International Journal of Pharmaceutics 422 (2012) 116–124

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Dissolution of solid lipid extrudates in biorelevant media

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ARTICLE INFO

Article history:
Received 13 July 2011
Received in revised form 13 October 2011
Accepted 18 October 2011
Available online 21 October 2011

Keywords:
Solid lipid extrudates
Biorelevant medium
Dissolution
Pancreatic lipase
Praziquantel
In vitro lipolysis

ABSTRACT

Solid lipid extrudates with the model drug praziquantel were produced with chemically diverse lipids and investigated regarding their dissolution behaviour in different media. The lipids used in this study were glyceryl tripalmitate, glyceryl dibehenate, glyceryl monostearate, cetyl palmitate and solid paraffin. Thermoanalytical and dissolution behaviour was investigated directly after extrusion and after 3 and 6 months open storage at 40 °C/75% RH. Dissolution studies were conducted in hydrochloric acid (HCl) pH 1.2 with different levels of polysorbate 20 and with a biorelevant medium containing pancreatic lipase, bile salts and phospholipids. Furthermore, the impact of lipid digestion on drug release was studied using in vitro lipolysis.

The release of praziquantel from cetyl palmitate and glyceryl monostearate in the biorelevant medium was much faster than in HCl, whereas there was hardly any difference for the other lipids. It was shown that drug release from glyceryl monostearate matrices is driven by both solubilisation and enzymatic degradation of the lipid, whereas dissolution from cetyl palmitate extrudates is dependent only on solubilisation by surfactants in the medium. Moreover, storage influenced the appearance of the extrudate surface and the dissolution rate for all lipids except solid paraffin.

1. Introduction

Dissolution testing is a well known standard in vitro method to simulate the release of drugs from oral dosage forms. In the simplest experimental setup digestive fluid is represented by a vessel with water. But rarely the results of such a simple dissolution test fully relate to *in vivo* drug release. The reason is that pH, volume, osmolarity, content of enzymes and surfactants of gastric and intestinal juice differ a lot from water and influence the release rate of drugs from oral dosage forms. Therefore, digestive fluids are simulated by development of complex media, so-called biorelevant media (Jantratid et al., 2008; Marques, 2004). Biorelevant dissolution media simulating intestinal fluids primarily contain bile salts and phospholipids at high or low levels, depending on whether the fed or the fasted state is simulated. When simulating the fed state lipid digestion products such as monoglycerides and free fatty acids can also be added.

For lipid dosage forms it is highly relevant to simulate the digestion in the gastro-intestinal tract, because lipids are important components of the human diet and substrates of digestion. In the western world 95% of dietary fats are triglycerides (Lowe, 1997) and almost all of these are digested and absorbed. In small intestine triglycerides are digested by the colipase dependent pancreatic lipase, secreted from pancreas together with many other digestive enzymes. Secretion is induced by presence of lipids in the gastrointestinal tract which also induces contraction of the gallbladder and secretion of bile. Bile contains bile salts and phospholipids, which are important for the solubilisation of lipids and drugs in the gastro-intestinal tract (Embleton and Pouton, 1997; Humberstone and Charman, 1997). Pancreatic lipase digests triglycerides to monoglycerides and free fatty acids. These digestion products are solubilised by bile salts and phospholipids and subsequently absorbed (Patton and Carey, 1979). Monoglycerides and free fatty acids on their part facilitate the emulsification of dietary fats in order to prepare them for digestion (Humberstone and Charman, 1997).

Apart from enzymatic digestion, different lipids interact with water to a variable extent. Non-polar lipids like long-chain paraffin do not interact with water at all. Polar lipids can be classified into insoluble non-swelling amphiphiles (class I), insoluble swelling amphiphiles (class II) and soluble amphiphiles (class III). Di- and triglycerides, long-chain fatty acids, cholesterol and waxes belong to class I, whereas class II contains for example monoglycerides and phospholipids. Class I and II lipids spread on aqueous surfaces and form stable monolayers. Class III, soluble amphiphiles, are typically emulsifiers, such as polysorbates, that can form micelles (Small, 1968).

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doi:10.1016/j.ijpharm.2011.10.037

Recently lipid dosage forms have attracted interest, because lipids can enhance the bioavailability of some drugs. Intake of a high-fat meal or a lipid dosage form results in stimulation of biliary and pancreatic secretion, stimulation of lymphatic transport, increased intestinal wall permeability and reduced metabolism, which significantly contribute in improving bioavailability (Chakraborty et al., 2009). For this purpose mainly liquid lipid dosage forms are used (Porter and Charman, 2001). Solid lipid dosage forms, on the other hand, can be used for controlled release systems (Hamdani et al., 2002; Reitz and Kleinebudde, 2007; Thomsen et al., 1994), for protective dosage forms with water-sensitive substances (Schulze and Winter, 2009; Windbergs et al., 2009) and for taste masking of bitter drugs (Krause et al., 2009; Michalk et al., 2008; Suzuki et al., 2003; Witzleb et al., 2011a).

Studies of lipid-based drug delivery systems in biorelevant media typically deal with liquid systems (Christensen et al., 2004; Ljusberg-Wahren et al., 2005; MacGregor et al., 1997; Zangenberg et al., 2001), whereas only very few articles about solid systems exist (Bergauer et al., 1977; Reitz, 2007). As for liquid systems, the most important factors influencing the drug release are the concentration of bile salts, pancreatic lipase and calcium in the medium. Bile salts solubilise drug and lipolytic products, the pancreatic lipase directly digests the lipid carrier and calcium removes free fatty acids from the surface of digesting oil droplets as calcium soap (MacGregor et al., 1997).

The aim of this work was to study the release rate of a poorly water soluble drug, praziquantel, from solid lipid matrices in different dissolution media: hydrochloric acid (HCl) with different levels of polysorbate 20 as standard medium and a biorelevant medium simulating the intestinal fluid. Further, in vitro lipolysis was used in order to study any influence of lipid digestion on the release of drug.

2. Materials and methods

2.1. Materials

Praziquantel was obtained from Bayer HealthCare (Leverkusen, Germany). The following powdered lipids were received from Sasol (Witten, Germany): glyceryl tripalmitate (Dynasan® 116) and glyceryl monostearate (Imwitor® 491). Solid paraffin (Sasolwax® 6403) was obtained from Sasol Wax (Hamburg, Germany). Powdered glyceryl dibehenate (Compritol® 888 ATO) and cetyl palmitate (Precifac®) were supported by Gattefossé (Weil am Rhein, Germany). Glyceryl tricaprylate was received from Hüls (Witten, Germany). Colloidal silicium dioxide (Aerosil® 200) was acquired from Degussa (Essen, Germany).

The following materials in alphabetical order were used for preparation of dissolution media: acetonitrile (HiPerSolv Chromanorm®, VWR, Darmstadt, Germany), bile salts for dissolution experiments (sodium taurocholate, PCA, Basaluzzo, Italy), bile salts for in vitro lipolysis (porcine bile extract, Sigma–Aldrich, Saint Louis, USA), calcium chloride ($CaCl_2 \cdot 2H_2O$, Riedel-de Haen, Seelze, Germany), hydrochloric acid (Merck, Darmstadt, Germany), tris-(hydroxymethyl)-aminomethane maleate (Trizma® Maleat, Sigma–Aldrich, Saint Louis, USA), maleic acid (Merck, Darmstadt, Germany), methylene chloride (Riedel-de Haen, Seelze, Germany), pancreatic lipase for dissolution experiments (Pankreatin porcine pancreas 1× USP spec., Sigma–Aldrich, Saint Louis, USA), pancreatic lipase for in vitro lipolysis (Pankreatin porcine pancreas 3× USP spec., Sigma–Aldrich, Saint Louis, USA), phospholipids for dissolution experiments (Lipoid® E PC S, >99% lecithine, Lipoid, Ludwigshafen, Germany), phospholipids for in vitro lipolysis (Epikuron® 200, >92% lecithine, Degussa, Hamburg, Germany), polysorbate 20 (Caelo, Hilden, Germany), sodium chloride (Merck, Darmstadt, Germany), and sodium hydroxide (Merck, Darmstadt, Germany).

Table 1
Composition of the biorelevant medium.

Bile salts	15 mM
Phospholipids	3.75 mM
Pancreatic lipase	20 USP units/ml
NaOH	52.5 mM
Maleic acid	28.6 mM
Sodium chloride	145.2 mM
Calcium chloride	5 mM

2.2. Production of lipid matrices

It has been shown in a previous study that solid lipid extrusion with the needle-shaped drug praziquantel is only possible if the drug powder is milled before extrusion, because the needle-shaped particles disturb the process (Witzleb et al., 2011b). Therefore, praziquantel was air jet milled before use. The particle size of milled drug powder was $d(0.9) = 3.6\,\mu m$, measured by laser diffraction.

The powdered lipids were mixed with praziquantel in a laboratory scale blender LM 20 (Bohle, Ennigerloh, Germany). All formulations contained 50% praziquantel/49% lipid/1% colloidal silicium dioxide. The latter was added in order to improve the flowability of the formulation and precision of dosing. The powder mixture was gravimetrically fed by a dosing device KT 20 (K-Tron Soder, Lenzhard, Switzerland) into the barrel of the co-rotating twin-screw extruder Mikro 27GL-28D (Leistritz, Nürnberg, Germany). The temperature of the extruder barrel was adjusted below the melting range of the lipid, so that the drug was dispersed in the solid lipid matrix. A constant extruder screw speed of 60 rpm and powder feed rate of 40 g/min were used, the die diameter was 0.3 mm.

After cooling down to room temperature, the extrudates were milled in an ultra centrifugal mill ZM 200 (Retsch, Haan, Germany) with a 12-tooth rotor at 6000 rpm and a sieve size of 1.5 mm with trapezoid holes. The extrudates were cut cross-sectional into short pieces and afterwards sieved in a sieve shaker AS 200 control (Retsch, Haan, Germany) in order to separate coarse and fine fraction. Sieve fraction 315–400 μm was used for further experiments.

2.3. Dissolution

2.3.1. Standard medium

Dissolution studies were performed in hydrochloric acid (HCl) pH 1.2 with 0.001% polysorbate 20 as standard medium. The low level of polysorbate 20 was added to improve the wettability of the extrudates, the concentration was set below the critical micelle concentration (CMC) of polysorbate 20 (Wan and Lee, 1974). Closed flow-through-cells (CE7 smart, Sotax, Switzerland) with tablet cells containing the milled extrudates dispersed in glass beads were used in order to ensure complete wetting of the extrudates. The flow rate was 16 ml/min. All dissolution experiments were conducted in triplicate. Praziquantel content was determined with ultraviolet detection at 210 nm with a Lambda 25 (Perkin Elmer, Waltham, USA).

2.3.2. Biorelevant medium

The FeSSIF (Fed State Simulated Intestinal Fluid) medium was prepared according to Marques (2004) with addition of pancreatic lipase and calcium chloride as per Jantratid et al. (2008) and the monograph Simulated Intestinal Fluid (USP 30). Table 1 shows the composition of the medium. At first a pH 6.5 buffer solution was prepared with sodium hydroxide, maleic acid, sodium chloride and calcium chloride. Further, bile salts were dissolved in the buffer solution, phospholipids were dissolved in methylene chloride and added to form an emulsion. The methylene chloride was eliminated under vacuum at about 40 °C with a rotary

Table 2
Composition of the in vitro lipolysis medium.

Bile salts	5 mM
Phospholipids	1.25 mM
Pancreatic lipase	800 USP units/ml
Maleate	2 mM
Sodium chloride	150 mM

evaporator (Janke & Kunkel, Staufen im Breigau, Germany) and vacuum pump MZ 2C (Vakuubrand, Wertheim, Germany) until formation of a clear micellar solution, having no perceptible odor of methylene chloride. After cooling to room temperature pancreatin was added, filled to the desired volume, pH adjusted to 6.5 again and dissolution started immediately.

Dissolution testing was performed in a paddle apparatus with 900 ml medium. A paddle rotation speed of 150 rpm was used in order to ensure complete wetting of the extrudates. Samples of 5 ml were taken manually, replaced by fresh medium and directly filtered through 20 μm polyethylene filter (Seal Analytical, Mequon, USA). Praziquantel content was determined by high performance liquid chromatography (HPLC) with a La Chrom Elite (Hitachi, Tampa, USA) and UV/vis-detection at 210 nm. A column Chromolith Performance RP-18e 100 mm × 4.6 mm with precolumn Chromolith RP-18e 5 mm × 4.6 mm (VWR, Darmstadt, Germany) was used for HPLC analysis, the mobile phase was acetonitrile/water 50:50 with a flow rate of 1 ml/min.

2.3.3. In vitro lipolysis

For preparation of the medium all compounds (Table 2) beside pancreatic lipase were weighed with water and stirred for at least 20 h until a clear solution was obtained. The pH was adjusted to 6.5. Pancreatin was suspended in purified water and centrifuged for 7 min at 4000 rpm at 37 °C in a Labofuge 400R (Thermo Scientific, Waltham, USA). The pH in the supernatant was adjusted to 6.5. Titration was performed in a pH-stat 842 Titrando (Metrohm, Herisau, Switzerland) with 0.05 M sodium hydroxide solution. Before starting the experiment 200 ml medium and 500 mg extrudate were mixed and equilibrated for 3 min. The lipolysis was initiated by addition of pancreatin suspension and simultaneously starting of the continuous addition of 1 M calcium chloride solution. A blank measurement was conducted in the same way, but without addition of extrudate.

Evaluation was done directly with the amount of fatty acids titrated. Lipolysis in % was calculated with the amount of fatty acids titrated related to the overall amount of ester groups in the sample. Ester values were taken from supplier certificates of analysis.

2.3.4. Similarity factor f_2

The dissolution profiles were compared using the similarity factor f_2 according to Moore and Flanner (1996) and O'Hara et al. (1998). In order to ensure comparability, the same time points of drug release in both media were used (0, 15, 30, 60, 90, 120, 150, 180, 210 and 240 min). The coefficient of variation was below 15% for all time points. The f_2 value is a number between 0 and 100. The higher f_2, the more the two compared dissolution profiles are similar. With a value below 50 the profiles are evaluated as different from each other.

2.3.5. Stability testing

Stability testing according to ICH was conducted under accelerated conditions. The extrudates were stored open at 40 °C/75% RH in a constant climate chamber KBF (Binder, Tuttlingen, Germany).

2.3.6. Differential scanning calorimetry (DSC)

Thermoanalytical measurements with extrudates and excipients were conducted with a DSC 1 (Mettler Toledo, Greifensee, Switzerland). Samples were prepared with 2–3 mg substance in sealed aluminium pans and measured with a heating rate of 10 K/min. All measurements were performed in duplicate, the diagrams exemplarily show one measurement.

2.3.7. Scanning electron microscopy (SEM)

The extrudates were visualised by the scanning electron microscope Leo 1430 VP (Leo Electron Microscopy, Cambridge, UK). The samples were gold sputtered by the Agar Manual Sputter Coater B7340 (Agar Scientific, Stansted, UK) prior to electron microscopic investigations.

3. Results and discussion

3.1. Dissolution of lipid matrices in different media

Dissolution studies with solid lipid extrudates containing the poorly soluble anthelmintic praziquantel and five different lipids were conducted in two media. The investigated lipids are glyceryl tripalmitate, glyceryl dibehenate, glyceryl monostearate, cetyl palmitate and solid paraffin. As standard medium hydrochloric acid pH 1.2 with 0.001% polysorbate 20 was used. The biorelevant medium was a modified FeSSIF at pH 6.5 containing bile salts, phospholipids, pancreatic lipase and calcium (Table 1). The pH difference between the two media can be disregarded, because the aqueous solubility of praziquantel and the lipids is nearly independent on pH. The stability of the extrudates was tested by dissolution in both media after 3 and 6 months open storage under accelerated conditions (40 °C, 75% RH).

Fig. 1 shows the results for all investigated formulations. Comparing the dissolution results in the two media, only glyceryl monostearate extrudates show a clearly enhanced drug release in biorelevant medium compared to standard medium. According to similarity evaluation the two profiles are clearly different with an f_2 value of 17.6. After 3 and 6 months storage the drug release was clearly slower and closer to the dissolution result in standard medium. Drug release from cetyl palmitate matrices was considerably accelerated after 3 months storage compared to dissolution in standard medium. Freshly prepared and 6 months stored extrudates showed similar drug release as in standard medium. The other lipids showed almost no difference between the two media. Even dissolution of glyceryl tripalmitate extrudates was hardly enhanced in biorelevant medium, the similarity factor directly after extrusion was 57.9, although triglycerides are a natural substrate of pancreatic lipase. Solid paraffin is an exception between the investigated lipids, because the extrudates show neither a difference in dissolution between the two media nor a deceleration of drug release during storage.

DSC and SEM studies of the extrudates were performed in order to examine the reasons for drug release deceleration during storage. Fig. 2 gives an overview of DSC-thermograms after 0, 3 and 6 months and SEM pictures of extrudate surfaces after 0 and 6 months. The surface of glyceryl tripalmitate, glyceryl dibehenate and glyceryl monostearate extrudates changed during storage. Growth of sharp crystals on glyceride surfaces is known as blooming and is caused by partial melting. Under certain conditions the lipid solidifies in its unstable α-modification, which transforms into the stable β-modification during storage (Hagemann, 1988; Khan and Craig, 2004). Crystals in β-form have a sharp needle-like morphology, clearly increasing the surface area and therefore

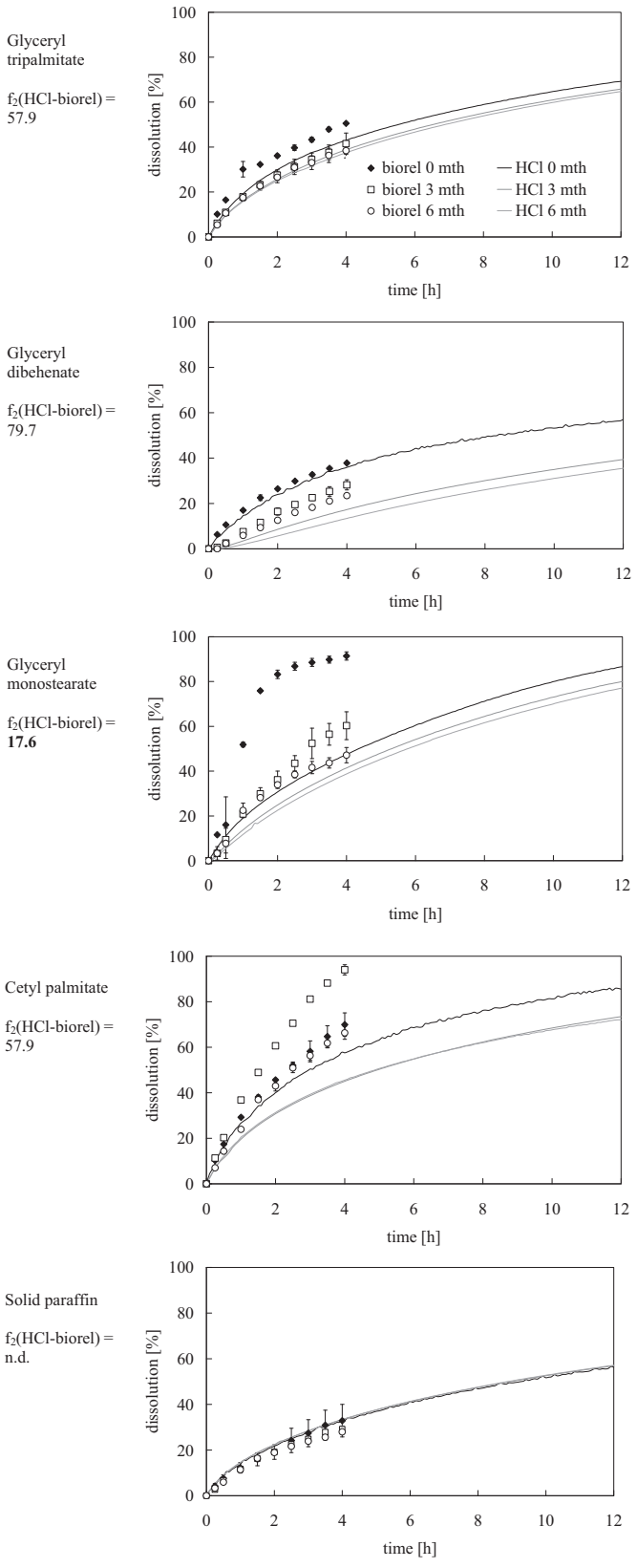

Fig. 1. Drug dissolution in biorelevant medium (♦) and in standard medium (—) and f_2 values for similarity analysis. n.d.: not determined, because CV > 15%, biorelevant medium: mean ± SD, standard medium: SD not shown (<3% at all time points), $n = 3$, formulation: 50% praziquantel/49% lipid/1% silicium dioxide.

decreasing the wettability (Fang et al., 2007; Sato, 2001; Windbergs et al., 2009). This worsening of wettability is the reason for drug release deceleration during storage.

The formation of unstable modifications of the lipid can be visualised by DSC measurements when the sample is heated twice. After first heating the lipid solidifies in its unstable form, whose melting peak becomes visible during a second heating. Fig. 2 shows not only thermograms of extrudates but also second heating thermograms of the pure lipids. Unstable forms are marked with arrows. Within the investigated lipids, only glycerol tripalmitate shows a melting peak of the α-form at 45 °C (small window), which was not detectable after 3 and 6 months. In glyceryl dibehenate and glyceryl monostearate extrudates no unstable modification peak could be measured, although all glyceride extrudates showed blooming on their surfaces. Probably the amount of α-form was below the detection limit.

Cetyl palmitate extrudates show an additional melting peak at 42 °C, which is not detectable after 3 and 6 months, and which corresponds to the melting peak of the unstable form in the second heating thermogram of pure cetyl palmitate. Nevertheless, no blooming occurred on the surface of cetyl palmitate extrudates, indicating a different mechanism underlying the recrystallisation of cetyl palmitate. Cetyl palmitate is a wax and not a glyceride, because the fatty acid is esterified with primary C14–C18 alcohols instead of glycerol. To our knowledge nothing is published about different modifications of cetyl palmitate yet. Apparently, the stable modification does not crystallise in sharp needle-like structures like glycerides do, but also causes a deceleration of drug release.

As expected, solid paraffin showed no unstable forms, first and second DSC heating curves looked exactly the same and no blooming occurred on the extrudate surface.

Windbergs et al. (2009) showed that extrusion with glyceryl tripalmitate is possible without development of blooming on the surface. They kept the extrusion temperature between the melting points of α- and β-form, in doing so partial molten lipid directly solidified in its stable β-form. Extrusion below the melting point of the α-form entailed solidification of the unstable form and blooming afterwards. Against this background, it seems contradictory that in this work blooming occurred on the surface of glyceryl tripalmitate extrudates, because extrusion was performed between the melting points of α- and β-form. The difference between the two studies is that Windbergs et al. (2009) worked with 1 mm die diameter, whereas during this study 0.3 mm dies were used. The determining factor for the solidifying form is the kinetic of cooling: a rapid cooling leads to formation of the α-modification (Hagemann, 1988), whereas a slow solidification in presence of β-crystals entails formation of the stable β-form (Sato, 1988). The heat capacity of 1 mm extrudates is higher than of 0.3 mm extrudates, so that possibly the small extrudates cool down faster and solidify below the melting point of the α-form. Further, the overall inner surface area of the dies is larger for smaller die diameter, which leads to an increase of friction during extrusion and a higher amount of molten lipid. Probably, during extrusion with 0.3 mm die diameter the development of unstable forms could be avoided by ensuring a controlled and slow cooling of the extrudates.

The results of stability testing in the two media show that blooming entails stronger deceleration of drug release in biorelevant medium compared to standard medium. Table 3 gives similarity factors for comparison of 0 and 3 months as well as 3 and 6 months dissolution testing for each lipid in both media. The f_2 (0–3) values for all lipids, with the exception of glyceryl dibehenate, are smaller in biorelevant medium, i.e. the deceleration of drug release is stronger than in standard medium. Biorelevant medium is more similar to the *in vivo* digestive fluid than standard medium,

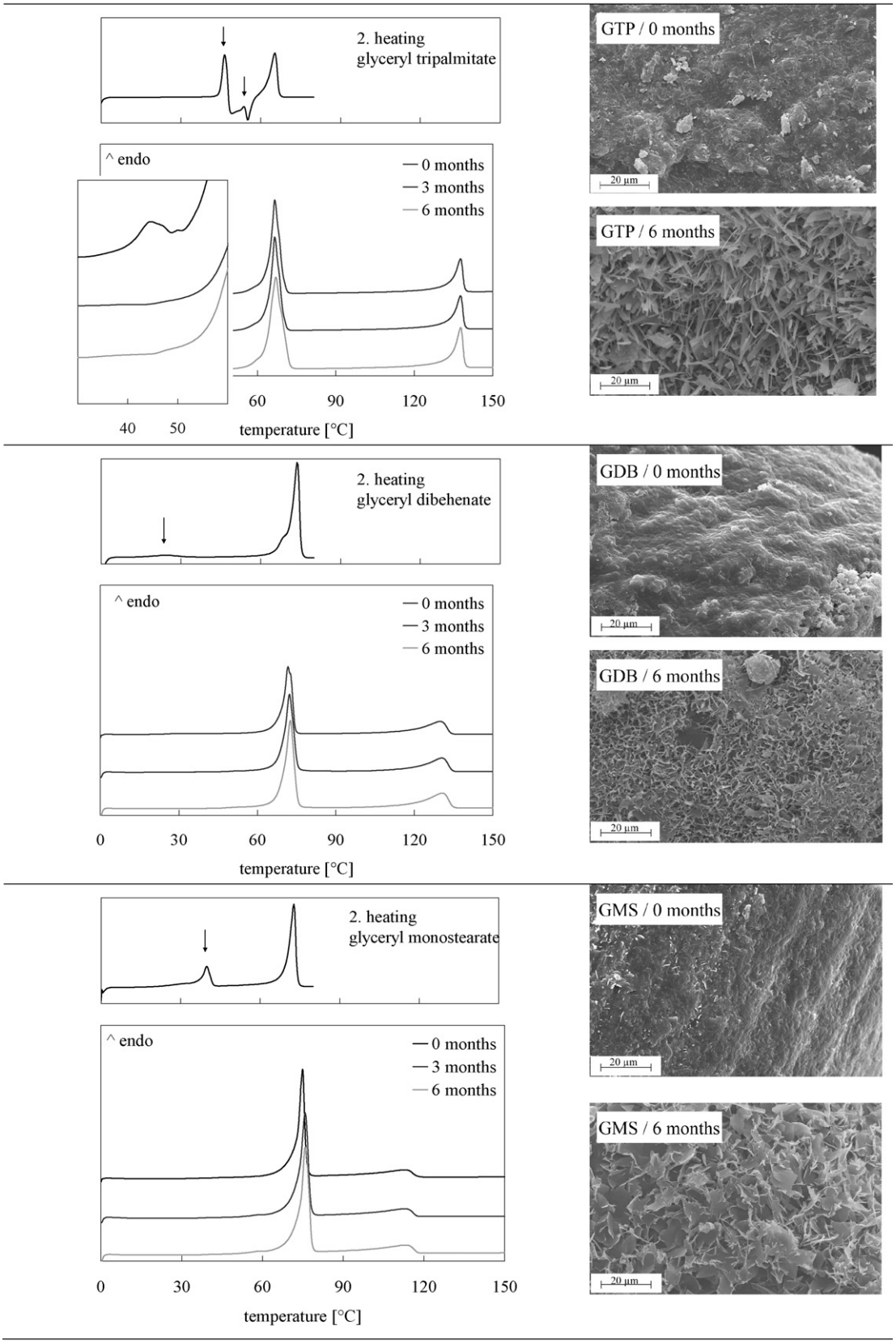

Fig. 2. DSC measurements of pure lipids and extrudates with 50% praziquantel/49% lipid/1% silicium dioxide and SEM-pictures of extrudates directly after extrusion and after 6 months open storage at 40 °C/75% RH.

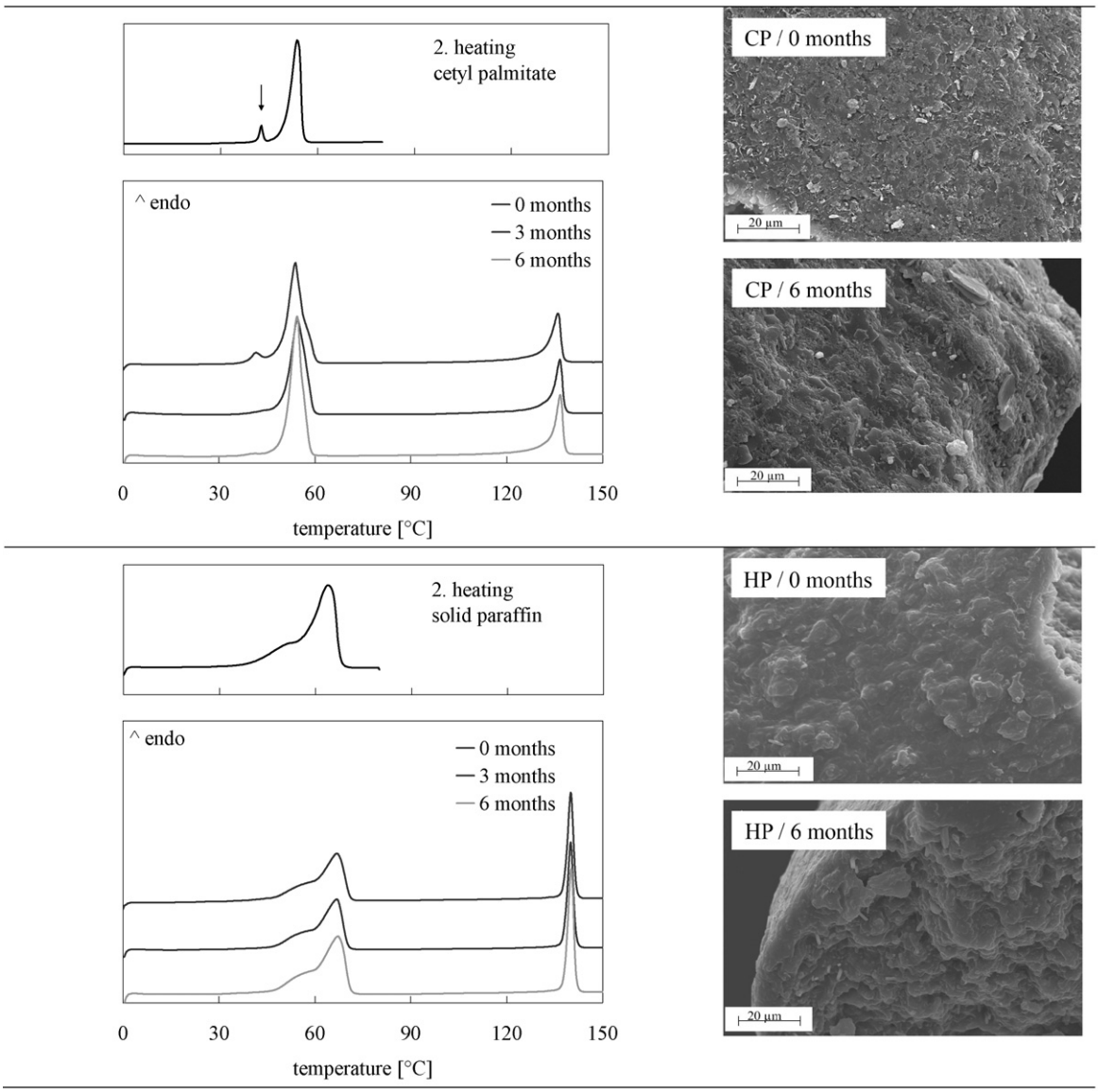

Fig. 2. (*Continued*).

therefore blooming probably also effects drug release *in vivo*. Also investigations on aging of lipid suppositories showed that the bioavailability of the incorporated drug was clearly reduced after storage (Kahela et al., 1987; Kanto, 1975).

Table 3
Similarity factors for comparison of 0 and 3 months and 3 and 6 months, respectively, dissolution testing for each lipid in both media.

		Biorelevant medium	Standard medium
Glyceryl tripalmitate	f_2(0–3)	52.7	70.3
	f_2(3–6)	89.3	93.3
Glyceryl dibehenate	f_2(0–3)	51.0	39.2
	f_2(3–6)	72.3	76.1
Glyceryl monostearate	f_2(0–3)	21.9	64.5
	f_2(3–6)	53.7	80.4
Cetyl palmitate	f_2(0–3)	39.3	50.9
	f_2(3–6)	36.4	96.5
Solid paraffin	f_2(0–3)	–	96.9
	f_2(3–6)	92.4	100.0

3.2. In vitro lipolysis of solid lipids

The results of dissolution testing in biorelevant medium show that with glyceryl monostearate and cetyl palmitate extrudates an acceleration of drug release occurs in comparison with dissolution in standard medium. The drug release from extrudates with other lipids was rarely affected by the medium. The reason could be an increased affinity of pancreatic lipase to these two lipids. To address this, the digestion of the five lipids was investigated directly with in vitro lipolysis according to Zangenberg et al. (2001). In order to ensure comparability, the same extrudates as for dissolution studies were used. The medium used for in vitro lipolysis (Table 2) was not the same as for dissolution in biorelevant medium (Table 1). The amount of pancreatic lipase was 800 USP units/ml in the lipolysis medium and 20 USP units/ml in the biorelevant medium. Bile salts and phospholipid concentration in the lipolysis medium were lower than in biorelevant medium and more related to a FaSSIF than to a FeSSIF. The intention was to work with pancreatic lipase in excess and to minimize the influence of surfactants on drug release during in vitro lipolysis experiments.

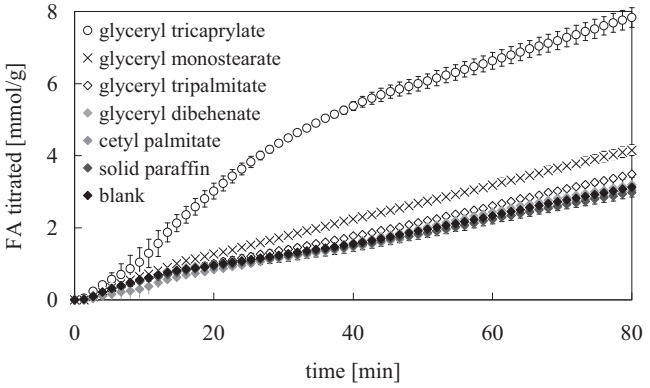

Fig. 3. Fatty acids (FA) titrated from solid lipid extrudates and a liquid glyceryl tricaprylate suspension with 50% praziquantel each, blank: titration without dosage form, mean ± SD, $n = 3$.

Fig. 3 shows the amount of fatty acids titrated, the blank was measured without addition of any dosage form or lipid. Obviously, there were fatty acids titrated during measurement of the blank due to hydrolysis of phospholipids in the medium. Phospholipids are added to simulate the composition of the bile and are believed to play an important role in terms of solubilisation of lipolytic products. The results show that, apart from glyceryl monostearate and glyceryl tripalmitate, the amount of fatty acids titrated were not different from the blank. A suspension with 50% praziquantel and 50% liquid glyceryl tricaprylate was investigated for comparison, because liquid lipids are well known for their quick lipolytic degradation (Christensen et al., 2004; Ljusberg-Wahren et al., 2005; Zangenberg et al., 2001). In vitro lipolysis of the liquid lipid occurred clearly faster compared to the solid lipids.

For a clear presentation, the amounts of fatty acids titrated were recalculated as percentage of titrated fatty acids related to the overall amount of ester groups in each specific lipid. Further, the blank measurement without addition of a lipid system was subtracted. Fig. 4 shows the in vitro lipolysis in percent. Solid paraffin and cetyl palmitate were not included, because solid paraffin does not have ester groups and cetyl palmitate shows a lower amount of fatty acids titrated than the blank. 79% glyceryl tricaprylate were digested in 80 min, fast during the first 40 min up to 65%, and more slowly afterwards. The amount of 65% exactly corresponds to the number of fatty acids in 1- and 3-position of glycerol, that are preferred to be liberated by pancreatic lipase (Morley and Kuksis, 1972). Lipolysis of solid lipids took place clearly slower than digestion of the liquid lipid. Glyceryl monostearate was digested to 38% in 80 min and was therewith the fastest between the investigated solid lipids. Only 10% glyceryl tripalmitate and 3% glyceryl dibehenate were digested in the same time.

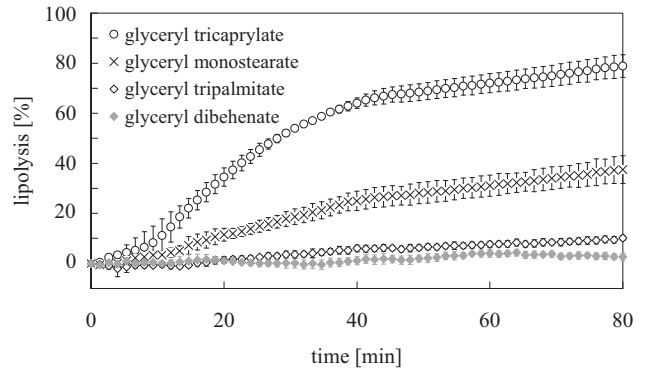

Fig. 4. In vitro lipolysis of lipids in extrudates with 50% praziquantel each, expressed as percentage of titrated fatty acids, mean ± SD, $n = 3$.

Olbrich et al. (2002) showed that in vitro lipolysis of triglycerides is slower, the higher their crystallinity. Liquid lipids are better solubilised and therefore better accessible for pancreatic lipase. Furthermore, glycerides are digested slower, the longer their fatty acid chain length, because the affinity of pancreatic lipase is higher to lipids with shorter fatty acid chains. This was investigated based on liquid lipids with different fatty acid chain length by Deckelbaum et al. (1990) and Christensen et al. (2004). The authors presume a higher solubility and mobility of lipids with medium-chain in comparison to long-chain fatty acids in the digestive interface. Therefore, the fast lipolysis of glyceryl tricaprylate compared to glyceryl tripalmitate can be explained not only by its liquid state, but also by its shorter fatty-acid chain length.

Unexpected is the obvious higher affinity of pancreatic lipase to glyceryl monostearate compared to glyceryl tripalmitate. Both lipids are solid, palmitic acid is 2 C-atoms shorter than stearic acid and triglycerides are the natural substrate of pancreatic lipase; even though lipolysis of glyceryl monostearate is faster compared to glyceryl tripalmitate. Ljusberg-Wahren et al. (2005) showed that glyceryl dioleate in contrast to glyceryl monooleate was not lipolysed. The authors presume that not the affinity of enzyme to substrate, but the extent of solubilisation of the substrate is the most important factor for the extent of lipolysis. Glyceryl monostearate has a higher aqueous solubility than glyceryl tripalmitate (Small, 1968) and is therefore better solubilised. Further, it should be noted that pancreatic lipase prefers to liberate fatty acids in 1- and 3-position (Morley and Kuksis, 1972). The glyceryl monostearate used in this study is esterified in 1-position to more than 90%, whereas the end product of triglyceride lipolysis is a 2-monoglyceride. It can be concluded that lipolysis of glyceryl monostearate is faster than lipolysis of glyceryl tripalmitate, firstly because the monoglyceride is better solubilised compared to the triglyceride. Secondly, the stearyl group in 1-position is a preferred substrate for pancreatic lipase.

3.3. Influence of surfactants on drug release from lipid matrices

The differences in drug release between standard and biorelevant medium can be explained for some lipids with knowledge of the results of the in vitro lipolysis experiments. But it remains unclear, why dissolution of praziquantel from cetyl palmitate matrices is much faster in biorelevant medium compared to standard medium, whereas dissolution from solid paraffin is nearly the same in both media. Both lipids show no degradation in presence of pancreatic lipase.

Beside pancreatic lipase, the main components of the biorelevant medium are bile salts and phospholipids. Both are surfactants. Therefore, the influence of surfactants on drug release from cetyl palmitate and solid paraffin matrices was investigated using polysorbate 20 as model surfactant. Dissolution tests were conducted in HCl with different concentrations of polysorbate 20. The lowest concentration used was 0.01 mM, equal to the concentration used in standard medium.

The results (Fig. 5a and b) show clear differences between the two lipids. Where drug release from cetyl palmitate matrices is clearly accelerated with increasing concentration of polysorbate, dissolution from solid paraffin remained the same in all experiments. On drug release from cetyl palmitate extrudates the addition of 0.01–0.1 mM polysorbate had only a small effect, but concentrations of 0.5 and 1 mM led to a clearly accelerated drug release of 100% praziquantel in 4 h. Obviously, the CMC of 0.05 mM polysorbate (Wan and Lee, 1974) is not the critical factor influencing drug release, because even the addition of 0.1 mM did not lead to a pronounced acceleration of dissolution.

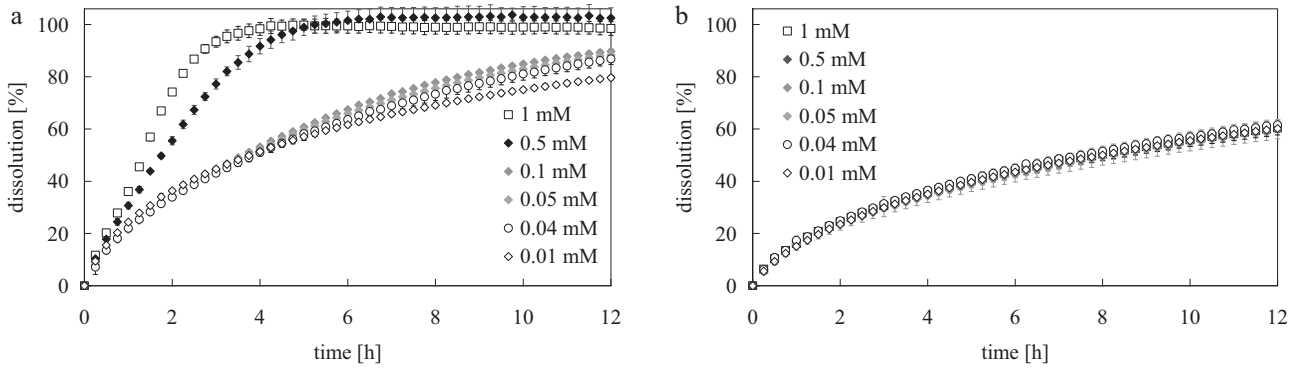

Fig. 5. Drug dissolution from extrudates with 50% praziquantel/49% lipid/1% silicium dioxide in standard medium with different polysorbate 20 concentrations, mean ± SD, $n = 2$. (a) Cetyl palmitate and (b) solid paraffin.

In experiments with different poorly soluble drugs it has been shown earlier that the addition of surfactants to dissolution media can lead to higher solubility of the drug, and that at surfactant concentrations above CMC poorly soluble drugs are enclosed in micelles. Therefore, the drug release can be enhanced by addition of surfactants (Galia et al., 1998; Humberstone et al., 1996; MacGregor et al., 1997). Shah et al. (1989) demonstrated that different surfactants like sodium taurocholate, polysorbate and sodium laurylsulfate in increasing concentrations lead to an acceleration of griseofulvin release from tablets. Reasons for the release enhancing effect of surfactants are an improved wetting and solubilisation of the drug in micelles.

There are no results published so far about the effect of surfactants in dissolution media on drug release from lipid matrices. But with respect to several studies where drugs with low aqueous solubility show enhanced dissolution in media with surfactants, the inertness of solid paraffin extrudates against polysorbate in the dissolution medium is remarkable.

Shah et al. (1989) describe that the addition of 1% (8.1 mM) polysorbate 20 and 1% (18,6 mM) sodium taurocholate led to similar griseofulvin release from tablets with 13% and 8% release after 1 h, respectively. Other surfactants like sodium laurylsulfate showed a much stronger dissolution enhancement with 94% drug release after 1 h. In this work the polysorbate 20 concentration was maximum 1 mM in all experiments. The content of sodium taurocholate in the biorelevant medium was 15 mM. Therefore, it can be concluded that the biorelevant medium had a clearly stronger solubilising effect than HCl with 1 mM polysorbate 20. Further, the enhanced drug dissolution from cetyl palmitate extrudates in biorelevant medium compared to standard medium can be explained by the high concentration of bile salts, i.e. taurocholate, and phospholipids.

Reitz (2007) showed that 90% theophylline was released from solid lipid matrices with glyceryl trimyristate in 8 h in a medium with pancreatin, bile salts and phospholipids, whereas 40% theophylline was released in the same time in a buffer solution with the same pH. The small degree of enzymatic degradation of solid lipids that was shown in this work, leads to the assumption that the release of theophylline in Reitz (2007) work was accelerated due to bile salts and phospholipids in the medium.

4. Conclusion

In this work it was demonstrated that drug loaded solid lipid matrices with a variety of lipids show not only differences in drug release among each other, but also in dependence on the dissolution medium. Drug release from lipid matrices with cetyl palmitate and glyceryl monostearate in biorelevant medium differs to dissolution in standard medium. The reason for the accelerated release from glyceryl monostearate matrices in biorelevant medium is degradation of the lipid by pancreatic lipase, because stearic acid is in position 1. Lipolytic digestion of the monoglyceride runs even faster than the degradation of glyceryl tripalmitate, which is a natural substrate of pancreatic lipase. Lipolytic digestion of liquid lipids runs clearly faster than degradation of solid lipids. The accelerated drug release from cetyl palmitate matrices on the other hand is not related to enzymatic digestion but to the high content of bile salts and phospholipids in the biorelevant medium. Possibly surfactants also contribute to the enhanced drug release from other lipid matrices. This has to be taken into account when interpreting results from dissolution experiments in biorelevant media.

Acknowledgements

The authors would like to thank the companies Sasol and Gattefossé for providing the lipids. Ms Annemarie Schmitz is gratefully acknowledged for performance of the HPLC analysis.

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