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Research paper

Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions

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ABSTRACT

The effect of low-molecular weight surfactants on the digestibility of lipids in protein-stabilized corn oil-in-water emulsions was studied using an *in vitro* digestion model. The impact of non-ionic (Tween 20, Tween 80, Brij35), anionic (SDS), and cationic (DTAB) surfactants on the rate and extent of lipid digestion was studied. All surfactants were found to inhibit lipid digestion at sufficiently high concentrations, with half-maximal inhibitory concentrations (IC50) of 1.2% for Tween 20, 0.7% for Tween 80, 2.8% for Brij35, 1.1% for SDS, and 1.4% for DTAB. The effectiveness of the surfactants at inhibiting lipid digestion was therefore not strongly correlated to the electrical characteristics of the surfactant head group, since the IC50 increased in the following order: Tween 80 > SDS > Tween 20 > DTAB > Brij35. The ability of these low-molecular weight surfactants to inhibit lipid digestion was attributed to a number of potential mechanisms: (i) prevention of lipase/co-lipase adsorption to the oil–water interface; (ii) formation of interfacial complexes; (iii) direct interaction and inactivation of lipase/co-lipase. Interestingly, DTAB increased the rate and extent of lipid digestion when present at relatively low concentrations. This may have been because this cationic surfactant facilitated the adsorption of lipase to the droplet surfaces through electrostatic attraction, or it bound directly to the lipase molecule thereby changing its structure and activity. A number of the surfactants themselves were found to be susceptible to enzyme digestion by pancreatic enzymes in the absence of lipids: Tween 20, Tween 80, Brij35, and DTAB. This work has important implications for the development of emulsion-based delivery systems for food and pharmaceutical applications.

1. Introduction

The increasing number of poorly water-soluble drug candidates in development in the pharmaceutical industry calls for advanced drug delivery systems (DDSs) that are able to increase bioavailability [1–3]. The administration of drugs *via* the oral route is usually preferred, with about 80% of the most common pharmaceutical products in United States and European markets being given orally [4]. Lipid-based DDSs offer a particularly convenient means of encapsulating, protecting, and delivering poorly water-soluble drugs and nutraceuticals *via* the oral route for pharmaceutical and food applications [5,6]. A variety of DDS based on lipids are available, including oil solutions, micelles, microemulsions, nanoemulsions, emulsions, and solid lipid nanoparticles [5,6]. Recently, there has been considerable interest in the utilization of self-micro-emulsifying (SMEDDS), self-nano-emulsifying (SNEDDS), and self-emulsifying (SEDDS) drug delivery systems, which spontaneously form microemulsions, nanoemulsions, or emulsions in the gastrointestinal tract (GIT) after ingestion. Many of these lipid-based DDS can be prepared using simple preparation procedures and have been shown to be effective at increasing the bioavailability of poorly water-soluble drugs [7,8]. The physicochemical mechanisms by which these different lipid-based DDS systems can increase bioavailability depend on their composition and structure.

In this manuscript, we focus on DDSs that are based on encapsulating highly lipophilic drugs within a carrier lipid phase, that is then homogenized to form an oil-in-water emulsion or nanoemulsion. The bioavailability of lipophilic drugs encapsulated within these emulsion-based DDSs is strongly influenced by the dispersion, digestion, and solubilization of the carrier lipid, digestion products, and encapsulated drug within the GIT. One of the most commonly used carrier lipids in emulsion-based DDSs are triacylglycerols, such as medium chain triglycerides (MCT) and long-chain triglycerides (LCT). Digestion of the triglyceride carrier lipid is usually initiated in the stomach due to gastric lipases, but then occurs predominately in the small intestine due to pancreatic lipases [5,9–11]. The digestion products formed by triacylglycerol digestion (free fatty acids [FFA] and monoacylglycerols [MAG]) participate in the formation of mixed micelles and other colloidal structures within the small intestine that are capable of increasing the solubilization and absorption of highly lipophilic components

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[12]. The rate and extent of the digestion of carrier triglycerides within the GIT is therefore one of the most important factors determining the performance of lipid-based DDSs [12–14]. If the digestion of the carrier triglycerides is inhibited, then there will be fewer mixed micelles available to solubilize and transport any encapsulated lipophilic drugs. In addition, an undigested triglyceride phase may act as a non-polar solvent for the lipophilic drugs that restricts their release into the surrounding intestinal fluids. For example, recent *in vitro* and *in vivo* studies have shown that increasing the ratio of surfactant to digestible triglycerides reduced the release and absorption of a lipophilic drug (danazol) from SEDDS [15,16].

In this study, we focus on the influence of small-molecule surfactants on the rate and extent of lipid digestion under simulated GIT conditions. Surfactants may be present in the GIT because they are an integral part of a lipid-based DDS or because they form part of a food matrix that is co-ingested with the DDS. For example, relatively high levels of surfactants are present in certain types of delivery systems (e.g., SMEDDS, SNEDDS, and SEDDS) and food products (e.g., dressings, sauces, and beverages). Some surfactants are known to interfere with lipid digestion because they can adsorb to lipid droplet surfaces, thereby altering the ability of lipase to come into contact with the lipid phase [17–20].

An *in vitro* digestion model ("pH stat") is used in this study to systematically examine the influence of surfactant type and concentration on lipid digestion under simulated small intestine conditions. The influence of various experimental variables on the rate and extent of lipid digestion have previously been studied using similar *in vitro* digestion models, including the composition of the simulated intestinal fluid [21], temperature, pH [22], lipid droplet size and composition [19,20,23–25], and polysaccharide addition [26–28]. However, few studies have focused on the influence of free surfactant on the activity of pancreatic lipase. Gargouri and co-workers showed that four kinds of non-ionic surfactants could strongly inhibit enzyme activity: Brij35, Triton-X 100, polysorbate 20, and polysorbate 80 [29]. It was proposed that the surfactant molecules bound to the lipid droplet surfaces, thereby disturbing the equilibrium between active and inactive forms of lipase. McGregor and co-workers showed that lipid digestion could be completely inhibited over a 90 min period when a lipid (medium chain triglycerides) and a non-ionic surfactant (Cremophor RH 40) were present in equal masses [13]. Johnston and co-workers showed that Poloxamer 407, a copolymer consisting of a central hydrophobic block of polypropylene glycol between two hydrophilic blocks of polyethylene glycol, was able to inhibit pancreatic lipase [30]. Finally, Chiristiansen and co-workers showed that a number of non-ionic surfactants could inhibit the digestion of triacylgylcerols by lipase (polysorbate 80, Cremophor EL, Cremophor 80 and D-α-Tocopheryl polyethylene glycol (1000) succinate), thereby reducing the amount of digestion products available for forming mixed micelles capable of enhancing drug solubilization [31].

In this study, we examined the effects of both non-ionic and ionic small-molecule surfactants on lipid digestion using simulated GIT conditions. In particular, we compared the impact of non-ionic (Tween 20), anionic (sodium dodecyl sulfate, SDS), and cationic (dodecyl trimethyl ammonium bromide, DTAB) surfactants with similar tail groups (lauric acid, C_{12}) so as to determine the influence of head group charge characteristics.

2. Experimental section

2.1. Materials

Powdered β-lactoglobulin (BLG) was obtained from Davisco Foods International (Lot # JE 002-8-415, Le Sueur, MN, USA). Tween 20 (T20, Lot# 1131 K) was purchased from MP Biomedicals LLC. (Solon, OH, USA). Tween 80 (T80, Lot# 50K0212) and lauryl sulfate (SDS, Lot# 118H0274) were purchased from the Sigma–Aldrich (St. Louis, MO). Brij35 (Lot # A0274549) and DTAB (Lot# A0221782) were purchased from Acros Organics (Morris Plains, NJ, USA). Corn oil was purchased from a local supermarket and used without further purification. Corn oil contains ⩾99% triacylglyceride, with proportions of approximately 59% polyunsaturated fatty acid, 29% monounsaturated fatty acid, and 13% saturated fatty acid [32]. Lipase from porcine pancreas, Type II (L3126, triacylglycerol hydrolase E.C. 3.1.1.3, PPL), and bile extract (porcine, B8613) were purchased from Sigma–Aldrich (St. Louis, MO, USA). It has been reported that lipase activity is 100–400 units/mg protein (using olive oil) and 30–90 units/mg protein (using triacetin) for 30 min incubation. The composition of bile has been reported: total bile salt content = 49 wt.%; with 10–15% glycodeoxycholic acid, 3–9% taurodeoxycholic acid, 0.5–7% deoxycholic acid, 1–5% hydrodeoxycholic acid, and 0.5–2% cholic acid; 5 wt.% phosphatidyl choline (PC); Ca^{2+} < 0.06 wt.%; CMC of bile extract 0.07 ± 0.04 mM; the mole ratio of BS to PC being around 15–1. Calcium chloride ($CaCl_2{\cdot}2H_2O$) and sodium chloride (NaCl) were obtained from Fisher Scientific (Pittsburg, PA, USA). Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich (St. Louis, MO). Purified water from a Nanopure water system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used for the preparation of all solutions.

2.2. Solution preparation

Emulsifier solutions were prepared by dispersing 1.0 wt.% of protein emulsifier (BLG) into 5 mM phosphate buffer solution (pH 7.0) and stirring for at least 2 h. The solutions were kept overnight at 4 °C to ensure complete hydration. Surfactant (T20, T80, Brij35, SDS, and DTAB), NaCl and $CaCl_2$ solutions were prepared by separately dissolving weighed amounts of chemicals in double-distilled water.

2.3. Emulsion preparation

A stock emulsion was prepared by homogenizing 10 wt.% corn oil with 90 wt.% aqueous emulsifier solution (1.0 wt.% BLG, pH 7.0) with a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland) followed by five passes at 9000 psi through a high pressure homogenizer (Microfluidics M-110Y, F20Y 75 μm interaction chamber, Newton, MA). Previous studies have shown that this level of lipid and emulsifier are convenient for forming protein-stabilized emulsions or nanoemulsions [33,34].

The stock emulsion was diluted by buffered surfactant solutions to obtain a series of 5% corn oil-in-water emulsions containing different types and amounts of surfactant. The particle size, surface charge, and physical stability of the samples were measured after overnight storage.

2.4. In vitro digestion model

The *in vitro* digestion experiment was carried out at 37 °C by mixing emulsion, phosphate buffer, and simulated intestine fluid (SIF). The final composition and physicochemical properties of the digestion medium are listed in Table 1. Lipid digestion kinetics was monitored by measuring the release of free fatty acids from the samples after lipase addition. Lipase converts triacylglycerols (and some other lipids with ester bonds) into monoacylglycerols (MAG) and free fatty acids (FFA), which would normally lead to a reduction in solution pH. However, the amount of FFA released can be quantified by adding NaOH solution (0.15 M) to maintain the pH at a constant level of 7.0 using an automatic titration unit,

Table 1
The final composition of materials in the simulated small intestine digestion medium (total volume = 37.5 mL, pH = 7.0).

NaCl (mmol/L)	$CaCl_2$ (mmol/L)	Bile salt extract (mg/mL)	Pancreatic lipase (mg/mL)	Corn oil (wt.%)	Phosphate buffer[a] (mmol/L)
150	5	5	1.6	1	5

[a] The phosphate buffer contained different types and concentrations of surfactants (0–5 wt.%).

.i.e., which is referred to as the "pH-stat" method (835_1 Titrino, Metrohm, Riverview, Florida). The amount of NaOH added is proportional to the amount of FFA produced. Blank experiments were also carried out without the addition of enzyme to account for any decrease in pH-value due to other factors. The hydrolysis of the surfactants was also analyzed in the absence of the corn oil-in-water emulsions using the pH Stat method. The *in vitro* digestion experiments were repeated two or three times for each sample.

2.5. Particle size and ζ-potential measurements

Particle size and ζ-potential were determined using a commercial dynamic light scattering and micro-electrophoresis device (Nano-ZS, Malvern Instruments, Worcestershire, UK). The samples were diluted 100 times in appropriate buffer solution at room temperature before measurement. The particle size data are reported as the *Z*-average diameter, while the particle charge data are reported as the ζ-potential. Measurements were conducted at ambient temperature (22 °C) and results are reported as the average of 5 measurements.

3. Results and discussion

3.1. Effect of surfactant addition on properties of protein-stabilized lipid droplets

Initially, we characterized the physicochemical properties of the protein-stabilized corn oil-in-water emulsion used as a model emulsion-based drug delivery system (DDS) in this study. The emulsions contained droplets that were negatively charged (−65.5 ± 2.3 mV), relatively small (d = 183.7 ± 1.6 nm) and had a narrow polydispersity index (PDI = 0.15 ± 0.01). The anionic nature of the protein-coated droplets can be attributed to the fact that BLG is negatively charged above its isoelectric point (pI ≈ 5) [35].

Before examining the impact of the small-molecule surfactants on the digestion of the emulsified lipid, we characterized their ability to interact directly with the protein-coated lipid droplets. This was achieved by mixing the protein-stabilized corn oil-in-water emulsion with solutions containing different types and concentrations of surfactants, and then measuring the resulting droplet charge and size to determine whether the surfactants had interacted with the droplets. The physicochemical properties of the various surfactants used in this study as reported in the literature are summarized in Table 2. The influence of surfactant type and concentration on droplet charge is shown in Fig. 1a. The droplet charge was around −65.5 mV in the absence of added surfactant, which is due to the presence of the adsorbed protein layer. The droplet charge changed appreciably when increasing amounts of surfactants were added to the emulsions, indicating that the surfactants interacted with the lipid droplet surfaces. The nature of the change in droplet charge was highly dependent on surfactant type. For the sake of comparison, we also prepared a series of 5 wt.% corn oil-in-water emulsions stabilized only by the surfactants (in the absence of BLG), and measured their physicochemical properties (Table 3).

Table 2
Molecular and physicochemical properties of the low-molecular weight surfactants used in this study. The HLB and CMC values were obtained from various literature sources.

Surfactant	Mw (g/mol)	Chemical name	Type, state	HLB	CMC (mM)
Tween 20	1227.5	Polyoxyethylene (20) sorbitan monolaurate	Nonionic, liquid	16.7	0.06 [48]
Tween 80	1310	Polyoxyethyene (20) sorbitan monooleate	Nonionic, liquid	15	0.012 [48]
Brij 35	1199.56	Polyethylene glycol dodecyl ether	Nonionic, solid	16.9	0.090 [48]
SDS	288.38	Sodium dodecyl sulfate	Anionic, solid	40	1.5–5 [49]
DTAB	308.34	Dodecyl trimethyl ammonium bromide	Cationic, solid	17.9	15 [50]

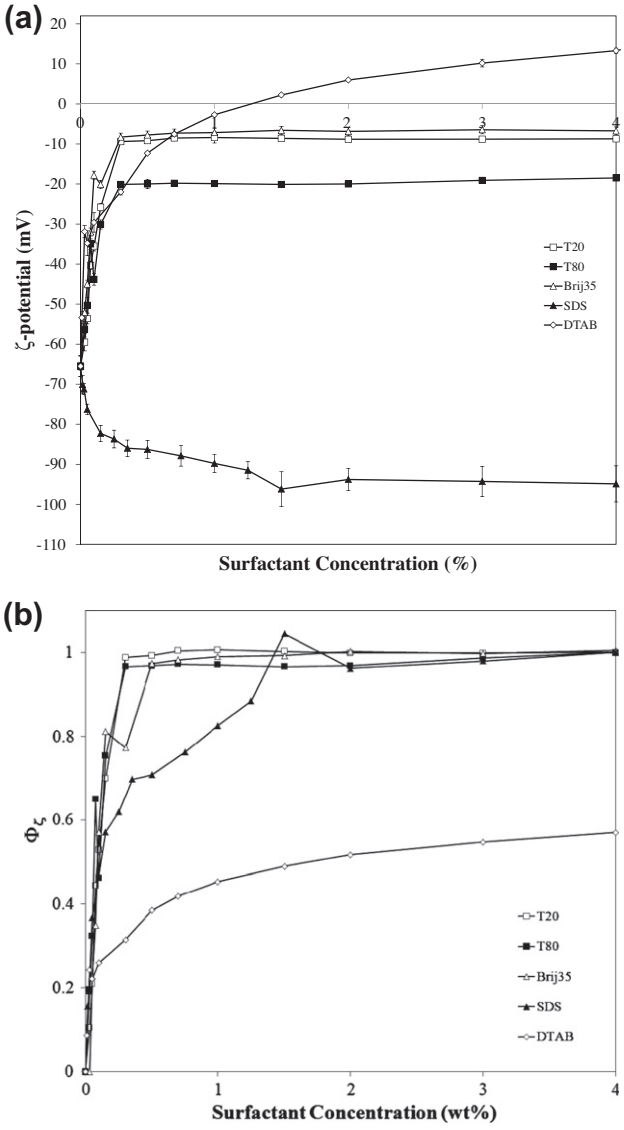

Fig. 1. (a) Effect of surfactant type and concentration on surface charge (ζ-potential) of lipid droplets in BLG-stabilized corn oil-in-water emulsions before digestion (pH 7). (b) Effect of surfactant type and concentration on normalized surface charge (Φ_ζ) of lipid droplets in BLG-stabilized corn oil-in-water emulsions (pH 7). Values are calculated from the data in Fig. 1a.

Table 3
Mean particle diameter (*Z*-average), polydispersity index (PDI), and ζ-potential of droplets in 5 wt.% corn oil-in-water emulsions prepared with 4 wt.% surfactant. Values presented as mean ± standard deviation ($n = 5$).

Surfactant	*Z*-average diameter (nm)		PDI		ζ-potential (mV)	
	Mean	SD	Mean	SD	Mean	SD
Tween 20	141.5	3.6	0.16	0.01	−8.4	0.3
Tween 80	143.9	2.8	0.18	0.04	−9.6	1.3
Brij 35	147.2	2.7	0.25	0.03	−3.4	0.9
SDS	137	4.8	0.18	0.04	−83.1	1.8
DTAB	187.7	2.9	0.23	0.05	39.6	4.9

For the anionic surfactant (SDS), the droplet charge became progressively more negative when increasing amounts of surfactant were added until a relatively constant value (≈−95 mV) was reached above about 2% SDS. This value was fairly similar to the value for the droplets coated only by SDS (Table 3), which suggested that the SDS had completely replaced the protein from the lipid droplet surfaces. For the cationic surfactant (DTAB), the droplet charge became less negative and then positive when increasing amounts of surfactant were added, however a much higher surfactant concentration was required to reach a plateau region, i.e., about 9% DTAB. Eventually, a very high positive charge (+73 mV) was measured on the droplets at high cationic surfactant concentrations (15% DTAB). At 4% DTAB, the positive charge on the droplets in the emulsions initially containing protein-coated droplets (+13 mV) was much lower than that in the emulsions only containing DTAB (+40 mV), which suggested that not all of the BLG had been replaced by the cationic surfactant at this concentration. For the non-ionic surfactants, the droplet charge went from highly negative to slightly negative (−8 to −18 mV) with increasing surfactant concentration, with the amount of surfactant required to reach the plateau region being relatively low <0.5%. The electrical charge on emulsions containing only non-ionic surfactant also had a slightly negative charge (−3 to 10 mV) (Table 3), which suggests that most of the protein in the BLG-coated lipid droplets had been displaced by surfactant. The relative affinity of the different surfactants for the droplet surfaces can be compared by plotting a normalized droplet charge versus surfactant concentration: $\Phi_\zeta = (\zeta - \zeta_0)/(\zeta_\infty - \zeta_0)$, where ζ is the droplet charge in the presence of surfactant, ζ_0 is the droplet charge in the absence of surfactant, and ζ_∞ is the droplet charge in the plateau region (Fig. 1b). The normalized droplet charge values indicate that the non-ionic surfactants had a higher affinity for the droplet surfaces than the ionic surfactants and that the anionic surfactant (SDS) had a higher affinity than the cationic surfactant (DTAB).

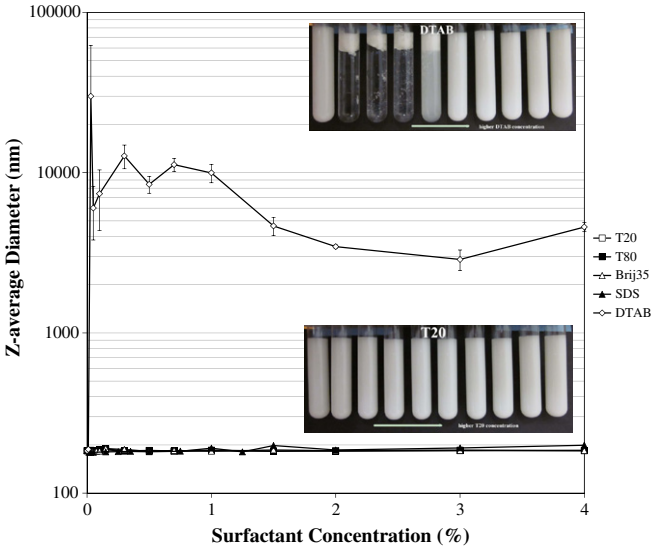

Fig. 2. Effect of surfactant type and concentration on mean particle diameter and appearance of protein-stabilized corn oil-in-water emulsions. Emulsions containing Tween 80, Brij, and SDS had similar appearances to the ones containing Tween 20, i.e., they were stable to creaming. Particle size data are presented as mean ± standard deviation ($n = 2$ or 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Measurements of the change in the mean particle diameter of the emulsions after addition of the surfactants indicated that there was no change in particle size when non-ionic or anionic surfactants were added, but that there was a large increase in particle size when cationic surfactant was added (Fig. 2). In addition, visual observation of the samples showed that emulsions containing non-ionic or anionic surfactants were stable to gravitational separation, whereas those containing intermediate levels of DTAB were prone to creaming (Fig. 2). The emulsions stabilized only by surfactants had relatively small droplet sizes (Table 3), which indicated that they were not susceptible to droplet aggregation.

The particle charge, particle size, and visual appearance measurements indicate that the small-molecule surfactants interacted with the protein-coated lipid droplets. Surfactants may interact with protein-coated oil–water interfaces in a number of different ways [36–39]: (i) they may bind directly to adsorbed proteins, which may alter the ability of the protein to adhere to the interface; (ii) they may co-adsorb with protein molecules forming a mixed protein-surfactant layer; (iii) they may completely displace the proteins from the interface. Previous studies indicate that non-ionic surfactants co-adsorb with globular proteins at relatively low surfactant concentrations, they cause two-dimensional phase separation into protein-rich and surfactant-rich regions at intermediate concentrations, and they promote complete protein displacement at high concentrations [38,39]. Similar results have been observed with ionic surfactants interacting with surfaces containing similarly charged proteins [40]. We therefore hypothesize that the non-ionic and anionic surfactants interacted with the protein-coated lipid droplets primarily by these mechanisms. This would account for the fact that little change in droplet size or appearance was observed (Fig. 2) when the protein layer was replaced by the surfactant layer (since both types of emulsifier were able to form stable emulsions) and that the droplet charge reached a final value determined largely by the electrical characteristics of the added surfactant.

The cationic surfactant (DTAB) appeared to behave quite differently from the other surfactants studied: it took much more surfactant to reach the plateau region (Fig. 1), and extensive droplet aggregation was observed (Fig. 2). We hypothesize that at relatively low surfactant concentrations, the cationic DTAB interacted with the anionic BLG molecules at the droplet surface forming an interfacial electrostatic complex that was more difficult to desorb than BLG alone. In addition, the DTAB may have promoted droplet aggregation through charge neutralization and bridging flocculation effects, leading to creaming instability [41]. At sufficiently high DTAB concentrations, we hypothesize that the electrostatic complex was displaced from the droplet surfaces so that the droplets were only covered by DTAB. This hypothesis is based on our droplet charge and size measurements, as well as previous neutron reflectivity and ellipsometry studies of the interactions of a cationic surfactant (TTAB) with a globular protein (lipase) at air–water and solid–water interfaces [42]. These studies showed that the cationic surfactant bound to the adsorbed proteins and formed a thick interfacial layer at relatively low concentrations, but that the complexes were eventually displaced from the surfaces when the surfactant concentration was sufficiently high.

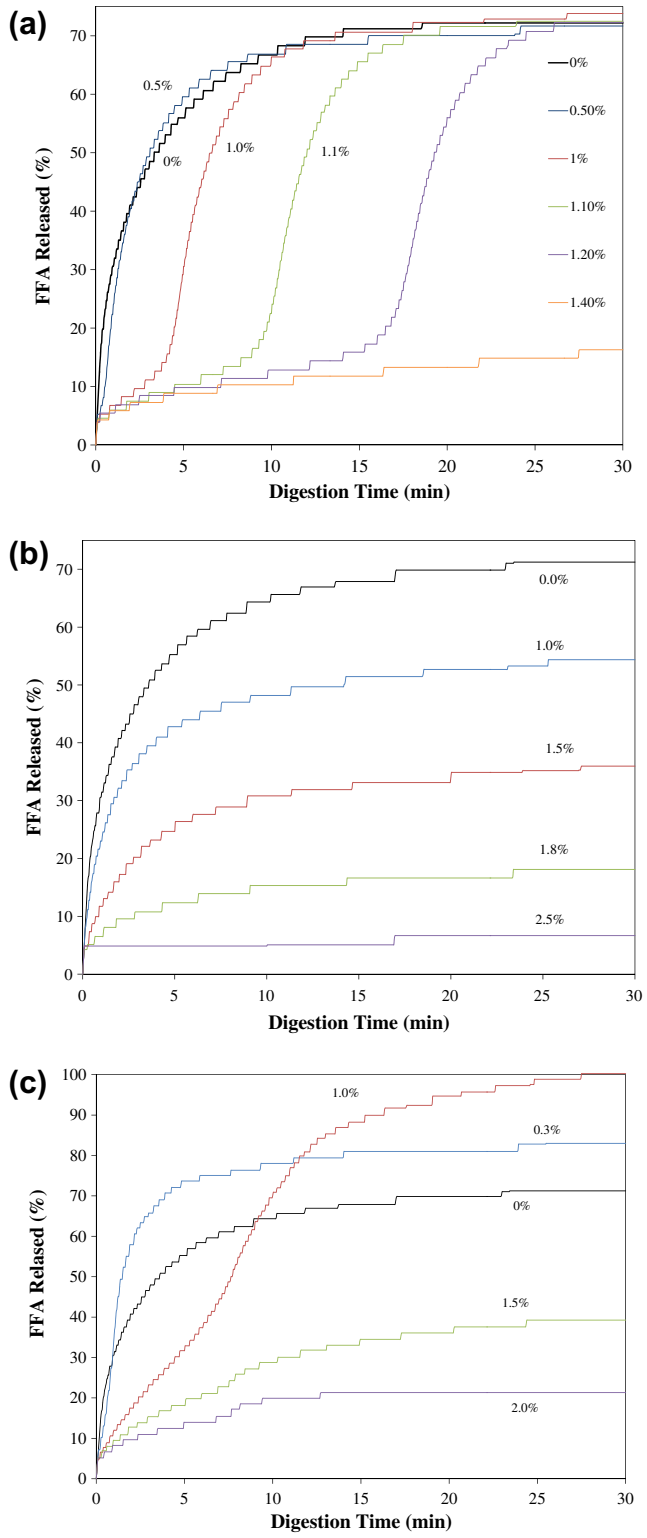

Fig. 3. Effect of surfactant type and concentration on the free fatty acid release profiles of oil-in-water emulsions during *in vitro* digestion by lipase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Effect of surfactant addition on lipid digestibility

The influence of surfactant type and concentration on the *in vitro* digestibility of the emulsified lipids was measured for all five surfactants. The free fatty acid (FFA) released versus digestion time profiles are shown for the anionic (SDS), cationic (DTAB), and a representative non-ionic (Tween 20) surfactant in Fig. 3. The percentage of FFA released after 30 min of digestion for all the surfactants is shown in Fig. 4. These results clearly indicate that lipid digestion can be inhibited above a certain surfactant concentration, which depended on surfactant type.

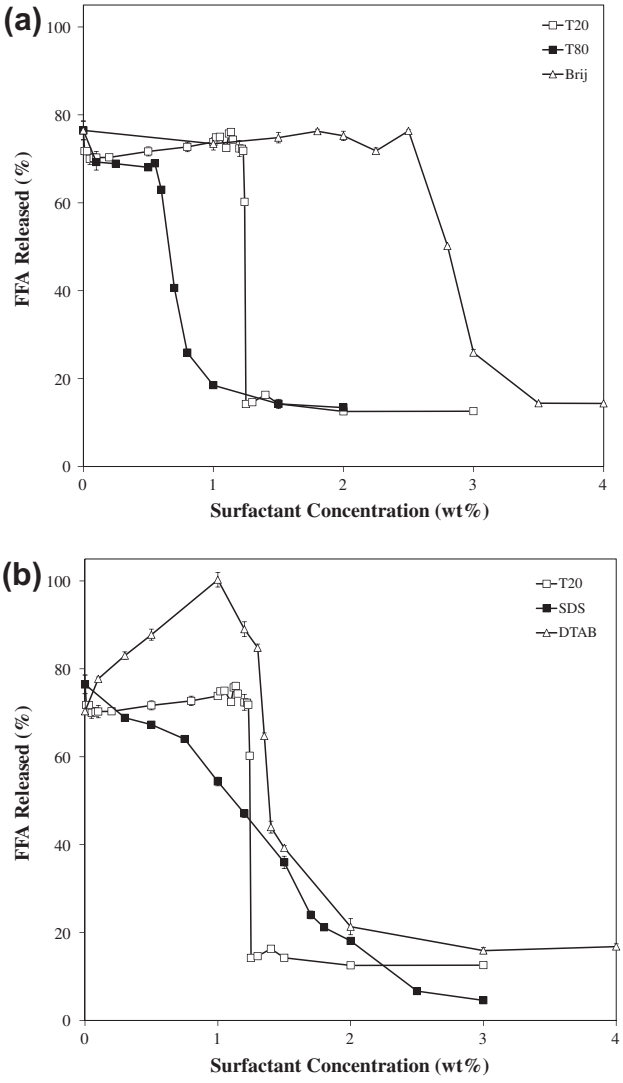

Fig. 4. Effect of surfactant type and concentration on the percentage of free fatty acids released after 30 min digestion: (a) Influence of non-ionic surfactant type (Tween 20, Tween 80, Brij); (b) Influence of head group charge (SDS, DTAB, Tween 20). Values are presented as mean ± standard deviations (n = 2 or 3).

3.2.1. Non-ionic surfactants

The FFA-time profile for the non-ionic surfactant is shown in Fig. 3a. In the absence of Tween 20, there was a rapid increase in FFA during the first 10 min; followed by a slower increase at longer times, until eventually a plateau value of around 73% was reached. This result suggests that lipase was able to rapidly attach to the surfaces of the lipid droplets and promote triacylglycerol digestion. Presumably, the layer of globular protein molecules (BLG) that initially coated the lipid droplet surfaces was rapidly displaced by bile salts, thus facilitating lipase attachment. At relatively low surfactant concentrations (Tween 20 < 0.8 wt.%), the presence of the surfactant had little impact on the rate or extent of lipid digestion. Over a fairly narrow range of intermediate surfactant concentrations (0.8–1.2 wt.%), the FFA-time profiles consisted of a number

of different regions: (i) a *lag period* during which little digestion occurred; (ii) a *digestion period* when the FFAs released increased rapidly with time; (iii) a *plateau region* where a relatively constant FFA concentration (≈72%) was reached. The length of the lag period increased with increasing surfactant concentration in this region. At relatively high surfactant concentrations (⩾1.5 wt.% Tween 20), the rate of lipid digestion was relatively slow with only a small increase in FFA released versus time. It is possible that these samples were still in the lag period, but no rapid increase in FFA occurred during a 30 min digestion period. Similar general trends in the FFA versus digestion time profiles were observed for the other two non-ionic surfactants studied (Tween 80 and Brij), but the range of surfactant concentrations where there was an appreciable inhibition in lipid digestion was different (Fig. 4a).

In the absence of small-molecule surfactants, the protein layer is rapidly displaced from the droplet surfaces by bile salts, thus enabling the lipase molecules to adsorb and come into close proximity to the lipid substrate (provided there is not too much bile salts) and rapidly generate FFA. On the other hand, in the presence of small-molecule surfactants there is a competition for the lipid droplet surfaces between the protein, bile salts, and surfactants. At sufficiently high concentrations, the surfactants will preferentially adsorb to the droplet surfaces and dominate their interfacial composition, which may restrict the adsorption of lipase and/or co-lipase molecules. Non-ionic surfactants may inhibit the adsorption of enzymes to the oil–water interface through a steric hindrance mechanism associated with the protrusion of their hydrophilic head groups into the aqueous phase [17]. The normalized droplet charge measurements discussed in the previous section suggest that all three surfactants were fairly similar in their ability to adsorb to the lipid droplet surfaces and displace BLG (Fig. 1b). This suggests that other factors must determine their effectiveness at inhibiting lipid digestion, such as differences in their abilities to prevent lipase molecules from attaching to the droplet surfaces through steric hindrance, differences in their abilities to directly interact with lipase and alter its adsorption or activity, or differences in their digestibility.

3.2.2. Ionic surfactants

FFA versus digestion time profiles were also measured for emulsions containing increasing amounts of either an anionic surfactant (Fig. 3b) or a cationic surfactant (Fig. 3c). The ionic surfactants exhibited quite different effects on the rate and extent of lipid digestion than the non-ionic surfactants (Fig. 4b). There was a progressive decrease in both the rate and extent of FFAs released when increasing amounts of SDS were incorporated into the emulsions (Fig. 3b). Unlike for non-ionic surfactants, the generation of FFAs occurred almost immediately after digestion was initiated, i.e., no lag period was observed. The FFAs increased most steeply during the first 10 min, then increased more gradually, and then reached a plateau region. However, the amount of FFAs released in the plateau region decreased progressively with increasing SDS concentration. SDS is known to bind both non-specifically and specifically to globular proteins [43,44], which promotes protein denaturation and would be expected to alter the activity of enzymes. Consequently, the decrease in lipid digestion observed in the presence of SDS may have been at least partly due to denaturation of the lipase, as well as partly due to its ability to displace lipase from the droplet surfaces.

The addition of cationic surfactant to the emulsions had a quite different effect on the FFA versus digestion time profiles (Fig. 3c). At relatively low concentrations (DTAB ⩽ 1.3 wt.%), the cationic surfactant actually increased both the rate and extent of lipid digestion (Fig. 4b). Previous studies have also found that DTAB can increase the activity of enzymes when present at relatively low concentrations, which was attributed to its ability to alter enzyme conformation and therefore activity [45,46]. We hypothesize that DTAB may also have promoted the adsorption of lipase to the oil–water interface through electrostatic attraction between the cationic surfactant and anionic lipase. A higher interfacial concentration of lipase could then lead to more efficient lipid digestion. At higher surfactant concentrations, the DTAB did decrease the rate and extent of lipid digestion (Figs. 3c and 4b), which can be attributed to its ability to denature the protein and/or displace it from the droplet surfaces. Previous studies have shown that DTAB denatured a globular protein (BSA) when present at concentrations exceeding about 4 mM (1.2 wt.%) [44], which is close to the value where the lipase activity started to decrease in this study (Fig. 4b). As mentioned earlier, neutron reflectivity and ellipsometry studies have shown that cationic surfactants (TTAB) can form complexes with lipase at interfaces [42]. At relatively low surfactant concentrations, these complexes remain at the interface, but at sufficiently high concentrations they are displaced into the surrounding aqueous phase. The DTAB may also have been able to alter the lipid digestion process due to its ability to interact with other anionic components in the simulated small intestinal medium. Cationic DTAB may have interacted with anionic bile salts forming insoluble complexes, thereby reducing their ability to adsorb to lipid droplet surfaces [47]. Cationic DTAB may also have interacted with any anionic free fatty acids generated at the lipid droplet surfaces due to triglyceride digestion, which could also alter the rate and extent of digestion [47].

3.2.3. Relative surfactant effectiveness

The relative effectiveness of surfactants at inhibiting lipase digestion was characterized in terms of the *half-maximal inhibitory concentration* (IC50), which is defined as the surfactant concentration where the free fatty acids (FFA) released was 50% after 30 min digestion (Fig. 4). The calculated IC50 values were 1.2% (Tween 20), 0.7% (Tween 80), 2.8% (Brij35), 1.1% (SDS), and 1.4% (DTAB). Hence, the ability of the surfactants to inhibit lipid digestion could be ranked in the following order: Tween 80 > SDS > Tween 20 > DTAB > Brij35. There therefore does not appear to be a good correlation between the nature of a surfactant's charge and its ability to inhibit lipid digestion. This is probably because there are a number of different physicochemical mechanisms by which a surfactant could alter lipase activity: competitive adsorption; complex formation; protein conformational changes.

Additional information about the ability of the surfactants to interact with the lipid droplets and alter their digestibility was obtained by measuring the ζ-potential and appearance of the emulsions after digestion (Fig. 5). In the absence of added surfactant, the electrical charge on the remaining droplets was relatively high after digestion (≈−90 mV), which can be attributed to the presence of bile salts and possibly free fatty acids. In the presence of surfactant, the droplet charge was changed appreciably, by an amount that depended on surfactant type and concentration. For the non-ionic surfactants (Tween 20, Tween 80, and Brij), the droplet charge became increasingly less anionic with increasing surfactant concentration until a relatively constant value around –30 mV was reached, which can be attributed to adsorption of non-ionic surfactants to the droplet surfaces. However, this charge was appreciably more negative than for the emulsions containing only non-ionic surfactants (Table 3), which suggests that at least some anionic surface-active species (presumably bile salts) remained at the lipid droplet surfaces. For the anionic surfactant (SDS), the droplet charge remained highly anionic as the surfactant concentration was increased, which is probably because both SDS and bile salts give highly negatively charged droplets. For the cationic surfactant (DTAB), the droplet charge became less negative and then became positive as the surfactant concentration was increased, suggesting that the DTAB adsorbed to the droplet surfaces. These

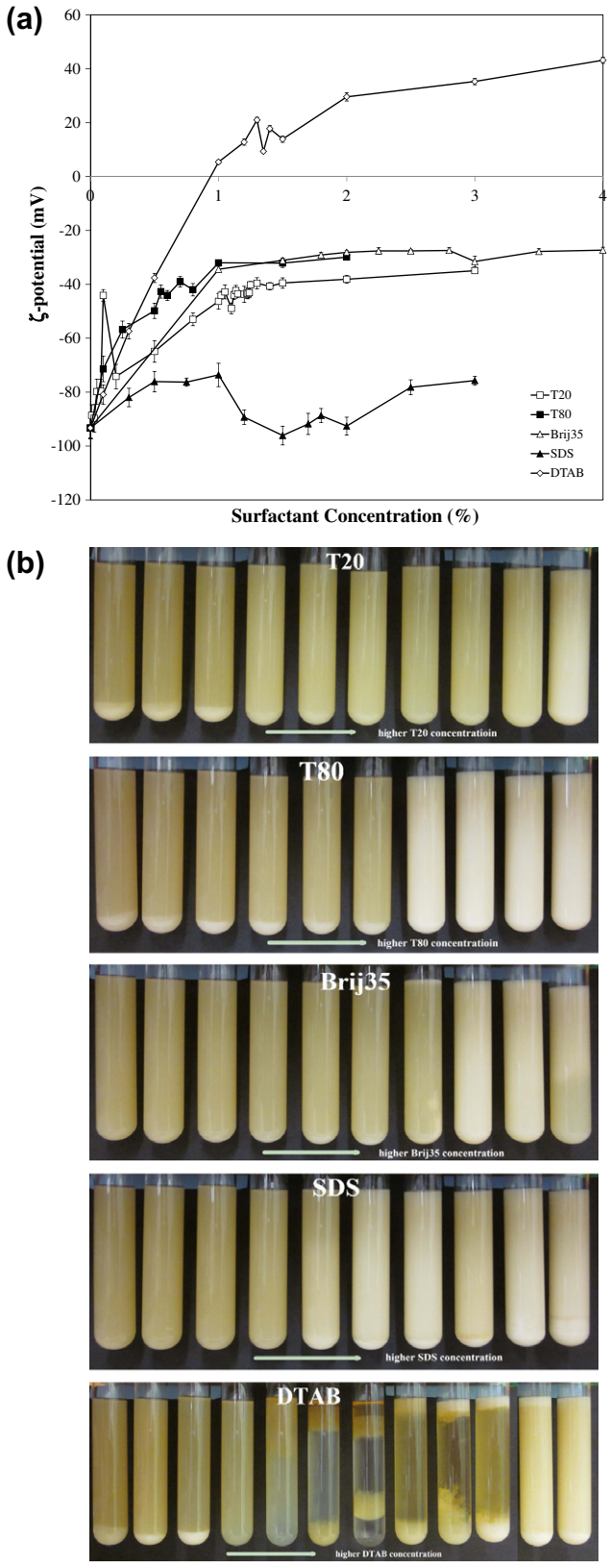

Fig. 5. (a) Effect of surfactant type and concentration on surface charge (ζ-potential) of lipid droplets in BLG-stabilized corn oil-in-water emulsions after digestion (pH 7). Values are presented as mean ± standard deviation (n = 5). (b) Effect of surfactant type and concentration on appearance of BLG-stabilized corn oil-in-water emulsions after digestion (pH 7). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

results suggest that at high surfactant concentrations the lipid droplets remaining after digestion are mainly coated by surfactant, which may have prevented adsorption of the lipase/co-lipase and thus inhibited digestion.

The appearance of the samples after digestion also provided some useful information about the impact of surfactant type and concentration on digestion (Fig. 5b). At relatively low concentrations of non-ionic or anionic surfactants, the samples consisted of a slightly turbid serum layer on top of an opaque sediment layer. Based on previous studies, this serum layer is likely to contain mixed micelles containing the lipid digestion products, whereas the sediment layer is likely to contain aggregated proteins and calcium salts of free fatty acids [47]. As the concentration of these surfactants was increased, the samples became cloudier in appearance, which suggests that an increasing fraction of the lipid droplets remained undigested. The samples containing cationic surfactants demonstrated a more complex dependence on surfactant concentration (Fig. 5b). At relatively low DTAB concentrations, the system separated into a turbid serum layer at the top and an opaque sediment layer at the bottom, suggesting that most of the lipid had been digested. At intermediate DTAB concentrations, the sediment layer was not observed and a creamy or oily layer was observed on the top of the samples, suggesting that some droplet flocculation or coalescence had occurred. At high DTAB concentrations, a sediment layer was observed again and the upper layer became cloudy suggesting that an appreciable amount of the lipid droplets had not been digested.

We should note that the maximum surfactant concentrations used in our *in vitro* digestion model were relatively high (5 wt.%). The surfactant levels found in the small intestine *in vivo* are likely to be appreciably less than this value due to dilution with digestive fluids in the mouth, stomach, and small intestine. Nevertheless, our results do suggest that certain types of surfactant have the potential to influence the lipid digestion process if they are present at sufficiently high levels.

3.3. Degradation of surfactants by pancreatic lipase

We also examined the ability of the lipase to digest the surfactants themselves. Many small-molecule surfactants have ester bonds between the non-polar tail group and the polar head group and may therefore be susceptible to enzymatic cleavage. Diges-

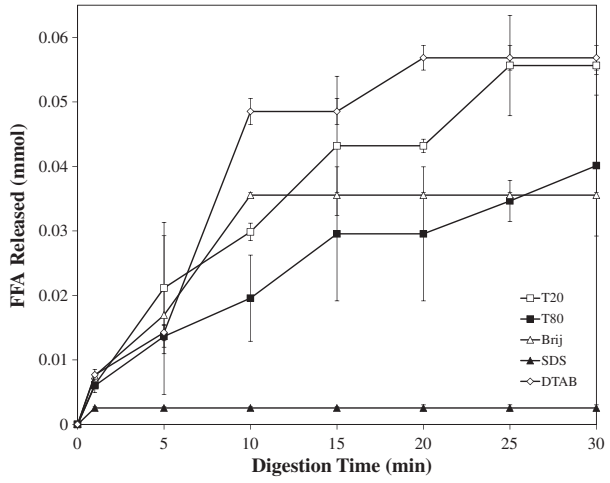

Fig. 6. Effect of surfactant type on the free fatty acid release profiles of 2 wt.% surfactant solutions during *in vitro* digestion by lipase. Values are presented as mean ± standard deviation (n = 2 or 3).

tion of a surfactant would be expected to have a major influence on its functionality, since dissociation of the head group and tail group would mean that the surfactant was no longer surface active.

The *in vitro* digestion model was used to measure the amount of free fatty acids released over time using similar conditions as for the emulsions (Fig. 6). All of the surfactants except SDS were susceptible to digestion by pancreatic lipase showing some increase in FFA over time. The percentage of surfactants that were hydrolyzed was calculated from these values and knowledge of their molecular weights: 8.9 ± 0.2%, 7.0 ± 1.9%, 10.10 ± 0.03%, and 2.3 ± 0.1% for Tween 20, Tween 80, Brij, and DTAB respectively. These measurements indicated that an appreciable faction of the non-ionic and cationic surfactants could be hydrolyzed under *in vitro* digestion conditions, which would contribute to the FFA values measured for the emulsions, as well as altering the functionality of the surfactants. The effect of surfactant digestion on the FFA values calculated for the lipid phase would be expected to be relatively small: the volume of alkali solution that had to be added to the surfactant-free BLG-stabilized emulsions to reach the plateau region was about 3.6 mL, whereas <0.2 mL per 1% surfactant solutions had to be added for the pure surfactant solutions.

4. Conclusions

This study has shown that a variety of small-molecule surfactants can impact the rate and extent of lipid digestion in emulsion-based drug delivery systems by an amount that depends on surfactant type and concentration. All surfactants were found to inhibit lipid digestion at sufficiently high concentrations. At relatively low surfactant concentrations, the non-ionic surfactants (Tween 20, Tween 80, and Brij 35) had little impact on lipid digestion, the anionic surfactant (SDS) inhibited digestion, and the cationic surfactant (DTAB) promoted digestion. A number of potential physicochemical mechanisms may account for the impact of the various surfactants on digestion: (i) prevention of lipase adsorption to the oil–water interface; (ii) formation of interfacial complexes; (iii) direct interaction with lipase; (iv) interaction with other components in the digestion medium, such as bile salts or phospholipids. We have also shown that a number of the surfactants themselves were susceptible to digestion by pancreatic enzymes in the absence of lipids: Tween 20, Tween 80, Brij35, and DTAB. This work has important implications for the development of emulsion-based delivery systems for food and pharmaceutical applications. Nevertheless, future work needs to be carried out on the impact of surfactant type and concentration using *in vivo* animal models to ascertain whether high surfactant levels do inhibit lipid digestion and the absorption of lipophilic drugs.

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