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Enzymatic characterization of lipid-based drug delivery systems

Helena Ljusberg-Wahren[a,b], Flemming Seier Nielsen[c], Mattias Brogård[a], Emma Troedsson[a,b], Anette Müllertz[c,*]

[a] *Camurus AB, Ideon, Gamma 2, Sölvegatan 41, SE-223 70 Lund, Sweden*
[b] *Division of Food Technology, Lund University, P.O. Box 124, SE 221 00 Lund, Sweden*
[c] *Department of Pharmaceutics, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark*

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Abstract

The present work introduces a simple and robust in vitro method for enzymatic characterisation of surface properties of lipid dispersions in aqueous media. The initial lipolysis rate in biorelevant media, using pancreatic lipase and a self-microemulsifying formulation (SMEDDS) containing digestible lipids as substrate, was determined. The impact of incorporating two sparingly water soluble model drugs, probucol and halofantrine, into the SMEDDS was studied. It was found that both model drugs reduced the initial rate of lipolysis compared with the vehicle, probucol having a larger effect than halofantrine. The reduction of initial lipolysis rate indicates that probucol and halofantrine are bound in the water/emulsion interface limiting the substrate availability.

Keywords: Lipolysis; Self-microemulsifying drug delivery system; Probucol; Halofantrine

The oral route is regarded by patients as the most convenient way for drug administration. However, an increasing number of poorly soluble drug candidates with low, variable and food-dependent bioavailability are facing the pharmaceutical industry today. A rate limiting step for the absorption of these drugs is often their solubilisation in the gastrointestinal tract. This has increased interest in lipid-based formulations which are able to maintain the drug in solution in the gastrointestinal tract, thus surpassing a dissolution step. Use of self-microemulsifying drug delivery systems (SMEDDS) containing digestible lipids and non-ionic surfactants, is one approach that has been applied.

Understanding the digestion- and absorption process of lipids is of great importance for interpretation of the biopharmaceutical properties of lipid-based formulations of lipophilic drugs intended for oral administration. Three sequential steps lie behind the efficient absorption of dietary long chained triglycerides in humans: dispersion of lipids, enzymatic hydrolysis

* Corresponding author. Tel.: +45 35306440; fax: +45 35306030.
E-mail address: amu@dfuni.dk (A. Müllertz).

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at the water/lipid interface and transport of digestion products (fatty acids and 2-monoglycerides) from the interface to the site of absorption (the epithelial cells). The enzymatic hydrolysis of dietary triglycerides in humans is mainly catalysed by pancreatic lipase. The importance of pancreatic lipase/colipase-mediated hydrolysis of di- and triglycerides for efficient absorption of lipophilic substances like dietary cholesterol has clearly been demonstrated (Young and Hui, 1999). The above steps described for triglyceride digestion are also expected to influence absorption of lipophilic drugs co-formulated with digestible lipid.

In this work, lipase from a readily available crude porcine pancreatic extract was used to characterise the interfacial regions of SMEDDS and dispersions of lipid excipients. The lipolysis reaction was monitored by continuous titration of fatty acids liberated during the reaction. The kinetics of lipase catalysed hydrolysis of long chained triglycerides is complicated by the fact that the reaction products (free fatty acids) are partly dissociated at physiological pH and locate themselves at the surface of the emulsion particles. In this study only the initial part of the lipolysis reaction was monitored and, therefore, any substantial build up of fatty acids at the interface of the dispersions would be avoided. Two sets of lipolysis experiments were carried out, one on aqueous dispersions of excipients and the other with self-microemulsifying drug delivery systems dispersed in bio-relevant media.

Lipolysis experiments were performed on dispersions of two lipids, glycerol monooleate GMO and glycerol dioleate GDO (gift from Danisco, Denmark) stabilized with a non-ionic surfactant Cremophor RH40® (CrRH 40) (BASF, Germany). Fig. 1 shows that the lipase was not able to hydrolyse the diacyl glyceride dispersion, while the monoacyl glyceride dispersion was hydrolysed under the chosen experimental conditions. In fact, the ratio of GDO to CrRH 40 had to be lower than 99:1 before any substantial rate of lipolysis was recorded. The partial phase diagrams presented in Fig. 2 show that the two compositions studied form an oil continuous isotropic phase (L_2) (GDO/CrRH 40) and a cubic liquid crystalline phase (Q) (GMO/CrRH 40) when dispersed in water. A difference in phase behaviour between the two samples is expected, since diglycerides of long chain fatty acids are non-swelling lipids while monoglycerides are swelling amphiphilic lipid according to Small's classification of biological lipids based on their interaction with water (Small, 1968). The emulsion (GDO/CrRH 40 75/25) was dispersed by ultrasonication (mean particle size (mean volume diameter) of 14.2 μm), while the liquid crystalline phase (GMO/CrRH 40 77/23) was dispersed with a microfluidizer (mean particle size (mean volume diameter) of 1.1 μm) (Fig. 3) (Gustafsson et al., 1997). The two dispersions display quite different hydrolysis profiles, even though they both contain esters of oleic acid, a non-ionic surfactant and were tested under experimental conditions where there was a surplus of

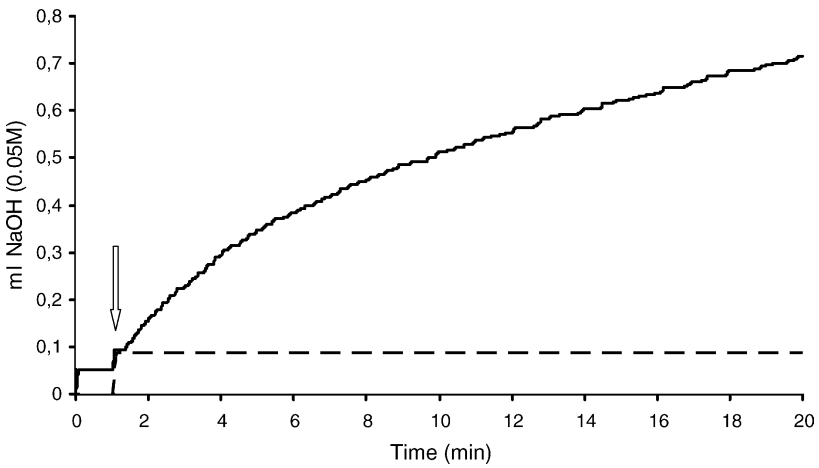

Fig. 1. Lipolysis of the systems GDO/CrRH 40 (75/25) (dashed line) and GMO/CrRH 40 77/23 (solid line). Lipolysis reaction is initiated by addition of lipase solution (arrow) and monitored as addition of NaOH (0.05 M) needed to neutralise liberated fatty acids as a function of time.

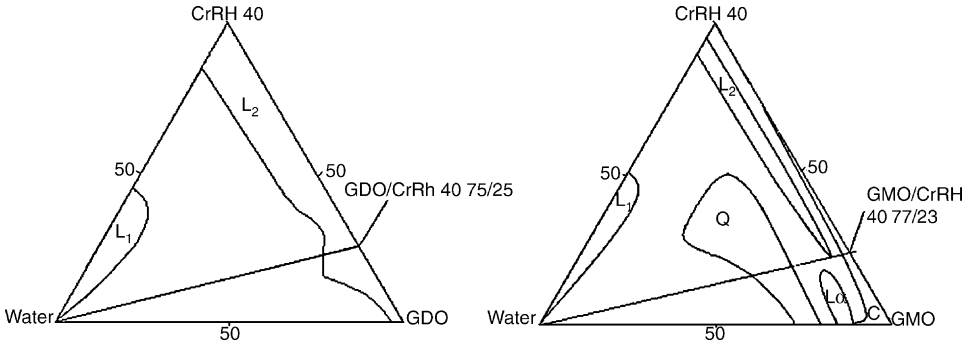

Fig. 2. Ternary phase diagrams of the systems GDO/CrRH 40/water and GMO/CrRH 40/water, at room temperature. Only the isotropic one phase regions are shown.

surface available for the lipase. These findings can be interpreted as follows: in the emulsion system where no hydrolysis occurs the diacyl glyceride molecule with just one hydroxyl group is shielded from contact with lipase at the interface by the non-ionic surfactant, while in the dispersed liquid crystalline system, the polar lipid monoglyceride (two hydroxyl groups) and the non-ionic surfactant form a mixed interface where hydrolysis of the lipid can occur.

Endogenous surfactants (bile salt/phospholipids) can interact with lipid-based formulations in the gastrointestinal tract, thereby changing the nature of drug solubilising aggregates. Hydrolysis of the vehicle and SMEDDS was therefore performed in a bio-relevant media (20 mM taurocholate, 4 mM phosphatidylcholine and 5 mM Ca^{2+}) (Sek et al., 2001, 2002). This type of experimental conditions has been used earlier to characterize the solubilisation of lipophilic drugs during lipolysis of triglycerides (Reymond and Sucker, 1988; Zangenberg et al., 2001; Kaukonen et al., 2004). In our experiments the initial rates of lipolysis were determined for both the pure SMEDDS and SMEDDS containing probucol and halofantrine. All samples were dispersed with gentle stirring (10 min and 100 rpm) before measurements were made. The results of the lipolysis experiments for the different self-dispersing systems are summarised in Table 1, together with the particle size distribution of the dispersions in saline solution, and dissolution media simulating intestinal conditions in fasted state (FaSSIF) and fed state (FeSSIF) (Galia et al., 1998). The inclusion of 7.6% (w/w) probucol and 3.1% (w/w) halofantrine

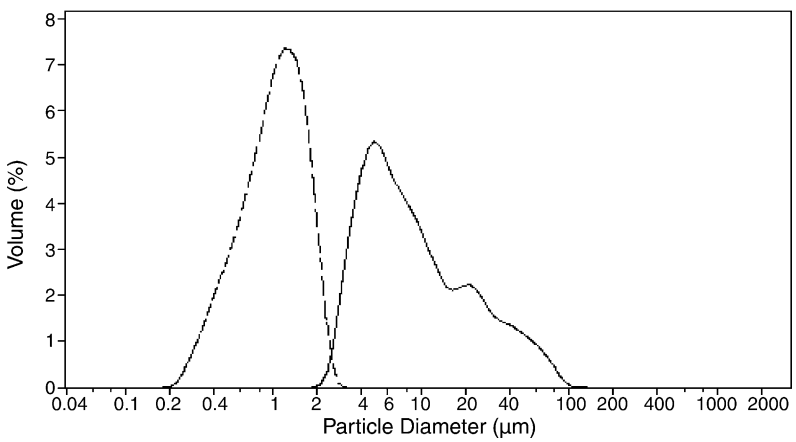

Fig. 3. Particle size distribution determined on Beckman Coulter, Model LS 230 (USA) instrument for two mixtures of excipients GDO/CrRH 40 75/25 (w/w) (solid line) and GMO/CrRH 40 77/23 (w/w) (dashed line) dispersed in phosphate buffer.

Table 1
Initial rate of lipolysis of self-microemulsifying drug delivery systems (SMEDDS) and the vehicle (lipids/CrRH 40/absolute ethanol, 60/30/10)

	Active substance (mg/g)	Particle diameter (nm)			Initial lipolysis rate (mmol NaOH/min)	Relative lipolysis rate
		Saline	FaSSIF	FeSSIF		
Vehicle	–	41.5 ± 0.2	38.4 ± 1.8	39.0 ± 3.2	0.042 ± 0.001	1
Halofantrine	31.3	41.7 ± 2.3	41.7 ± 2.4	56.2 ± 3.1	0.034 ± 0.001	0.81
Probucol	76.1	45.0 ± 3.4	42.6 ± 1.5	44.1 ± 1.0	0.029 ± 0.002	0.69

The lipid component is 1:1 mixture of Maisine 35-1 and Sesame oil. The amount of active substance is 80% of maximum drug load. FaSSIF (3 mM NaTC; 0.75 mM PC; pH 6.5) and FeSSIF (15 mM NaTC; 3.75 mM PC; pH 5.0) (Galia et al., 1998).

in the SMEDDS vehicle are equivalent to 80% of the maximum drug load. Inclusion of both drugs reduced the initial rate of hydrolysis compared to the vehicle, with probucol having a greater effect than halofantrine.

Drug substances with low water solubility like probucol and halofantrine can either be solubilised in the hydrophobic core of microemulsion particles or in the palisade layer that contains the polar head groups and is covering the hydrophobic core.

The decrease in the initial lipolysis rate found in this study indicates that halofantrine, which is partly ionized at the pH (6.5) used in the lipolysis experiment (Taillardat-Bertschinger et al., 2003), is solubilised in the interface region of the microemulsion and therefore limits substrate availability. The presence of ionized halofantrine at the interface is further supported by differences in particle size of dispersions made in saline solution and FeSSIF (Table 1) suggesting that halofantrine promotes the binding of bile salt to the emulsion particles.

A highly lipophilic compound like probucol with a calculated ClogP of 11 (ChemDraw Ultra, version 8.0.3) is expected to favour the hydrophobic environment of the core of the microemulsion, but our results indicate that inclusion of probucol in the self-dispersing system effects the interface region. In addition, it can be noted that the relative rates of hydrolysis of the two studied SMEDDS, one containing 3.2% (w/w) probucol and the other 7.6% (w/w) probucol, were equivalent (data not shown). The equivalent reduction of hydrolysis rate obtained with the two different probucol loadings points towards a saturation of the interface region at a lower concentration of probucol than 3.2% (w/w) probucol load.

We have in this work demonstrated a simple and robust method to characterize an emulsion surface with respect to susceptibility to enzymatic degradation by pancreatic lipase. The results show that the initial rate of lipolysis, and hence, release of drug load from a self-microemulsifying drug delivery system is influenced by inclusion of drug. The described method can give useful information on self-microemulsifying drug delivery systems when combined with physicochemical data such as phase studies and particle measurements in bio-relevant media.

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