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Controlling lipolysis through steric surfactants: New insights on the controlled degradation of submicron emulsions after oral and intravenous administration

Miguel Wulff-Pérez*, Juan de Vicente, Antonio Martín-Rodríguez, María J. Gálvez-Ruiz

Biocolloid and Fluid Physics Group, Department of Applied Physics, Faculty of Sciences, University of Granada, 18071 Granada, Spain

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ABSTRACT

In this work we have investigated how steric surfactants influence the metabolic degradation of emulsions (lipolysis). To do so, we have prepared submicron emulsions stabilized with Pluronic F68, Pluronic F127, Myrj 52 or Myrj 59, four non-ionic surfactants with key differences on their structure. Submicron emulsions have been prepared also with mixtures of these surfactants with different proportions between them. Then, in vitro methods have been applied to analyze the lipolysis of these emulsions, both under duodenal and intravenous conditions, to simulate lipolysis after oral and intravenous administration. Our results show that the properties of the surfactant influence dramatically the lipolysis rates observed both under duodenal and intravenous conditions, e.g., intravenous lipolysis was completely blocked when Pluronic F127 was used, while it was almost complete within 6 h when using Myrj 52. The reason for this seems to be the steric hindrance that the surfactant produces around the droplet and at the interface. As a result, we can modify the lipolysis patterns by changing some characteristics of the surfactant, or by varying the proportion between two surfactants in a mixture. These findings may be applied in the development of novel strategies to rationally design submicron emulsions as lipophilic drug carriers.

1. Introduction

It has been estimated that nearly 50% of new potential drugs have low solubility in water, which leads to poor bioavailability and makes the control of their release a challenging issue (Tamilvanan, 2009). In order to bypass these difficulties, the use of lipid dispersion carrier systems, such as lipid emulsions or liposomes, has attracted particular interest in recent years, since they have significant and beneficial effects on the absorption and exposure of co-administered lipophilic drugs, leading to higher bioavailability (Porter et al., 2008). Submicron emulsions are one especially interesting drug delivery system, due to their biocompatibility, biodegradability, ease of preparation on a large scale, large surface area, and proven long-term stability against destabilizing phenomena such as creaming or sedimentation (Tamilvanan, 2004).

Traditionally, phospholipids have been used as surfactants to prepare submicron emulsions for drug delivery applications. However, phospholipids are rapidly displaced from interfaces by bile salts in the duodenum when phospholipid-stabilized emulsions are administered orally (Torcello-Gómez et al., 2011). Also, if these emulsions are administered by the intravenous route, they are rapidly eliminated according to their colloidal foreign body character by the mononuclear phagocyte system (MPS) (Lucks et al., 2000). In both cases, the use of phospholipids as the only surfactant does not allow us to control the processes involved in the metabolic degradation of the emulsions in order to use lipid emulsions as controlled-release drug carriers (Lucks et al., 2000; Kurihara et al., 1996). Non-ionic, steric surfactants containing polyethylene oxide (PEO or PEG) have been proposed to overcome those problems, both for oral (Wulff-Pérez et al., 2010) and parenteral applications (Tamilvanan, 2009). However, there is still a lack of understanding of how these surfactants affect the metabolic processing of lipid carrier systems.

The release of hydrophobic drugs from lipid based delivery systems is strongly related with the natural degradation of these emulsions in the body (Larsen et al., 2011), i.e., the lipolysis or enzymatic hydrolysis of the triglycerides composing the drug delivery system. Therefore, a better understanding of how to alter the lipolysis rate should provide us with a finer control of the drug release from submicron o/w emulsions when administered both orally and parenterally. By oral route, this lipolysis occurs mainly in the upper small intestine. When oil droplets arrive to the duodenum, they are mixed with pancreatic lipase, colipase and bile salts. Bile salts displace molecules adsorbed at the oil–water interface, removing potential inhibitors of pancreatic lipase, while colipase helps the lipase to anchor to a bile salt-covered interface and activates the lipase through a conformational change (Miled et al., 2001; Reis et al., 2008). Then, pancreatic lipase starts the hydrolysis of

* Corresponding author at: Dpto. Física Aplicada, Universidad de Granada, Facultad de Ciencias, Fuentenueva s/n, Granada 18071, Spain. Tel.: +34 958246175; fax: +34 958243214.
E-mail address: mwulff@ugr.es (M. Wulff-Pérez).

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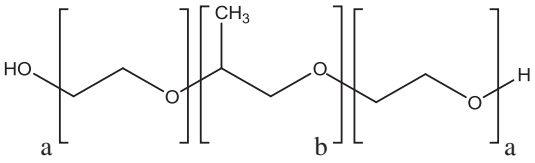
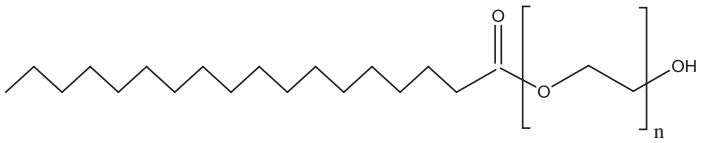
Table 1
Chemical structure of the surfactants used in this study to prepare and stabilize the submicron emulsions.

Pluronic F68 (PF68)		$a = 75$
Pluronic F127 (PF127)		$b = 29$ $a = 100$ $b = 65$
Myrj 52 (M52)		$n = 40$
Myrj 59 (M59)		$n = 100$

triglycerides into free fatty acids and monoglycerides, which are small, more soluble molecules. These lipolysis products leave the droplet forming a series of colloidal structures, including multilamellar and unilamellar vesicles, mixed micelles and micelles. Hydrophobic drugs leave the droplets within these species, which significantly increase the solubilization capacity of the small intestine for these drugs (Porter et al., 2007). Therefore, the lipolysis rates influence directly the release and absorption of the hydrophobic drugs delivered in lipidic systems.

Regarding the intravenous (IV) route, emulsions can be recognized as foreign bodies by the MPS and retired from circulation, or they can behave as chylomicrons and enter the fat metabolism pathway (Buszello and Müller, 2000). Chylomicrons are the oil droplets responsible for the transport of dietary lipids from the intestines to other locations in the body. In the blood they acquire apolipoproteins, and with the help of apolipoprotein C-II (apoC-II), the hydrolysis of lipids begins via the action of the endothelial cell bound lipoprotein lipase (LpL). Some LpL dissociates from endothelial cells during lipolysis and continues its enzymatic action in the bloodstream (Yamamoto et al., 2003; Rensen and Van Berkel, 1996; Goldberg, 1996) while the free fatty acids leave the droplets, being these fatty acids now available for the body cells or for binding to the serum albumins (Lucks et al., 2000). Again, the release of highly lipophilic drugs will be directly related with the lipolysis rate. Surprisingly, there is a vast literature on the relation between stability against MPS and control of the drug release, but very little regarding lipolysis under IV conditions and lipidic drug delivery systems, even for emulsions that have already proven to be stable against MPS uptake.

In this work, we have focused on the first stage of the enzymatic degradation of the drug delivery system, i.e., the lipolysis. Four different steric surfactants were used to prepare submicron emulsions, in order to study how the structure and properties of these surfactants affect the lipolysis, both under in vitro duodenal and intravenous conditions. These surfactants were Pluronic F68, Pluronic F127, Myrj 52 or Myrj 59, as well as mixtures with different proportions between them. A better understanding on the relation between the structure of the surfactant and the lipolysis rate should help us to control the time when the hydrophobic drug is released. This knowledge may be applied in the development of novel strategies to rationally design submicron emulsions as lipophilic drug carriers.

2. Materials and methods

2.1. Materials

Surfactants: The poloxamer Pluronic F68 (PF68) and Pluronic F127 (PF127), also known as Poloxamer 188 and 407 respectively, were obtained from Sigma–Aldrich and used without further purification. They are based on a poly(ethylene oxide)-block-poly(propylene oxide)-block- poly(ethylene oxide) structure (see Table 1). The central polypropylene oxide (PPO) block links to the oil because of its hydrophobic character, while the two lateral hydrophilic chains of polyethylene oxide (PEO) remain in the aqueous phase, stabilizing the droplets by steric hindrance. PF127 has been approved as inactive ingredient for oral intake by U.S. Food and Drug Administration (FDA), whereas PF68 has been approved for both oral and intravenous administration. Myrj surfactants consist of stearic acid esterified with polyethylene oxide, with Myrj 52 (M52) having 40 subunits of PEO and Myrj 59 (M59) having 100 subunits. M52 is approved by both FDA and European Food Safety Authority as a food ingredient (number E431) and was obtained from Sigma–Aldrich. M59 was a kind gift from Croda Ibérica S.A. Epikuron 145V is a deoiled phosphatidylcholine enriched fraction of soybean lecithin. This phospholipids mixture was used for a comparative analysis and was kindly provided by Cargill Ibérica S.L.

Oils: Sunflower oil was obtained from Sigma–Aldrich. This oil was purified to eliminate free fatty acids as described elsewhere (Wulff-Pérez et al., 2011).

Gastric conditions: Pepsin (924 U/mg), sodium chloride and hydrochloric acid (38%) were obtained from Sigma–Aldrich to prepare the simulated gastric fluid.

Duodenal conditions: Sodium chloride (99.5%), calcium chloride (96%), Tris–Maleate (>99.5%), pancreatin (from porcine pancreas, lipase activity 4.9 USP/mg), and bile salt extract (B8631, porcine) were also purchased from Sigma. Pancreatin is assumed to have equivalent moles of lipase and colipase whereas bile salt extract contains 49 wt% of bile salts. The water was purified in a Milli-Q Academic Millipore system.

IV conditions: Purified bovine milk LpL (4510 U/mg, 0.37 mg/mL), suspended in 3.8 M ammonium sulfate, 0.02 M Tris–HCl at pH 8.0, was obtained from Sigma. Human serum plasma, heparin ammonium salt (196 U/mg), albumin from bovine serum (lyophilized powder, ≥98, essentially fatty acid free, essentially globulin free) and Tris-Base were also obtained from Sigma.

2.2. Preparation of submicron emulsions

Sunflower oil-in-water emulsions of 30 mL were prepared with a volume fraction of 25%. Different types of emulsions were obtained, according to the surfactant used to stabilize the droplets: (a) only Pluronic F68 as surfactant, (b) Pluronic 127, (c) Myrj 52 and (d) Myrj 59. Emulsions were also prepared with mixtures PF68/PF127 and M52/M59 with different proportions between them (3:1, 1:1 and 1:3 in weight), as well as with phospholipids for comparison purposes. The final surfactant concentration was 1% (w/v) for polymeric surfactants and 1.5% (w/v) for phospholipids.

In all cases, the surfactant was dissolved in water and then the resulting solution pre-mixed with oil using a high speed Heidolph Diax 900 stirrer for 4 min at 13,000 rpm. This coarse emulsion was immediately homogenized using the high pressure Emulsiflex C-3 (Avestin, CA) homogenizer for 10 passes at 100 MPa.

2.3. Measurement of droplet size

The mean droplet size of the different emulsions was measured by dynamic light scattering (DLS) using an ALV-NIBS/HPPS particle sizer with an ALV 5000 multiple digital correlator (ALV-Laser Vertriebsgesellschaft GmbH, Langen, Germany) at 37 °C. The emulsions were diluted to a final volume fraction below 1%, in order to reach optimal measurement conditions.

2.4. Stability of submicron emulsions under gastric conditions

Emulsions were mixed with simulated gastric fluid in order to achieve a final oil volume fraction of 10%, with 3.2 g/L of pepsin, 2 g/L NaCl and pH adjusted to 1.2 with HCl, according to the compositions given by the U.S. Pharmacopeia for simulated gastric fluid. This mixture was immediately transferred to the measuring cell, where the stability of the emulsions was measured for 2 h. Gastric lipase is not commercially available for research and was not used. In any case, gastric lipases play a small role in comparison to duodenal lipase in the overall lipid digestion in healthy human adults (Sarkar et al., 2009) and hydrolyze the medium-chain triglycerides (predominantly those with 8- to 10-carbon chain lengths) much better than they hydrolyze the long-chain triglycerides of vegetable oils (Porter et al., 2007), as the sunflower oil used in this study.

The stability of the emulsions under simulated gastric fluid was evaluated using a Turbiscan MA 2000 (Formulaction, Toulouse, France). The emulsion is placed in a flat-bottomed cylindrical glass cell and scanned from the bottom to the top with a light source (near infrared, λ_{air} = 850 nm) and the light backscattered by the sample (45° from the incident radiation) is detected. The Turbiscan works in scanning mode: the optical reading head scans the length of the sample acquiring transmission and backscattering data every 40 μm. The corresponding curves provide the backscattered (BS) light flux as a function of the sample height, and scans are repeated over time. The BS light intensity is related with the amount and size of droplets present in the dispersion. BS remains unchanged in the whole height of the cell when the number of particles and interfaces is not changing, i.e. if no coalescence, flocculation, creaming or sedimentation occurs (Wulff-Pérez et al., 2009). Emulsions in which this behavior was observed were considered stable. This stability was later confirmed by DLS measurements.

2.5. In vitro duodenal lipolysis

Solutions of NaCl, $CaCl_2$, bile salt extract and Tris–maleate were mixed and maintained at 37 °C in order to mimic the chemical conditions of duodenal digestive fluids. The final compositions were chosen in order to produce final concentrations similar to those used by other authors and in previous works (Hur et al., 2009; Versantvoort et al., 2005; Zangenberg et al., 2001): pH 6.5, NaCl 150 mM, $CaCl_2$ 2 mM, 12 g/L of sunflower oil and 4.18 g/L bile salt extract. The final volume was 7.5 mL. The lipase and colipase were obtained from pancreatin as previously reported (Wulff-Pérez et al., 2010) obtaining a final lipase activity of 136 U/mL.

The in vitro duodenal lipolysis was evaluated by measuring the time evolution of BS light with the Turbiscan MA 2000 as described in detail elsewhere (Wulff-Pérez et al., 2010). Aliquots of 0.4 mL of the freshly prepared emulsion, 3.6 mL of Milli-Q water and 2.5 mL of duodenal juice were mixed together. Then, 1 mL of fresh pancreatin suspension was added. This mixture was inverted four times and immediately transferred to the measuring cell of the Turbiscan. For small Rayleigh–Debye scatterers (diameter <0.3 μm), a decrease in particle diameter produces a decrease in BS (Mengual, 1999). According to their droplet size, our emulsions belong to this type of scatterers, and therefore the generalized decrease in BS observed through the whole height of the cell can be attributed to a decrease in particle size, which in this system can only be caused by the action of the lipase, as the oil from the droplets is digested and solubilized in mixed micelles, vesicles, etc. (Hur et al., 2009). Thus, measuring BS changes in the center of the tube and plotting these changes against time gives us a quick qualitative estimation of the lipolysis rate: the faster the lipolysis, the sharper the decrease in BS observed. The lipolysis rate was characterized using the parameter ΔBS, defined as the difference in mean BS value (recorded for the emulsion sample in the central region that went from 20 to 40 mm of its height) between the first scan and *n* scan.

This experiment was repeated three times for each type of emulsion. Also a blank experiment for each emulsion was performed, by adding 1 mL of Milli-Q water instead of the pancreatin suspension.

2.6. In vitro intravenous lipolysis

A buffer containing the main components involved in intravenous lipolysis was prepared, based on the compositions and concentrations used by other authors (Yamamoto et al., 2003; Rensen and Van Berkel, 1996; Ton et al., 2005; Deckelbaum et al., 1990) in order to obtain a final composition for the experiments with pH 8.6, NaCl 150 mM, Tris-Base 10 mM, 4% albumin and 66.7 μg/mL heparin. 10% (v/v) of human plasma, heat-inactivated (30 min at 56 °C), was used as a source of apolipoprotein C-II.

Emulsions were diluted with the buffer previously described until a final oil/water volume fraction of 0.1%, being the total volume 400 μL, and incubated at 37 °C for 30 min. Then, 4.0 μg/mL LpL were added, starting the lipolysis experiment, and the released free fatty acids from the lipid emulsions were measured at predetermined time intervals using an enzymatic kit (Non-esterified fatty acid (NEFA) Kit; RANDOX Laboratories Ltd., United Kingdom). Studies were performed in triplicates. A blank experiment was performed in order to correct the possible FFA coming from plasma, impurities, etc., adding Milli-Q water instead of LpL solution.

3. Results and discussion

3.1. Characterization of the emulsions

The droplet sizes were between 200 and 230 nm for the emulsions stabilized with polymeric surfactants and between 275 and 290 nm for the phospholipid-stabilized emulsions. The standard deviations were ±20 nm and ±35 nm, respectively. All the emulsions remained stable for 24 h, i.e., under the time frame of the experiments.

3.2. Stability under gastric conditions

No changes (ΔBS <0.3%) in backscattering were observed under gastric conditions for 2 h for any of the polymer-stabilized emulsions, i.e., the droplets remained unaffected under these conditions, with no destabilizing phenomena such as coalescence or flocculation. This was confirmed by DLS measurements, where no change in particle size was observed. Therefore, these emulsions are expected to protect effectively any drugs dissolved in them against the harsh gastric conditions, allowing these hydrophobic drugs to arrive mostly unaffected to the intestine, where most of these drugs are absorbed when administered orally (Porter et al., 2007).

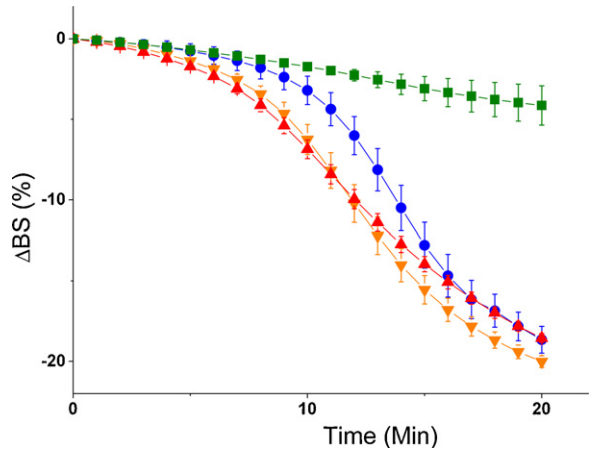


Fig. 1. Changes in backscattering measured during a lipolysis experiment under duodenal conditions for four submicron emulsions stabilized with different steric surfactants: -■-, Pluronic F127; -●-, Pluronic F68; -▼-, Myrj 52; -▲-, Myrj 59. $n = 3$.

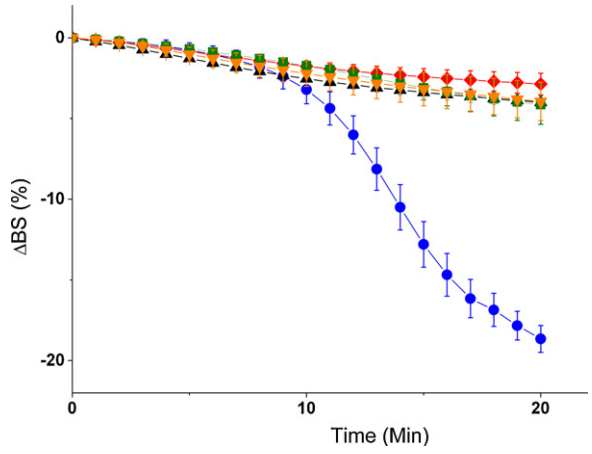


Fig. 2. Changes in backscattering measured during a lipolysis experiment under duodenal conditions for five submicron emulsions stabilized with different mixtures of Pluronics: -■-, only Pluronic F127; -●-, only Pluronic F68; -▲-, PF68-PF127 3:1; -◆-, PF68-PF127 1:1; -▼-, PF68-PF127 1:3. $n = 3$.

3.3. *In vitro duodenal lipolysis*

3.3.1. *Pure PF68, PF127, M52 and M59*

The results of the experiments are shown in Fig. 1. Regarding the experiments performed without lipase (the blanks), no changes in backscattering are observed for 2 h, i.e., the presence of duodenal juice does not affect in any manner the stability of the emulsions (ΔBS <0.3%, data not shown). When lipase is added, lipolysis takes place in all cases, with the typical lag-phase previously reported for emulsions stabilized with steric surfactants, in contrast to the fast, almost instantaneous lipolysis observed when phospholipids are used as the only surfactant (Wulff-Pérez et al., 2010; Chu et al., 2009). It is also worth to note here that the decrease of backscattering was equal both at the bottom and at the top part of the measuring cell, meaning that no phase separation was happening within the time frame of the experiment. This indicates that no coalescence is taking place, i.e., the observed changes can be attributed to the lipolysis of the oil droplet and not to the hydrolysis of the surfactants stabilizing the droplet.

However, the lipolysis observed is not equal for all the surfactants, being this lipolysis notably slower for the biggest surfactant of all of them, PF127. If we check the curves for M52, PF68 and PF127, we may think on a relation between the length of the PEO chain (i.e., the length of the steric barrier around the droplet) and the lipolysis observed: M52, having a PEO chain of 40 subunits, presents the fastest lipolysis, while PF127, having a 100 PEO chain, presents the slowest lipolysis. The lipolysis rate for an emulsion stabilized with PF68, having a PEO chain of 75 subunits, is intermediate between the other two. However, the lipolysis observed for emulsions stabilized with M59 suggests a more complex explanation. This lipolysis is slower than that of PF68 and PF127, in spite of the fact that M59 has a PEO chain of 100 subunits, i.e., longer than that of PF68 and equal to PF127. This leads us to think that the mechanism by which steric surfactants delay the lipolysis rate under duodenal conditions is not only influenced by the length of the steric barrier around the droplet, but also by the architecture of the surfactant at the interface, i.e., how the surfactant occupies that interface. The reported area per molecule at a water/air interface for M52 is between 0.4 and 1.2 nm^2/molecule (Lee et al., 2001; Shen et al., 2008). Unfortunately, to the best of our knowledge, this value has not been reported for M59. However, we may think on a similar value to that of M52, due to the fact that the hydrophobic part that links to the droplet is exactly the same for the two surfactants (see Table 1). Regarding the Pluronics, the area per molecule reported for PF68 lies between 3 and 3.4 nm^2/molecule (Chang et al., 2005; Noskov et al., 2006), while this value increases to 6.5–7.21 nm^2/mol for PF127 due to its longer hydrophobic PPO block (Phipps et al., 1993; Blomqvist et al., 2005). Therefore, PF127 occupies double area at the interface than PF68, and approximately five times that area occupied by M52 and M59, which is consistent with the structure and size of the hydrophobic part of the surfactants (see Table 1). Lipolysis is an interfacial process, where bile salts have to displace adsorbed molecules from the lipid–water interface before colipase and lipase are adsorbed (Wickham et al., 2002). As bile salts tend to lie flat on that interface (Maldonado-Valderrama et al., 2008), it should be more difficult for them to remove molecules that adsorb strongly to that interface and that occupy a high amount of its area. This combination between steric hindrance and high occupation of the interface could explain the strong inhibition of the duodenal lipolysis by PF127 observed in our experiments. The same inhibition has been previously observed in the in vivo experiments performed by Johnston and Goldberg following an oral administration of PF127 solution to mice (Johnston and Goldberg, 2006).

3.3.2. *Mixtures of PF68 and PF127*

The results of the experiments performed with mixtures of PF68 and PF127 are depicted in Fig. 2. It can be noted that the behavior observed for all the mixtures is almost undistinguishable from that of PF127 alone, even for that mixture with less PF127 (3:1 mixture). This remarks the great influence of PF127 on the interfacial processes related to duodenal lipolysis, as it still dominates the interfacial behavior when its concentration is just a quarter of the total surfactant concentration.

3.4. *In vitro intravenous lipolysis*

3.4.1. *Pure PF68, PF127, M52 and M59*

In vitro experiments about IV lipolysis without using phospholipids as surfactant are very scarce in the literature (Arimoto et al., 1998a,b; Kurihara et al., 1996). In order to check our experimental conditions (lipase concentration and activity, amount of plasma added, etc.) and to compare our results with other authors, we have prepared emulsions stabilized only with phospholipids, obtaining similar lipolysis rates to that observed by other authors under similar conditions (Ton et al., 2005; Arimoto et al., 1998a,b), i.e., the lipolysis was complete within the first 30–60 min. For the emulsions stabilized with steric surfactants, a slower lipolysis is observed, as depicted in Fig. 3.

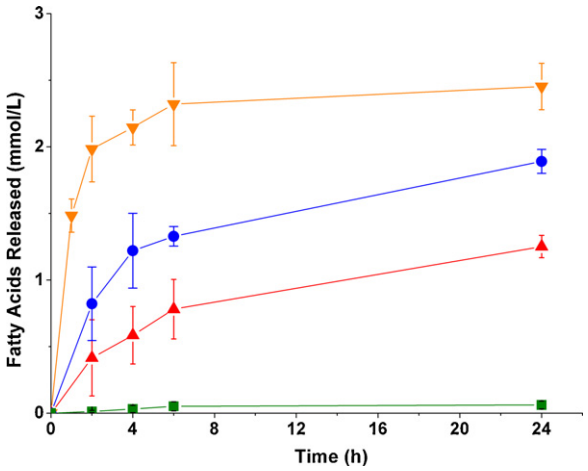


Fig. 3. Free fatty acids released during a lipolysis experiment under intravenous conditions for four submicron emulsions stabilized with different steric surfactants: -■-, Pluronic F127; -●-, Pluronic F68; -▼-, Myrj 52; -▲-, Myrj 59. $n = 3$.

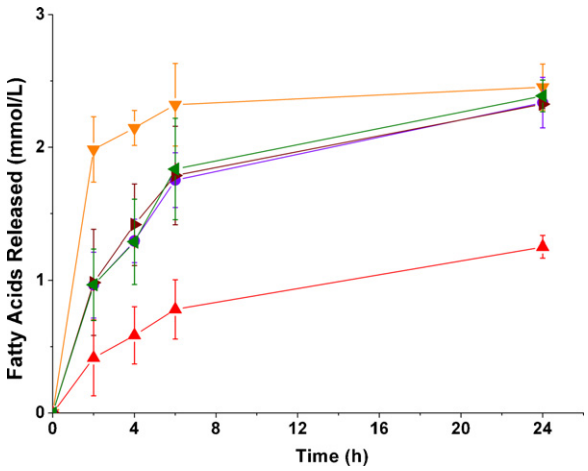


Fig. 4. Free fatty acids released during a lipolysis experiment under intravenous conditions for five submicron emulsions stabilized with different mixtures of Myrj surfactants: -▼-, only M52; -▲-, only M59; -►-, M52-M59 3:1; -●-, M52-M59 1:1; -◄,- M52-M59 1:3. $n = 3$.

It can be observed that the surfactant employed to prepare the emulsions influences critically the lipolysis rate under IV conditions: lipolysis is almost complete within the first 4–6 h for the emulsions stabilized with M52, the smallest surfactant, while this lipolysis is almost completely blocked when PF127 is used as surfactant. For the other two surfactants, an intermediate behavior is observed, and no plateau indicating the end of lipolysis is observed within 24 h. It is also worth to mention that under IV conditions the inhibition by steric hindrance seems to prevail over that caused by occupation of the interface, since M59 in this case reduces more efficiently the lipolysis rate than PF68. However, the coverage of the interface by the surfactant still plays an important role, since M59 does not block completely the lipolysis, as PF127 does, although both surfactants have the same PEO chain and therefore should present a similar steric barrier. These results show some similarities with the trends observed for the different surfactants under duodenal conditions. This is not surprising, as in both cases structurally similar lipases are being used, as well as indispensable co-factors (colipase under duodenal conditions, and apolipoprotein-CII under IV conditions). However, the intravenous lipolysis is considerably slower, and the influence of each surfactant is clearly more pronounced under IV conditions. This could be explained by the differences in concentrations and compositions between duodenal fluids and IV fluids: the concentrations of lipase and co-factor are notably higher under duodenal conditions, and the presence of a high concentration of bile salts will undoubtedly lead to a faster lipolysis, taking into account their ability to displace other surfactants from surfaces and to bind co-lipase to that interface.

The mechanism for this inhibition seems to be then the steric hindrance that these polymeric surfactants produce both around the droplet and at the interface. The access to the triglycerides of the interface is difficult to a greater degree for apolipoprotein-CII and lipoprotein lipase, and therefore the lipolysis is slowed-down or even completely blocked when the surfactant used has a proper size. This mechanism would explain the very low adsorption of apoliproteins to emulsions partially covered by PF127 (Harnisch and Müller, 2000) in the same way that Pluronics prevent the adsorption of major plasma proteins (Tamilvanan et al., 2005; Jackson et al., 2000). These results and the mechanism suggested are in good agreement with the in vivo works of Johnston and Palmer, where the in vivo LpL activity was reduced more than 90% as long as 24 h after a single injection of a PF127 solution (Johnston and Palmer, 1993).

3.4.2. Mixtures of M52 and M59

The lipolysis rates observed for emulsions stabilized with different mixtures of M52 and M59 are shown in Fig. 4. For the three different mixtures used, 3:1, 1:1 and 1:3, the behavior observed was quite similar, and different from that of pure M52 or pure M59. In other words, by combining two surfactants we are able to obtain a new lipolysis pattern, intermediate between that of the original surfactants: within the first 2 h is slower than with M52 alone, but it leads also to completeness within 24 h.

3.4.3. Mixtures of PF68 and PF127

The results of the lipolysis experiments performed using mixtures of PF68 and PF127 as surfactant under IV conditions are depicted in Fig. 5. Depending on the proportion between the two surfactants, different lipolysis rates are observed. For a 3:1 mixture, the lipolysis is slower than that with PF68 alone. When the content of PF127 is increased up to a 1:1 mixture, the lipolysis is slowed-down, but still takes place. If a mixture 1:3 is used as surfactant, i.e., the amount of PF127 triplicates that of PF68, the lipolysis observed is almost undistinguishable from that obtained when PF127 was the only surfactant. Again, we are able to obtain different lipolysis rates under IV conditions just by changing the proportions between

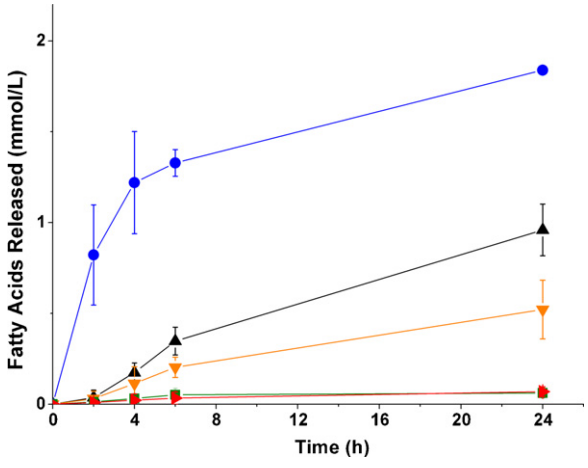


Fig. 5. Free fatty acids released during a lipolysis experiment under intravenous conditions for five submicron emulsions stabilized with different proportions of Pluronics: -■-, only Pluronic F127; -●-, only Pluronic F68; -▲-, PF68-PF127 3:1; -▼-, PF68-PF127 1:1; -►-, PF68-PF127 1:3. $n = 3$.

the two surfactants used to prepare the emulsions. This could be a relatively easy manner to modify the lipolysis of lipid carrier systems, and therefore to achieve a better control of the release of the hydrophobic drugs dissolved in them.

4. Conclusions

In this paper, we have studied how polymeric non-ionic surfactants modify the enzymatic degradation (i.e., lipolysis) of submicron emulsions both under duodenal and intravenous conditions. It has been depicted that the chosen surfactant influences critically the lipolysis rates, being able even to completely block this lipolysis, as occurs with Pluronic F127 under IV conditions. Thus, the election of the surfactant used to prepare such lipid carrier systems is not a trivial issue, since it will modify the hydrolysis of the carrier and therefore the release of the hydrophobic drugs dissolved in it. The mechanism of this inhibition seems to be the steric hindrance produced by these surfactants both around the droplet and at the interface. As a consequence, it is possible to modify the lipolysis pattern by selecting a surfactant with the desired properties (size, disposition at the interface, etc.), e.g., we could slow-down the lipolysis by using a surfactant that produces a longer steric barrier or that occupies a higher amount of lipid/water interface.

In addition, we have shown that under intravenous conditions it is possible to alter the lipolysis rate only by changing the proportions between the two surfactants used, obtaining intermediate lipolysis patterns than those of pure surfactants.

These findings give us new insights on the rational design of controlled-release submicron emulsions, for both oral and intravenous administration, and emphasize the influence of the characteristics of the surfactant on this control as an active agent.

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