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Stability of emulsions stabilised by two physiological surfactants: L-α-phosphatidylcholine and sodium taurocholate

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Abstract

The emulsion phase formed within the stomach and duodenum during digestion of a fatty meal has been modelled using two physiological surfactants, the phospholipid L-α-phosphatidylcholine (PC) and the bile salt sodium taurocholate (NaT). Upon dilution of the phospholipid stabilised emulsions with a solution of NaT the bile salt became incorporated into the oil/water interface imparting a negative charge to the droplet surface. The magnitude of the droplet microelectrophoretic mobility for the mixed PC and NaT system was 47% of that found for emulsion droplets stabilised by NaT alone. But the electrostatic repulsion between droplets was not sufficient to account for the observed improvement in emulsion stability to coalescence. It is suggested that a residual liquid crystalline phospholipid interface is present imparting a significant steric component to the stabilisation of the emulsions droplets.

Keywords: Emulsion stability; Surfactants; L-α-phosphatidylcholine; Sodium taurocholate

1. Introduction

After ingestion dietary lipid passes through a number of colloidal phases prior to absorption by the mucosa of the small intestine. These phases are stabilised by surfactants either produced by the gut itself or intrinsic to the diet. Principal amongst these are phospholipids, fatty acids, cholesterol and the bile salts. The physicochemical properties of these surfactants, in isolation, have been investigated in some depth [1] but their possible synergistic interactions have not attracted so much interest. We have undertaken a study of the surfactants available during digestion of lipid in the mammalian gut.

Upon ingestion of lipid, a crude emulsion is formed by mastication and subsequent travel to the stomach. The principal emulsifiers are thought to be phospholipids either intrinsic to the diet or supplied by the gastric mucin. These have a complex chemistry, Fig. 1 and they do not form true solutions but display lyotropic mesomorphism [2]. Phase diagrams illustrating the forms these liquid crystalline phases can take are readily available in the literature [3].

The emulsion is held within the stomach for a number of hours before ejection, via the pylorus sphincter, into the duodenum where it is exposed to the surface-active components of the bile (i.e. the bile acids, cholesterol and more phospholipid). The bile acids are conjugated with either taurine or glycine. This amidation lowers the pK_a value significantly resulting in a pK_a approaching 0 in the

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taurine conjugates. Thus, at the pH of the duodenum, the acids are in an ionised form. They have a rigid 5β ring structure, Fig. 1, allowing a hydrophilic (α-)face and a hydrophobic (β-)face to be maintained. This unusual structure leads to the formation of micelles but over a range of concentrations which is broader than found with conventional surfactants and micelle formation tends to be stepwise [4]. Upon contact with the lipid emulsion, the bile salts are thought to become incorporated into the interface imparting a negative charge to it, but, as yet, no in vitro quantification of this has been attempted.

There are two factors which play an important role during the subsequent digestion of the droplets by the pancreatic enzyme lipase; (1) the extent to which bile molecules become incorporated into the droplet interface and (2) the effect of the resultant mixed interface on the subsequent stability of the droplets [5]. For optimum lipase activity the droplets must be non-flocculated and of small diameter i.e. stable and of large *accessible* surface area. In many cases competitive adsorption or the formation of a mixed interfacial layer of surfactants enhances colloid stability [6], but such studies have not been undertaken on surfactants present in the gut lumen during digestion. Our in vitro study investigates the behaviour of two representative surfactants; the phospholipid, L-α-phosphatidylcholine, and the bile salt, sodium taurocholate, to determine whether the addition of bile to model duodenal emulsions improves their stability. The mechanisms of emulsion stabilisation with each surfactant both in isolation and in combination, at physiological concentrations, are discussed. The migration of bile acids to the phospholipid interface has been investigated semi-quantitatively by measurement of the droplet micro-electrophoretic mobility.

2. Experimental

2.1. Materials

2.1.1. Emulsifiers

The emulsifiers used were purified L-α-phosphatidylcholine (PC), grade 1 quality (chromatographically pure (TLC) in a number of solvent systems) derived from egg yolk and supplied by Lipid Products, South Nutfield, Surrey, UK; and 98% sodium taurocholate (NaT), molecular weight 537.7 daltons, supplied by Sigma. The phospholipid was initially dried under a stream of purified N_2 and the drying continued in vacuo (48–72 h), until a constant dry weight was obtained. The purified phospholipid was hydrated in 0.1 M citric acid, 0.2 M disodium orthophosphoric acid buffer (40 mM, pH 6.6) for 1 hour on a microid flask shaker (at room temperature, under N_2 and in the absence of light) to give a 10% w/w dispersion.

2.1.2. Olive oil

The free fatty acid content of the olive oil (highly refined, low acidity Sigma Chemical Company 01500) was estimated to be 0.13% [7]. The oil was used without further purification following evidence that this level of impurities has been found to have no effect on phospholipid adsorption at the interface [8].

2.1.3. Emulsion preparation

For each emulsifier a concentrated premix emulsion was prepared of 8% w/w olive oil by emulsification of an aqueous dispersion of the surfactant with the oil. Unless otherwise stated, emulsification was achieved with a reverse-flow microfluidiser model M-1206 (Christiansen Scientific Equipment Ltd. Gateshead, UK) at 100 bar pressure to produce a size distribution of approximately 1.0 μm weight mean droplet diameter.

The premix was diluted with a continuous phase of 0.56 M sodium chloride (Analar grade BDH, Poole, UK) and sodium azide (0.8% w/w) in diluted stock 0.1 M citric acid, 0.2 M disodium orthophosphoric acid buffer solution (31.2 mM, pH 6.6), and a 58.8 mM stock sodium taurocholate solution to produce a series of 2% wt. oil emulsions containing 6.5 mM PC and 0, 2.4, 4.8, 7.2, 9.6, 12 mM NaT at 0.15 M NaCl and 0.2% w/w sodium azide. Following mixing (by inverting the container ten times) the emulsions were kept continually agitated for 1000 hours on a Gallenkamp thermostatted orbital shaker, setting number 4, at 37°C. At appropriate time intervals samples were drawn from the centre of the agitated emulsion for size analysis. The oil content of the emulsions was determined from density measurement.

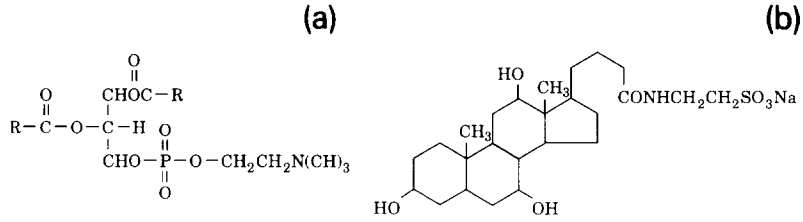

Fig. 1. Chemical structure of; (a) L-α-phosphatidylcholine (where R = 32% palmitic, 13% stearic, 31% oleic and 15% linoleic acid) and (b) sodium taurocholate.

2.2. Methods

2.2.1. Droplet size distribution

The droplet size distributions of the pre-mix emulsions and subsequent monitoring of droplet size distributions were determined using a Malvern Mastersizer (Malern Instruments) light diffraction sizer.

2.2.2. Density

The density of all continuous phases and the dispersed phase were measured using a Anton Paar DMA60 vibrating tube density meter with a DMA602 measuring cell. Changes in the oil content of the test emulsion with time, as a result of the gradual breaking of the emulsion, were calculated from density measurements.

2.2.3. Droplet microelectrophoretic mobility

The mobility of the emulsion droplets was measured at 20.0°C using either a Rank Mark II apparatus using a rectangular cell in two electrode operation or a Malvern Instruments Zetasizer 3 with a AZ4 capillary electrophoresis cell. Unless otherwise stated, the continuous phase employed as diluent for each emulsion was prepared to the exact aqueous composition of the emulsion. No attempt was made to correct the surfactant composition for adsorption onto the oil/water interface. Although the phospholipid was homogenised prior to emulsion preparation, the continuous phase, containing bile salt concentrations below 6 mM, remained too turbid for accurate mobility measurement. At bile concentrations equal to or greater than 6 mM, this turbidity decreased (within 24 hours at 37°C) with the production of mixed micelles.

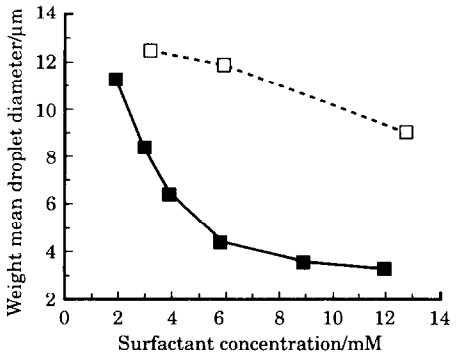

Fig. 2. The weight mean droplet diameter obtained using a fixed shear cycle with varying concentrations of either NaT or PC. (–) NaT; (---) PC.

3. Results

3.1. Initial droplet size distribution

3.1.1. Single emulsifier system

For each emulsifier the droplet size distribution of the premix was determined for a range of concentrations analogous to those found in vivo. Fig. 2 shows the weight mean droplet diameters obtained from emulsions formed using each surfactant at 2% w/w oil, prepared using a fixed shear cycle of a Waring blender. For NaT stabilised emulsions the mean droplet diameter was found to plateau above 6 mM NaT. This concentration corresponds to the critical micelle concentration, c.m.c., of NaT in 0.15 M NaCl [9]. Such behaviour is expected for an anionic surfactant [10]. Unlike short chain phospholipids (C6–C9) which form micellar aggregates in dilute solution those with chains > C10 do not form micelles but insoluble liquid crystalline bilayers when added to water. Thus with PC stabilised emulsions a gradual decline in weight mean droplet diameter was observed with increasing surfactant concentration.

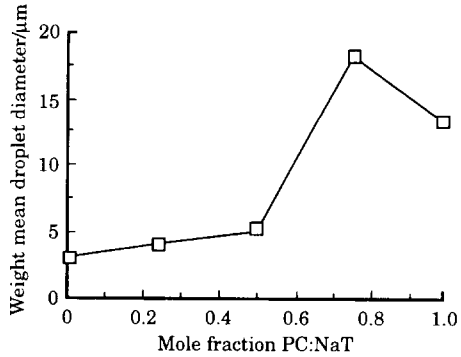

Fig. 3. The weight mean droplet diameter obtained for varying ratios of PC:NaT at a constant surfactant concentration.

3.1.2. Mixed emulsifier systems

Under the same shear cycle as above, a mixture of the two surfactants, PC and NaT, was used at constant total surfactant concentration (molarity), to produce a 2% w/w emulsion. Fig. 3 shows the weight mean droplet diameter obtained for varying ratios of PC:NaT. Only above the c.m.c. of NaT (6 mM) was the droplet size smaller then when only PC was present.

3.2. Microelectrophoretic mobility

3.2.1. Single emulsifiers

The microelectrophoretic mobility of emulsion droplets stabilised with varying concentrations of NaT are shown in Fig. 4 for a continuous phase at 0 M NaCl and 0.15 M NaCl. The mobility is shown to rise significantly upon inclusion of NaT reaching a plateau at and above the c.m.c. in parallel with the changes found in the initial weight mean droplet diameter. Upon investigation of the behaviour of the droplets to electrolytes i.e. NaCl and $CaCl_2$, the mobility of the droplets formed at 6 mM NaT was found to decrease with increasing ion concentration, Fig. 5. The concentration range over which this decrease was observed was significantly lower for divalent calcium ions compared to sodium ions. This behaviour is expected for indifferent electrolytes interacting with a electrostatically stabilised system.

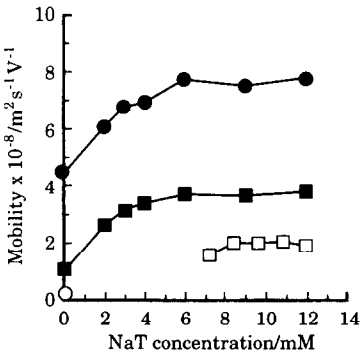

Fig. 4. The microelectrophoretic mobility of emulsion droplets with increasing NaT concentration and in the presence or absence of 6.5 mM PC. (■) NaT/salt; (●) NaT/water; (□) PC/Nat; (○) PC.

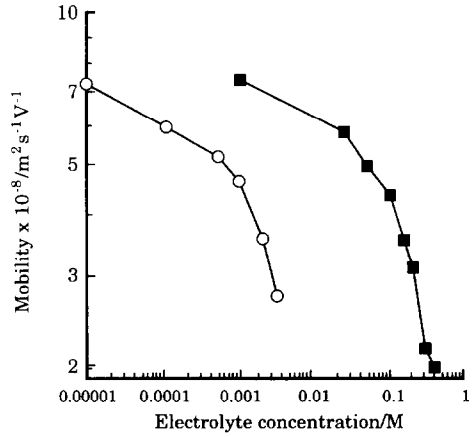

Fig. 5. Variation of microelectrophoretic mobility with increasing indifferent electrolyte for NaT emulsions (12 mM). (■) NaCl; (○) $CaCl_2$.

For PC stabilised emulsions, the zwitterionic headgroup of the surfactant allows pH to be the potential-determining ion as reported by other workers [11]. For our system, an isoelectric point at approximately pH 5 was found but throughout the pH range of 3–8 the magnitude of the zeta potential was no more than 3 mV.

3.2.2. Mixed surfactant system

Similar results are found irrespective of the method of preparation of the emulsion i.e. whether the emulsion has been homogenised in the presence of both surfactants or if it has been prepared with the phospholipid and diluted by addition of NaT. Fig. 4 shows the mobility measured for droplets at greater than 6 mM NaT but at a constant PC concentration of 6.5 mM. The mobility of the emulsion droplets prepared with both surfactants is 47% of the value for those prepared with NaT at the same concentra-

tion. This would suggest that the inclusion of NaT into the oil/water interface has been restricted by the presence of the PC and it does not reach the same interfacial concentration as observed when the only surfactant present is NaT.

3.3. Stability to coalescence of emulsions at varying concentrations of surfactant

For all emulsions prepared with NaT, either as the sole surfactant or in combination with PC, no persistent flocculation was observed when the emulsions were monitored by optical microscopy whereas for PC stabilised emulsions flocculation was present. By combining the droplet size distribution measured at a given time with the corresponding oil volume fraction determination, it was possible to estimate the number of droplets present in the emulsion. The rate of decrease in droplet number was then used to monitor emulsion instability. This indirect method of droplet counting does not take into account the composition of any flocs but treats the flocs as single particles. All emulsions were monitored continuously at approximately 12 hour intervals; the data given here is a representative sample of those results.

3.3.1. Single surfactant

For both single surfactant species the stability to coalescence of emulsions formed was strongly dependent upon the concentrations of the surfactant present. For NaT the stability increased only gradually at concentrations below the c.m.c. but improved dramatically above the c.m.c. reaching a maximum at 12 mM NaT [12]. For PC at 6.5 mM measurable coalescence was observed from the onset of the monitoring, and at higher concentrations enhanced stability was observed, as in previous work [12].

3.3.2. Mixed surfactant

Exposure of a phospholipid (6.5 mM) stabilised emulsion to NaT (by appropriate dilution of the concentrated premix) at concentrations as low as 2.4 mM resulted in a significant improvement in stability to coalescence (Fig. 6). But the stability was not improved by further increase in NaT concentration. A significant lag phase was observed prior to the onset of measurable coalescence for all emulsions containing NaT. When both surfactants were present this lag increased significantly.

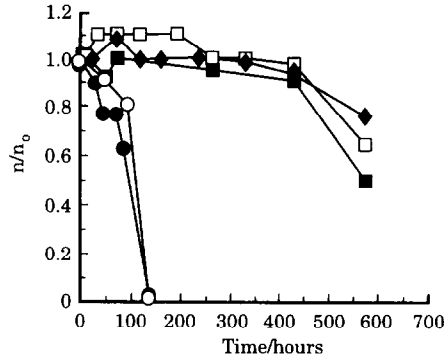

Fig. 6. Variation in droplets number concentration with time for PC (6.5 mM) stabilised emulsions exposed to varying NaT concentration. (○) 12 mM NaT; (□) 6.5 mM PC/2.4 mM NaT; (◆) 6.5 mM PC/7.2 mM NaT; (■) 6.5 mM PC/12 mM NaT; (●) 6.5 mM PC.

3.4. Observation of birefringence

When viewed under cross-polarisers all emulsions prepared with PC, either as the sole surfactant or in combination with NaT displayed birefringence at the droplet interface. No comment can be made as to the variation in intensity of the birefringence observed with different surfactants, due to the changes in size distribution and stability of the emulsions. Calculation of the surface area of the PC stabilised emulsions indicate that there is sufficient PC present to form multilayers at the emulsion interface. A value of 75 Å^2 was taken as the effective surface area of the PC molecule when adsorbed at an oil–water interface.

4. Discussion

The free energy changes associated with emulsion breakdown, ΔG_{break} can be considered as a combination of those associated with flocculation and those of coalescence [10].

$$\Delta G_{break} = \Delta G_{flocc} + \Delta G_{coal} \quad (1)$$

For all emulsions containing NaT there was no evidence of persistent flocculation implying a very small or zero barrier to coalescence from the flocculated state. But for the emulsions stabilised only by PC flocculation was present indicating this barrier to be significant.

The factors influencing the free energy of flocculation can be discussed by consideration of the interaction free energy between two approaching droplets. In the absence of polymeric species within the continuous phase, an interaction potential, G_t, may be calculated from a linear combination of the attractive van der Waals potential, G_a and the repulsive electrostatic potential, G_r, present by virtue of the surface charge of the droplets [13].

$$G_t = G_a + G_r \tag{2}$$

Each of these components is dependent upon the droplet size distribution. The electrostatic factor is calculated from the surface electrical potential, approximated by the zeta potential. Addition of an electrolyte shields the electrostatic repulsion and hence decreases the repulsive term. Calculation of G_t for NaT and the PC stabilised emulsions, using only these electrostatic and van der Waals contributions with appropriate Hamaker constants, has shown neither system to have a sufficiently high electrostatic contribution to overcome the van der Waals attraction and a potential energy minimum is predicted. Due to the lower electrostatic repulsion of the PC stabilised system, a larger potential energy minimum was predicted than for a NaT stabilised system but at PC concentrations > 6.5 mM considerable stability has been found [14]. This suggests that a steric component to the interaction potential is present and/or the phospholipid interface has a significant resistance to rupture once the droplets have flocculated. Absorption of surfactants of sufficient size can result in the formation of a 'steric' barrier to flocculation. Alternatively an emulsifier system can form a strong interfacial layer which will resist rupture and hence coalescence upon close approach of another interface. This layer may be formed by strong intermolecular attractions as seen with proteins or metaphase formation [10]. The observation of birefringence for lecithin stabilised emulsions indicates the origin of the improved stability to be the formation of a liquid crystalline interface surrounding the emulsion, this is in accord with previous work [14]. Such LC layers are known to change the distance dependence of the van der Waals interaction energy between the droplets reducing the effective attractive interaction [10]. The observation of persistent flocculation would also suggest a significant barrier to coalescence.

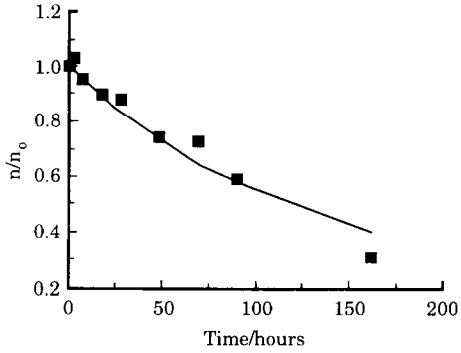

Fig. 7. Variation in droplet number concentration with time for PC stabilised emulsions (6.5 mM) and expected behaviour calculated from Van den Tempel formulation (Eq. 3). (■) Measured; (–) calculated.

Attempts to determine the relative importance of these two effects by fitting a rate equation for the observed instability of the emulsions have been partially successful. A treatment based on Van den Tempel's [15] formulation:

$$n = \frac{n_0}{1 + kn_0 t} + kn_0^2 \frac{t}{(1 + kn_0 t)^2} \times \left[\frac{kn_0}{K} + \left(1 - \frac{kn_0}{K}\right) \exp(-Kt) \right] \tag{3}$$

was used where n_0 is the initial number of droplets present and n those present at time t. The first term on the right-hand side represents the number of particles which would have been present if each droplet had been counted as a single particle and the second term takes into account the composition of the aggregates. This gives two rate constants; for aggregation, k, and for coalescence, K. It can be seen that Eq. 3 reduces to the Smoluchowski relationship if K approaches infinity. With PC stabilised emulsions the fit was good as shown in Fig. 7 but the solution was insensitive to k, the form of the curve being dominated by K which was found to be 10^{-6} s^{-1}. This is consistent with the breakup of flocs during droplet size analysis and a relative stability towards coalescence.

The bile salt NaT behaves as a simple anionic surfactant; producing charge stabilised emulsions of

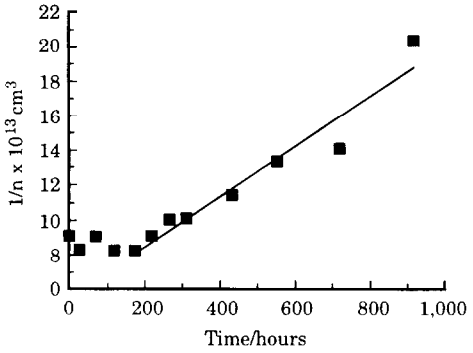

Fig. 8. Variation of reciprocal droplet number concentration with time for emulsion prepared with PC (6.5 mM) and exposed to NaT (12 mM). (■) Measured; (–) calculated.

limited stability above its c.m.c.. No evidence for the formation of persistent flocs was found; a separate oil phase formed upon detection of a significant increase in the droplet size distribution and microscopic examination of the emulsion revealed no persistent flocs. Attempts to fit the rate Eq. (3) were unsuccessful mainly due to a significant 'lag time' prior to the onset of measurable coalescence. Once coalescence had been detected, however, the data fitted the Smoluchowski relationship well i.e. $1/n$ varied linearly with time (Fig. 8). This indicates that K approaches infinity and the interface offers little resistance to rupture and coalescence. The origin of the 'lag phase' could be an initially slow rate of floc formation (these break up upon analysis) hence coalescence is slow. Once flocculation is established coalescence proceeds at a significant rate.

When the phospholipid interface was exposed to NaT, however, cooperative adsorption of the bile salt onto the interface was evident by the increase in the microelectrophoretic mobility of the droplets. The observed stability of the resulting emulsion was enhanced over that obtained at equal total surfactant concentrations of single surfactants. Thus, the improved stability can not be only a consequence of an increase in the electrostatic repulsion between droplets as it is far beyond that shown by emulsions stabilised by 12 mM NaT. No persistent flocculation was observed in the emulsions but, as with the NaT stabilised emulsions, a significant 'lag' was observed prior to measurable coalescence. Once coalescence was established Smoluchowski-type kinetics were observed.

It is suggested that the improved stability of the emulsions prepared with both surfactants over those containing only one, is a consequence of a combined steric/electrostatic stabilisation mechanism. The retention of a birefringent interface would suggest a residual LC layer but one which has a significant surface charge. The reduction in the barrier to coalescence, however, would indicate the LC layer to be significantly reduced. There are a number of studies within the literature on the NaT/PC phase behaviour in the absence of oil. Perhaps the most comprehensive is a study by Mazer et al. [16] in which quasielastic light scattering was used to characterise the aggregated structures formed at lipid concentrations from 0.625–10 g/dl. These figures suggest that at NaT concentrations below 6.5 mM the mixed surfactant system would consist of bilayers and liposomes whereas at higher NaT concentrations a mixed micellar phase would exist. This is in agreement with our own observations of the turbidity of the emulsion continuous phase when studied in the absence of oil. Those for which the NaT concentration was above 6.5 mM produced a clear dispersion upon standing for 4 days whereas those at lower concentration remained turbid. This would suggest that the residual LC layers observed at the higher NaT concentrations may be a consequence of the system not having achieved a steady state at the interface. This is indicated by the complex transport processes required for the growth of liquid crystalline interfaces requiring days or even weeks for completion [17].

5. Conclusions

We have shown the physiological surfactants, L-α-phosphatidylcholine and sodium taurocholate, to have a synergistic interaction at the emulsion interface which significantly enhances its stability to flocculation. The bile salt has been shown to become incorporated into the droplet interface imparting a surface charge. But the increased electrostatic repulsion between droplets would not be sufficient to explain the observed improvement in stability. It is suggested that a residual LC layer to the interface is present imparting a significant steric stabilisation to

the emulsion droplets. This suggests that in the gut lumen the incorporation of bile salts into the emulsion interface reduces the tendency of the droplets to flocculate thereby maintaining a maximum surface area. The reduction in resistance to coalescence suggests significant modification to the droplet interface with possible consequences for lipase activity at the interface. Whether these changes to the interface are responsible for the 'bile salt activation' of the lipase is being investigated at present.

Acknowledgements

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