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A new standardized lipolysis approach for characterization of emulsions and dispersions

M. Brogård [a,*], E. Troedsson [a,b], K. Thuresson [a,c], H. Ljusberg-Wahren [a,b]

[a] *Camurus AB, Ideon Science Park, 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] *Physical Chemistry 1, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*

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Abstract

A new standardized lipolysis approach is presented where the focus is on the initial rate of lipolysis. An advantage is that data obtained in this way reflect degradation before growing amounts of lipolysis products retard the process. The method can be used to rank different lipase substrates. In particular, the method can be used to obtain information about the susceptibility to degradation of various emulsions and dispersions that are used in technical applications. We present how the method is standardized to facilitate comparison of various substrates. This involves (i) lipase substrate in excess, i.e., the amount of lipase is rate limiting, and (ii) expressing rate of degradation relative to that of a reference substrate, tributyrin. Under such conditions, with the amount of lipase substrate held constant, an increase in enzymatic activity will generate a proportional increase in the lipolysis rate. This enables comparison of results obtained from different enzyme batches and corrects for day-to-day variability. Examples illustrating the potential of the method to discriminate and rank different lipase substrates with regard to enzymatic degradation are presented.

Keywords: Structured lipids; Lamellar; Lipid-based drug delivery; Lipid digestion; Lipolysis; Self-emulsifying drug delivery systems (SEDDS); Triglyceride; Dispersion; Emulsion

1. Introduction

Drug delivery systems in the form of colloidal-sized aggregates have played a prominent and growing role in the past decades. In particular, lipid-based drug carriers have been found to increase the bioavailability (BA) of poorly water-soluble lipophilic drug molecules and to reduce the variability of systemic exposure. Lipid-based drug carriers are likely to have influence on BA through enhanced solubilization of the drug in the gastrointestinal tract (GIT) [1–4]. Often medium-chain triglycerides (MCT) or long-chain triglycerides (LCT), together with a surfactant and a cosolvent, have been used for such formulations. In fact, drug vehicles ranging from crude emulsions [5], with large and often ill-defined oil droplet size distributions, to self-emulsifying drug delivery systems (SEDDS) [6], as well as more elaborate structures [7,8] can be obtained by relatively small variations in lipid composition.

Both in vitro and in vivo studies indicate that the size of the carriers as well as the rate of enzymatic hydrolysis of drug-carrying lipid aggregates can be important to the extent to which lipophilic drugs reach the systemic circulation [9]. Although it appears obvious that the fate of a drug vehicle in the GIT is essential for understanding the limitations and improving the performance of medicines, this field is far from understood and substantial research activities are ongoing [10]. To fully realize the potential of lipid-based drug delivery systems, improvements in the knowledge of dispersion properties in aqueous media, diffusion and degradation of drug-containing aggregates within the lumen, and absorption of lipids and drug molecules is required [3]. Understanding these processes would facilitate design of formulations and drug vehicles with properties tailor-made for specific substances. Enzymatic degradation in the GIT can be expected to be of particular importance because

* Corresponding author. Fax: +46 46 2865739.
E-mail address: mattias.brogard@camurus.com (M. Brogård).

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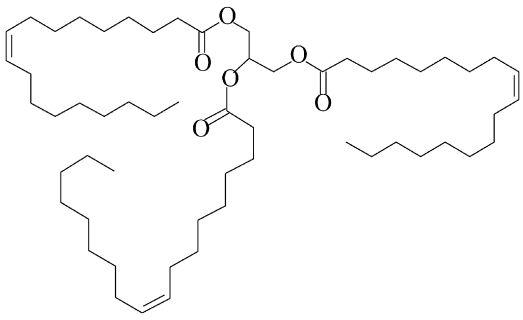
this process changes the composition of the self-assembled drug vehicles [11–14]. A changed composition may alter both the structure of the drug vehicles [15] as well as their drug-solubilizing capacity [16]. For this purpose various in vitro models, such as dissolution, particle sizing, and lipolysis of drug vehicle precursors in relevant media, have been developed with the goal to achieve laboratory test methods providing sufficient in vitro–in vivo correlation [17–19].

After oral administration of a lipid-based drug formulation the various pancreatic lipases start to hydrolyze the lipid ester bonds. For a triglyceride (TG), a two-step reaction is expected to take place. First the ester bond in position 1 is cleaved and a diglyceride (DG) and a free fatty acid (FA) are liberated. In the subsequent step the FA located in the 1 position of the DG is hydrolyzed. The net reaction produces two FA and one 2-monoglyceride (2-MG). After hydrolysis the products (MG and FA) are solubilized in bile-salt aggregates followed by absorption [3,20,21]. The latter process, which continuously removes products formed during lipolysis, has a bearing on the present methodological approach where the initial lipolysis rate is followed. Representative chemical structures of substrates and products that have been used in the present investigation are shown in Fig. 1.

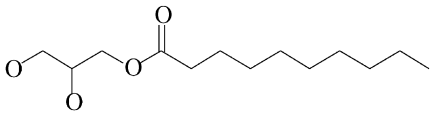
Several different in vitro models can be found in the literature that have been used to study enzymatic hydrolysis of dispersed lipids in various aqueous media [10,14,22–26]. In the present study we have used a novel and slightly different approach, and we argue that the initial degradation rate, before any substantial build-up of reaction products occurs, can be used to study interfaces formed from different lipid mixtures in various media. For this reason our main focus has been on the first couple of minutes of the degradation process. To be able to compare data from different lipid substrates, conditions producing a maximal rate of lipolysis were chosen (compare Michaelis–Menten behavior). This means that in the present experimental setup the amount of lipase is rate-limiting and a particular substrate's susceptibility to lipase degradation can be expressed relative that of any other substrate. Furthermore, to ensure balance between a robust method with a stable signal and yet high sensitivity, the digestion experiments were conducted in a simple phosphate buffer with low buffering capacity. The measurements were performed at pH 6.5, simulating the duodenum and the upper part of the small intestine [19].

Since the method can be used to benchmark formulations, it is important that the reproducibility is high, and substantial efforts have therefore been directed to standardizing both equipment and procedures. A triglyceride, tributyrin (TB), has been used before as reference standard in similar methods [10,14]. The major advantage with TB is that the degradation product (butyric acid) is water-soluble and accumulation of lipolysis products at the oil/water interface, which could have a negative influence on the rate of lipolysis, is negligible. Using TB as reference, the comparison of data from different analysis occasions is facilitated and problems arising from variations in activity in between lipase batches are minimized.

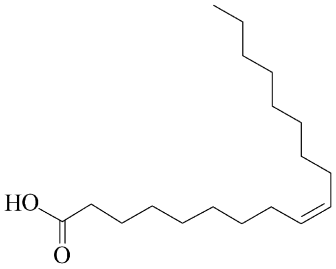
After correct procedures and conditions were identified, the lipolysis method was applied to aqueous lipid dispersions of



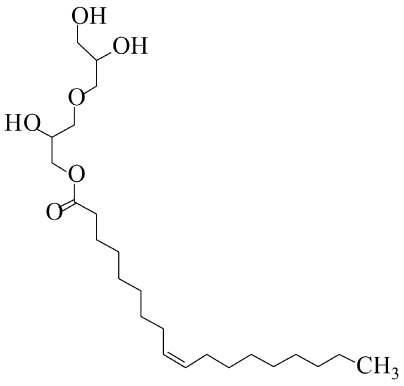
(a) Glycerol trioleate



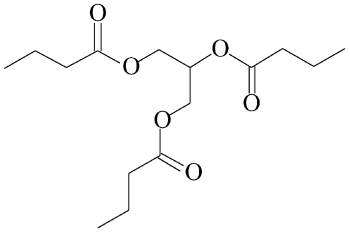
(b) Clycerol monocaprinate



(c) Oleic acid



(d) Diglycerol monooleate



(e) Tributyrin

Fig. 1. The figure shows chemical structures of (a) glycerol trioleate (GTO), (b) glycerol monocaprinate (GMC), (c) oleic acid (OA), (d) diglycerol monooleate (DGMO), and (e) tributyrin (TB).