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Digestion of lipid excipients and lipid-based nanocarriers by pancreatic lipase and pancreatin

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ABSTRACT

The digestion behaviour of lipid-based nanocarriers (LNC) has a great impact on their oral drug delivery properties. In this study, various excipients including surfactants, glycerides and waxes, as well as various drug-delivery systems, namely self-emulsifying drug delivery systems (SEDDS), solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were examined via the pH-stat lipolysis model. Lipolysis experiments with lipase and pancreatin revealed the highest release of fatty acids for medium chain glycerides, followed by long chain glycerides and surfactants. Waxes appeared to be poor substrates with a maximum digestion of up to 10% within 60 min. Within the group of surfactants, the enzymatic cleavage decreased in the following order: glycerol monostearate > polyoxyethylene (20) sorbitan monostearate > PEG-35 castor oil > sorbitan monostearate. After digestion experiments of the excipients, SEDDS, SLN and NLC with sizes between 30 and 300 nm were prepared. The size of almost all formulations was increasing during lipolysis and levelled off after approximately 15 min except for the SLN and NLC consisting of cetyl palmitate. SEDDS exceeded 6000 nm after some minutes and were almost completely hydrolysed by pancreatin. No significant difference was observed between comparable SLN and NLC but surfactant choice and selection of the lipid component had an impact on digestion. SLN and NLC with cetyl palmitate were only digested by 5% whereas particles with glyceryl distearate were decomposed by 40–80% within 60 min. Additionally, the digestion of the same SLN or NLC, only differing in the surfactant, was higher for SLN/NLC containing polyoxyethylene (20) sorbitan monostearate than PEG-35 castor oil. This observation might be explained by the higher PEG content of PEG-35 castor oil causing a more pronounced steric hindrance for the access of lipase. Generally, digestion experiments performed with pancreatin resulted in a higher digestion compared to lipase. According to these results, the digestion behaviour of LNC depends on both, the type of nanocarrier and on the excipients used for them.

1. Introduction

Lipid-based nanocarriers (LNC) such as nanoemulsions, self-emulsifying drug delivery systems (SEDDS), solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and liposomes [1] are interesting tools for oral drug delivery as drugs being poorly soluble in aqueous media [2,3] can be dissolved in their lipophilic phase [4]. Furthermore, therapeutic peptides and proteins can be protected towards a presystemic metabolism when they are incorporated in LNC [5–7].

All these nanocarriers themselves have a broad compositional spectrum although typically they consist of a molecularly dispersed drug in a mixture of different oils/glycerides with divergent polarity, surfactants and co-solvents. Within the gastrointestinal tract these formulations undergo various changes in their physical and chemical properties. Most important is the influence of bile salts and pancreatic enzymes within the small intestine. When lipids are digested, their products are solubilized by bile salts, phospholipids and cholesterol. Together they shape micelles which transport the digestion products to the intestinal membrane [8]. This principle can be used for delivering the drug together with the digestion products to the site of absorption [1,9–11]. In case of SEDDS, SLN and NLC, the drug is already presented to the gastrointestinal tract in a solubilized state. Therefore, digestion of LNC has a great impact on drug delivery, as continuing decomposition could lead to undesired drug release, drug precipitation or inactivation [5,6,9,12–15].

To achieve a better understanding on how these formulations are

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digested, enzymatic digestion models simulating small intestinal conditions have been established [8]. Pancreatin is a mixture of different enzymes including proteases, amylases and lipases all playing a crucial role in digestion. Regarding lipid digestion, pancreatic lipase is the main enzyme mediating decomposition of triglycerides. Nevertheless, pancreatin possesses several other lipolytic enzymes as well [16]. Lipase belongs to the esterases and builds up a complex with co-lipase acting on the oil–water-interface. The main substrates of lipase are triglycerides but it also has an impact on other compounds containing ester bonds [17]. Several parameters like droplet size, inductive effects and steric hindrance affect the extent and velocity of enzymatic degradation of these compounds [18].

Simulating digestion in the upper intestine, usually the pH stat lipolysis model is utilized [19]. It quantifies free fatty acids (FA) released from the substrates after treatment with pancreatic lipase or pancreatin by titration with NaOH. The quantity of fatty acids correlates to the extent of digestion [8]. Most lipolysis experiments conducted by other research groups were performed with pancreatin [9,20–23]. Any comparisons between the digestion of the same excipients or formulations by lipase and pancreatin, however, are rare [24–26]. Glycerides [20,23], surfactants [21,27] and LNC [6,7,26,28] were widely investigated within these experiments but there is a lack of information about commonly used waxes. Additionally, only the decomposition of LNC and the released digestion products have been investigated [6,7,26,28,29] but to our knowledge there is no information about size changes during decomposition. Moreover, SEDDS consist of liquid nanodroplets whereas SLN and NLC are composed of solid nanoparticles. This difference in physical state could as well result in divergent degradation properties. It was therefore the aim of this study to compare the stability of different excipients towards lipase and pancreatin and their digestion properties within LNC. Furthermore, size measurements of LNC during lipolysis should give more information about its decomposition or resistance against the enzyme. The results could predict the behavior of the formulation in the intestine and if it is suitable for drug delivery without causing massive changes in size and composition. Especially a direct comparison between SEDDS (liquid nanocarrier) and SLN/NLC (solid nanocarriers) could reveal significant differences in digestibility. For a comparison of digestion by lipase and pancreatin, several excipients including surfactants, oils and waxes were investigated via the pH stat lipolysis model. Since triglycerides are the main substrates of lipase [17], their digestion is predicted to be highest among the chosen excipients. Neighbouring groups to ester linkages should have an impact on their digestion and might result in different digestion patterns between lipase and pancreatin. A space-saving group like PEG chains might even inhibit an enzymatic attack by lipase [28]. Since pancreatin contains a mixture of enzymes, it might be able to hydrolyze excipients whereupon lipase has no effect on. Digestion of the excipients solely probably differs from decomposition in LNC. For this purpose, SEDDS, SLN and NLC were prepared consisting of some of the earlier investigated excipients and characterized for their stability against pancreatin. Additionally, size development of these formulations was measured during lipolysis.

2. Materials and methods

2.1. Materials

Pancreatin from porcine pancreas [4x USP specifications], lipase from porcine pancreas [Type II; 100–500 units/mg protein using olive oil 30 min incubation; 30–90 units/mg protein using triacetin], L-alpha-phosphatidylcholine from egg yolk [60%] (PC), $CaCl_2 \cdot H_2O$, Tris–maleate, Span® 60 (sorbitan monostearate), Kolliphor EL® (PEG-35 castor oil), poloxamer 407 and eugenol were all purchased from Sigma-Aldrich (Vienna, Austria). Sodium taurodeoxycholate hydrate (NaTDC) was bought from Biosynth (Bratislava, Slovakia). Glycerol monostearate, soybean oil and all waxes were obtained from Carl Roth (Karlsruhe, Germany). Tween® 60 (polyoxytheylene (20) sorbitan monostearate, polysorbate 60) was acquired from Gatt Koller (Absam, Austria), Imwitor 742® (medium chain partial glycerides, glyceryl caprylate/caprate Type I) from IOI Oleo GmbH (Hamburg, Germany) and Miglyol® 812 (medium chain triglycerides) from Caesar & Loretz GmbH (Bonn, Germany). Capmul® MCM (mono/di-glyceride of caprylic and capric acid) was kindly provided by Abitec (Columbus, USA), Precirol® ATO 5 (glyceryl distearate) and Maisine™ 35–1 (glyceryl monolinoleate) by Gattefossé (Saint-Priest, France) as free samples.

2.2. Preparation of SEDDS, SLN and NLC

For SEDDS, components as listed in Table 1 were mixed and homogenized in a Thermomixer (Eppendorf ThermoMixer® C, Eppendorf AG, Germany) at 700 rpm and 37 °C for 30 min. SLN and NLC were prepared via the hot homogenization method [30] and ultrasonication [31] (Table 2). Thereby the solid lipid components were melted at 70 °C. Surfactants were dissolved in demineralized water and heated to the same temperature. The hot surfactant-water mixture was added to the melted lipids and stirred for 10 min at 70 °C to form a pre-emulsion. Thereafter, the pre-emulsion was homogenized via ultrasonication (UP 200H, Hielscher Ultrasonics GmbH, Germany) with an ultrasonic frequency of 24 kHz and an amplitude of 100% for 5 min. Storage on ice for 20 min allowed the formation of solid particles.

2.3. Characterization of SEDDS, SLN and NLC

SEDDS were diluted in a ratio of 1:100 in distilled water and 1:40 in digestion medium. A dilution ratio of 1:40 in digestion buffer was applied for the SLN and the NLC. Samples were heated up to 37 °C and vortexed before each measurement. Size and polydispersity index (PDI) of SEDDS, SLN and NLC were determined at 37 °C via dynamic light scattering using a Zetasizer Nano ZSP (Malvern Panalytical Ltd., United Kingdom).

2.4. Preparation of digestion medium and enzyme solution

Digestion medium was prepared as described by Williams et al. 2012 with some minor changes [9]. Briefly, the digestion buffer consisted of 150 mM NaCl, 1.4 mM $CaCl_2 \cdot H_2O$, 2 mM Tris–maleate, 3 mM NaTDC and 0.75 mM PC in demineralized water. The medium was stirred for 3–5 h until PC was completely dissolved and pH was adjusted to 6.5 with 1 M NaOH. These parameters were chosen to simulate conditions of the small intestine. Tris–maleate was used as buffer for initial pH stabilization having a negligible effect on decrease of pH caused by the release of fatty acids during titration. NaTDC and PC contributed as emulsifiers to the dispersion of lipid components. Ca^{2+} forms insoluble complexes with the released fatty acids during titration. In this way, fatty acids can be removed from the emulsion surface that might otherwise hinder lipolysis [8]. Several research groups have shown the impact of different calcium concentrations on the release of fatty acids in lipolysis experiments [12,32]. Since the duodenum shall be simulated in these studies, physiological calcium levels were chosen.

For the lipase solution, 50 mg of pancreatic lipase was vortexed in 11 ml of digestion medium (without NaTDC and PC) [6]. To avoid a loss in enzymatic activity, cold digestion medium (2–8 °C) was used and enzyme solutions were prepared freshly before each titration. After 10 min the suspension was centrifuged at 4000 rpm for 10 min at 4 °C (Sigma 3-18KS, Sigma Laborzentrifugen GmbH, Germany). The supernatant was collected and pH adjusted to 6.5 with approximately 50 µl of 1 M NaOH. Pancreatin solution was prepared in the same way, except for suspending 1 g of pancreatin in 5 ml of digestion medium [9]. After pH adjustment, 10 ml of the pancreatic lipase solution or 4 ml of the pancreatin solution were used for the titration experiment.

Table 1
Composition of SEDDS in % (V/V).

	Kolliphor EL®	Imwitor® 742	Miglyol® 812	Capmul® MCM EP/NF	Soybean oil	Isopropylmyristate	Eugenol
SEDDS 1	35	32.5	32.5				
SEDDS 2	35		15	40			10
SEDDS 3	35			40	15		10
SEDDS 4	35			40		15	10

Table 2
Composition of SLN and NLC in mg. Weights refer to a production in 10 ml water.

	Poloxamer 407	Kolliphor EL®	Tween® 60	Cetyl palmitate	Precirol® ATO 5	Miglyol® 812
SLN 1	75		75	150		
SLN 2	75		75		150	
SLN 3	75	75			150	
NLC 1	75		75	120		30
NLC 2	75		75		120	30
NLC 3	75	75			120	30

2.5. pH stat lipolysis model

The lipolysis protocol was modified from Williams et al. 2012 [9]. In brief, 1 g of substrate (lipid component/SEDDS) was dissolved or suspended in 30 ml of digestion medium for the titration with lipase or 36 ml for the titration with pancreatin. SLN and NLC were prepared according to the method described above, except that water was replaced by digestion medium. The mixtures were stirred for at least 10 min at 37 °C before starting the titration to allow a homogenous distribution of the excipients. The pH value was set to 6.5 ± 0.05 by 1 M HCl or 1 M NaOH.

The digestion was initiated by the addition of the enzyme to the substrate solution. The enzyme caused the release of fatty acids which led to a decrease in pH. After 5, 10, 20, 30, 40, 50 and 60 min 0.1 M NaOH was added in order to lift pH back to 6.5. Blank experiments without any substrate in the digestion buffer were performed to exclude any reduction of pH due to other factors. These factors might be hydrolysis of PC, impurities in the bile salts or pancreatic extract [13]. The lone amount of PC, which was hydrolyzed by mainly lipase and phospholipase A2 [18,33] in the blank digestion medium experiments, was subtracted from the original results for the substrates.

After 60 min, back titrations were performed because only fully ionized fatty acids are detectable during titration at pH 6.5. As especially long chain fatty acids are only partially ionized at this pH, the complete amount of released fatty acids could not be measured by the previously described method. Thus, pH was raised rapidly to 9 with 1 M NaOH at the end of each titration. The amount of 1 M NaOH represents the partially ionized fatty acids [34]. Nevertheless, experiments were executed to determine the volume of 1 M NaOH which had to be added to a blank sample to increase pH to 9. As a result, the total release of fatty acids consisted of the fully ionized fatty acids titrated at pH 6.5 and the only partially ionized ones detected at pH 9. The extent of digestion can be determined by the following equation:

$$Extent\ of\ digestion[\%] = \frac{FA_{ionized} + FA_{nonionized}}{theoretical\ maximum\ amount\ of\ released\ FA} \times 100$$

2.6. Size determination during lipolysis

SEDDS, SLN and NLC were diluted in a ratio of 1:40 in digestion medium. 70 µl of pancreatin solution prepared as described above were added to 630 µl of the diluted formulation [9]. Directly after addition of the enzyme, the size measurement using the Zetasizer Nano ZSP was started. Measurements took place every 5 min proceeding for one hour. Measurements for time point zero were performed without the enzyme.

2.7. Statistical design and analysis of data

All experiments were performed at least in triplicates and results were presented as mean ± standard deviation. Statistical analysis was performed via a two-way ANOVA (GraphPad Prism 5) with $p < 0.05$ considered as level of significance.

3. Results

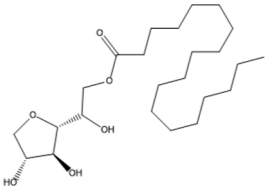
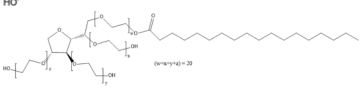
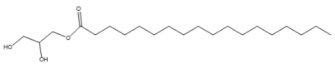
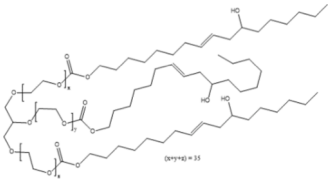
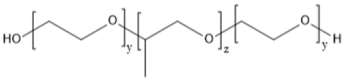
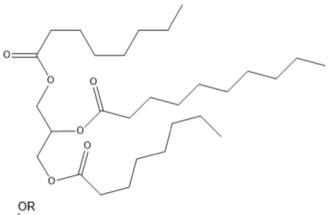
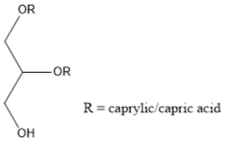
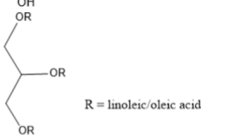
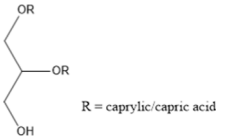
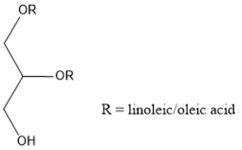
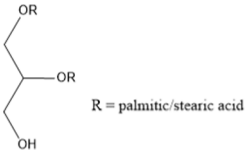
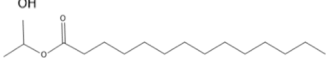
3.1. Excipients

Digestion of several excipients was investigated via the pH stat lipolysis method. Table 4 summarizes all of the excipients used within these experiments. Medium chain triglycerides, glyceryl caprylate/caprate Type I, glyceryl monolinoleate, methyl palmitate, sorbitan monostearate and glycerol monostearate were dispersed completely in the digestion medium resulting in a cloudy white emulsion. Glycerol monostearate was melted at 60 °C before forming a proper emulsion. Polyoxyethylene (20) sorbitan monostearate and PEG-35 castor oil were entirely soluble in the digestion medium. Addition of soybean oil, isopropyl myristate and cetyl palmitate to the medium arose in a suspension with most of the oil/wax causing phase separation and layering as oily droplets on the buffer's surface. Titration with cetyl palmitate was performed at 60 °C, as at lower temperatures solid wax assembled on the surface. Results for cetyl palmitate are therefore not completely comparable to the other titrations which were performed at 37 °C. Thereby, the optimum temperature of lipase is at 37 °C and decreases afterwards [35].

The extent of digestion was calculated using the equation described above. Other publications [11,18,23,36] proved that triglycerides are split into two fatty acids and a 2-monoglyceride. For the oils (medium chain triglycerides, soybean oil) and PEG-35 castor oil it was assumed that they release two fatty acids and a 2-monoglyceride which does not show any lipolysis. For the mixtures of mono-, di- and triglycerides (glyceryl caprylate/caprate Type I, glycerol monolinoleate and glyceryl distearate) only the digestion of the diglyceride was taken into account, since the share of triglycerides within all excipients was remarkably low (<10%). Diglycerides are the main compound in glycerol monolinoleate and glyceryl distearate, whereas the monoglycerides are predominant in glyceryl caprylate/caprate Type I. As all other tested excipients contained one ester bond, they were able to release just one fatty acid. Poloxamer 407 and eugenol did not possess any ester bonds at all. They were excluded from the calculation since theoretically no decomposition by lipase can take place. For SEDDS, SLN and NLC only excipients with ester linkages were utilized for the calculation.

The release of fatty acids and the extent of digestion for the titration

Table 4
Characterization of single components in terms of chemical name, composition, hydrophilic-lipophilic-balance (HLB), regulatory status and chemical structure referring to Ph. Eur., USP, company's product information and [51].

Trade name (Company)	**Chemical name**	**Composition**	**HLB**	**Regulatory status**	**Structure** (example)
Span® 60 (Sigma Aldrich)	Sorbitan mono-stearate	Monoester between sorbitan and stearic (C_{18}) acid	4.7	Ph. Eur. (Sorbitan stearate), USP NF (Sorbitan monostearate)	
Tween® 60 (Sigma Aldrich)	Polyoxy-ethylene (20) sorbitan mono-stearate	Ester between sorbitan and stearic (C_{18}) acid reacting with 20 mol ethylene oxide	14.9	Ph. Eur. (polysorbate 60), USP NF (polysorbate 60)	
Glycerol mono-stearate	Glycerol mono-stearate	Mainly monoesters between glycerol and stearic (C_{18}) acid (40–55%); small amounts of di- and triglycerides	4	Ph. Eur. (glycerol mono-stearate 40–55), USP NF (glyceryl mono-stearate)	
Cremophor EL/ Kolliphor EL® (BASF)	PEG-35 castor oil	Product from the reaction by castor oil and 35 mol ethylene oxide	12–14	Ph. Eur. (macrogol-glycerol ricinoleate), USP NF (polyoxyl 35 castor oil)	
Kolliphor® P 407 (BASF)	Poloxamer 407/ Polyoxy-ethylene polyoxy-propylene glycol	Polyoxy-ethylene polyoxy-propylene glycol	18–23	Ph. Eur. (poloxamer 407), USP NF (poloxamer 407)	
Miglyol® 812 (Caelo)	Medium chain tri-gylcerides	Triglycerides with mainly caprylic (C_8) and capric (C_{10}) acid	1	Ph. Eur. (tri-glycerides, medium-chain), USP NF (medium-chain tri-glycerides)	
Capmul® MCM EP/NF (ABITEC)	Glyceryl caprylate/ caprate Type I	Mono/Di-glycerides with caprylic (C_8) and capric (C_{10}) acid; 1-mono-glyceride > 48%	5–6	Ph. Eur. (glycerol mono-caprylo-caprate type I), USP NF (glyceryl monocaprylocaprate type I; mono- and diglycerides)	
Soybean oil	Soybean oil	Trigylcerides with mainly linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acid; small amounts of palmitic (C_{16}) and linolenic ($C_{18:3}$) acid	–	Ph. Eur. (soya-bean oil – refined, hydro-genated), USP NF (soybean oil – refined, hydro-genated)	
Imwitor® 742 (IOI Oleo GmbH)	Glyceryl caprylate/ caprate Type I	Mono-, di- and triglycerides with caprylic (C_8) and capric (C_{10}) acid; mono-glycerides ~ 45–75%, diglycerides ~20–50%	5–6	Ph. Eur. (glycerol monocaprylocaprate type I), USP NF (glyceryl monocaprylocaprate type I; mono- and diglycerides)	
Maisine™ 35–1 (Gatte-fossé)	Glyceryl mono-linoleate	Mono-, di- and triglycerides of mainly linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids; the diester fraction being predominant	1	Ph. Eur. (glycerol mono-linoleate), USP NF (glyceryl mono-linoleate)	
Precirol® ATO 5 (Gatte-fossé)	Glyceryl distearate	Esters between glycerol and palmitic (C_{16}) and stearic (C_{18}) acid; the diester fraction being predominant	2	Ph. Eur. (glycerol distearate type I), USP NF (glyceryl distearate)	
Isopropyl myristate	Isopropyl myristate	Ester between isopropanol and myristic (C_{14}) acid	–	Ph. Eur. (isopropyl myristate), USP NF (isopropyl myristate)	
Cetyl palmitate	Cetyl palmitate	Mixture of esters between C_{14} to C_{18} alcohols and lauric (C_{12}), myristic	–		

(*continued on next page*)

Table 4 (*continued*)

Trade name (Company)	Chemical name	Composition	HLB	Regulatory status	Structure (example)
		(C_{14}), palmitic (C_{16}) and stearic (C_{18}) acid		Ph. Eur. (cetyl palmitate), USP NF (cetyl palmitate)	
Methyl palmitate	Methyl palmitate	Ester between methanol and palmitic (C_{16}) acid	–	–	

with lipase and pancreatin are shown in Fig. 1 and Fig. 2. Generally, higher release of fatty acids and higher digestion was observed for most of the compounds in the experiment with pancreatin. There was no significant increase in the extent of digestion for some of the less digestible excipients, namely waxes, soybean oil, PEG-35 castor oil and sorbitan monostearate. Since pancreatin is a mixture of enzymes, it contains some enzymes that might as well act on ester structures usually less affected by lipase [37]. Pancreatic lipase-related protein 2 (PLRP2) is able to cleave the ester linkages in triglycerides, phospholipids, galactolipids and vitamin esters. Carboxyl ester hydrolase (CEH) has an

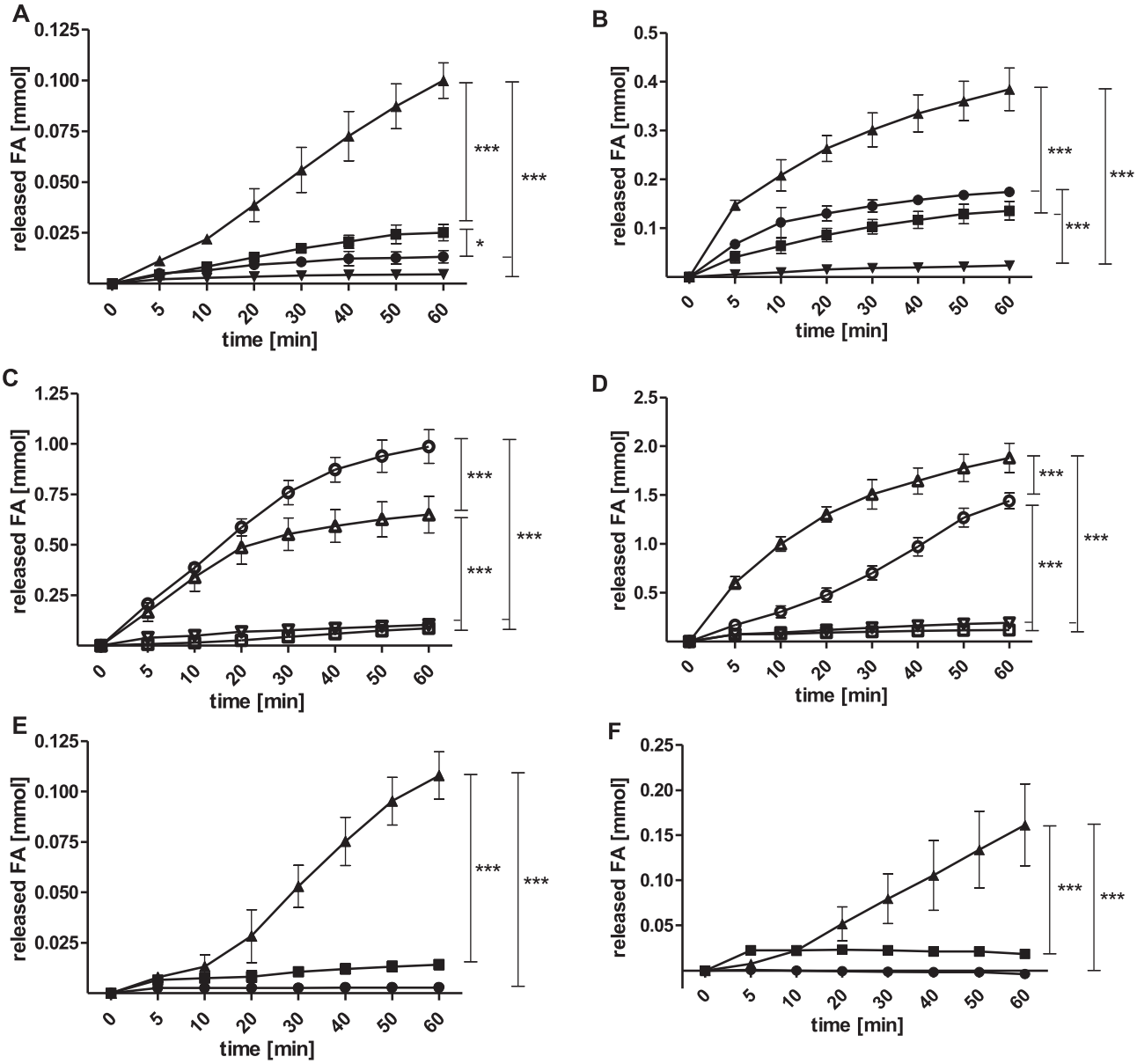


Fig. 1. Released fatty acids (FA) for excipients. Data is presented as mean ± standard deviation for at least three experiments. Significant differences are indicated as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Released FA [mmol] for the surfactants (*A*), glycerides (*C*) and waxes (*E*) by pancreatic lipase. Released FA [mmol] for surfactants (*B*), glycerides (*D*) and waxes (*F*) by pancreatin. Surfactants (*A* and *B*) are divided into ▲ glycerol monostearate, ■ polyoxyethylene (20) sorbitan monostearate, ● sorbitan monostearate and ▼ PEG-35 castor oil. Glycerides (*C* and *D*) are divided into ○ medium chain triglycerides, □ soybean oil, ▲ (unfilled) glyceryl caprylate/caprate Type I and ▼ (unfilled) glyceryl monolinoleate. Waxes (*E* and *F*) are divided into ● isopropyl myristate, ■ cetyl palmitate and ▲ methyl palmitate.

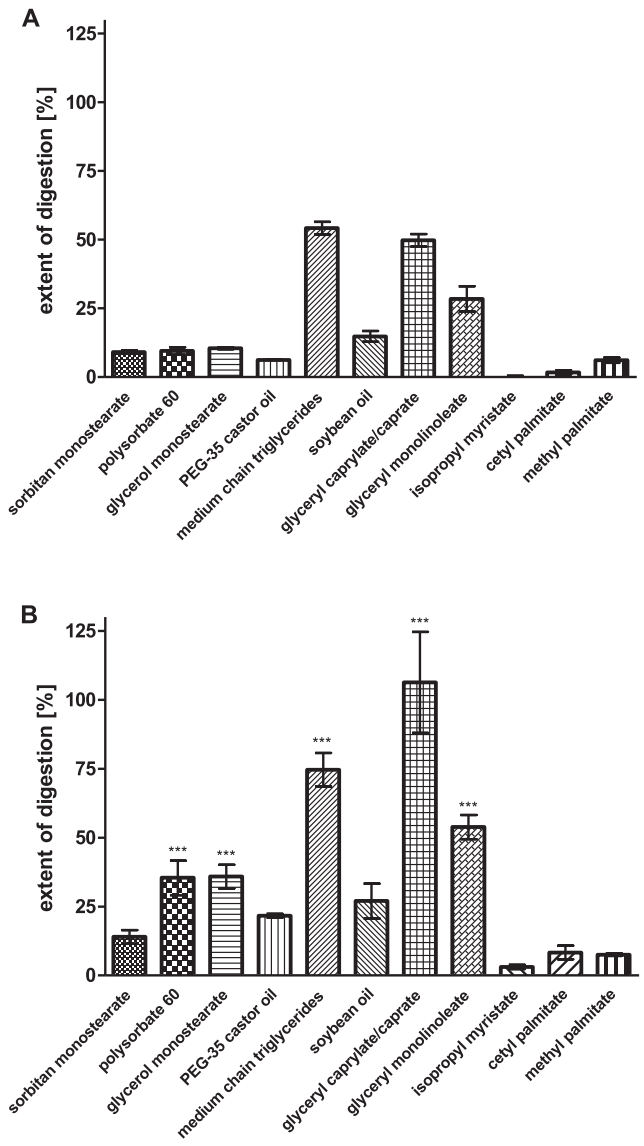


Fig. 2. Extent of digestion [%] based on the theoretical release of fatty acids for the single components. Data is presented as mean ± standard deviation for at least three experiments. *A*: Extent of digestion for the experiment with pancreatic lipase. *B*: Extent of digestion for the experiment with pancreatin, significant differences to the digestion with lipase are indicated as $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$.

effect on many substrates including mono-, di- and triglycerides, vitamin esters, cholesterol esters and phospholipids [16,26]. Another reason is that lipase needs co-lipase to build an active complex. Without co-lipase, bile salts seem to cause a desorption of lipase from the interface [38,39]. Pancreatin includes co-lipase which mediates the binding of lipase to the interface, although bile salts are present [12,16].

There were comparable results of NaOH consumption within the group of surfactants, glycerides and waxes, whereas the glycerides showed a significant higher lipolysis than the surfactants and waxes. Generally, the curves start levelling off after different time values. The digestion behaviour of the glycerides when utilizing pancreatin has already been investigated by other research groups [20,23]. Medium chain triglycerides and glyceryl caprylate/caprate Type I (Imwitor® 742) have already been investigated by Arnold et al. 2012 [20]. They titrated 500 mg of the excipients and used a pancreatin solution with a final activity of 10,000 tributyrin units/ml. The values for the release of fatty acids for both substances are comparable to the results obtained in the experiments made here. Moreover, the digestion behaviour of soybean oil (long-chain triglyceride), glyceryl monolinoleate (long-chain mono/diglyceride mixture), medium chain triglycerides and glyceryl caprylate/caprate Type I (Capmul® MCM, medium chain mono/diglyceride mixture) has already been explored by Sek et al. 2002 [23]. They revealed a higher release of fatty acids for the medium chain glycerides. In addition, the mono/diglyceride mixtures showed a slightly higher release than the corresponding triglycerides. Comparable results were obtained here for the titration with pancreatin. Additionally, Arnold et al. 2021 [20] also studied the digestion of polyoxyethylene (20) sorbitan monooleate and polyoxyl-40-hydrogenated castor oil. They are quite similar to polyoxyethylene (20) sorbitan monostearate and PEG-35 castor oil, with the differences of higher PEGylation or the composition of divergent fatty acids. They revealed an almost identical NaOH consumption for polyoxyethylene (20) sorbitan monooleate and polyoxyl-40-hydrogenated castor oil. In contrary, the results obtained in this study showed a higher consumption for polyoxyethylene (20) sorbitan monostearate than for PEG-35 castor oil. In other studies [21,27,29], PEG-35 castor oil is hydrolysed to a lower extent than polyoxyethylene (20) sorbitan monooleate [27,29] and the digestion rate of sorbitan monooleate was the lowest for the surfactants [21]. Results in the experiments here present a decreasing digestion rate from polyoxyethylene (20) sorbitan monostearate > PEG-35 castor oil > sorbitan monostearate which corresponds with those of other studies. The results for the digestion of PEG-35 castor oil by Koehl et al. 2020 [21] are higher compared to the investigations made here. Main reason for this observation might be the usage of pancreatin with 8x USP specifications.

3.2. LNC

SEDDS, SLN and NLC were characterized for their size, stability against pancreatin and size development during lipolysis. The results for the size measurements of SEDDS, SLN and NLC are presented in Fig. 3 and Fig. 4. SEDDS were diluted in a ratio of 1:100 in demineralized water to simulate typical dilution conditions for oral drug delivery. The PDI of SEDDS containing eugenol as co-solvent was above 0.3 being considered too broad for various applications [40,41]. The dilution of 1:40 in digestion medium was chosen to reflect the conditions in the lipolysis experiments. Measurements were carried out at 37 °C in order to simulate in vivo conditions. If temperature is increased, particles size might decrease due to greater molecular mobility [42]. On the other hand, an increase in particle size could as well be observed in case of thermal instability. The size of LNC containing glycerides was smaller than of those containing waxes.

Digestion experiments for the LNC were solely performed with pancreatin. Release of fatty acids did as well level off for the SLN and NLC (Fig. 5) and for the SEDDS (Fig. 6). This curve flattening has also been investigated while measuring size during lipolysis for the SLN and NLC (Fig. 7). Size measurements for the SEDDS could not proceed for 60 min because the size increase of SEDDS quickly exceeded 6000 nm (Table 3), which was the limit of detection of the instrument. Hence, measurements were aborted after passing 6000 nm. Regarding lipolysis, the LNC with waxes were decomposed to a lower extent than the ones with the glycerides. Furthermore, the digestion of SLN/NLC with polyoxyethylene (20) sorbitan monostearate was higher than with PEG-35 castor oil. Related SLN and NLC have already been investigated [26]. These particles were composed of polyoxyethylene (20) sorbitan monooleate, poloxamer 407, glyceryl distearate and Labrafac® (medium chain triglycerides, only in NLC) and the ratio between surfactants and lipids was 1:1. In their experiment, the SLN were digested to a higher extent than the NLC, which differs from the results obtained here.

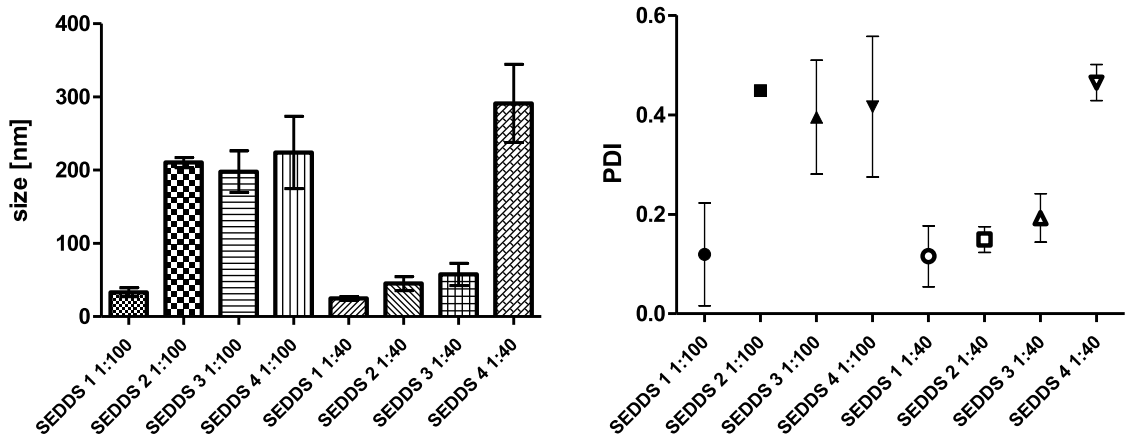


Fig. 3. Sizes [bars] and polydispersity indices (PDI) [points] of SEDDS in a dilution of 1:100 in demineralized water and 1:40 in digestion medium presented as mean ± standard deviation.

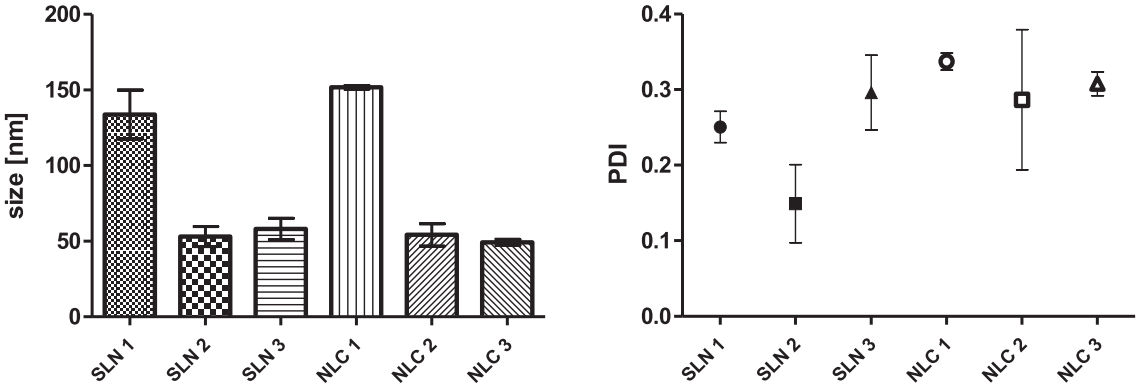


Fig. 4. Sizes [bars] and polydispersity indices (PDI) [points] of SLN and NLC in a dilution of 1:40 in digestion medium presented as mean ± standard deviation.

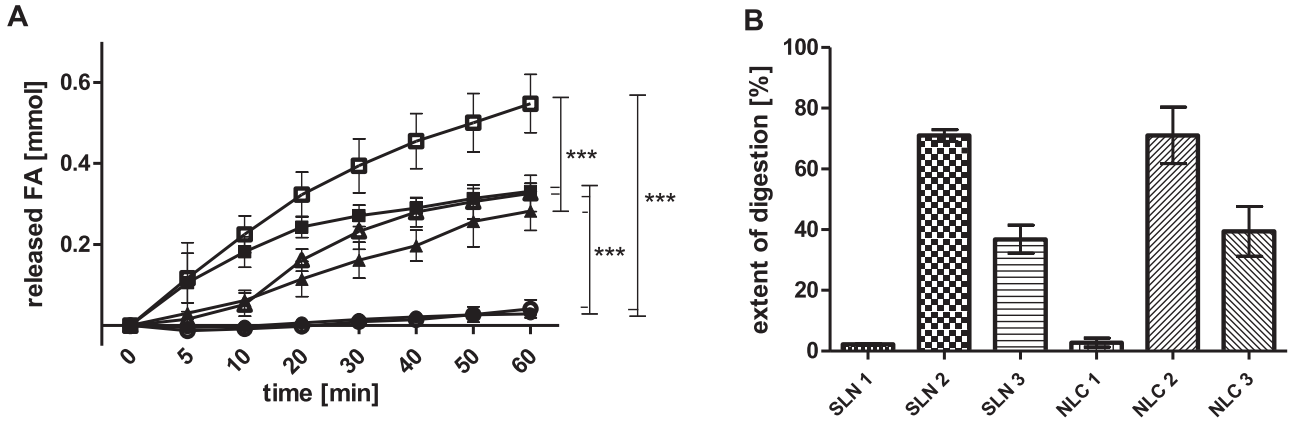


Fig. 5. Release of fatty acids (FA) and extent of digestion [%] for SLNs with ● SLN 1, ■ SLN 2, ▲ SLN 3 and NLCs with ○ NLC 1, □ NLC 2, ▲ (unfilled) NLC 3. Data is presented as mean ± standard deviation for at least three experiments. Significant differences are indicated as: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. *A*: Released fatty acids [mmol] from SLNs and NLCs caused by pancreatin. *B*: Extent of digestion [%] based on the theoretical release of fatty acids from SLNs and NLCs by pancreatin.

4. Discussion

4.1. Surfactants

Among the group of surfactants, glycerol monostearate showed the highest release of fatty acids. The substance used here contained > 35% monoglyceride but probably included side products like di- and triglycerides. These impurities were hydrolysed more easily than the monoglycerides. Additionally, lipase preferentially acts on 1-monoglycerides than on 2-monoglycerides [18]. This might be the reason why it was not digested completely, as both isomers of monoglycerides were present. Since the enzymes PLRP2 and CEH can hydrolyse monoglycerides as well [24], the digestion rate when using pancreatin doubled in comparison to lipase alone. The same outcome occurred for the two PEGylated surfactants (polyoxyethylene (20) sorbitan monostearate and PEG-35 castor oil). This suggests that lipase is not the main

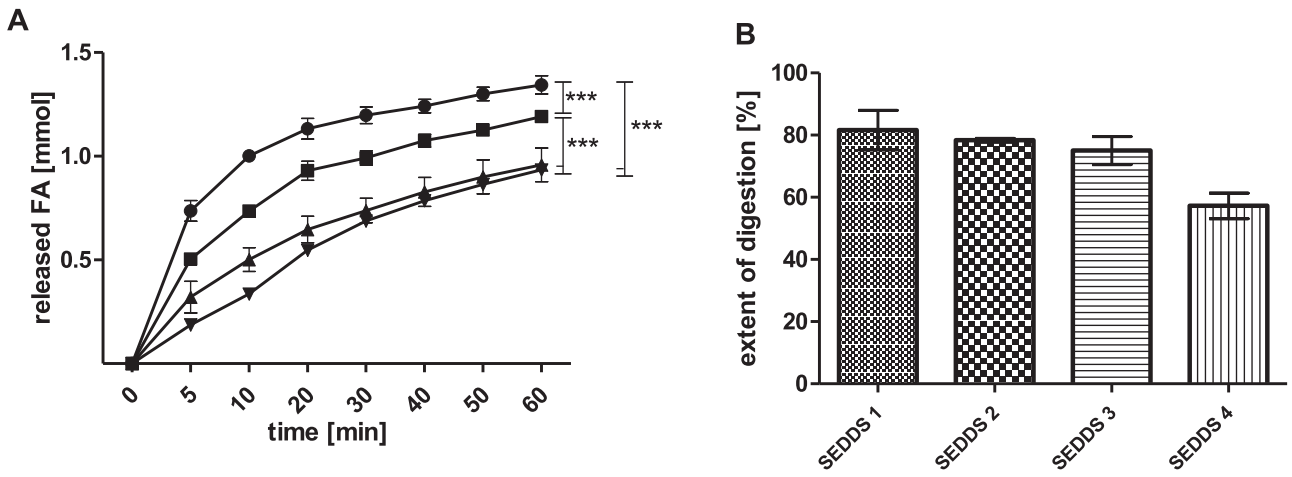


Fig. 6. Release of fatty acids (FA) and extent of digestion [%] for the SEDDS with ● SEDDS 1, ■ SEDDS 2, ▲ SEDDS 3 and ▼ SEDDS 4. Data is presented as mean ± standard deviation for at least three experiments. Significant differences are indicated as: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. *A*: Released fatty acids [mmol] from SEDDS caused by pancreatin. *B*: Extent of digestion [%] based on the theoretical release of fatty acids from SEDDS by pancreatin.

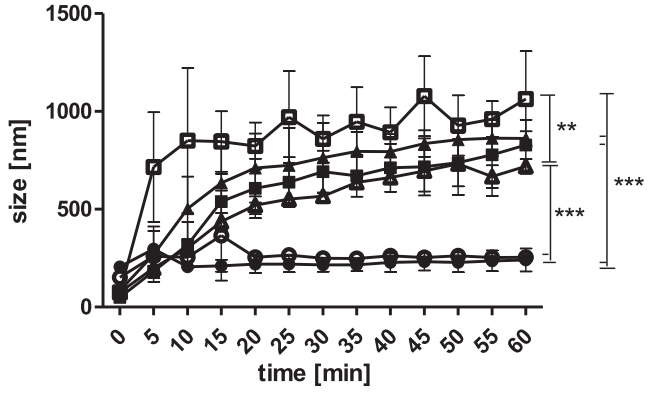


Fig. 7. Size development of SLNs with ● SLN 1, ■ SLN 2, ▲ SLN 3 and NLCs with ○ NLC 1, □ NLC 2, ▲ (unfilled) NLC 3 in nm after addition of pancreatin for 60 min. Data is presented as mean ± standard deviation for at least three experiments. Significant differences are indicated as: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Table 3
Size development of SEDDS in nm after addition of pancreatin for 60 min. Data is presented as mean ± standard deviation for at least three experiments. 6000 nm was the instrument's limit of detection for size measurement. When size exceeded 6000 nm, the measurement was aborted.

time [min]	SEDDS 1	SEDDS 2	SEDDS 3	SEDDS 4
0	24.75 ± 4.76	45.08 ± 9.41	57.74 ± 15.18	291.03 ± 53.36
5	166.51 ± 88.76	712.07 ± 278.24	485.13 ± 199.76	1367.33 ± 181.66
10	> 6000	3125.67 ± 756.17	2646.02 ± 974.08	3491.12 ± 802.02
15	–	> 6000	5461.05 ± 887.17	> 6000
20	–	–	> 6000	–

enzyme involved in the process of their decomposition. In another study, human pancreatic lipase-related protein 2 (HPLRP2) and CEH proved to have a higher impact on the digestion of PEG esters [24,25]. It might be possible that these enzymes in pancreatin are predominantly responsible for the ester cleavage in PEGylated surfactants. Furthermore, some of these PEGylated surfactants act as inhibitors of pancreatic lipase e.g. polyoxyl-40-hydrogenated castor oil, PEG-35 castor oil, polyoxyethylene (20) sorbitan monooleate or polyoxyethylene (20) sorbitan monostearate [21,22,28]. The PEG chains hinder bile salts and lipase sterically from adsorption to the surface of the particle and therefore hydrolysis is retarded. This effect seems to increase with growing PEG chain length [14,28,43]. Moreover, the digestion rate of sorbitan monostearate was lower than those of polyoxylethylene (20) sorbitan monostearate and PEG-35 castor oil. When using solely lipase, the digestion of polyoxylethylene (20) sorbitan monostearate and sorbitan monostearate almost equaled which implicates that lipase itself has the same affinity on both substrates. Pancreatin instead contains further enzymes which seem to have a higher impact on the lipolysis of polyoxylethylene (20) sorbitan monostearate. Generally, surfactant digestion can have a great impact on drug delivery by LNC. Using surfactants which inhibit pancreatic lipase might stabilize SLN or NLC. Furthermore, surfactants play a crucial role in self-emulsification of SEDDS. With increasing decomposition, the formulation might become unstable and the drug could precipitate. Therefore, surfactants with low digestibility should be chosen to decrease the risk of formulation destabilization [21].

4.2. Glycerides

Regarding the digestion of glycerides, the long chain glycerides (soybean oil, glyceryl monolinoleate) were digested to a lower extent than the medium chain glycerides (medium chain triglycerides, glyceryl caprylate/caprate Type I) which corresponds with previous studies [9,23]. Soybean oil and medium chain triglycerides are hydrolysed in a two-step reaction. Firstly, the triglyceride is separated into a fatty acid and a diglyceride. Within the second step, the decomposition of the diglyceride continues into another fatty acid and a 2-monoglyceride [44]. As known, the digestion rate decreases in the following ratio: triglyceride > 1,2-diglyceride > 1,3-diglyceride > 1-monoglyceride > 2-monoglyceride [18,36]. The final 2-monoglyceride could isomerize to the 1-monoglyceride, whereupon the lipase has a higher affinity. Unfortunately, this isomerization is limited in vivo [45]. The digestion of soybean oil probably stops at the stage of the 2-monoglyceride, whereas the digestion of the monoglyceride in glyceryl monolinoleate could continue. This depends on the isomer of monoglyceride being present in glyceryl monolinoleate. Most part of the monoglyceride fraction consists of the 1-monoglyceride [Product information, Gattefossé] which is hydrolyzed more efficiently than the 2-monoglyceride. Additionally, the mono- and diglycerides in glyceryl monolinoleate and glyceryl caprylate/caprate Type I (Imwitor® 742) have some emulsifying properties per se and thus led to an improved dispersibility within the buffer.

Accordingly, the oil–water interface was enlarged so that the enzyme could affect a higher amount of the substrate. In contrary, soybean oil dispersed poorly in the medium, forming a smaller interface for lipase to act on. Most of the long chain fatty acids present in soybean oil were only detected within the back titration. Looking at glyceryl caprylate/caprate Type I and medium chain triglycerides, glyceryl caprylate/caprate Type I showed a higher release of fatty acids within the titration with pancreatin. glyceryl caprylate/caprate Type I mainly (>50%) consists of monoglycerides [Product information, IOI Oleo GmbH]. The pancreatic enzymes PLRP2 and CEH could act on monoglycerides which increased the digestion of glyceryl caprylate/caprate Type I compared to lipase. Since the digestion of the monoglyceride was excluded from the digestion calculation, the digestion rate for glyceryl caprylate/caprate Type I was above 100%. There are several explanations why medium-chain glycerides show higher digestion rates. Firstly, pancreatic lipase implements a higher activity on chain lengths from C2 to C8. It is estimated that the mechanism of lipase includes the catalytic triade where a serine group performs a nucleophilic attack at the sn1 or sn3 position of the triglyceride to start the reaction. In theory, the reaction would speed up if the electrophilicity of the carbonyl carbon is increased and the more stable the emerged carboxylate is [46]. Moreover, medium-chain fatty acids have a lower pKa (apparent 6.8) than long-chained ones (7.8–8.5) [23,47]. The medium chained ones are ionized to a higher extent at pH 6.5 and thus more soluble. Their increased ionization also has an influence on the formation of calcium soaps. The formation of these ion pairs is facilitated and thus medium chain lipolytic products can desorb from the digestion interface more rapidly. In contrast, long chain lipolytic products usually stay at the interface where they can hamper hydrolysis [47] and need the help of bile salts for proper emulsification. For this reason, their solubility is limited by the capacity of the digestion buffer. When the saturation of the system is reached, lipolysis has to slow down. The same results have already been presented for medium chain triglycerides and soybean oil in other studies [23,29]. To summarize, the reasons for higher lipolysis of medium chain glycerides include droplet size/emulsification properties, pKa and ionization, solubility of the lipolytic products and stability of the produced fatty acids as products arising from the ester hydrolysis. On the other hand, tested on fenofibrate, digestion products of long chain glycerides seem to keep the drug within a soluble state during gastrointestinal digestion superior to the products of medium chain glycerides [29].

4.3. Waxes

It is known that triglycerides are hydrolysed much faster than waxes by pancreatic lipase [48]. Waxes are esters between alcohols and fatty acids. As cetyl palmitate owns the longest aliphatic chains, there must be a higher potential of electron donation in direction of the carbonyl group which makes it less reactive for a nucleophilic attack by pancreatic lipase. Another reason might be that cetyl palmitate and isopropyl myristate did not form a stable emulsion with the digestion buffer. Since they accumulated on the surface of the medium, lipase could act on less substrate. In contrary, methyl palmitate formed an emulsion. Its emulsification properties and an its structure causing less electron donation in direction of the carbonyl group are the explanation for the highest lipolysis amongst the waxes. Isopropyl myristate possesses a small branched aliphatic substructure. Regarding the titration with pancreatin, the results for the release of fatty acids were lower than for the blank titration of the digestion buffer. Probably the enzyme was hindered sterically from proper adsorption to the substrate due to the branched isopropyl substructure. In 1968, Brockerhoff has already demonstrated the same effect on isopropyl oleate [18]. A small amount of the substance might have been emulsified within micelles composed of bile salt and phospholipid. There, isopropyl myristate might have prevented the action of lipase on phosphatidylcholine and thus the titration values were below blank.

4.4. SLN and NLC

Comparable SLN and NLC had the same size, so the small amount of medium chain triglycerides in NLC did not cause a significant change in particle size and additionally, no significant difference was observed concerning the extent of digestion. The size measurements during lipolysis revealed an increase in size for the more digestible particles, namely SLN/NLC 2 and SLN/NLC 3. During the experiment, the sizes levelled off within 60 min, indicating that the particles were not decomposed completely. The size of SLN/NLC 1 remained constant. These results match with the release of the fatty acids. SLN 1 and NLC 1 show the least effect which is probably due to their composition of cetyl palmitate. As discussed earlier, waxes are poor substrates for pancreatic enzymes, especially compared to glycerides like glyceryl distearate. The same has been demonstrated by comparing SLN composed of cetyl palmitate to SLN consisting of Dynasan® 116 or 118 (glyceryl tripalmitate or tristearate) [49]. Another explanation would be the increased size in comparison to the other particle formulations. SLN/NLC 2 and SLN/NLC 3 were about the same size and were composed of the same excipients, except for the replacement of polyoxyethylene (20) sorbitan monostearate by PEG-35 castor oil. SLN/NLC 3 were digested slower and to a lower extent than SLN/NLC 2 which might be due to PEG-35 castor oil being higher PEGylated than polyoxyethylene (20) sorbitan monostearate and thus hindering adsorption of lipase more efficiently. Poloxamer 407 inhibits the adsorption of lipase to the particle surface as well [49,50]. SLN 1 and NLC 1 are both only degraded by about 2–3% whereas cetyl palmitate itself was decomposed by 8% utilizing pancreatin. This proves that PEGylated surfactants inhibit lipolysis in the particle formulations. Taken all, drug delivery by SLN and NLC can be optimized by choosing a sterically stabilizing surfactant and a corresponding lipid with required lipolysis properties. Furthermore, SLN and NLC can lead to a sustained drug release within the gastrointestinal tract [31].

4.5. SEDDS

Glycerides seem to form more stable and smaller LNC in comparison to waxes as their size was smaller. Some formulations possessed smaller or bigger droplet sizes in digestion medium which probably depends on interactions between SEDDS and ions and surfactants being present in digestion medium. The digestion medium also had an impact on SEDDS 2 and SEDDS 3 causing a decrease in PDI. SEDDS 2–4 only differed in the lipid component. Highest release of fatty acids was shown for SEDDS 2, due to medium chain triglycerides being the most digestible substrate for pancreatic lipase in comparison to soybean oil and isopropyl myristate. Glyceryl caprylate/caprate Type I (Capmul® MCM) can be hydrolysed to a high extent [20]. Its medium chain diglycerides and the 1-monoglycerides are both substrates for pancreatic lipase and other pancreatic enzymes. SEDDS 1 possessed the highest amount of lipids and in addition, medium chain triglycerides and glyceryl caprylate/caprate Type I (Imwitor® 742) were both substrates with high lipolysis. The digestion rate also corresponded with the size of the SEDDS, resulting in a slightly higher digestion in order of reduced droplet size. Droplet size increased much quicker for the SEDDS than for the particle formulations and were beyond the detection limit of 6000 nm. This indicates that SLN and NLC were more stable systems and show less degradation than SEDDS.

5. Conclusion

In vitro lipolysis experiments were performed with several excipients, SEDDS, SLN and NLC. Generally, pancreatin led to higher lipolysis than lipase. The additional enzymes being present in pancreatin had an effect on substrates usually less affected by lipase. Excipients containing medium chain fatty acids were hydrolysed faster and to a higher extent than long chained substrates. Moreover, waxes and LBFs consisting of

waxes were hydrolysed to a low extent. Within the group of surfactants, the lipolysis of sorbitan monostearate was the lowest, followed by PEG-35 castor oil and polyoxylethylene (20) sorbitan monostearate. SLN/NLC composed of waxes and sterically stabilizing surfactants were less digestible than the excipients itself. In contrary, there was no such difference between the digestion of SEDDS and their single components. Size measurements during lipolysis presented an increasement of size for the particles levelling off after a certain time whereas SEDDS were decomposed completely. This indicates that lipolysis can be influenced by the type of nanocarrier and by the excipients used for them.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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