

Influence of Emulsifier Structure on Lipid Bioaccessibility in Oil–Water Nanoemulsions

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ABSTRACT: The influence of several nonionic surfactants (Tween-20, Tween-40, Tween-60, Span-20, Span-60, or Span-80) and anionic surfactants (sodium lauryl sulfate, sodium stearyl lactylate, and sodium stearyl fumarate) showed drastic differences in the rank order of lipase activity/lipid bioaccessibility. The biophysical composition of the oil and water interface has a clear impact on the bioaccessibility of fatty acids (FA) by altering the interactions of lipase at the oil–water interface. It was found that the bioaccessibility was positively correlated with the hydrophilic/lipophilic balance (HLB) of the surfactant and inversely correlated to the surfactant aliphatic chain length. Furthermore, the induction time in the jejunum increased as the HLB value increased and decreased with increasing aliphatic chain length. The rate of lipolysis slowed in the jejunum with increasing HLB and with increasing aliphatic chain length.

KEYWORDS: nanoemulsion, surfactant, lipid bioaccessibility

INTRODUCTION

With diet related diseases surging in the United States, and one-in-three adults being obese, we urgently need to understand how foods can be formulated or processed so that they are healthier.^{1–3} High concentration of FAs in the blood impairs bodily responses to insulin, resulting in the potential development of type II diabetes and coronary heart disease.⁴ These changes have resulted in, for the first time, noncommunicable diet-related diseases being responsible for a larger percentage (46.8%) of the mortality rate than communicable disease (41.0%).^{5,6} Perhaps, what is even more shocking is that noncommunicable diseases are responsible for nearly two-thirds of deaths in the Americas.⁶ Fat digestion in humans can be highly efficient with as high as 95–97% of some ingested lipids being hydrolyzed and absorbed.^{7,8} Controlling digestion, by means of reducing the rate at which FAs are absorbed, may reduce spikes in blood FAs and aid in the regulation of appetite.⁹ A mechanism to limit fat absorption is to control enzyme activity in the gastrointestinal (GI) track by using a lipase inhibitor such as Orlistat.¹⁰ As well, adjusting the physical system may also be used to limit lipase activity. It has been shown that for nonemulsified oils, an increase in cholecystokinin (CCK) hormone was detected, along with an initial increase in the rate of gastric emptying relative to an emulsified system.¹¹ Another possible mechanism to regulate enzyme activity is to use appropriate emulsifiers, which compete at the oil and water interface with the enzymes altering the amount of lipase at the interface that decreases the rate of lipolysis.¹²

The rate of fat digestion is controlled by the ability of lipase to interact with emulsion interfaces occurring primarily in the small intestine but also to a significant extent in the stomach.^{13,14} In the small intestine, pancreatic lipase works in conjunction with colipase, bile salts, and calcium, enabling

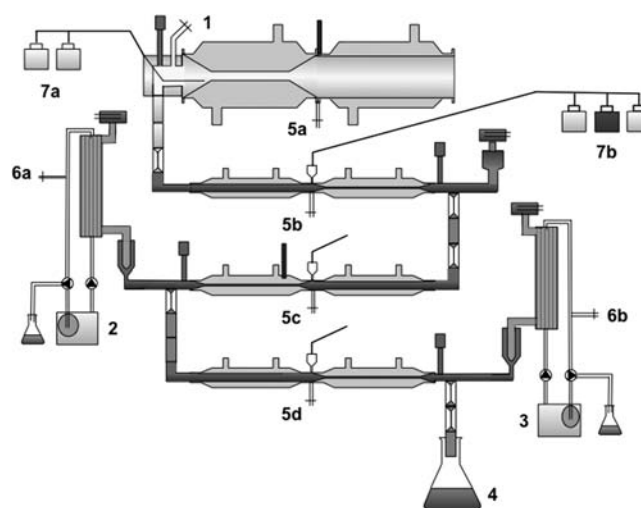


Figure 1. Schematic diagram of the in vitro gastrointestinal model, TIM-1: (1) food inlet, (2) jejunum filtrate, (3) ileum filtrate, (4) ileal colorectal valve, (5a) gastric compartment, (5b) duodenal compartment, (5c) jejunum compartment, (5d) ileal compartment, (6a) hollow fiber membrane from jejunum, (6b) hollow fiber membrane from ileum, (7a and 7b) secretion pumps.

access to the oil–water interface.¹⁵ Surfactants are commonly used in numerous processed food products, such as beverages, salad dressings, and sauces, increasing the emulsion stability

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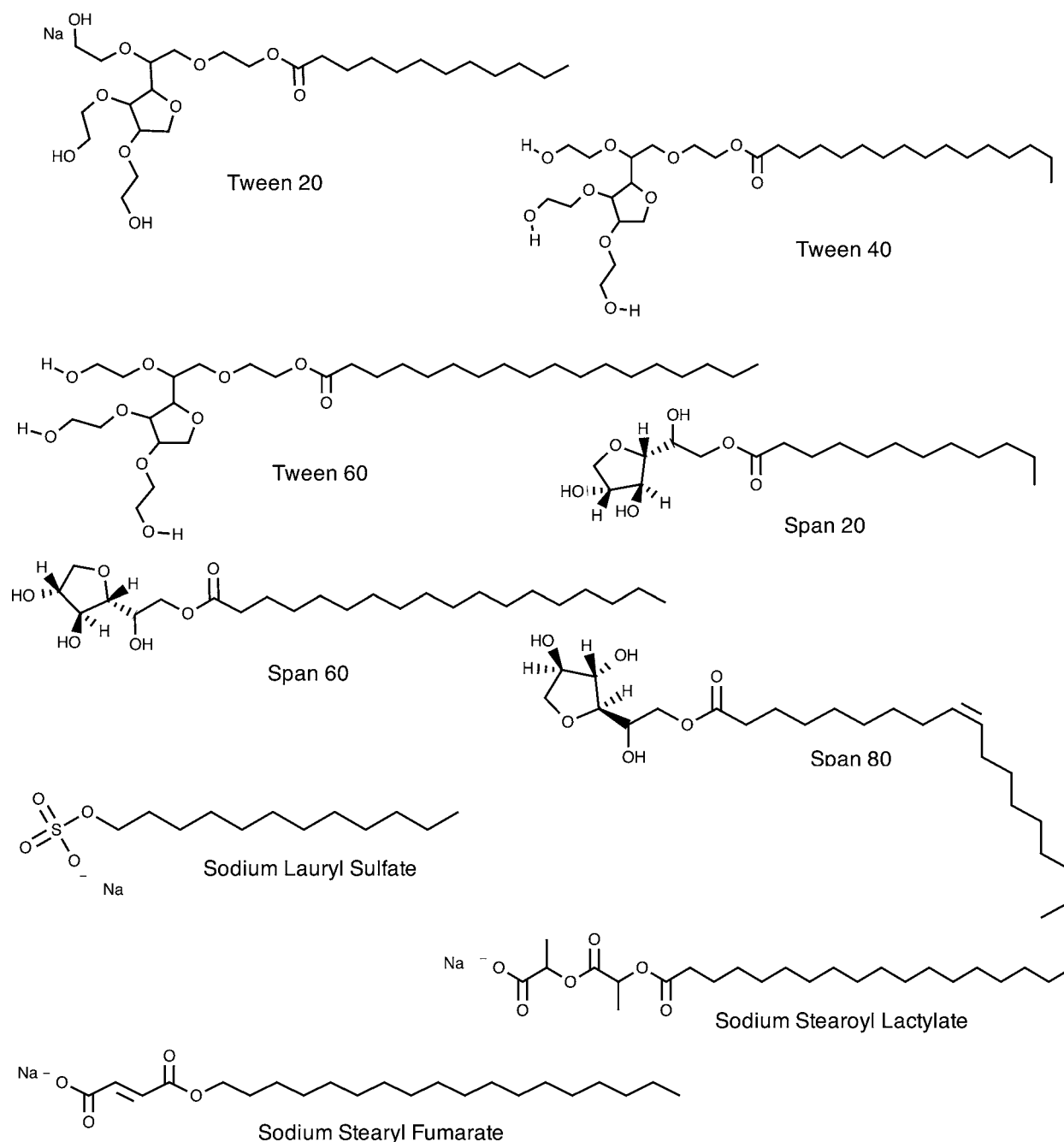


Figure 2. Chemical structures of different food grade emulsifiers.

and thereby reducing undesirable separation and improving textural quality.¹⁶ Certain surfactants inhibit the formation of the colipase/pancreatic lipase complex, altering the lipase activity. Bile salts can remove such inhibitory surfactants from the interface via an orogenic displacement mechanism.¹⁷ Once lipase reaches the interface, the lipolytic activity cleaves triglycerides into 2-monoglycerides and FAs, resulting in the generation of more surface-active molecules, further limiting lipase adsorption at the interface. These surface-active products are eliminated from the interface by bile salts and phospholipid micelles.

In emulsions, there are multiple modes in which surfactants inhibit lipase including the following: steric prevention of lipase at droplet interface, interfacial complex formation, direct interaction of the surfactant with lipase, and interaction with other nonlipase digestive components such as bile.^{8,13,18–21} Elevated

levels of serum FAs have also been associated with insulin resistance, inflammation, and predisposition for metabolic syndrome.²² Therefore, structured emulsions act as alternative method to eliminate some of the negative health implications of fats in processed foods.²³ The effect of emulsifier structure, at the lipid droplet interface in oil-in-water emulsions, on lipolytic activity will be studied using the TIM-1 simulated gastrointestinal track.

■ MATERIALS AND METHODS

Materials. Pancrex V powder (lipase activity = 25 000 units/g, protease activity = 1400 units/g, and amylase activity = 30 000 units/g) was obtained from Paines & Byrne, UK. Fresh pig bile was obtained from TNO Zeist, Netherlands. The bile was collected and standardized from a slaughterhouse, pooled together, aliquoted for individual TIM

Table 1. Classes, Molecular Weights (MW), Hydrophilic–Lipophilic Balance (HLB), and Applications of Individual Surfactants Used in This Study in the Structuring of Tricaprylin-in-Water Emulsions³³

class	surfactant	HLB	common uses
anionic surfactant	sodium stearyl lactylate	8.3	dough strengthener
	sodium stearyl fumarate	19	tablet lubricant
	sodium lauroyl sulfate	40	tapioca
nonionic Spans	sorbitan monolaurate (Span-20)	8.6	mouth drops
	sorbitan monostearate (Span-60)	4.7	cakes
	sorbitan monooleate (Span-80)	4.3	icing, moist pet food
nonionic Tweens	polyoxyethylenesorbitan monolaurate (Tween-20)	16.7	mouth drops
	polyoxyethylenesorbitan monopalmitate (Tween-40)	15.6	cosmetics
	polyoxyethylenesorbitan monostearate (Tween-60)	14.9	whipped topping

experiments, and stored at $-20\text{ }^{\circ}\text{C}$ until use. Rhizopus lipase (150 000 units/mg F-AP-15) was obtained from Amano Enzyme Inc. (Nagoya, Japan). Trypsin from bovine pancreas (7500 *N*- α -benzoyl-L-arginine ethyl ester (BAEE) units/mg, T9201) was obtained from Sigma Aldrich. Tricaprylin (T9126, >99%), nonanoic acid (N5502, >97%), Tween 20 (P1379), Tween 40 (P1504), Tween 60 (P1629), Span 20 (S6635), Span 60 (S7010), and Span 80 (S6760) were obtained from Sigma-Aldrich. Sodium lauryl sulfate (S1331, 98%), sodium stearyl fumarate (S1451, 99%), and sodium stearyl lactylate (S1297, NA) were obtained from Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ).

Gastrointestinal Model. A dynamic, *in vitro* gastrointestinal model, TIM-1, developed by TNO (Zeist, The Netherlands), was utilized to simulate digestion. The TIM-1 system models the human digestive tract utilizing four compartments mimicking the stomach, duodenum, jejunum, and ileum, peristaltic movements, nutrient and water absorption, gastric emptying, and transit time as would be observed *in vivo* (Figure 1). Compartments are infused with formulated gastric secretions, bile, and pancreatic secretions to modify pH and reproduce digestive conditions respective of a fed or fasted state. In the fed state, the pH of the gastric compartment is 5.5 upon feeding and decreases to 1.5 with a half-life of 40 min and the gastric emptying rate has a half-life of 70 min.

A 7% pancreatin solution (Pancrex V powder, Paines & Byrne, UK) and small intestinal electrolyte solution (SIES: NaCl 5 g/L, KCl 0.6 g/L, CaCl₂ 0.25 g/L) were prepared. Duodenal start residue (60 g; 15 g of SIES, 30 g of fresh porcine bile, 2 mg trypsin solution, 15 g of pancreatin solution), jejunal start residue (160 g: 40 g of SIES, 80 g of fresh porcine bile, 40 g of pancreatin solution), and ileal start residue (160 g of SIES) were injected into respective compartments prior to heating the system to physiological temperature ($37\text{ }^{\circ}\text{C}$) in preparation for feeding.

Emulsion “meals” were tested during 4-h experiments in the TIM-1 model, simulating fed-state physiological conditions following ingestion of a high fat meal. To simulate the initial amount of gastric juice, 5 g of gastric enzyme (NaCl 4.8 g/L, KCl 2.2 g/L, CaCl₂ 0.22 g/L, and 7% pancreatin solution) was added to the gastric compartment followed immediately by the “meal”. The meal was prepared by combination of 100 g of prepared emulsion with 95 g of gastric electrolyte solution. A 50 g amount of water and 11 mg of amylase were added to the meal, and the pH was adjusted to 5.5 by dropwise addition of 0.1 M HCl. This was immediately placed into the gastric compartment followed by a 50 g water rinse. Secretion of HCl (1 M) into the gastric compartment during digestion was controlled to follow a preprogrammed computer protocol, which regulates gastric emptying (half-life = 70 min), intestinal transit times, pH (half-life = 40 min adjusted from pH 5.5 to 1.5), and secretion fluid amounts.²⁴

Secretion of digestive fluids were set up based on the following: Duodenal secretion consisting of fresh porcine bile at a flow rate of

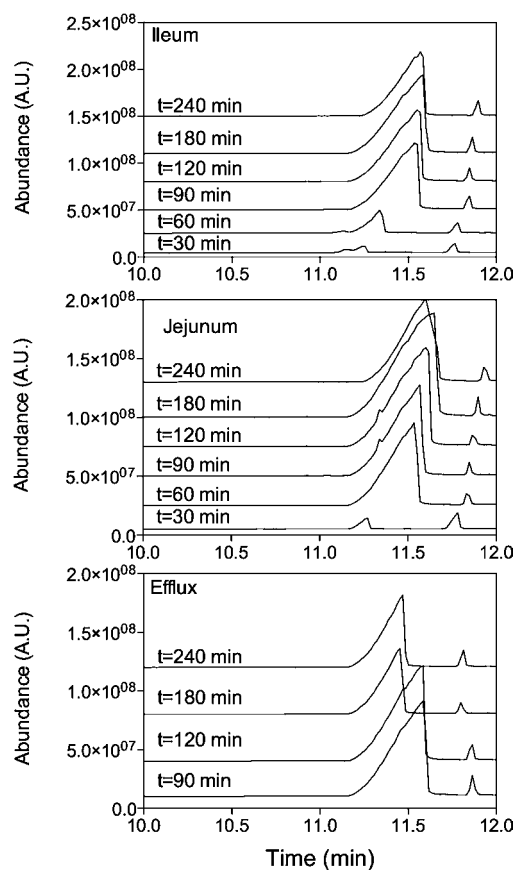


Figure 3. GC–mass spectroscopy elution times for the methylene chloride-extracted free FA for the oil-in-water emulsions stabilized with Tween 20. Curves are offset to distinguish peaks. The internal standard elutes are ~ 11.8 min.

0.5 mL/min, a 7% pancreatin solution at a flow rate of 0.25 mL/min, and SIES at a flow rate of 3.2 mL/min. Jejunal secretion consisting of SIES and 10% fresh porcine bile at a flow rate of 3.2 mL/min. Ileal secretion consisting of SIES at a flow rate of 3.0 mL/min. Secretion of HCl (1 M) into the gastric compartment during digestion was controlled to follow a preprogrammed computer protocol that regulates gastric emptying, intestinal transit times, pH values, and secretion fluid amounts. The pH of duodenal, jejunal, and ileal compartments was maintained at 6.5, 6.8, and 7.2, respectively, by controlled secretion of sodium bicarbonate solution (1 M).

Absorption of available micellar fractions was observed by collection of dialysate fluids, which were passed through semipermeable capillary membranes (Spectrum Milikros modules M80S-300-01P) with 0.05 μm pores at the ileal and jejunal compartments. Jejunal and ileal filtrates and ileal efflux were cooled on ice to reduce residual lipase activity once the samples passed through the capillary membranes, and samples were gathered at 30, 60, 90, 120, 180, and 240 min. This allows the individual compartments of the upper GI to have their isolated effects on lipid digestion studies. Residues were not collected for analysis following experiment termination at 240 min. Bioaccessibility of FAs was evaluated for each of the nine surfactant-stabilized emulsions and a control emulsion in duplicate, and each duplicate was analyzed in duplicate, providing two sample duplicates and two technical duplicates for each surfactant and control (i.e., absence of any surfactant).

Emulsion Preparation. Several food-grade surfactants (Figure 2) have been selected for oil-in-water emulsion preparation with tricaprylin (trioctanoyl glycerol). Tricaprylin was selected as the model lipid because none of the surfactants had caprylic acid as the aliphatic chain; therefore, if FAs are cleaved from the surfactant by lipase it will not influence the FA bioaccessibility measurement.

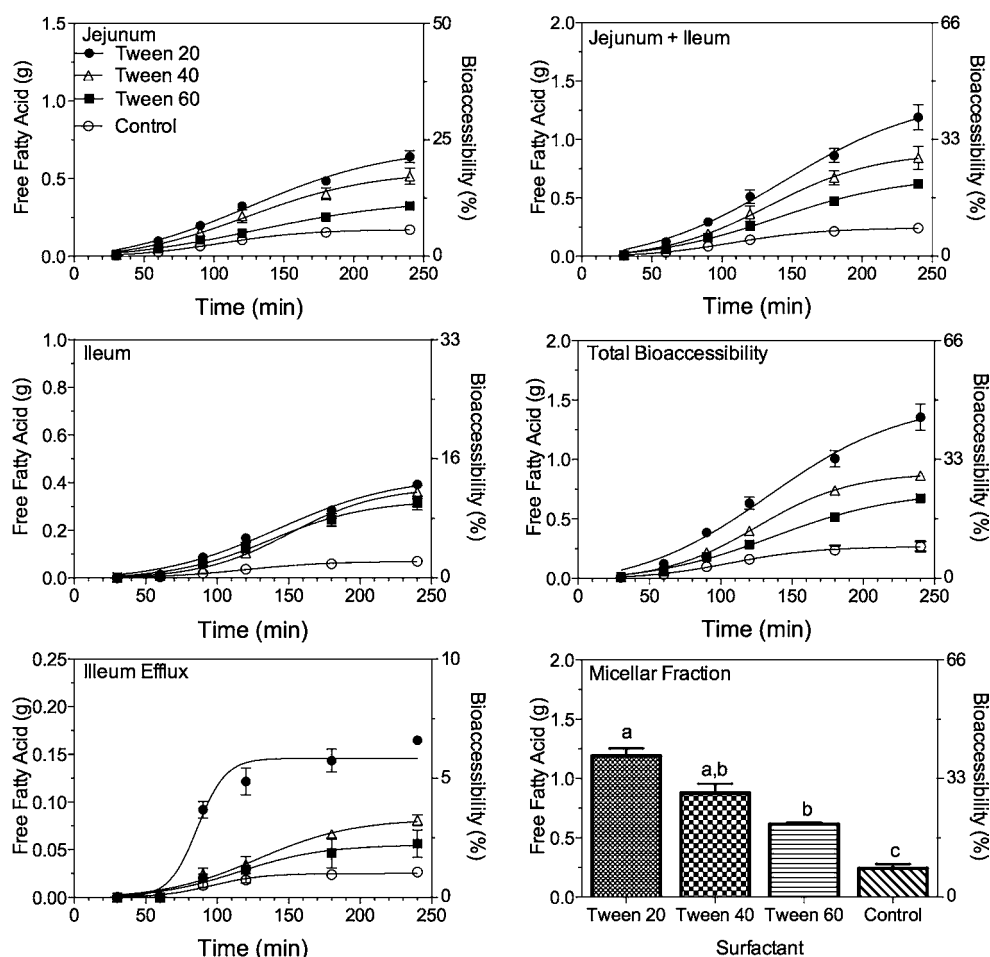


Figure 4. Free FA concentration cleaved from tricaprilyn in oil-in-water emulsions stabilized with different Tween emulsifiers for different regions of the TIM-1 system. The micellar fraction was obtained from sum of the jejunum and ileum after 240 min. Statistical differences were determined using a one-way ANOVA ($p < 0.05$).

As well, tricaprilyn was selected because it has three identical, short chain FAs (8:0), which simplified analysis, using GC-MS because there is no need to create methyl esters. Finally, caprylic acid is not typically a major component of physiological systems such as bile.

Simple, monodispersed tricaprilyn-in-water (o/w) emulsions were used in this study, each prepared with a single surfactant. Several surfactants were selected to cover a wide range of HLB values and were subdivided into three categories: polysorbates (Tweens), sorbitans (Spans), and charged (anionic) surfactants (Table 1). Additional compounds were not added to avoid complex formation and potential interactions in an attempt to observe only the biophysical behavior of the surfactant at the droplet. Concentration of surfactant was maintained constant across all samples (1.5 wt %). Emulsion meals were prepared as follows: 5.00 g of tricaprilyn was combined with 1.50 g of surfactant and homogenized with 93.5 g of sodium bicarbonate buffer (1.5 g/L) using an Ultraturax high speed homogenizer for 2 min at 20 000 rpm. Pre-emulsions were then high pressure homogenized (Emulsiflex-C3, Avestin) at 80 °C for 20 min (10 cycles) at 1000 bar to ensure uniform droplet size. At the time of feeding, emulsion with an average droplet diameter of 90 ± 20 nm was fed to the TIM-1. Particle size was evaluated using light scattering (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation, Holtsville, NY) following 10-fold dilution.

Extraction and Analysis of Free FAs. The pH of 5 mL samples from each compartment was raised between to 10 and 12 by dropwise addition of sodium hydroxide (10 N). An internal standard of nonanoic acid in dichloromethane (5 mg/mL) was added to each sample (10 μ L). Aliquots were extracted with 15 mL of dichloromethane. Following partitioning, acidification by dropwise addition of HCl (1 N) to

a pH between 2 and 3 was performed, the FAs were extracted with 2.5 mL of dichloromethane, and the samples were collected and stored at -20 °C until further analysis.

The degree of lipolysis was determined by measuring the FA concentration using GC-MS (6890N Agilent Technologies). One milliliter of sample was placed into autosample vials and 10 μ L was injected in splitless mode into a gas chromatograph (model 6890N, Agilent Technologies)—mass spectrometer (model 5973, Agilent Technologies) equipped with a 60 m, 0.25 mm DB-xLB fused silica capillary column (J&W Scientific, Folsom CA). For convenient identification of mass spectra, software was programmed with the Wiley Registry of Mass Spectra, 7th ed. Chromatographic parameters were as follows: injection temperature at 150 °C, initial oven temperature of 50 °C for 5 min and successive temperature raise of 30 °C per min for 4 min. The mass spectrometer was operated in electron ionization (EI) mode, scanning from 50 to 650 m/z . The retention time of octanoic and nonanoic acids was found to be 11.5 and 11.8 min with primary ions of 158 and 144, respectively. Standard curves were constructed based on the peak area corresponding to known quantities of each compound.

Mathematical Modeling. FAs generated by the lipolytic activity of lipase, under simulated gastrointestinal conditions, followed a sigmoidal pattern. This profile may be characterized by an initial lag phase followed by a steep increase in released FA that approaches an ultimate plateau. The lag phase, observed early in digestion, has been described by Troncoso, Aguilera, and McClements,²⁵ as lipids being inaccessible to enzymes due to a surfactant barrier at the interface of the emulsion lipid droplet. However, this lag phase is unaccounted for by current models in which a steep monotonical increase in released free FAs is assumed from the start of the experiment.²⁶

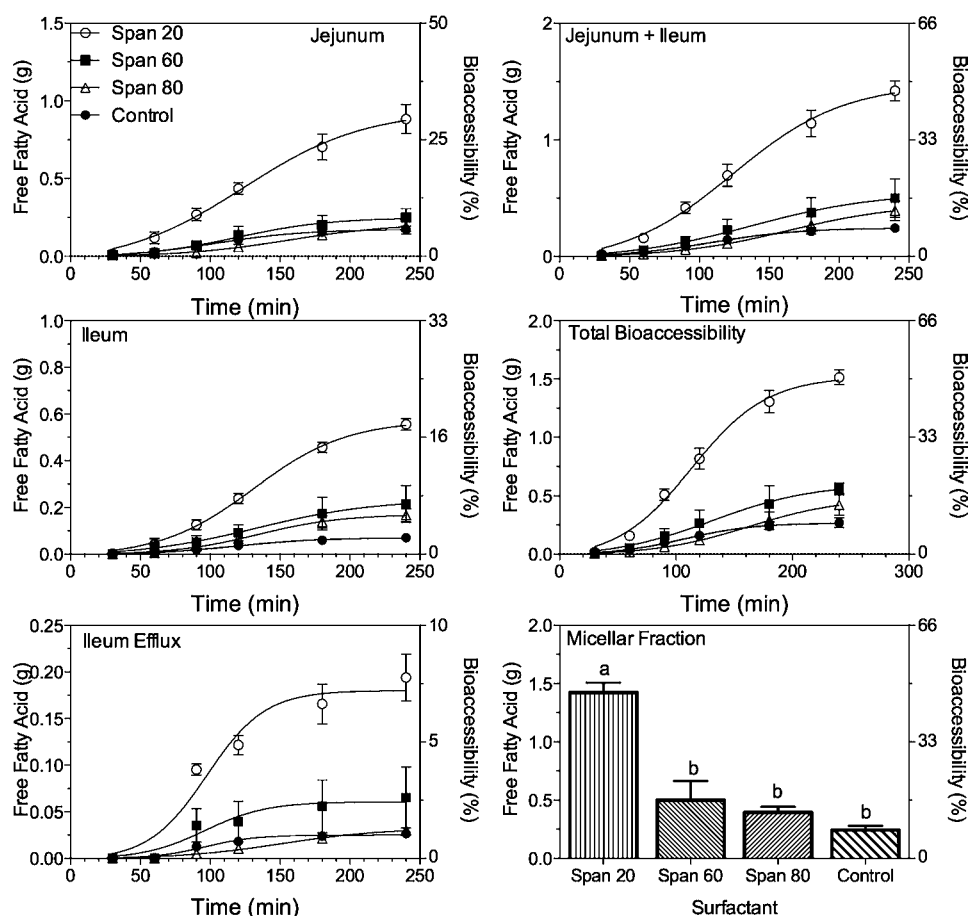


Figure 5. Free FA concentration cleaved from tricaprylin in oil-in-water emulsions stabilized with different Span emulsifiers for different regions of the TIM-1 system. The micellar fraction was obtained from sum of the jejunum and ileum after 240 min. Statistical differences were determined using a one-way ANOVA ($p < 0.05$).

Based on this understanding, a three-parameter shifted logistic model has been proposed to illustrate lipolytic generation of FA in each digestive compartment as a function of time (t). The amount of FA released respective to the degree of digestion may be described in the model as the momentary concentration $C(t)$ of FAs released at time t . The shifted logistic model is expressed as:

$$C(t) = \frac{C_{\text{asympt}}}{1 + e^{[k(t_c - t)]}} - \frac{C_{\text{asympt}}}{1 + e^{(kt_c)}} \quad (1)$$

for which C_{asympt} is the asymptotic level at the plateau region and is indicative of the total amount of FAs released, k is the rate of FA release per unit time, and t_c is the critical time at which half the total amount of FA released is achieved. The additional term of the logistic model shifts the curve to force it through the origin (i.e., $t = 0$ min). The FA concentration, in each digestive compartment, was fitted to eq 1 using a nonlinear analysis in Graphpad Prism (La Jolla, CA). Adjusted R^2 and mean square error were utilized to evaluate the goodness of fit and determine the model's ability to illustrate the data. The parameters of eq 1 were used to compare FA bioaccessibility at each compartment and over the course of digestion. The dependence of the three bioaccessibility parameters, C_{asympt} , k , and t_c , on physicochemical properties of the emulsion are assessed along this study.

RESULTS AND DISCUSSION

Samples were analyzed using GC-MS to determine the concentration of octanoic acid (Figure 3). The peaks, for octanoic (elution time ~ 11.5 min) and nonanoic acids (elution time ~ 12 min) were integrated, and the area under the curve was calibrated using nonanoic acid as an internal standard.

Concentrations of octanoic acid increased with transit time, and the concentration tapered toward the end of the 4 h run.

The concentration of octanoic acid, as a function of time in the jejunum, in the ileum, and at the ileum efflux, was plotted for the nonionic polyethoxylated sorbitan monoesters (Tweens) (Figure 4). The bioaccessibility was interpreted as the FAs generated via the lipolytic activity of lipase in the jejunum, in the ileum, and at the ileum efflux. Differences in the overall bioaccessibility arise when the aliphatic chain length of the Tweens vary (i.e., Tween 20, 40, and 60 having 12, 16, and 18 carbons, respectively). As the aliphatic chain length of the Tweens increases, there is a decrease in the HLB value (i.e., Tween 20 has an HLB value of 16.7, Tween 40 has an HLB of 15.6, and Tween 60 has an HLB of 14.9). The FAs, generated by the lipolytic activity of lipase, were significantly lower (determined using a two-way ANOVA ($p < 0.05$)) in the control compared to all Tween formulated emulsions (Figure 4). Furthermore, irrespective of the location of sampling (i.e., jejunum vs ileum vs efflux), Tween 20 resulted in a significantly greater release of FAs after 4 h of digestion. Compared to the control, addition of 1.5 wt % Tweens 60 resulted in a 2-fold increase in the bioaccessibility of octanoic acid while for Tween 20 the increase in the bioaccessibility was 5 fold.

The overall bioaccessibility for the nonionic acid using Span surfactants were similar to that of the Tween surfactants (Figure 5). Oil-in-water emulsions stabilized by Tween surfactants have been shown to be acid stable, which undergo less phase

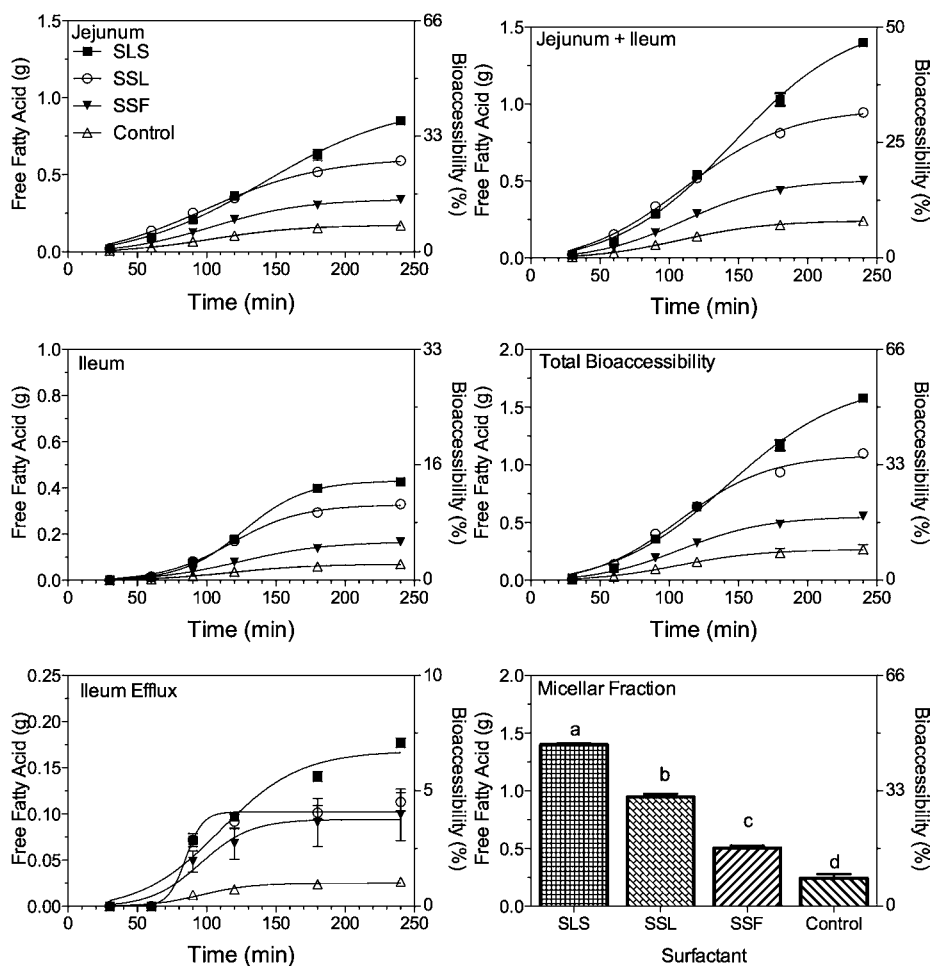


Figure 6. Free FA concentration cleaved from tricapylin in oil-in-water emulsions stabilized with different charged emulsifiers for different regions of the TIM-1 system. The micellar fraction was obtained from the sum of the jejunum and ileum after 240 min. Statistical differences were determined using a one-way ANOVA ($p < 0.05$).

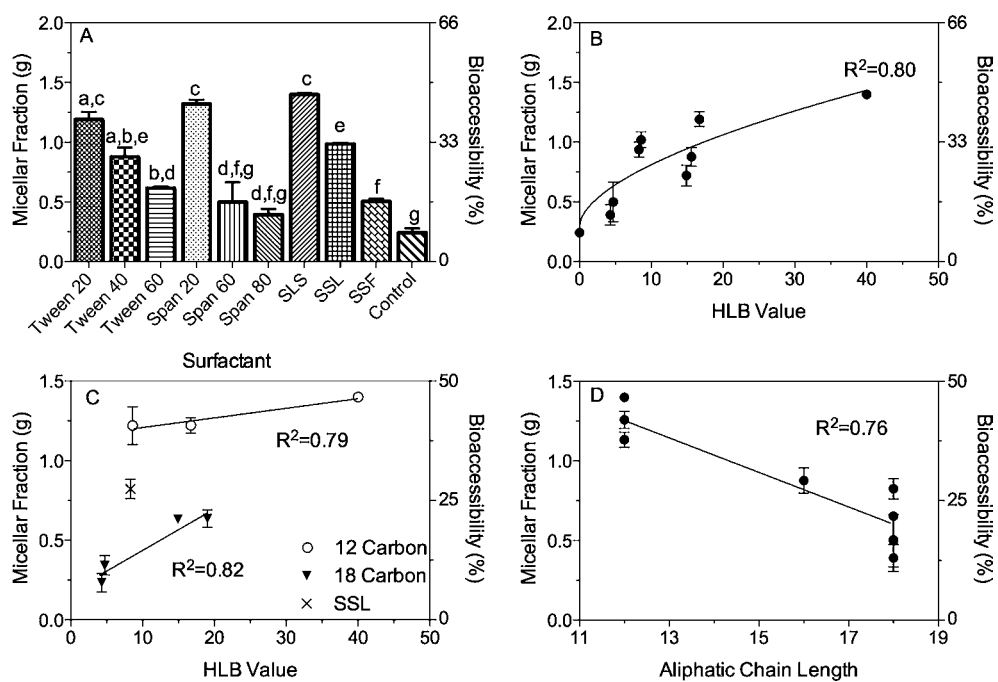


Figure 7. The micellar fraction, obtained from sum of the jejunum and ileum after 240 min, versus the hydrophilic–lipophilic balance (HLB) values. Statistical differences were determined using a one-way ANOVA ($p < 0.05$).

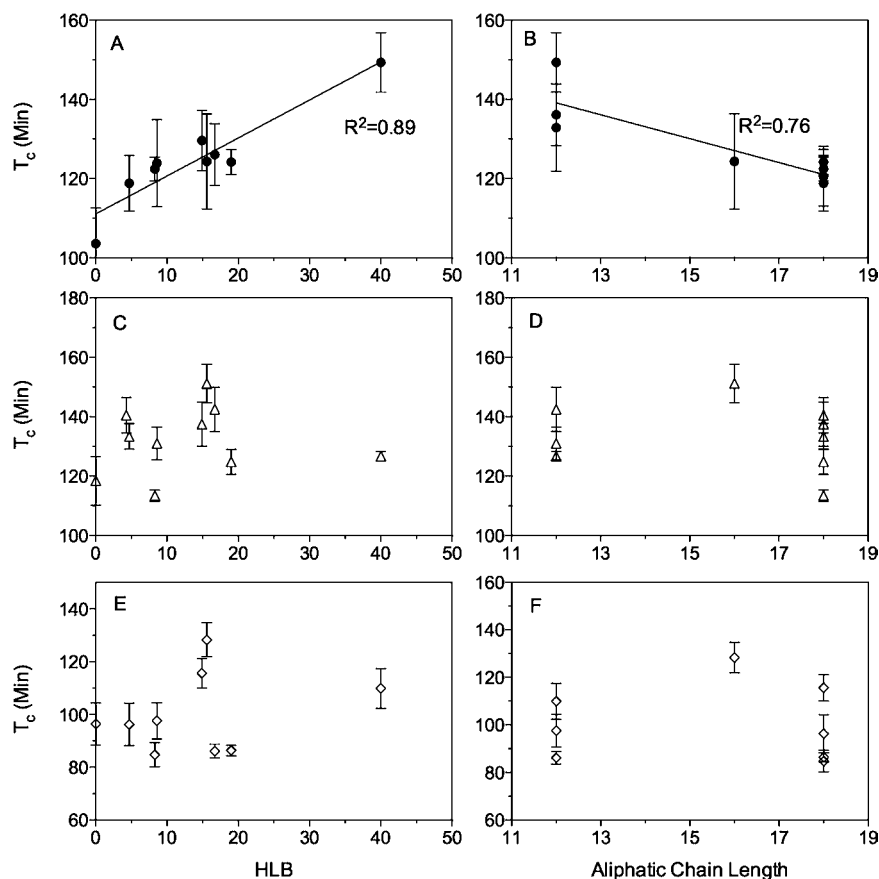


Figure 8. Inflection point of the curve at which half of the net release is reached, calculated using the shifted logistic model proposed to describe the amount of FAs released at different compartments (jejunum A, B), (ileum C, D) and (efflux E, F) of the GI tract as a function of digestion time which is correlated to the HLB value (A, C, E) and the aliphatic chain of the surfactant (B, D, F).

separation in the stomach compared to emulsions stabilized with the acid unstable Spans.²⁷ However, the overall bioaccessibility did not seem to be affected by the acid stability. As the chain length of the aliphatic tail increased, there was a significant decrease in the FA bioaccessibility in the jejunum, in the ileum, and at the ileum efflux. Also for the Spans, when the headgroup and aliphatic chain length were consistent, and only the saturation of the aliphatic chain varied (i.e., stearic versus oleic acid), there was no significant effect on the accessibility of the octanoic acid (Figure 5). Furthermore, when the aliphatic chain length of the Span was 18 carbons, the bioaccessibility was not statistically different than that of the control.

The FA bioaccessibilities were determined for sodium stearyl lactylate (SSL), sodium stearyl fumarate (SSF), and sodium lauroyl sulfate (SLS), which are all anionic surfactants (Figure 6). The FA bioaccessibility for the anionic surfactants were all significantly higher than that of the control with no emulsifier irrespective of the compartment where analysis occurred. SSF also had a significantly higher bioaccessibility than the SLS and SSL, suggesting again that the aliphatic chain plays a central role in limiting FA lipolysis.

Overall, the bioaccessibility varied depending on the polar headgroup of the surfactant, aliphatic chain length of the surfactant, and the presence or absence of surfactant (Figure 7A). The FA bioaccessibility was lowest in the control, Span 60, and Span 80, which were all statistically the same. Previously, it has been shown that plant-derived lipase on its own can easily hydrolyze oil-bodies (protein/phospholipids coated oil) in vitro.²⁸ The lower bioaccessibility of the control corresponds

well to human studies where it was found that nonemulsified long-chain triglycerides induced a smaller CCK release compared to emulsified long-chain triglycerides.²⁹ Typically, the aliphatic chain length determined the bioaccessibility as Tween 20, Span 20, and SLS were all statistically the same (Figure 7A,C,D). Similarly, Tween 60, Span 60, Span 80, and SSF were not statistically different with the exception of SSF and Tween 60. The increase of the length of aliphatic chain causes a decrease in the HLB value and a subsequent decrease in the overall lipid bioaccessibility (Figure 7D). Although the chain length was central in modifying lipid bioaccessibility, the HLB value also seemed to play a role (Figure 7B). It was found that as the HLB value increased, there was a positive correlation with lipid bioaccessibility. While the overall bioaccessibility is useful in understanding the overall amounts of lipid digestion, it provides little insight into the kinetics of digestion because the rate of free FA release is not linear (Figures 4–6).

In an attempt to better understand the mechanism and rate of lipase activity for oil-in-water emulsions, a three-parameter shifted logistic model was applied to the FA concentration versus time profiles (Figures 4–6). Using the shifted logistic model, the coefficient of determination (R^2) was greater than 0.88 for each sample fitted using a least-squares method. T_c , the time to reach effective lipolysis/digestion, can be considered as an indirect measure of the induction or lag time, observed early in digestion and described by Troncoso, Aguilera, and McClements²⁵ as the result of lipids being inaccessible to enzymes because of a surfactant barrier at the interface of the emulsion lipid droplet. It is important to note that not all

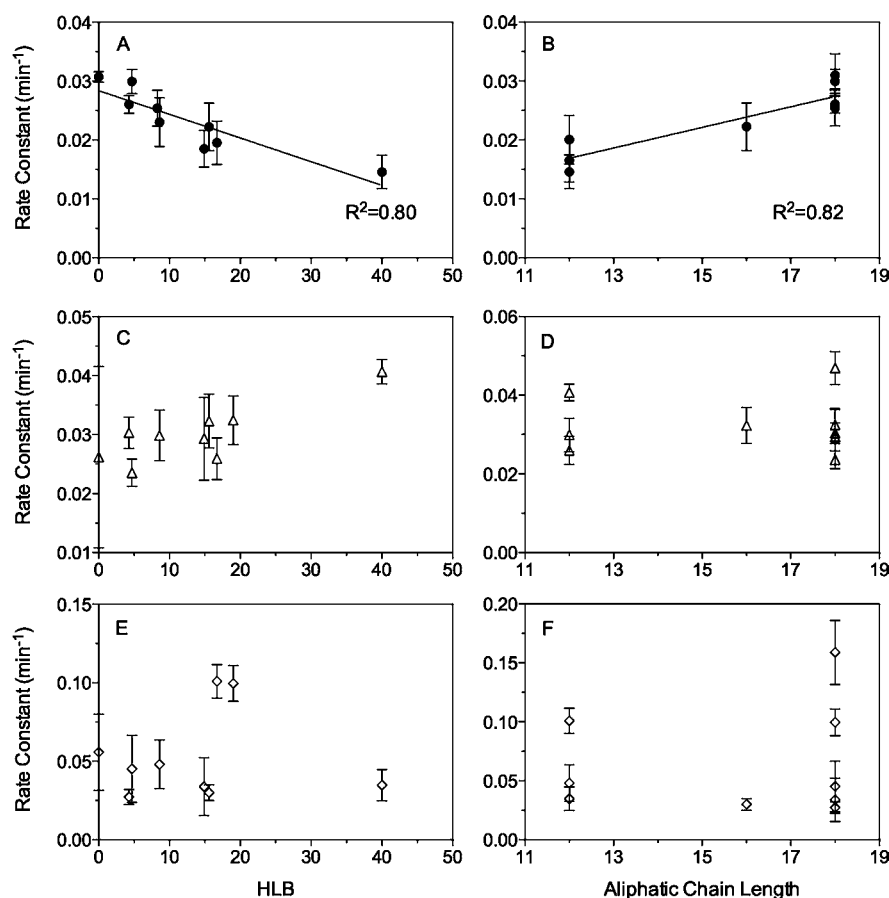


Figure 9. Rate constants calculated using the shifted logistic model proposed to describe the amount of FAs released at compartments (jejunum A, B), (ileum C, D), and (efflux E, F) of the GI tract as a function of digestion time which is correlated to the HLB value (A, C, E) and the aliphatic chain of the surfactant (B, D, F).

emulsifiers follow this kinetic behavior.²⁵ It was obvious that the critical time, t_c , for effective lipolysis varied based on the HLB value and the chain length of the surfactant in the jejunum (Figure 8A,B). However, no change in t_c was observed in either the ileum (Figure 8C,D) or the ileum efflux (Figure 8E,F). This is not surprising because a delay in digestion will be only observed upon initial exposure of bile and lipase that occurs in the jejunum. Once the material arrives at the ileum, lipolysis is already rapidly progressing. In the jejunum, the surfactant's HLB value is positively correlated to the values of t_c (Figure 8A). It is important to note that there is a significant delay in t_c compared to the study by Troncoso, Aguilera, and McClements.²⁵ There are numerous reasons why we have significant delays compared to their study, some of which include sampling intervals, differing enzyme activities, differing shear forces (peristalsis), and bile sources. Although the absolute values differ, comparison in the rank order may still be made between the two studies. The surfactant acts as an oil-in-water emulsifier at low HLB values, and at higher HLB values, the molecule behaves like a detergent. When no surfactant is present, the shortest critical time to effective lipolysis (~ 100 min) is observed and the critical time increases by over 40 min as the HLB value of the surfactant increases. The surfactant aliphatic chain length was negatively correlated with t_c values, suggesting that bile more easily displaces the surfactant with longer aliphatic chains. The negative correlation between the aliphatic chain length and t_c may also be explained by the triglyceride–surfactant interactions.³⁰ In their study, they found that surfactants interact with triglyceride

through the FA regions of the molecules.³⁰ When the hydrophobic regions of the surfactant and triglyceride are the same (i.e., the aliphatic chain lengths and unsaturation degree), then there is a greater intermolecular interaction. The greater the intermolecular interactions, the more difficult the surfactant will be to displace, hence causing a longer t_c .

Once lipolysis has initiated, a continual displacement of surface-active molecules from the interface, by bile, allows lipase continued access to the interface. FAs are cleaved from the triglyceride backbone at the sn-1 and -3 positions and 2-monglyceride remains. Because FAs and monoglycerides are both surface-active molecules, they will compete for the interface with the original surfactant, bile salts, and lipase. Therefore, the interfacial composition is dynamic, undergoing constant exchange with the aqueous phase and resulting in excess FAs, monoglycerides, bile salts, and surfactants forming micelles. The rate of lipolysis depends not only on the lipase access to the interface but also on the displacement of surface-active molecules from the interface, which are formed during lipolysis, via the creation of micellar structures from the shrinking interface.¹⁶ Using the three-parameter shifted logistic model, it is found that the kinetics of lipolysis proceeds most quickly at the unprotected interface where no surface-active compound is initially present (Figure 9A). Although the rate of lipolysis is the fastest in a nonemulsified emulsion, it has the lowest bioaccessibility because of the long lag time and the reaction reaches the plateau the quickest (i.e., shortest reaction time). When surfactant is present, it may act as a detergent,

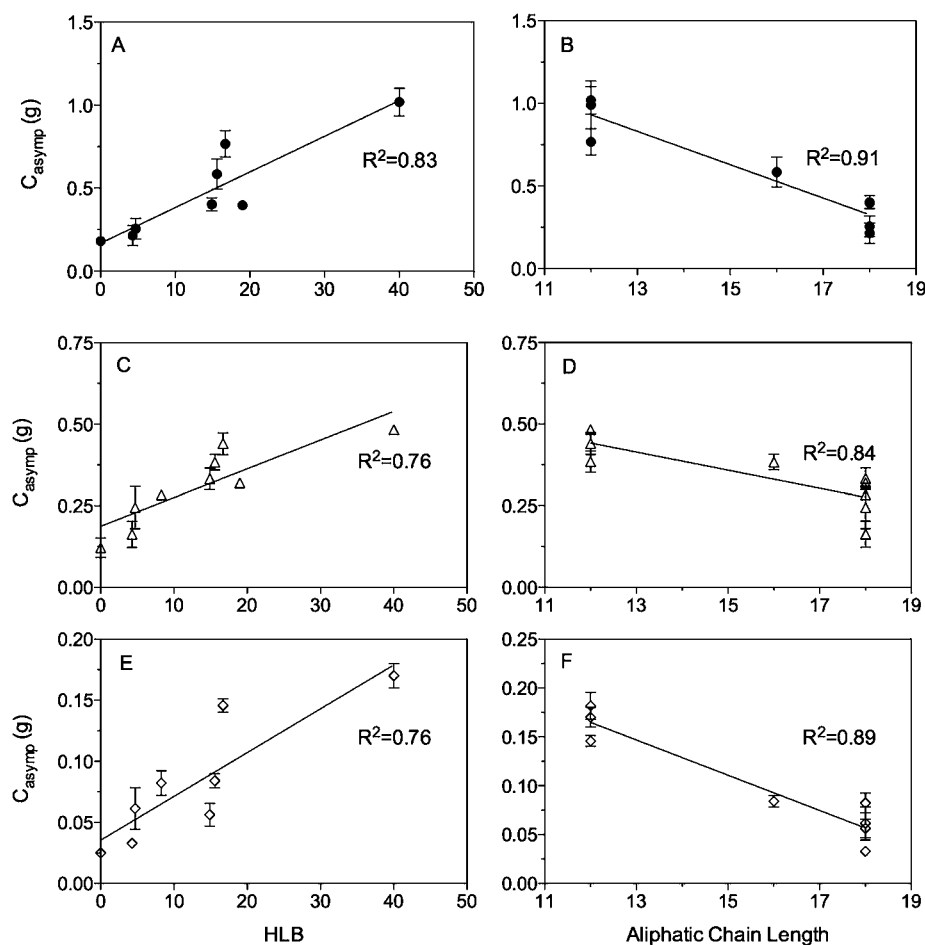


Figure 10. Maximum amount of free FA released, calculated using the shifted logistic model proposed to describe the amount of FAs released at different compartments (jejunum A, B), (ileum C, D), and (efflux E, F) of the GI tract as a function of digestion time which is correlated to the HLB value (A, C, E) and the aliphatic chain of the surfactant (B, D, F).

which then can displace the 2-monoglycerides generated during lipolysis from the interface, allowing lipase to continue to be active for a longer duration. As surfactant is added to the system, the rate of lipolysis decreases and the HLB value is negatively correlated with the rate of lipolysis (Figure 9A). The negative correlation between HLB and rate of lipolysis is attributed to the increased hydrophilic nature of the surfactant, making the surfactant reside more in the water phase at the interface than in the oil phase. Put simply, when the surface active molecule resides more on the water side of the interface, it limits lipase access to the interface, which is approaching the interface from the continuous aqueous phase. As the chain length of the surfactant increased, there was an increase in the rate of lipolysis (Figure 9B). The positive correlation between surfactant chain length and rate of lipolysis is less simple to explain. Because the correlation between induction time and chain length is negative, the rate of lipolysis may increase because of a less efficient packing of the monolayer, preventing inhibition of lipase at the interface. This is also reflected in the time to effective lipolysis, which is negatively correlated. The long aliphatic chain, with a large degree of freedom, will have a molecular volume much larger than that of the short aliphatic chains. This has been shown to impede micellar formation because of ineffective packing at the interface.³¹ In other words, as the molar volume increases for the aliphatic chain, the surfactant hydrophilic head groups must be further apart to

accommodate and prevent the side chains from overlapping. This inefficient packing makes the surfactants more easily to displace by the bile salts, allowing lipase to access the interface.

Once in the jejunum, bile salts displace the original surfactant and lipase begins to generate new surface-active molecules that compete with the bile salts and emulsifier for the interface. It has previously been suggested that 2-monoglycerides at the interface are very effective at limiting lipase activity.¹⁶ Hence, by the time the emulsion reaches the ileum, the original surfactant has been displaced by bile salts, monoglycerides, and FAs; therefore, the interface at this point is similar in all systems and therefore no differences are observed in the rate of lipolysis either at the ileum or at the ileum efflux.

The overall bioaccessibility, or the level of the plateau in FA concentration, C_{asymp} , is dependent not only on the rate of lipolysis but also on the effective time to lipolysis and on the duration which lipase can interact with the changing oil–water interface. The overall lipid bioaccessibility increased as the HLB value of the surfactant increased in the jejunum, in the ileum, and at the ileum efflux (Figure 10A,C,E). Previously, it was found that pluronic F68, a nonionic surfactant, is more resistant to bile salt adsorption than Epikuron 145 V, an anionic surfactant, at higher concentrations of bile salts.³² Our study found similar results, where the nonionic surfactants had a bioaccessibility lower than that of the anionic surfactants. The increase in the bioaccessibility correlated to a decrease in the

rate of the lipase activity and an increase in the t_c values. Therefore, the mechanism, which results in a higher lipid bioaccessibility, cannot be explained simply by a more accessible interface for lipase. Previous work by Reis et al. illustrated 2-monoglycerides that formed by the lipolytic activity of lipase effectively displace lipase from the interface.¹⁶ Molecules with high HLB values act as detergents and solubilizers, which may aid in the solubilization of the 2-monoglycerides from the oil and water interface. By displacing the 2-monoglyceride from the interface, lipase will continue to be active at the interface, continuously cleaving FAs from the triglycerides. This would be a simple explanation for why lipid bioaccessibility is positively correlated with the HLB value. Conversely, the aliphatic chain length of the amphiphilic molecule is negatively correlated with lipid bioaccessibility in all zones of simulated gastrointestinal track (Figure 10B,D,F). Because the aliphatic chain length of the surfactant is correlated with the HLB value, the same previously proposed mechanism that enhances lipid bioaccessibility explains the effect of surfactant chain length.

Drastic differences in the rank order of lipase activity/lipid bioaccessibility in oil-in-water emulsions were found using nonionic surfactants and anionic surfactants. The biophysical makeup of the oil-and-water interface clearly has an impact on the bioaccessibility of lipids. It was found that the bioaccessibility was positively correlated with the HLB of the surfactant and inversely correlated with the surfactant aliphatic chain length. Furthermore, the induction time in the jejunum increased as the HLB value got larger and decreased with increasing aliphatic chain length. The rate of lipolysis slowed in the jejunum with increasing HLB and with decreasing aliphatic chain length.

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