At MeC concentrations < 0.1 mg ml⁻¹ the emulsion centrifugate had much the same viscosity as the control (5 mg ml⁻¹ BSA with or without MeC) solutions, according to the sensitivity of this technique. Very minor changes in the droplet interface composition would not be highlighted by this technique. We rely on the creaming measurements on complete systems (Fig. 2(a) and (b)) to highlight very small differences between bulk and interfacial effects. At higher MeC concentrations the emulsion centrifugate was considerably less viscous than the corresponding control samples, indicating MeC had been adsorbed in these emulsions. UV absorbance measurements (325 nm) indicated the presence of insignificant levels of oil droplets in these samples as the absorbance changed over the range 0.02–0.06 with increasing MeC concentration (BSA control 0.015). A much

Table 1
Measurement of the interaction of BSA with MeC^a

Sample	Complex viscosity, η^* (mPa.s) 0.1 Hz	Complex modulus, G^* (mPa) 0.1 Hz	Loss angle, ϕ (°) 0.1–0.7 Hz
MeC alone	35	20	80 ± 5
MeC and 5 mg ml ⁻¹ BSA	50	31	55 ± 4

^a By oscillation shear viscometry performed on solutions containing 10 mg ml⁻¹ MeC at 20°C and a frequency of 0.1–0.7 Hz.

Fig. 4. Effect of MeC concentration on the surface adsorbed concentration and specific surface area (*) of 5 mg ml⁻¹ BSA-based *n*-hexadecane/water emulsions prepared with the Polytron blender (droplet diameter, $d_{43} \approx 20$ nm) and measured after a sample equilibration of 1 h. Other symbols represent the surface concentration of MeC (Δ), BSA ($\bullet$) and total MeC/BSA present at the interface ($\square$). The emulsion oil phase volume (ϕ) was 0.375.

greater absorbance would be expected in the presence of significant amounts of droplets, thus validating our measurements but the results did point to the presence of more protein in centrifugates from emulsions with higher MeC concentrations.

3.2. Surface loading and concentration

Fig. 4 shows the reduction in the surface concentration of BSA as a function of increasing the MeC concentration in coarse (20 μ m diameter) emulsions. The most significant reductions started at 0.25 mg ml⁻¹ MeC. Reductions of adsorbed BSA could not be probed below this small-scale change. However, minor adsorption was highlighted in the drop in specific surface area, which was not due to bulk viscosity changes. This provides evidence for the mounting competitive displacement of BSA and adsorption of MeC that results almost exclusively from augmentation of MeC concentration because the concentration of BSA adsorbed tended to zero but the MeC concentration rose sharply surpassing that of BSA at 2.5 mg ml⁻¹ MeC (molar ratio MeC:BSA, $R_{cp} = 0.24$). MeC adsorption itself did not start at an

appreciable level until a concentration of 0.5 mg ml⁻¹ MeC in the emulsion. This anomaly of an apparent adsorption of either MeC or BSA corresponded to a minimum in the total adsorbed surfactant concentration in the concentration range 0.25–1 mg ml⁻¹ MeC and notably a minimum in the specific surface area possibly as a result of the formation of larger droplets. The minima indicates that inclusion of increasing concentrations of MeC weakened the droplet, in terms of its ability to form smaller droplets. This takes place until such a concentration that the volume of MeC available to the interface overcome this inadequacy. At low MeC concentrations the surface was dominated by a MeC–BSA complex which was displaced by MeC at higher concentrations. Such data provides strong circumstantial evidence for the mutual incompatibility of adsorbing MeC and BSA by a competitive displacement mechanism [11]. At concentrations > 1 mg ml⁻¹ MeC the augmentations of surface adsorbed MeC and displacement of BSA took place at a highly significant level. The concentration of surface adsorbed MeC began to stabilize over 5 mg ml⁻¹ MeC. Notably the surface concentration of MeC, on a weight basis, was much greater than that of BSA. This possibly indicates that only a small part of the MeC molecule, relative to its total size, was adsorbed at the interface [1] or that greater surface packing had taken place with each 1.49×10^{-5} mol m⁻² of BSA being replaced by 2.32×10^{-5} mol m⁻² of the larger MeC at the optimum surface loading for each substrate, corresponding to a MeC concentration of 1.6 times that of BSA.

The area per molecule of adsorbed surface active agent, determined from a surface balance technique, is presented in Fig. 5. The area per molecule of MeC was much greater than that of BSA at the same surface pressure values, except at compressions corresponding to very small molecular areas. Here the surface pressure and area per molecule were very similar for both MeC and BSA. On compression of the adsorbed surface film (decrease in area per molecule) little initial change in surface pressure was observed for MeC, whereas a sharp increase was noted for BSA. Both samples showed a sharp increase in surface

pressure when the compression corresponded to an area per molecule of 10–18 nm². With the MeC sample an apparent collapsing or large scale conformational change was observed at a pressure of 32 mN m⁻¹ corresponding to 6 nm². This peak followed by a plateau was not observed for BSA. This behavior may corresponds to desorption, but interdigitation and reptation of the MeC chains would explain the higher surface concentration of MeC compared with BSA (Fig. 4) because the surface pressures of 10–20 mN m⁻¹, corresponding to surface tension values of 60–50 mN m⁻¹ (Fig. 1), are far from this apparent collapse pressure [1]. This collapse pressure corresponded to a state where the adsorbed species were highly compressed and very closely packed, further compression in the interface of MeC is apparently not possible. Here the formation of a morphology resembling ‘compact sphere’ structures was evidenced by the displacement of MeC from the interface and the resulting drop in surface pressure on continued compression of the surface film. These structures, however, remained highly surface active because a plateau was observed in surface pressure on continued compression of the interface.

Fig. 5. Results of Langmuir trough measurements (air–water interface) with a compression of the surface that show the average area occupied per molecule of the surface adsorbed surfactant plotted against the resulting surface pressure after a thermal equilibration of 4 h. The samples tested were MeC alone (Δ) and BSA alone ($\bullet$).

Fig. 6. Changes in the average droplet diameter, d_{43} (circles) and specific surface area (squares) of *n*-hexadecane/water emulsions as a function of MeC concentration. The open symbols represent 5 mg ml⁻¹ BSA samples with or without the incorporation of MeC and the filled symbols represent MeC alone. The emulsion oil phase volume (ϕ) was 0.318. Errors bars are presented where they exceed the symbol size.

3.3. Competitive adsorption, flocculation and coalescence

The MeC-induced changes in droplet size (d_{43}) of emulsions made with the jet-homogenizer are presented in Fig. 6. We used the d_{43} volume mean droplet diameter to characterize our emulsions as the value characterized more fully the average of bell-shaped droplet size distributions than Sauter volume-surface mean (d_{32}) values which were biased toward the smaller droplet sizes. Emulsions formed from 5 mg ml⁻¹ BSA alone had an average droplet diameter of 2 ± 0.15 μ m. Incorporation of MeC into the emulsion increased droplet size but only with concentrations greater than an initial ‘lag’ range of concentrations of 0–0.1 mg ml⁻¹ MeC. This increase in droplet size when MeC concentrations over 0.1 mg ml⁻¹ were incorporated in the mixture also corresponded to the onset of the most significant decrease in emulsion specific surface area. Ultimately, the specific surface area of BSA/MeC mixtures and MeC alone were congruous, as expected from data showing that BSA was displaced from interface by high concentrations of MeC (Fig. 4). The deviation about an average droplet size was also

related to the MeC concentration above 0.75 mg ml^{-1} . This variation was reflected in the droplet size distributions, which showed a broadening at higher ($1\text{--}10 \text{ mg ml}^{-1}$) concentrations. The droplet size variation was particularly large when 5 and 10 mg ml^{-1} MeC was incorporated into the emulsions.

Measurements of MeC emulsions consolidate the results of creaming studies when only MeC was used to prepare emulsions. In the concentration range $0.1\text{--}5 \text{ mg ml}^{-1}$, the average droplet diameter changed from 38.0 to $5.8 \mu\text{m}$, respectively, showing the poor emulsification properties of MeC at low concentrations. Despite having larger droplets, viscosity increases appear to have effected large behavioural differences, such as those manifested in slowing of creaming (Fig. 2(b)) at higher MeC concentrations. Values of the specific surface area of MeC emulsions also indicated extremely poor emulsification properties since the specific surface area was in any case lower than with BSA. The discrepancy between moderate droplet size and an apparently large specific surface area at 5 mg ml^{-1} MeC resulted from a non-Gaussian size distribution with a large numbers of small and large droplets and that is congruous with poor emulsification. This was further evidenced at 10 mg ml^{-1} MeC where the specific surface area did not change much but average droplet size was halved, due to the creation of a higher proportion of very small droplets. A general broadening of the 'bell-shaped' droplet size distribution was observed with increasing concentrations of MeC. Moreover, change from an essentially mono (center of the size range, $\sim 2 \mu\text{m}$) to a more bidispersed-like system (centers of the size range, ~ 2 and $\sim 10 \mu\text{m}$) followed the incorporation of MeC into the mixture or by itself. In addition, the Mastersizer droplet distribution also indicated an increase in the number of submicron droplets as a function of MeC concentration.

Qualitative surface rheological data are presented in Fig. 7 and show shear stress versus shear rate for BSA, MeC and a BSA/MeC mixture. Interfacial shear stress for the same interfacial shear rate was greater for the individual components than for the mixture that indicates the in-

consistency of the adsorbed layer upon incorporation of the two competing surfactants. This evidently reflects the mutual adsorption of both amphiphiles but does not indicate a synergistic interaction between the two. Had such an interaction existed then the interface would have offered more resistance to the measure body and would have required a greater applied shear stress to achieve the same interfacial shear rate of the BSA or MeC systems alone [28]. In the case of BSA and MeC alone the shear stress at a given shear rate was higher for BSA than MeC. However, not much difference between the two individual components was noted indicating little difference in rigidity between the adsorbed layers. This was perhaps a reflection of the experiment equilibration time since BSA would usually be very slow to develop intermolecular interactions [1] which would be covalent in nature and should have produced a much more rigid interfacial network. The results of emulsion and interfacial studies are not directly comparable in that the relative surface areas to volumes are different. Consequently, it is possible that in studies at the macroscopic oil–water interface, the MeC dominated more than in emulsion studies. Thus, the process of emulsification would be expected to

Fig. 7. Surface rheological data presented as shear stress against shear rate of the adsorbed interfacial layer. These experiments on *n*-hexadecane-water macroscopic interfaces were performed on 0.1 mg ml^{-1} MeC alone (Δ), 5 mg ml^{-1} BSA alone ($\bullet$) and a 0.1 mg ml^{-1} MeC + 5 mg ml^{-1} BSA mixture ($\blacktriangle$) after an equilibration of 1 h.

Table 2

Measurement of flocculation in *n*-hexadecane/water emulsions using a bulk viscometry technique applied to solutions and emulsions at 20°C, 2 h after formation^a

Sample	Bulk viscosity solution, η_b (mPa.s)	Bulk viscosity emulsion, η_e (mPa.s)	Bulk viscosity normalized (η_e/η_b) (mPa.s)
BSA alone	1.02	4.2	4.1
BSA and 0.1 mg ml ⁻¹ MeC	1.03	4.2	4.1
BSA and 1 mg ml ⁻¹ MeC	1.51	6.6	4.3

^a The emulsion oil phase volume (ϕ) was 0.318.

change protein conformation radically, by means of shear-induced denaturation, facilitating more effective surface stabilization. Therefore, to replicate the equivalent conditions in emulsions with macroscopic interface studies we may have needed to perform experiments at adjusted MeC concentrations with respect to that of BSA. Further studies are needed to clarify this point. The relative surface composition at a bulk MeC:BSA molar ratio of $R_{cp} = 0.01$ in the studies here may not therefore fully correspond to the same surface composition when an identical formulation was used to prepare emulsions. It is perhaps for this reason and also in terms of surface composition that results of Table 1, which suggest a positive 'elasticity-inducing' interaction takes place between BSA and MeC in bulk solutions appear to conflict with interfacial studies. Indeed, it is unwise to make direct comparisons between bulk and surface behavior, given the competition for space at the interface. A lack of interfacial interaction seems highly plausible because hydrophobic regions of the protein adsorbed alone at the interface would be oriented away from the bulk solution, and thus unable to interact with hydrophobic regions of the MeC. Further studies are required to investigate this phenomenon. However, we are sure that addition of significant quantities of MeC to a BSA stabilized adsorbed layer reduces the 'rigidity' of the surface as in the case of milk proteins and emulsifiers [2,8,11,12].

In addition, we measured a rigidity and since all the flow curves demonstrated an apparent yield stress of the interface 0.05, 0.06 and 0.035 N m⁻¹ for MeC, BSA and BSA/MeC, respectively, selecting appropriate viscosity modeling conditions and

prediction of the contributions of viscous and elastic components were difficult. Shear flow curves were most revealing at shear rates $< 1 \text{ s}^{-1}$ since data indicated a disruption of structure in the adsorbed layer that seemed to be largely independent of shear rate at higher shear rate values, i.e. all the curves showed a tendency to merge. Quantification of viscoelastic components remains possible but beyond the scope of this article. Since measurements were performed to identify gross properties of the interface, comparisons of flow curves was sufficient for the purpose of this study.

In order to test the hypothesis that the increase in droplet size was possibly due to an increase in size due to the formation of flocculates and not to individual droplet growth, selected emulsions were assessed using bulk viscometry (Table 2). When emulsion viscosities were normalized for changes in the stock solution viscosity no significant difference was found between the samples. Formation of flocculates would be indicated by an increase in viscosity [34], whereas the contribution of minor changes in average droplet size for the samples measured (diameters between 2 and 4 μm) to the bulk viscosity would be negligible. Samples examined represented the control (BSA alone) and BSA in the presence of 0.1 and 1 mg ml⁻¹ MeC. The latter two samples produced an increase in the creaming rate (Fig. 2(b)). Apparently this increased creaming was related to droplet size differences and not to enhanced flocculation.

Droplet coalescence was investigated after sample storage. Experiments were performed at 20°C, where the *n*-hexadecane was fully liquid, to facilitate droplet fusion as it occurred. Gentle but thorough mixing of the samples prior to measure-

ment produced homogeneous samples and was insufficient to initiate mixing-induced coalescence. In a separate series of confirmatory experiments, no change in d_{43} as a result of this mixing (over a range of time periods) was observed. Coalescence presumably took place at a most significant level in the concentrated creamed fraction [33], on standing when droplets were in relatively close proximity to one another. The increase of droplet size (d_{43}) as a function of time is presented in Fig. 8. An insignificant change in droplet size was observed for BSA stabilized emulsions over a period of 0–7 days, indicating no significant droplet coalescence had taken place. BSA emulsions, which had 0.01 and 0.1 mg ml⁻¹ MeC incorporated in the mixture, showed an increase in droplet size that was greater at the higher MeC concentration. Emulsions which were formed from solutions containing 1 mg ml⁻¹ MeC showed a rapid and the largest change in average droplet size. In some instances the absolute values of droplet sizes were marginally different to those presented in Fig. 6, due to unavoidable day-to-day variation. Variations in the range of droplet size at higher MeC concentrations (> 0.75 mg

Fig. 8. Measurement of droplet coalescence expressed in terms of average droplet size (diameter, d_{43}) as a function of the time after *n*-hexadecane/water emulsion preparation. The initial measurement (day 0) was taken immediately after emulsion formation. Experiments were performed on 5 mg ml⁻¹ BSA based emulsions in the absence (●) and presence of 0.01 (Δ), 0.1 (□) and 1 mg ml⁻¹ (*) MeC. The emulsion oil phase volume (ϕ) was 0.318.

ml⁻¹) in BSA/MeC emulsions also indicated differing emulsifying properties. In any case, results of Fig. 8 were directly comparable, since these emulsions were prepared with the same stock solutions, same equipment and measured at the same time under identical conditions. All samples showed an initial lag to coalescence-induced changes which was in the order of a day and which scaled in magnitude as a function of MeC concentration. This along with preliminary surface rheological data, surface concentration measurements, surface tension measurements and bulk rheology indicated that the rigidity and anti-coalescence properties of the adsorbed layer were unfavorably correlated with increased MeC adsorption and the corresponding displacement of BSA from the interface.

The experimental data indicated rather complicated surface adsorption properties of methylcellulose and we proposed explanations for this behavior. In our studies we found the effect of MeC and BSA in the same emulsion to be antagonistic and showed that use of these two surface active materials to be generally detrimental to emulsion stability. In industrial applications there still exists the possibility for use of MeC and protein emulsifiers in the same mixture but only at very low concentrations of MeC. Enhancement of long-term stability or product 'shelf-life' by using MeC is not without its firm disadvantages unless used as the principal surfactant.

4. Conclusions

Methylcellulose (MeC) was investigated for its possible role as an emulsion stabilizing agent. Being hydrophobic in nature, the starting point of our study was to test the use of MeC as an agent that would enhance adsorbed layer rigidity via co-adsorption. Having used air–water measurements for some key experiments, the data were considered not to be directly applicable to emulsions in terms of the magnitude of values but very similar in modes of action and general behaviour. For coalescence and creaming studies we have been able to delineate bulk viscosity effects from

droplet size-related effects. Flow curves of shear stress versus shear rate were used to assess the properties of the macroscopic interface.

A destabilization of O/W emulsions, stabilized by BSA in the presence of MeC was measured. This destabilization was related to three phenomena. Firstly, the competitive displacement or competitive adsorption of MeC at the oil–water interface over that of BSA. Secondly, the increase in oil droplet size which accompanied the increasing adsorption of MeC at concentrations $> 0.1 \text{ mg ml}^{-1}$ and thirdly, the decrease in surface rigidity that was congruous with an increase in the ratio of adsorbed MeC to BSA. This increase in adsorbed MeC facilitated increased droplet coalescence. No evidence of flocculation enhancement in the presence of MeC was found.

Inclusion of low concentrations of MeC (0.01 mg ml^{-1}), in BSA solutions reduced the creaming rate of emulsions presumably by altering the buoyancy properties of the droplets as a consequence of limited MeC co-adsorption with binding of MeC by BSA, also indicated in surface tension studies. Further adsorption of MeC reduced surface rigidity and thus facilitated droplet fusion and inefficient emulsification that produced an increase in average droplet size. Such an increase in droplet size favoured enhanced creaming rates but, at concentrations in the range $1\text{--}10 \text{ mg ml}^{-1}$ MeC, it was superimposed on mounting bulk viscosity changes, attributed to the MeC alone, that actually retarded creaming. This viscosity related reduction in creaming was most pronounced at $5\text{--}10 \text{ mg ml}^{-1}$ MeC.

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