Emulsifier Solubilization in the Bile Micelles
Controls Lipase Adsorption and Fat Lipolysis

ACS Publications
MOST TRUSTED. MOST CITED. MOST READ.

www.acs.org

Effects of Emulsifier Charge and Concentration on Pancreatic Lipolysis: 2. Interplay of Emulsifiers and Biles

Zahari Vinarov,[†] Slavka Tcholakova,*[,†] Borislava Damyanova,[†] Yasen Atanasov,[†] Nikolai D. Denkov,[†] Simeon D. Stoyanov,[‡] Edward Pelan,[‡] and Alex Lips[§]

[†]Department of Chemical Engineering, Faculty of Chemistry and Pharmacy, Sofia University, 1164 Sofia, Bulgaria
[‡]Unilever R&D, Vlaardingen, The Netherlands
[§]Unilever Discover, Port Sunlight Laboratory, Quarry Road East Bebington, CH63 3JW, Wirral, U.K.

S *Supporting Information*
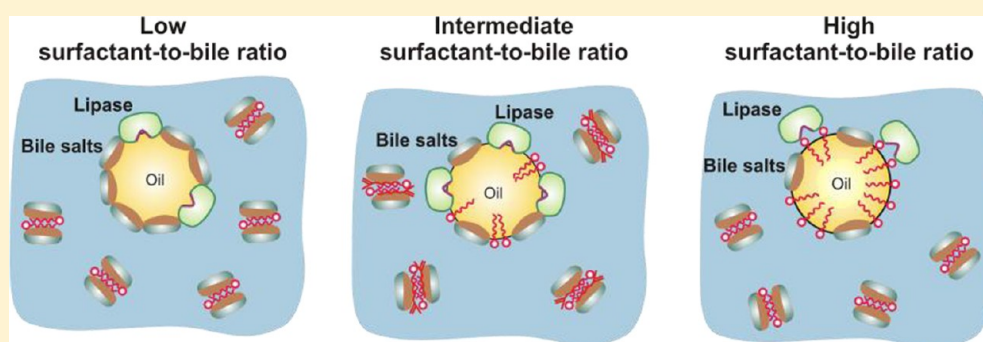
ABSTRACT: As a direct continuation of the first part of our in vitro study (Vinarov et al., *Langmuir* **2012**, *28*, 8127), here we investigate the effects of emulsifier type and concentration on the degree of triglyceride lipolysis, in the presence of bile salts. Three types of surfactants are tested as emulsifiers: anionic, nonionic, and cationic. For all systems, we observe three regions in the dependence degree of fat lipolysis, α, versus emulsifier-to-bile ratio, f_s: α is around 0.5 in Region 1 ($f_s < 0.02$); α passes through a maximum close to 1 in Region 2 ($0.02 < f_s < f_{TR}$); α is around zero in Region 3 ($f_s > f_{TR}$). The threshold ratio for complete inhibition of lipolysis, f_{TR}, is around 0.4 for the nonionic, 1.5 for the cationic, and 7.5 for the anionic surfactants. Measurements of interfacial tensions and optical observations revealed the following: In Region 1, the emulsifier molecules are solubilized in the bile micelles, and the adsorption layer is dominated by bile molecules. In Region 2, mixed surfactant-bile micelles are formed, with high solubilization capacity for the products of triglyceride lipolysis; rapid solubilization of these products leads to complete lipolysis. In Region 3, the emulsifier molecules prevail in the adsorption layer and completely block the lipolysis.

1. INTRODUCTION

Fat digestion is widely studied in the literature[1−20] due to its importance for human health, and for the food and pharmaceutical industries. In recent years, there has been a growing interest in the understanding of the factors controlling the rate of fat absorption in the gastrointestinal tract (GIT), due to its relation to various diseases such as obesity, type-2 diabetes, artherosclerosis, tuberculosis, and so forth.[17]

One possible way to control fat absorption is to regulate enzyme activity in the GIT by using enzyme inhibitors. An example for such control is Orlistat, a globally licensed antiobesity drug.[17]

Another way to regulate enzyme activity is to use appropriate emulsifiers, which compete with the enzymes for adsorption on the fat−water interface, thus decreasing the rate of fat lipolysis. This possibility was examined in various in vitro studies, in which the rate of fat lipolysis was determined for different types of emulsifiers.[4,12−34] It was shown in these studies that the digestion rate can be significantly reduced using appropriate emulsifiers, such as biopolymers,[31] sn-2 monoglycerides (MGs),[14] digalactosyldiacylglycerol,[20] poloxamer Pluronic F68,[21] lysophosphatidylcholine (LysoPC),[32] green tea extracts,[33] and dietary fibers.[34]

It was found in these studies that, besides the effect of the emulsifiers used, the rate of fat lipolysis depends significantly on the concentration of the reaction products formed (fatty acids and MGs),[9,13,14,35] and on the concentrations of bile salts and calcium in the reaction mixture.[10,11,16,36,37] The accumulating reaction products were found to inhibit the enzyme activity,[9] while the micelles of bile salts solubilize these products, thus removing them from the fat−water interface and restoring lipase activity.[6] Furthermore, it was established that the lipase activity may be suppressed strongly by the bile salts themselves, if the reaction mixture does not contain a pancreatic colipase, a

small protein that adsorbs on the fat-water interface even in the presence of complete adsorption layer of bile molecules and thus promotes lipase activity in the presence of bile salts.[17] These studies revealed rather complex interactions between the various components involved in the fat digestion process.

The effects of the specific emulsifiers and their interaction with the bile salts have been studied extensively in literature.[4,7,8,10,20,24,25,38] Thus, Gargouri et al.[4] observed inhibition of the pancreatic lipase by various surfactants in the absence of bile salts, and subsequent lipase reactivation after addition of bile salts. Wickham et al.[7,8] showed that the addition of bile salts to a phospholipid stabilized emulsion destroys the packed phospholipid adsorption layer, and a mixed phospholipid/bile layer is formed. This mixed layer allows greater binding of the lipase to the fat−water interface.[7,8] The ability of bile salts to displace proteins from the fat−water interface was also found to facilitate the lipolysis of protein-stabilized emulsions.[24] These studies convincingly demonstrate that the bile salts can significantly facilitate the lipolysis of emulsions stabilized by low-molecular-mass surfactants, phospholipids, and proteins. Interestingly, in other studies, it was shown[21] that less surface active molecules, compared to lecithin, such as Pluronic F68, can significantly delay emulsion digestion, even in the presence of bile salts, whereas the lecithin-stabilized emulsions are easily digested by the enzyme under similar conditions.[22] Therefore, no direct correlation between the surface activity of the emulsifiers and their ability to inhibit enzyme activity has been established in the literature. Also, no clear conclusions about the mechanisms of these effects were formulated.

Recently, Li et al.[25] studied the effect of the concentration of low-molecular weight surfactants on the lipase activity. These authors found that, at sufficiently high concentrations, all surfactants studied were able to inhibit the pancreatic lipase. To explain this effect, the authors suggested several possible mechanisms, without defining which mechanism is operative in the systems studied by them.

Summarizing this literature review, many interesting effects related to the interactions between emulsifiers and bile salts were reported in the literature. However, there is a lack of understanding of the specific mechanisms that govern these effects.

The aim of the current study is to systematically investigate the effect of low-molecular mass surfactants (as emulsifiers) on the triglyceride (TG) lipolysis by pancreatic lipase and to provide a mechanistic explanation of the main effects observed. The factors studied are the surfactant concentration, charge, and length of the hydrophobic tail (varied between 12 and 16 carbon atoms). Purposely, to clarify the effect of surfactant type, we have chosen a series of surfactants that are not necessarily of food grade; in this way, we could focus on the physicochemical factors involved in the process of lipase inhibition by surfactants and in the related process of lipase reactivation by bile salts.

In the first part of this study,[39] we presented experimental results for the effect of emulsifier type and concentration, C_S, on the degree of fat hydrolysis, α, by pancreatic lipases, in the absence of bile salts in the reaction mixture. As substrate for the lipase action, we used sunflower oil (SFO)-in-water emulsions, stabilized by different types of surfactants as emulsifiers: anionic (sodium laurylethersulfate (SLES) and α-olefinsulfate (AOS)), nonionic (Tween 20 and Tween 80), and cationic (cetyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB)). Experiments aimed to

determine the coverage on the drop surface, θ, by the emulsifier molecules were also performed. For all studied emulsifiers, three different regions in the dependence of α on θ were determined: At low surface coverage, up to $\theta \approx 0.6$, the degree of fat lipolysis remained almost constant $\alpha \approx 0.5\%$ (Region 1). At higher surface coverage (between 0.6 and 0.7), a significant decrease of the degree of fat lipolysis was observed (Region 2). At high surface coverage and high surfactant concentration ($C_S \gg$ CMC), the lipolysis was suppressed (Region 3). Lipolytic experiments with (partially) water-soluble substrate tributirin demonstrated that the lipase was still very active in Region 3, which was a direct proof that the surfactants did not deactivate the used lipase and colipase (e.g., by denaturing them).[39] Thus we concluded that the lipase inhibition in the studied systems was due to the formation of almost completed surfactant adsorption layers, which blocked the direct contact of the pancreatic lipase (and/or the colipase) with the insoluble SFO substrate.[39]

In the current paper we continue these studies by performing experiments in the presence of bile salts. The same types of surfactants were used, and the degree of fat transformation was determined as a function of surfactant-to-bile ratio. To clarify the reasons for the observed apparent discrepancy between the surface activity of the emulsifiers and their ability to inhibit the lipase activity in the presence of bile salts, the interfacial tensions of the studied solutions were measured, and optical observations of the oil drops were performed. The experimental results are analyzed by considering the roles of the competitive adsorption of emulsifiers and bile acids on the oil−water interface, the solubilization of the reaction products in the aqueous phase, and the precipitation of these products on the drop surface.

The article is structured as follows: in section 2 we describe the materials and methods used, in section 3 we present the experimental results, in section 4 we discuss the main factors controlling the observed phenomena, and in section 5 we summarize the main conclusions.

2. MATERIALS AND METHODS

2.1. Materials. As the source of bile salts we used porcine bile extract, obtained from Sigma-Aldrich (cat. no. B-8631, no. 038K0014). This extract contains 50 wt % bile acids, 6 wt % phosphatidylcholine, and less than 0.06 wt % Ca^{2+}.[10] Our analysis by gas chromatography (GC) also showed the presence of 1.8 wt % cholesterol and 4.3 wt % fatty acids.[40] According to the producer, the composition of the bile salts in this extract is 13 wt % hyodeoxycholic acid, 18 wt % deoxycholic acid, 5 wt % cholic acid, 39 wt % glycodeoxycholic acid, and 24 wt % taurodeoxycholic acid. The percentages of these bile acids and the corresponding molecular masses were used to calculate an average molecular mass of 442 g/mol; the latter was used to define the average molar concentration of bile salts in our experiments.

Pancreatin from porcine pancreas (Sigma-Aldrich, cat. no. P8096), containing a range of enzymes including amylase, trypsin, lipase, ribonuclease, and protease, was used as the source of pancreatic lipase and colipase (at 1:1 molar ratio in the pancreatic source[21,41]). The lot numbers of the used pancreatin samples were 088K0764 and 087K1888. According to the producer, both lots were with activity of 4.9 USP units/mg, defined as follows: pancreatin releases 4.9 μequiv of fatty acids/min/mg of pancreatin from olive oil at pH = 9.0 at 37 °C.

Pepsin from porcine gastric mucosa (Fluka, cat. no. 77160) with lot number 1238420 was used. The activity of this pepsin is 643 U/mg. One unit here corresponds to the amount of enzyme, which increases the absorbance at 280 nm by 0.001 per minute at pH = 2.0 and 37 °C Hemoglobin.

The emulsions were prepared with SFO, purified as described in ref 39. As emulsifiers, we used the nonionic surfactants Tween 20 (Fisher Scientific, cat. No. PP337) and Tween 80 (Fluka, cat. no. 93780), the cationic surfactant CTAB (product of Sigma, cat. no. H9151) and the anionic surfactants SLES (commercial name CS-170, product of Stepan Co.) and AOS (product of Teokom). These emulsifiers were used as received.

As electrolytes we used NaCl (Riedel de Haen, cat. no. 13423), KCl (Merck, cat. no. 1.04936.1000), Tris (hydroxymethyl)-aminomethane (Sigma, cat. no. T87602), $CaCl_2.6H_2O$ (Fluka, cat. no. 21110), hydrochloric acid (Merck, cat. no. 1.00318.1000) and NaOH (Sigma, cat. no. 82730).

Xenical (Roche), which contains tetrahydrolipstatin (Orlistat), was purchased from local pharmacies and used as a pancreatic lipase inhibitor.[17,41,42]

All solutions were prepared with deionized water, which was purified by an Elix 3 water purification system (Millipore). The organic solvents used for extraction of lipolysis products and for thin-layer chromatography (TLC) were chloroform (Sigma, cat. no. 32211), diethyl ether (Sigma, cat. no. 32203), petroleum ether (Merck, cat. no. 1.01775.5000), and acetic acid (Merck, cat. no. 511 Z96982).

2.2. In Vitro Lipolysis Model. First, we prepared the following basic (stock) solutions, which were afterward mixed to prepare the final solutions for the actual experiments: Saline solution containing 150 mM NaCl + 5 mM KCl in water, and electrolyte solution containing 5 mM Tris +15 mM $CaCl_2$ + 40 mM NaCl in water. These solutions were prepared and stored at room temperature. Before using them for dissolving bile salts or mixing with pancreatic solution, we thermostatted these solutions at T = 37 °C.

The pepsin solution was prepared by dissolving 12.5 mg of pepsin in 2.5 mL of 0.25 M HCl, upon stirring for 5 min at T = 37 °C. Bile salt solution was prepared by dissolving 250 mg of bile extract in 5 mL of electrolyte solution, and pancreatic solution was prepared by dissolving 30 mg of pancreatin in 5 mL of saline solution. Pepsin, bile salts, and pancreatic solutions were directly prepared at 37 °C, just before their use in the actual experiments.

The order of solution mixing is schematically shown in Figure 1. First, 109 μL of 60 vol % SFO emulsion (see section 2.3 for its preparation) was added to 17.5 mL of saline solution by a micropipet. Second, 2.5 mL of pepsin solution was added, and the obtained mixture with pH = 2.0 ± 0.1 was stirred for 1 h. This stage of mixing is aimed at mimicking the conditions in the stomach, where pepsin is one of the major enzymes and the pH is low.[35]

Afterward, the pH was adjusted to 7.5 with NaOH, and then 5 mL of bile solution was added. Since the addition of bile caused a slight decrease of pH, it was readjusted with NaOH to 7.5. Finally, pancreatic solution was added, and the whole mixture was stirred for 0.5, 2, or 4 h. The final composition of the reaction mixture is 0.2 wt % SFO, 0.1 wt % pancreatin, 0.042 wt % pepsin, 0.833 wt % bile extract (9.5 mM bile salts), 0.83 mM Tris, 2.5 mM $CaCl_2$, 140 mM NaCl, and 3.75 mM KCl. This stage of mixing is aimed at mimicking the conditions in the duodenum and small intestine, where pH in the fasted state is measured to be between 5.6 and 8.1.[43−45] In our experiments, it starts from 7.5 and, after the addition of enzyme, gradually decreases to 6.5, due to the formation of reaction products. The latter value is in the range of values measured in vivo (pH between 5.0 and 6.6) for the fed state.[45]

To stop the lipolysis after a certain reaction time, we added 24 mg of granules of Xenical (Roche), which contained 12 mg of the pancreatic lipase inhibitor tetrahydrolipstatin. The extraction of the reaction products (fatty acids, MGs, and diglycerides (DGs)) and nontransformed triglyceride in the chloroform was performed by the procedure described in ref 39. The obtained chloroform phase was further analyzed by TLC as described in ref 39.

The extent of lipolysis reaction was characterized by the overall degree of TG transformation, α, defined as

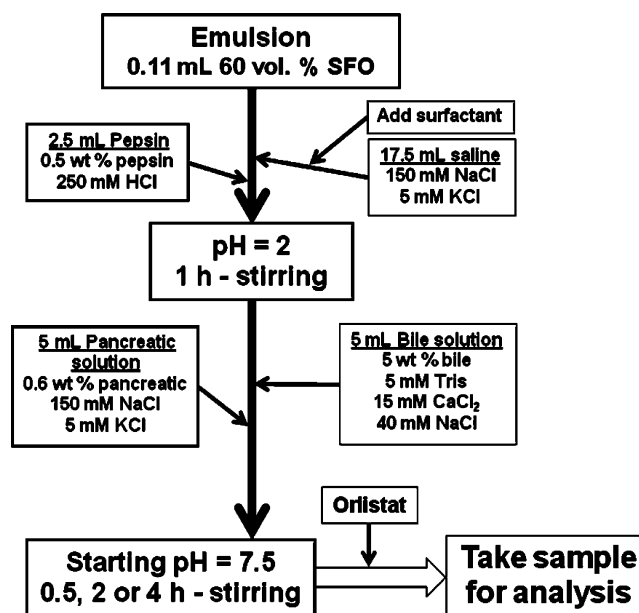$$\alpha = \frac{C_{TG}^{INI} - C_{TG}^{END}}{C_{TG}^{INI}} \qquad (1)$$

Figure 1. Schematic presentation of the used in vitro model for triglyceride lipolysis. The first stage, involving pepsin and stirring at low pH = 2, mimics the stomach conditions. The second stage, involving pancreatic and bile solutions and stirring at pH = 7.5 (which gradually decreases down to 6.5 with the advance of the lipolysis reaction), mimics the conditions in the duodenum and the small intestine.

Here C_{TG}^{INI} is the initial concentration of TGs in the reaction mixture and C_{TG}^{END} is the final concentration of the TGs remaining intact after the reaction time.

Let us note that the overall degree of TG transformation measured in our experiments is affected by the accumulation of reaction products (free fatty acids, MGs, and DGs) with the advance of the lipolysis reaction. However, because the major aim of our study is to investigate the complex interplay between all components involved in the lipolysis and the related physicochemical phenomena (competitive adsorption, solubilization in bile micelles, and transfer of the reaction products across the oil−water interface), we have chosen to measure this integral quantity, which reflects all occurring interrelated phenomena.

2.3. Emulsion Preparation. Stock oil-in-water emulsions were prepared by stirring 20 mL of emulsifier solution and 30 mL of SFO for 5 min, to obtain a 60 vol % emulsion. A rotor-stator homogenizer Ultra Turrax T25 (Janke & Kunkel GmbH & Co, IKA-Labortechnik), operating at 13 500 rpm, was used for emulsification. The emulsifier solution contained 1 wt % surfactant, 10 mM NaCl, and 0.1 g/L of the antibacterial agent NaN_3. The formed emulsions were stored in glass jars for no more than 1 week, at room temperature. Before usage, these stock emulsions were rehomogenized by a gentle hand-shaking. For performing the actual lipolysis experiments, the necessary amount of the stock emulsion was taken by a pipet and diluted in the electrolyte−enzyme solutions. The drop size distribution in these emulsions was determined by video-enhanced optical microscopy, as described in ref 39.

2.4. Interfacial Tension Measurements. The interfacial tension of the SFO with the studied aqueous solutions was measured by the pendant drop method on a DSA 100 M instrument (Krüss, Germany) at T = 37 °C, by using a thermostatting chamber (Krüss, Germany). These measurements were performed in the presence of all electrolytes, as described in section 2.2, and in the absence of enzymes. The obtained dependences of the interfacial tension on time, $\sigma(t)$, were converted to $\sigma(t^{-1/2})$ and fitted with linear dependence to extrapolate the values of the surface tension to infinite times; from the intercept of the fit, the equilibrium value of the interfacial tension was determined and used for construction of the plots $\sigma_{EQ}(f_s)$, where f_S denotes the surfactant-to-bile ratio in the reaction mixture.

2.5. Optical Observations. The studied emulsions, containing enzymes and electrolytes as described in section 2.2, were introduced in a Petri dish and placed in a thermostatted cell with temperature $T = 37\ °C \pm 1\ °C$. The oil drops were observed in transmitted light with a Axioplan microscope (Zeiss, Germany) and objectives with magnification of ×20 or ×50. These observations provided information about the possible formation of solid precipitates on the surface of the SFO drops, in the course of the lipolysis reaction. Indeed, the formation of solid precipitates leads to visible corrugations of drop surface and to deviations of the drop shape from the spherical one.

3. EXPERIMENTAL RESULTS

3.1. Effect of the Reaction Time on the TG Lipolysis. In this series of experiments, we studied the kinetics of TG lipolysis for Tween 80-stabilized emulsions at two surfactant concentrations, $C_S = 0.005$ and 0.1 wt %, in the presence of 9.5 mM bile salts in the solution. The mean volume−surface diameter of the emulsion droplets was $d_{32} = 18\ \mu m \pm 5$ in these emulsions. The obtained results are shown in Figure 2. One

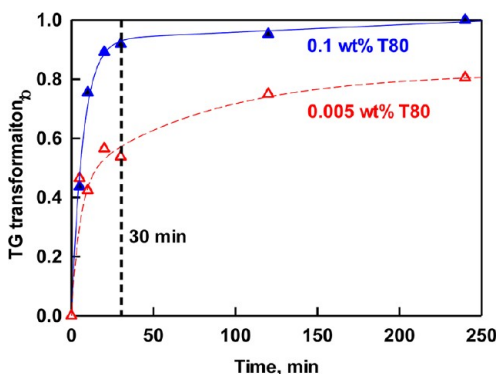**Figure 2.** Degree of TG lipolysis, α, as a function of reaction time, for emulsions stabilized by Tween 80, with different Tween concentrations in the reaction mixture: 0.1 wt % (filled triangles, solid line) or 0.005 wt % (open triangles, dashed line). The lines are eye guides.

sees a steep increase in α during the first 15 min, followed by a much slower increase of α in the next 4 h for both surfactant concentrations studied. At $C_S = 0.1$ wt %, the TG hydrolysis is almost completed after 30 min, $\alpha \approx 90\%$ (and $\alpha \approx 100\%$ after 4 h). At $C_S = 0.005$ wt %, the TG transformation increases up to $\alpha \approx 55\%$ during the first 30 min and reaches 80% after 4 h. We can conclude from these experiments that, under these conditions, the lipolysis is faster during the first 30 min and becomes much slower afterward. Therefore, most of the experiments described below, aimed to compare the various emulsifiers and to clarify the effect of surfactant concentration, are performed at 30 min reaction time.

3.2. Effect of Bile Salts on TG Lipolysis for Different Emulsifiers. The major aim of this series of experiments was to determine the effect of bile salts on the degree of fat lipolysis for the different emulsifiers studied (SLES, AOS, CTAB, Tween 20, and Tween 80) at fixed concentrations of the surfactant ($C_S = 0.005$ wt %) and bile salts ($C_B = 9.5$ mM) in the reaction mixture. The mean volume−surface diameter of the studied emulsions was as follows: $d_{32} = 13 \pm 3\ \mu m$ for AOS, SLES, and Tween 20, and $18 \pm 5\ \mu m$ for CTAB and Tween 80. The reaction time was 30 min. In the experiments without bile salts, this surfactant concentration corresponds to different surface coverage for the studied emulsifiers,[39] and, as a consequence, the degree of fat lipolysis varies between 0 (for

CTAB) and 40% (for Tween 20 and Tween 80 stabilized emulsions) (see the open symbols in Figure 3).
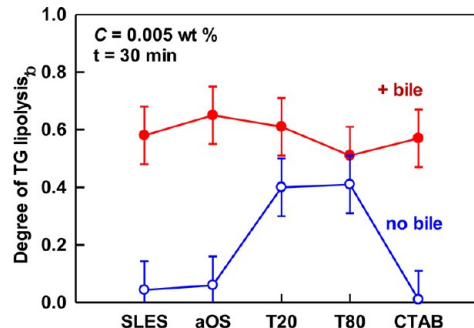

Figure 3. Dependence of the degree of TG lipolysis on the type of used emulsifier, in the absence (open symbols) and in the presence of bile salts (filled symbols). The emulsifier concentration is 0.005 wt %, and the reaction time is 30 min for all systems. The experiments are performed in the presence of 9.5 mM bile salts, as described in section 2.2. The results for the systems without bile salts are taken from the first part of this study.[39] All data points and error bars are determined from at least four independent experiments.

In contrast, the degree of TG transformation for all studied emulsifiers was almost the same ($\alpha \approx 60\% \pm 10\%$) in the experiments performed with bile salts in the reaction mixture (see the filled symbols in Figure 3). Thus we see that the enzyme activity is recovered when bile salts are present in the reaction mixture, which is in a good agreement with the results reported in the literature for similar surfactant−bile mixed systems.[4] It is worth mentioning that the surfactant concentration in this experimental series varied between 0.04 mM (for Tween 80 and Tween 20) and 0.15 mM (for SLES), which are all much lower than the bile concentration in the solution, $C_B = 9.5$ mM. This estimate shows that the surfactant-to-bile ratio, defined as $f_S = C_S/C_B$, is below 0.02 for all emulsifiers used in this series of experiments. Thus, at $f_S < 0.02$, we do not see any significant effect of the used emulsifier on the degree of TG hydrolysis.

3.3. Effect of Surfactant-to-Bile Ratio on TG Lipolysis. The next series of experiments was aimed at determining the degree of TG lipolysis, α, as a function of emulsifier concentration, C_S, at a fixed bile concentration of 9.5 mM. The emulsifier concentration was varied in the range between 0.005 and 2 wt %.

The obtained results for the different emulsifiers are compared in Figure 4. Three important features of the obtained results should be emphasized: (1) At low emulsifier concentration, $C_S = 0.005$ wt %, the degree of lipolysis is not affected by the type of emulsifier and is $\alpha \approx 0.6 \pm 0.1$ (Region 1). (2) For all emulsifiers, the dependence $\alpha(C_S)$ passes through a maximum with $\alpha \approx 0.8$−1.0 (Region 2), followed by a decrease and complete inhibition of the lipolysis, $\alpha = 0$, at high emulsifier concentrations (Region 3). (3) The threshold surfactant concentration for complete inhibition of enzyme activity is much higher for the anionic surfactants, compared to the cationic and nonionic ones.

To determine the effect of bile salt concentration, C_B, on the observed dependence $\alpha(C_S)$, we performed an additional series of experiments with Tween 20-stabilized emulsions, in which C_B was increased twice to 19 mM. The obtained results for the two bile salt concentrations are compared in Figure 5. One sees
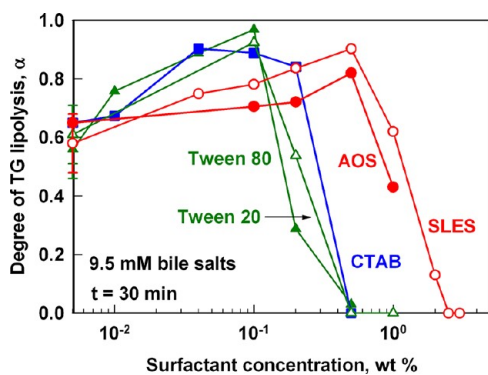
Figure 4. Degree of TG lipolysis, α, as a function of surfactant concentration in the reaction mixture, for emulsions stabilized with CTAB (filled squares), Tween 80 (filled triangles, solid line), Tween 20 (open triangles, dashed line), AOS (filled circles, solid line), or SLES (open circles, dashed line). The concentration of bile salts is 9.5 mM, and the reaction time is 30 min. The experimental data for Tween 80 are average from three independent series of experiments, for Tween 20 and SLES from two independent series of experiments, and for CTAB and AOS from a single series of experiments. The scattering of the data for a given system is small and is represented by the size of the symbols, except for the lowest surfactant concentrations where error bars are shown.
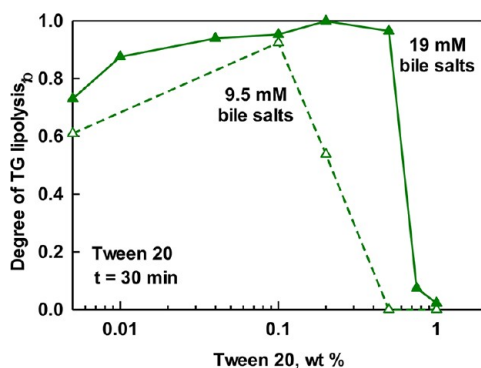

Figure 5. Degree of TG lipolysis, α, as a function of surfactant concentration in the reaction mixture, for Tween 20 stabilized emulsions, in the presence of 9.5 mM (dashed line) or 19 mM bile salts (solid line). The reaction time is 30 min. The data are averages from two independent series of experiments for 9.5 mM bile salts, whereas a single experimental series was performed at 19 mM bile salts.

that the dependences $\alpha(C_S)$ are very similar in shape for the two values of C_B. However, the values of α in Regions 1 and 2 are higher at the higher bile concentration, and the threshold surfactant concentration that corresponds to complete inhibition of enzyme activity is almost twice higher for the solutions containing 19 mM bile salts. The latter result indicates that a more relevant physicochemical parameter, which controls the overall shape of the transformation curves, is the surfactant-to-bile molar ratio, f_S, rather than the surfactant concentration *per se*.

To further check the latter suggestion, we rescaled the data shown in Figures 4 and 5 and represent them as dependence $\alpha(f_S)$ in Figure 6. One sees three well-defined regions for all emulsifiers studied: (1) In large excess of bile salts, corresponding to $f_S < 0.02$, the degree of lipolysis is not affected by the type of emulsifier at fixed C_B, while α depends on C_B. Namely, $\alpha \approx 0.6$ at $C_B = 9.5$ mM and $\alpha \approx 0.75$ at $C_B =$
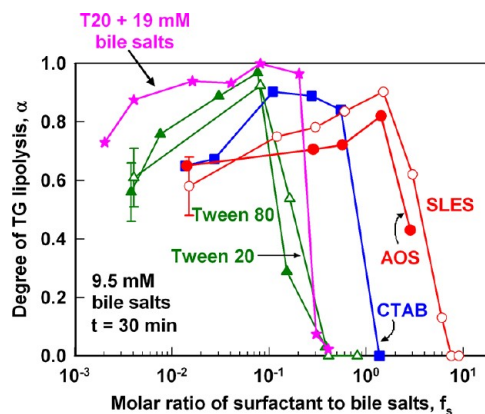

Figure 6. Degree of TG lipolysis, α, as a function of surfactant-to-bile salts molar ratio for emulsions stabilized by CTAB (filled squares), Tween 80 (filled triangles, solid line), Tween 20 (open triangles, dashed line), AOS (filled circles, solid line), or SLES (open circles, dashed line). The concentration of bile salts is 9.5 mM for all experiments, except for one series of experiments with Tween 20, where $C_B = 19$ mM (asterisks, solid line). The reaction time is 30 min. The experimental data for Tween 80 are averages from three independent series of experiments, for Tween 20 and SLES from two independent series of experiments, and for CTAB and AOS from a single series of experiments. The scattering of the data for a given system is small and is represented by the size of the symbols, except for the lowest surfactant concentrations where error bars are shown.

19 mM. (2) At intermediate surfactant-to-bile ratios, the dependence $\alpha(f_S)$ passes through a pronounced maximum. The value of f_S at which the maximal transformation is observed, denoted as f_{MAX}, depends significantly on the type of used emulsifier, whereas the value of α in the maximum depends weakly on the type of emulsifier and bile concentration. (3) The further increase of the emulsifier concentration and of the related surfactant-to-bile ratio, f_S, leads to a steep decrease in the degree of fat lipolysis, until a complete inhibition ($\alpha = 0$) is obtained at a certain value of f_S, denoted hereafter as f_{TR}. The threshold value f_{TR} depends significantly on the type of emulsifier used: it is ≈ 0.4 for the nonionic surfactants, ≈ 1.4 for the cationic CTAB, and ≈ 7.5 for the anionic surfactants, while f_{TR} does not depend on the total bile concentration in the solution. Summarizing, the total bile concentration significantly affects the TG transformation at low f_S, but does not affect the value of f_{TR}, which depends mostly on the type of emulsifier used.

To check whether the TG lipolysis at $f_S > f_{TR}$ is permanently or kinetically inhibited, we performed experiments at high concentrations of CTAB and Tween 80, and at a longer reaction time of 240 min. The obtained results showed that $\alpha = 0$ for both emulsifiers, even after 240 min. Thus we conclude that the pancreatic lipase inhibition at $f_S > f_{TR}$ is permanent, in the physiologically relevant time scale[46] of several hours.

To further check whether the onset of Region 3 (complete enzyme inhibition) depends mainly on f_S, we performed experiments with AOS−bile mixtures at $C_S = 10.5$ mM and a lower bile concentration of $C_B = 4.2$ mM. Again we observed that TG lipolysis is completely inhibited at $f_S = 2.3 > f_{TR}$, despite the fact that at higher surfactant concentration ($C_S = 14$ mM) we observed a maximum in TG transformation when C_B was also higher ($C_B = 9.5$ mM). Therefore, the molar surfactant-to-bile ratio, f_{TR}, is a well-defined value for given emulsifier type and does not depend significantly on the total

concentration of bile salts in the physiologically relevant range (between ca. 5 and 15 mM bile salts).

We should emphasize here that the complete inhibition, $\alpha = 0$, corresponds to no any reaction products formed (MGs, DGs, or fatty acids). This conclusion was verified by TLC and GC analyses of the final reaction mixture. This result shows unambiguously that the inhibition of the lipolysis at $f_S > f_{TR}$ is not related to the accumulation of reaction products.

3.4. Effect of Emulsifier-to-Bile Ratio on the Interfacial Composition.
To check how the dependence $\alpha(f_S)$ for the various emulsifiers is related to the composition of the adsorption layer formed on the surface of the emulsified oily drops, we measured the interfacial tension, σ, of bile–surfactant mixtures with $C_B = 9.5$ mM. These measurements were performed by the pendant drop method at 37 °C.

As seen from Figure 7, there is a significant difference in the shape of the curves $\sigma(f_S)$ for the anionic surfactants (AOS and
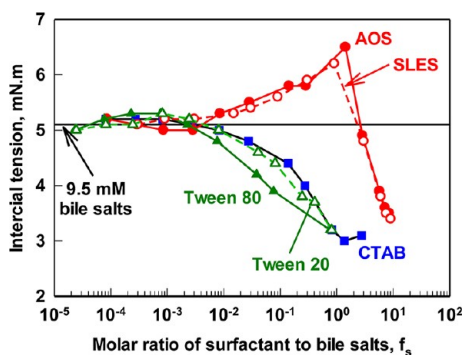

Figure 7. Oil–water interfacial tension, σ, as a function of the surfactant-to-bile salts molar ratio, for solutions of CTAB (filled squares), Tween 80 (filled triangles, solid line), Tween 20 (open triangles, dashed line), AOS (filled circles, solid line), or SLES (open circles, dashed line). The concentration of bile salts is 9.5 mM, and the temperature is 37 °C. The experimental data are averages from two independent series of experiments; the scattering of the data is represented by the symbol size.

SLES) on one side, and the cationic and nonionic surfactants on the other side. For the bile–anionic surfactant mixtures, $\sigma(f_S)$ curves first increase with f_S, pass through a maximum at $f_S \approx 1$, and afterward decrease down to $\sigma \approx 3.5$ mN/m at $f_S > 8$. By contrast, for bile–CTAB and bile–nonionic surfactant mixtures, $\sigma(f_S)$ decreases monotonously with increasing f_S. The values of σ measured with these mixtures are ≈ 3 mN/m at $f_S \approx 1$ (viz., at equal molar ratio of surfactant to bile).

At low surfactant-to-bile ratios, $f_S < 0.01$, the measured interfacial tensions for all studied bile–surfactant mixtures (anionic, cationic, and nonionic) are almost the same, $\sigma \approx 5.1$ mN/m. Therefore, below we discuss together all emulsifiers at $f_S < 0.01$, while we discuss separately the anionic surfactants on one side, and the nonionic and cationic surfactants on the other side, at $f_S > 0.01$.

The fact that $\sigma \approx 5.1$ mN/m for all studied emulsifiers at $f_S < 0.01$ can be easily explained by assuming that the adsorption layers at such low surfactant-to-bile ratios are dominated by the bile salt molecules. This result is far from trivial (as it might appear at a first glance), because the values of σ for CTAB and Tween 80 are rather different in the absence of bile salts for the same surfactant concentration (1.4 mN/m for CTAB and 8.4 mN/m for Tween 80). In other words, the bile salts are able to displace from the drop surface not only the Tween 80

molecules, which give higher σ at this surfactant concentration, but also the CTAB molecules, which give much lower σ than the bile salts. In other words, the bile salts dominate the adsorption layer at $f_S < 0.01$ for all studied emulsifiers, independently of the interfacial tension of the surfactant solutions at the same surfactant concentration (as measured in the absence of bile salts).

The latter result can be explained by considering the fact that the bile micelles can efficiently solubilize the surfactant molecules, thus competing with the adsorption layer for the location of the surfactant molecules available in the reaction mixture (see Figure 8). The obtained results evidence that, at
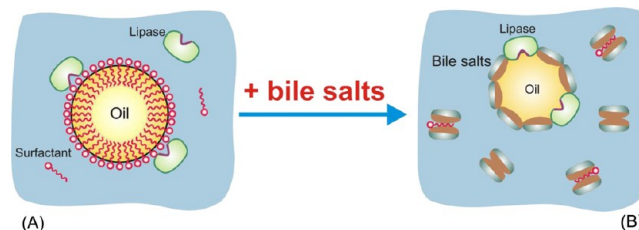

Figure 8. Mechanism of pancreatic lipase reactivation by bile salts, at low surfactant-to-bile ratio: (A) As shown in ref 39, in the absence of bile, the surfactant molecules adsorb on the oil–water interface and block the direct contact of the lipase (and/or colipase) molecules with the TG oil. (B) At the same surfactant concentration, in the presence of bile acids, the surfactant molecules are solubilized in the bile aggregates, and the drop surface is covered by bile molecules, which do not block the lipase–colipase adsorption and the TG lipolysis.

large excess of bile molecules, the surfactant is solubilized in the bile micelles and is thus removed from the oil–water interface. As a consequence, the adsorption layers are dominated by bile molecules, and the degree of TG lipolysis is very close to that without added surfactant. The above mechanism is expected to be operative only at low surfactant-to-bile ratios.

It should be clarified here that our bile extract also contains phospholipids, cholesterol, and fatty acids, which can compete with the surfactants and bile molecules for adsorption on the oil–water interface. The molar concentration of the phospholipids (PC) is 13-times lower than the concentration of bile salts in the used bile extract.[10] Taking into account that 63 wt % of the bile salts are tauro- and glycine-conjugates of deoxycholic acid, we can estimate that the molar ratio of the PC to deoxycholic conjugates is 1:8. For this ratio of PC to sodium tauro-deoxycholate (NaTDC), Wickham et al.[8] showed that the interfacial tension of the mixed solution PC+NaTDC is very similar to that of the NaTDC system (without PC), which allowed these authors to conclude that NaTDC almost completely removes the PC molecules from the oil–water interface.[8] We can expect similar effect in our experiments, because it is known from the literature that the respective glycine conjugate (NaGDC) has similar or higher surface activity than NaTDC.[47] Cholesterol is also known to be solubilized in the bile micelles[48,49] without forming mixed adsorption layers under these conditions.[50] Thus we can conclude that the adsorption layer and the respective interfacial tension measured at $f_S < 0.01$ are dominated by the bile salts. On the other hand, the available experimental data do not allow us to completely exclude the possibility for the presence of some other minor components (such as PC, cholesterol, and fatty acids) in the adsorption layers. To simplify the following explanations, this latter possibility will not be explicitly

mentioned below, a simplification that does not affect any of the final conclusions of this study.

At higher surfactant-to-bile ratio ($f_S > 0.01$), the interfacial tension depends on the type of used emulsifier: σ decreases for the nonionic (Tween 20 and Tween 80) and the cationic (CTAB) surfactants, while it increases for the anionic surfactants (SLES and AOS) (see Figure 7). Note that the degree of fat lipolysis increases in this region for all emulsifiers studied (see Figure 6 above). Thus we see that there is no direct correlation between the interfacial tension and the degree of lipolysis for the bile−surfactant mixtures.

Let us now discuss the results for the nonionic−bile and cationic−bile mixtures at $f_S > 0.02$. In this range, the bile micelles contain a noticeable fraction of these surfactants (Tween 20, Tween 80, and CTAB), and some non-negligible amount of surfactant molecules adsorbs on the drop surface, forming a mixed adsorption layer with the bile molecules, as evidenced from the decreased interfacial tension of these solutions. The further increase of surfactant concentration leads to gradual decrease of the interfacial tension, due to the increased adsorption of surfactant molecules. It should be emphasized that even at the highest surfactant concentrations studied, mixed adsorption layers of bile and surfactant were formed. The latter conclusion is based on the fact that the interfacial tensions of the surfactant solutions (without bile salts) are significantly lower, as compared to the mixed solutions. The experimental data indicate that, above a certain fraction of surfactant in the mixed adsorption layer, the enzyme activity is completely suppressed (as in the systems without bile).[39] However, it is not obvious why the TG transformation in the intermediate range of surfactant-to-bile ratios should pass through a maximum, as seen in Figure 6. This effect is further discussed and explained in section 3.5.

The effect of the anionic surfactants on the interfacial tension of mixed solutions is qualitatively different. The observed maximum in the interfacial tension (see Figure 7) is nontrivial and could be explained only by assuming that the addition of anionic surfactants in the bile solutions leads to less adsorbed molecules on the oil−water interface. This statement follows directly from the Gibbs adsorption isotherm, which relates the decrease of interfacial tension of surfactant solution with the amount and chemical potential of the adsorbed molecules: higher adsorption leads to lower interfacial tension and vice versa.[51] The only mechanism we could envisage for such reduced adsorption is that the mixed bile−anionic surfactant micelles strongly favor the bulk aggregation of the bile molecules (as compared to the bile micelles without surfactants), so that the chemical potential of the bile molecules is significantly reduced in the mixed solutions; as a result, they are less prone to adsorb on the oil−water interface. In other words, the mixed bile−anionic surfactant micelles must be very appropriate hosts for the bile molecules, so that these bile molecules prefer to be incorporated in the mixed micelles, instead of adsorbing on the oil−water interface. As a result, the bile adsorption is decreased, and the interfacial tension increases upon addition of anionic surfactants in the bile solution.

Two additional observations strongly support the idea that the bile molecules are incorporated very efficiently in the mixed bile−anionic surfactant micelles. The bile solutions in our experiments (without surfactants) were slightly turbid due to a partial precipitation of the bile salts with the Ca^{2+} ions present in the reaction mixtures (in the absence of calcium ions, these solutions were only slightly translucent). In addition, upon decrease of pH of these solutions down to pH ≈ 2, we observed the formation of a heavy precipitate of bile acids. By contrast, the solutions containing anionic surfactants with $f_S > 10$ were transparent (see, for example, Figure S2 in the Supporting Information) and did not precipitate upon decrease of pH down to 2. These two observations are clear indications that the bile acids are so well accommodated in the mixed micelles containing anionic surfactants, that their precipitation in the presence of Ca^{2+} ions or upon decrease of pH is not favored thermodynamically anymore, because the precipitation would require the bile molecules to leave the mixed micelles.

When the surfactant-to-bile ratio is above $f_S > 1$, the solubilization capacity of the bile micelles is saturated, and the anionic surfactant molecules compete with the bile molecules for incorporation in the adsorption layer. As a consequence, the interfacial tension decreases significantly (see Figure 7). At $f_S > 8$, a mixed adsorption layer of surfactant and bile molecules is formed on the drop surface ($\sigma \approx 3.5$ mN/m), which completely blocks the lipase activity.

From all these experiments, we can conclude that in Region 1 ($f_S < 0.01$), the interfacial properties are controlled by the bile molecules for all emulsifiers studied. At high surfactant-to-bile ratio, a mixed adsorption layer of bile and surfactants is formed, which prevents the lipase and/or colipase from direct adsorption on the drop surface.[39] This mixed adsorption layer has interfacial tension around 3.5 mN/m for all emulsifiers studied. In the intermediate range of surfactant-to-bile ratios, a maximum in the dependence $\alpha(f_S)$ is observed. For the anionic surfactants, this maximum in TG transformation corresponds to the maximum in the interfacial tension, whereas for the nonionic and cationic surfactants, gradual decrease of the interfacial tension is observed. This intermediate region, which is characterized with highest degree of TG lipolysis, is discussed again in section 4.

3.5. Effect of Emulsifier-to-Bile Ratio on Precipitate Formation on Drop Surface.

To gain information about the conditions under which solid precipitates are formed on the surface of the TG drops, we observed the lipolysis process by optical microscopy. In the first series of experiments, we observed emulsion droplets, placed in contact with the enzyme solution, at three CTAB concentrations: −0.005 wt % (when $\alpha \approx 0.5$), 0.2 wt % (when $\alpha \approx 1$), and 0.5 wt % ($\alpha \approx 0$). Representative images taken 30 min after the contact of the oil drops with the enzyme solution are compared in Figure 9. One sees that, at the lowest CTAB concentration, solid shells of precipitates are formed on the drop surface in the course of the lipolysis reaction. This solid shell is evidenced by the corrugated surface of the oil drops and by their nonspherical shape. The formation of these precipitates reduces the rate of the lipolysis reaction, so that $\alpha \approx 0.5$ is reached after 30 min (with much lower rate at longer times). No such precipitates are seen for 0.2 wt % CTAB solutions in which the lipolysis reaction is almost completed for 30 min ($\alpha \approx 0.9$). At the highest CTAB concentration (with $\alpha \approx 0$), virtually all drops remain unaffected by the enzyme during the observation.

Summarizing, solid precipitates are formed on the surface of the TG drops at low emulsifier concentrations only. Additional experiments in the absence of Ca^{2+} ions showed no formation of precipitates in either of these solutions (including those at the lowest CTAB concentration). The fact that the precipitates are formed in the presence of Ca^{2+} ions only, indicates that
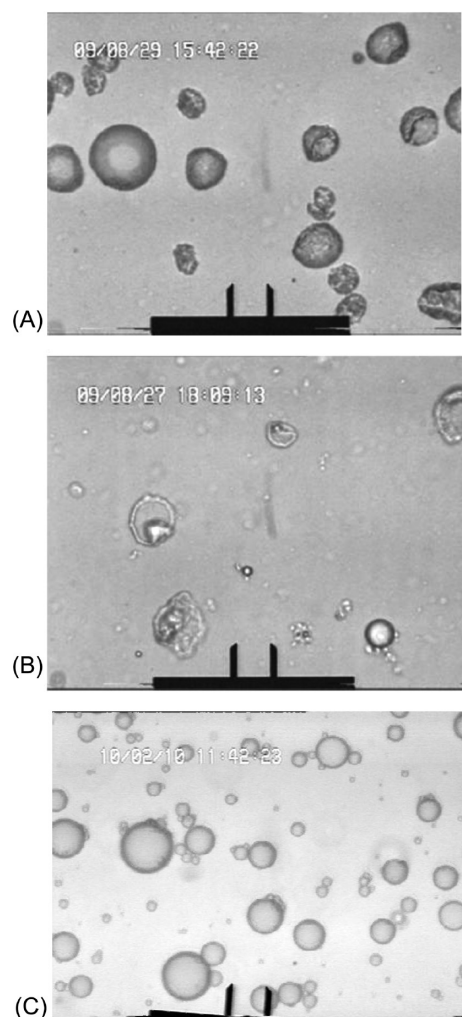
(A)

(B)

(C)

Figure 9. Representative images of SFO drops, after 30 min of their contact with lipase−bile solutions, containing CTAB of different concentrations: (A) 0.005 wt %; (B) 0.2 wt %; (C) 0.5 wt %. The distance between the vertical dark bars is 20 μm.

(A)

(B)

Figure 10. Representative images of oil drops (90 wt % OA + 10 wt % SFO) after 2 min in contact with electrolyte solution containing (A) 9.5 mM bile salts, and (B) 0.2 wt % CTAB + 9.5 mM bile salts (no lipase is present in any of these solutions). One sees in panel B the rapid transfer of OA from the oil drop into the aqueous phase via the formation of liquid crystalline phases around the drop surface. The distance between the vertical bars is 20 μm.

these precipitates are Ca salts of the fatty acids, formed in the lipolysis.

To clarify why Ca precipitates are formed on the drop surface at low CTAB concentrations only, whereas no precipitates are formed at the intermediate CTAB concentration of 0.2 wt % (when the lipase is very active and fatty acids are produced), we performed optical observations of TG drops, in which we initially dissolved oleic acid (OA). The concentration of OA in the TG drops was 90 wt %, which represented the contents of a TG drop with ≈90% degree of lipolysis. The optical observations with electrolyte solutions (without enzymes) showed a very fast dissolution of the OA into the aqueous phase containing 0.2 wt % CTAB, whereas this transfer was much slower at the lower CTAB concentration (0.005 wt %). Representative images of this process are compared in Figure 10. We can conclude from these observations that the maximum in the dependence $\alpha(C_S)$ corresponds to emulsifier concentrations, which are optimal for the fast solubilization of the reaction products into the aqueous solution, thus preventing the formation of solid precipitates on the drop surface.

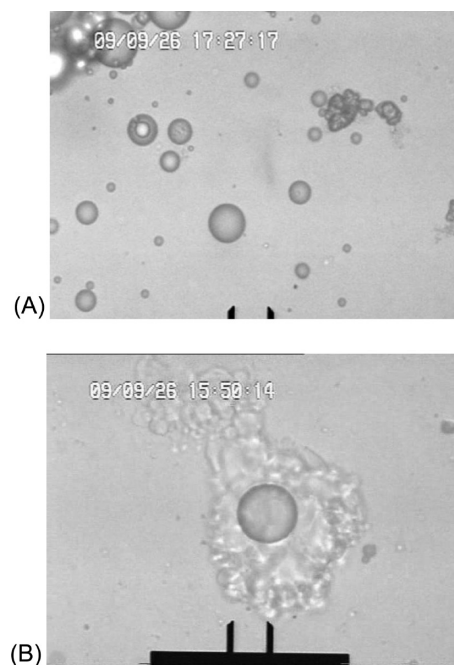The lack of precipitates at the highest surfactant concentrations shows that the mechanism of lipolysis suppression in these systems is through preventing the lipase and/or colipase adsorption on the drop surface (as in the systems without bile salts)[39] rather than blocking the drop surface by reaction products, as is the case at low surfactant concentrations.

4. DISCUSSION: CONTROLLING PHYSICOCHEMICAL FACTORS

From all performed experiments we conclude that the observed regions in the curves $\alpha(f_S)$ are explained as schematically in Figure 11: (1) In Region 1, at $C_S \ll C_B$ corresponding to $f_S < 0.01$, the adsorption layer contains exclusively bile acids, and the TG transformation is not affected by the surfactants. In these systems, the lipolysis reaction becomes very slow when the reaction products form a precipitated shell on the drop surface, thus blocking the enzyme access to the interface and impeding the transfer of reaction products into the aqueous phase. (2) At $0.02 < f_S < f_{TR}$, very efficient transfer of the reaction products across the oil−water interface is realized, resulting in fast solubilization of these products into the aqueous solution, without the formation of precipitates on the drop surface. (3) At $f_S > f_{TR}$, a mixed adsorption layer of surfactant−bile molecules is formed on the solution surface, which prevents the adsorption of enzyme on the drop surface and thus suppresses the enzyme activity. The above explanations are in accordance with all experimental results obtained in our study, including those from ref 39.

On the basis of this mechanistic understanding, let us now summarize the physicochemical factors that affect the TG transformation in these three regions:

Because all properties in Region 1 (micelles and composition of the adsorption layer) are governed by the bile salts, one could expect that the bile salt concentration will significantly
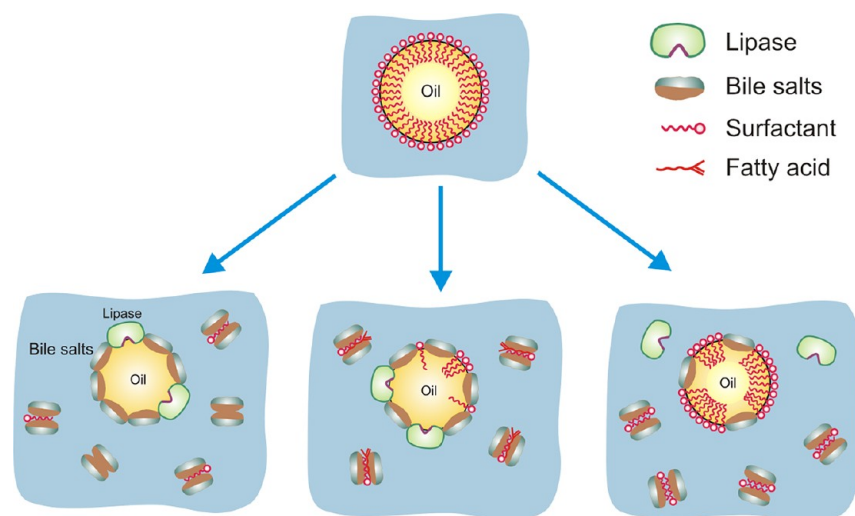
Figure 11. Schematic presentation of the system when a TG drop is placed in solution with (A) low surfactant-to-bile salts ratio; (B) intermediate surfactant-to-bile salts ratio; (C) high surfactant-to-bile salts ratio.

affect the value of α. Indeed, as seen in Figure 5, the value of α increases with the increase of C_B, which is due to the increased concentration of bile micelles, which solubilize the reaction products and prevent the formation of Ca precipitates on the drop surface. The type of used emulsifier has no any impact on the TG transformation in this region.

According to the proposed mechanism and the obtained results, both the surfactant and bile salts are very important in Region 2. Here, the mixed surfactant−bile micelles have higher solubilization capacity than that of the bile micelles (without surfactant), and the TG transformation is virtually completed within 30 min (α is between 90 and 100%). The value f_{MAX} at which the TG transformation is maximal depends significantly on the type of emulsifier used (see Figure 6). This experimental fact is related to the solubilizing capacity of the mixed micelles, which is different for the various surfactants: lowest for the nonionic surfactants (Tween 20 and Tween 80), intermediate for the cationic CTAB, and highest for the anionic AOS and SLES. Further experiments and analysis of the aqueous phase are needed to reveal the detailed molecular mechanisms behind these interesting differences.

Region 3 is related to the formation of mixed adsorption layers, containing surfactant and bile molecules of comparable surface concentrations. These mixed adsorption layers have interfacial tension of ≈3.5 mN/m for all surfactants studied, which falls between the values of bile alone (5.1 mN/m) and surfactants alone (which could be as low as 1.8 mN/m). Therefore, in this region there are some bile molecules adsorbed on the drop surface, but they are not sufficient to ensure lipase/colipase adsorption. As a consequence, the TG transformation is blocked (as shown in ref 39, the enzyme is still very active at such high surfactant concentrations).

5. CONCLUSIONS

Systematic experiments with several low-molecular mass emulsifiers (two anionic, two nonionic, and one cationic) revealed three typical regimes of TG lipolysis, depending on the emulsifier-to-bile salts ratio (see Figure 11):

At emulsifier concentration below ca. 0.1 mM (less than 1:50 molar ratio of emulsifier to bile), the adsorption layer on the oil drop surface is dominated by the bile molecules, because the emulsifier molecules are predominantly incorporated (solubi-

lized) in the bile micelles. As a result, fat lipolysis occurs like in the absence of emulsifiers.

At intermediate emulsifier concentrations of 0.2 to 2 mM (molar ratio of emulsifier to bile between 1:50 and 1:5), the solubilization capacity of the micelles is significantly increased, which allows fast transfer of the reaction products into the aqueous phase and prevents the formation of precipitated shell from reaction products on the oil drop surface. As a result, the degree of lipolysis passes through a maximum around these intermediate concentrations of emulsifier.

At higher emulsifier concentration (above ca. 1:5 ratio of emulsifier to bile, but this ratio depends on the emulsifier type), the emulsifier molecules prevail in the adsorption layer, and the lipase activity is completely suppressed, as in the case without bile salts.[39]

The anionic emulsifiers show some specific features: Higher interfacial tension (lower adsorption) was observed at the intermediate emulsifier-to-bile ratios (between 1:3 and 3:1). As a result, the degree of lipolysis was very high in this concentration range. At even higher concentration of the anionic emulsifiers, the surface layer is dominated by the emulsifier molecules, and the lipolysis is completely suppressed, as with all other surfactants studied.

The obtained results clearly show that the surfactant-to-bile ratio in the mixed solutions, f_S, is the governing parameter, whereas the interfacial activity of the surfactant (often considered to be important) has no direct relation to the lipase activity in these mixed systems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Molecular structure of the most important bile salts and their glycine and taurine conjugates. Photos with visual appearance of the bile solutions in the presence of different concentrations of the anionic surfactant SLES. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Address: Department of Chemical Engineering Faculty of Chemistry and Pharmacy, Sofia University 1 James Bourchier

Ave., 1164 Sofia Bulgaria. Phone: (+359-2) 962 5310. Fax: (+359-2) 962 5643. E-mail: SC@lcpe.uni-sofia.bg.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

AOS = α-olefinsulfonate
CMC = critical micelle concentration
CTAB = cetyltrimethylammonium bromide
DG = diglycerides
DTAB = dodecyltrimethylammonium bromide
MG = monoglycerides
OA = oleic acid
SFO = sunflower oil
SLES = sodium laurylethersulfate
TG = triglycerides

■ REFERENCES

(1) Patton, J. S.; Carey, M. C. Watching fat digestion. *Science* **1979**, *204*, 145.

(2) Verger, R.; De Haas, G. H. Interfacial enzyme kinetics of lipolysis. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 77.

(3) Carey, M. C.; Small, D. M.; Bliss, C. M. Lipid digestion and absorption. *Annu. Rev. Physiol.* **1983**, *45*, 651.

(4) Gargouri, Y.; Julien, R.; Bois, A. G.; Verger, R.; Sarda, L. Studies on the detergent inhibition of pancreatic lipase activity. *J. Lipid Res.* **1983**, *24*, 1336.

(5) Verger, R. "Interfacial activation" of lipases: Facts and artifacts. *Trends Biotechnol.* **1997**, *15*, 32.

(6) MacGregor, K. J.; Embleton, J. K.; Lacy, J. E.; Perry, E. A.; Solomon, L. J.; Seager, H.; Pouton, C. W. Influence of lipolysis on drug absorption from the gastro-intestinal tract. *Adv. Drug Delivery Rev.* **1997**, *25*, 33.

(7) Wickham, M.; Garrood, M.; Leney, J.; Wilson, P. D. G.; Fillery-Travis, A. Modification of a phospholipid stabilized emulsion interface by bile salt: Effect on pancreatic lipase activity. *J. Lipid Res.* **1998**, *39*, 623.

(8) Wickham, M.; Wilde, P.; Fillery-Travis, A. A physicochemical investigation of two phosphatidylcholine/bile salt interfaces: Implications for lipase activation. *Biochim. Biophys. Acta* **2002**, *1580*, 110.

(9) Pafumi, Y.; Lairon, D.; De La Porte, P. L.; Juhel, C.; Storch, J.; Hamosh, M.; Armand, M. Mechanisms of inhibition of triacylglycerol hydrolysis by human gastric lipase. *J. Biol. Chem.* **2002**, *277*, 28070.

(10) Zangenberg, N. H.; Mullertz, A.; Kristensen, H. G.; Hovgaard, L. A dynamic in vitro lipolysis model I: Controlling the rate of lipolysis by continuous addition of calcium. *Eur. J. Pharm. Sci.* **2001**, *14*, 115.

(11) Zangenberg, N. H.; Mullertz, A.; Kristensen, H. G.; Hovgaard, L. A dynamic in vitro lipolysis model II: Evaluation of the model. *Eur. J. Pharm. Sci.* **2001**, *14*, 237.

(12) Reis, P. M.; Raab, T. W.; Chuat, J. Y.; Leser, M. E.; Miller, R.; Watzke, H. J.; Holmberg, K. Influence of surfactants on lipase fat digestion in a model gastro-intestinal system. *Food Biophys.* **2008**, *3*, 370.

(13) Reis, P.; Holmberg, K.; Miller, R.; Krägel, J.; Grigoriev, D. O.; Leser, M. E.; Watzke, H. J. Competition between lipases and monoglycerides at interfaces. *Langmuir* **2008**, *24*, 7400.

(14) Reis, P.; Holmberg, K.; Watzke, H.; Leser, M. E.; Miller, R. Lipases at interfaces: A review. *Adv. Colloid Interface Sci.* **2009**, *147–148*, 237.

(15) McClements, D. J.; Decker, E. A.; Park, Y. Controlling lipid bioavailability through physicochemical and structural approaches. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 48.

(16) Klinkesorn, U.; McClements, D. J. Impact of lipase, bile salts, and polysaccharides on properties and digestibility of tuna oil multilayer emulsions stabilized by lecithin-chitosan. *Food Biophys.* **2010**, *5*, 73.

(17) Delorme, V.; Dhouib, R.; Canaan, S.; Fotiadu, F.; Carrière, F.; Cavalier, J.-F. Effects of surfactants on lipase structure, activity, and inhibition. *Pharm. Res.* **2011**, *28*, 1831.

(18) Wilde, P. J.; Chu, B. S. Interfacial & colloidal aspects of lipid digestion. *Adv. Colloid Interface Sci.* **2011**, *165*, 14.

(19) Chu, B.-S.; Rich, G. T.; Ridout, M. J.; Faulks, R. M.; Wickham, M. S. J.; Wilde, P. J. Modulating pancreatic lipase activity with galactolipids: Effects of emulsion interfacial composition. *Langmuir* **2009**, *25*, 9352.

(20) Chu, B.-S.; Gunning, A. P.; Rich, G. T.; Ridout, M. J.; Faulks, R. M.; Wickham, M. S. J.; Morris, V. J.; Wilde, P. J. Adsorption of bile salts and pancreatic colipase and lipase onto digalactosyldiacylglycerol and dipalmitoylphosphatidylcholine monolayers. *Langmuir* **2010**, *26*, 9782.

(21) Wulff-Pérez, M.; Gálvez-Ruíz, M. J.; de Vicente, J.; Martín-Rodríguez, A. Delaying lipid digestion through steric surfactant Pluronic F68: A novel in vitro approach. *Food Res. Int.* **2010**, *43*, 1629.

(22) Torcello-Gómez, A.; Maldonado-Valderrama, J.; Martín-Rodríguez, A.; McClements, D. J. Physicochemical properties and digestibility of emulsified lipids in simulated intestinal fluids: Influence of interfacial characteristics. *Soft Matter* **2011**, *7*, 6167.

(23) Li, Y.; McClements, D. J. Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions. *Eur. J. Pharm. Biopharm.* **2011**, *79*, 423.

(24) Maldonado-Valderrama, J.; Woodward, N. C.; Patrick Gunning, A.; Ridout, M. J.; Husband, F. A.; Mackie, A. R.; Morris, V. J.; Wilde, P. J. Interfacial characterization of β-lactoglobulin networks: Displacement by bile salts. *Langmuir* **2008**, *24*, 6759.

(25) Li, Y.; McClements, D. J. Inhibition of lipase-catalyzed hydrolysis of emulsified triglyceride oils by low-molecular weight surfactants under simulated gastrointestinal conditions. *Eur. J. Pharm. Biopharm.* **2011**, *79*, 423.

(26) Sandra, S.; Decker, E. A.; McClements, D. J. Effect of interfacial protein cross-linking on the in vitro digestibility of emulsified corn oil by pancreatic lipase. *J. Agric. Food Chem.* **2008**, *56*, 7488.

(27) Hur, S. J.; Decker, E. A.; McClements, D. J. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion. *Food Chem.* **2009**, *114*, 253.

(28) Mun, S.; Decker, E. A.; Park, Y.; Weiss, J.; McClements, D. J. Influence of interfacial composition on in vitro digestibility of emulsified lipids: Potential mechanism for chitosan's ability to inhibit fat digestion. *Food Biophys.* **2006**, *1*, 21.

(29) Lesmes, U.; Baudot, P.; McClements, D. J. Impact of interfacial composition on physical stability and in vitro lipase digestibility of triacylglycerol oil droplets coated with lactoferrin and/or caseinate. *J. Agric. Food Chem.* **2010**, *58*, 7962.

(30) Diaz, J. C. M.; Cordova, J.; Baratti, J.; Carriere, F.; Abousalham, A. Effect of nonionic surfactants on Rhizopus homothallicus lipase activity - A comparative kinetic study. *Mol. Biotechnol.* **2007**, *35*, 205.

(31) Tsujita, T. Inhibiting lipid absorption using basic biopolymers. *Future Lipidol.* **2007**, *2*, 547.

(32) Tsuzuki, W.; Ue, A.; Nagao, A.; Endo, M.; Abe, M. Inhibitory effect of lysophosphatidylcholine on pancreatic lipase-mediated hydrolysis in lipid emulsion. *Biochim. Biophys. Acta* **2004**, *1684*, 1.

(33) Juhel, C.; Armand, M.; Pafumi, Y.; Rosier, C.; Vandermander, J.; Lairon, D. Green tea extract (AR25®) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *J. Nutr. Biochem.* **2000**, *11*, 45.

(34) Pasquier, B.; Armand, M.; Castelain, C.; Guillon, F.; Borel, P.; Lafont, H.; Lairon, D. Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium in vitro. *Biochem. J.* **1996**, *314*, 269.

(35) Borel, P.; Armand, M.; Ythier, P.; Dutot, G.; Melin, C.; Senft, M.; Lafont, H.; Lairon, D. Hydrolysis of emulsions with different triglycerides and droplet sizes by gastric lipase in vitro. Effect on pancreatic lipase activity. *J. Nutr. Biochem.* **1994**, *5*, 124.

(36) McClements, D. J.; Li, Y. Review of *in vitro* digestion models for rapid screening of emulsion-based systems. *Food Funct.* **2010**, *1*, 32–59.

(37) Hu, M.; Li, Y.; Decker, E. A.; McClements, D. J. Role of calcium and calcium-binding agents on the lipase digestibility of emulsified lipids using an in vitro digestion model. *Food Hydrocolloids* **2010**, *24*, 719.

(38) Torcello-Gómez, A.; Maldonado-Valderrama, J.; de Vicente, J.; Cabrerizo-Vílchez, M. A.; Gálvez-Ruiz, M. J.; Martín-Rodríguez, A. Investigating the effect of surfactants on lipase interfacial behaviour in the presence of bile salts. *Food Hydrocolloids* **2011**, *25*, 809.

(39) Vinarov, Z. P.; Petkova, Y. S.; Tcholakova, S.; Denkov, N. D.; Stoyanov, S.; Pelan, E.; Lips, A. *Langmuir* **2012**, *28*, 8127.

(40) Vinarov, Z. P.; Petrova, L. I; Tcholakova, S.; Denkov, N. D.; Stoyanov, S.; Lips, A. *Food Funct.* **2012**, submitted for publication.

(41) Patton, J. S.; Albertsson, P. A.; Erlanson, C.; Borgstrom, B. Binding of porcine pancreatic lipase and colipase in the absence of substrate studied by two-phase partition and affinity chromatography. *J. Biol. Chem.* **1978**, *253*, 4195.

(42) Drent, M. L.; Larsson, I.; William-Olsson, T.; Quaade, F.; Czubayko, F.; Von Bergmann, K.; Strobel, W.; Sjostrom, L.; Van der Veen, E. A. Orlistat (RO 18-0647), a lipase inhibitor, in the treatment of human obesity: A multiple dose study. *Int. J. Obes.* **1995**, *19*, 221.

(43) Lindahl, A.; Ungell, A.-L.; Knutson, L.; Lennernäs, H. Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharm. Res.* **1997**, *14*, 497.

(44) Armand, M.; Borel, P.; Pasquier, B.; Dubois, C.; Senft, M.; Andre, M.; Peyrot, J.; Salducci, J.; Lairon, D. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1996**, *271*, G172.

(45) Di Maio, S.; Carrier, R. L. Gastrointestinal contents in fasted state and post-lipid ingestion: In vivo measurements and in vitro models for studying oral drug delivery. *J. Controlled Release* **2011**, *151*, 110.

(46) Daugherty, A. L.; Mrsny, R. J. Regulation of the intestinal epithelial paracellular barrier. *Pharm. Sci. Technol. Today* **1999**, *2*, 281.

(47) Maldonado-Valderrama, J.; Wilde, P.; Macierzanka, A.; Mackie, A. The role of bile salts in digestion. *Adv. Colloid Interface Sci.* **2011**, *165*, 36.

(48) Staggers, J. E.; Hernell, O.; Stafford, R. J.; Carey, M. C. Physical—chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* **1990**, *29*, 2028–2040.

(49) Hernell, O.; Staggers, J. E.; Carey, M. C. Physical—chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* **1990**, *29*, 2041.

(50) Yamamoto, S. K.; Shibata, O.; Sakai, M.; Sasaki, Y.; Lee, S.; Sugihara, G. Mixed monolayer of deoxycholic acid with cholesterol on aqueous NaCl solution at pH 1.2 and 25°C. *Colloids Surf. B: Biointerfaces* **1995**, *5*, 249.

(51) Kralchevsky, P. A.; Danov, K. D.; Denkov, N. D. In *Handbook of Surface and Colloid Chemistry*, Chemical Physics of Colloid Systems and Interfaces; Birdi, K. S., Ed.; CRC Press: New York, 1997, Chapter 11.