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Lipid-Based Oral Formulations

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1.1 Introduction

Lipid-based formulations have been used for oral dosage forms for many years, as there have always been drugs to be formulated which are oil-soluble, e.g. the fat-soluble vitamins and their derivatives. Whereas 25 years ago drugs that are poorly water-soluble but very oil-soluble were few and far between, these days drugs in this category are encountered more and more frequently in drug discovery. As a result, the need for lipid-based oral formulations is on the increase. But lipid-based formulations can be used for other purposes than to adapt the vehicle to the drug's solubility characteristics. For example, preparing a drug in a lipid-based suspension can also be a good way of avoiding safety problems due to dusting during the manufacturing process for drugs that are toxic.

Several years ago, David Hauss published a book that was devoted to oral lipid dosage forms [1]. In this book the lipid-based dosage forms on the market at the time of writing [2] were summarized and some key examples are shown in Table 1.1.

Lipid-based dosage forms may contain a pure oil as the vehicle, a combination of lipids and surfactants, and in some cases consist primarily of surfactants with some co-solvents added. These possibilities have been described by Colin Pouton in his Lipid Formulation Classification System, LFCS [3, 4]. Table 1.2 summarizes the various categories of the LFCS.

These formulations range in composition from self-emulsifying through "self-micro emulsifying" to "self-nanoemulsifying" in nature (Types I through IIIb). Somewhat surprising at first glance in this classification system is that it also covers compositions that contain no classical lipids (Type IV formulations). Because of the huge spectrum of properties covered in the LFCS, there is obviously no "one size fits all" approach to the study of release and absorption from such formulations. In fact, in 2008, Pouton and Porter summarized the properties of lipid formulations as a function of their LFCS classification, including expectations of the importance of lipolysis to drug release, ability of

In Vitro Drug Release Testing of Special Dosage Forms, First Edition. Edited by Nikoletta Fotaki and Sandra Klein.

Table 1.1 Some examples of drugs which are formulated in lipid-based dosage forms.

Drug	Trade name and manufacturer	Date introduced to the market	Indication and dose in adults	Type of formulation
Amprenavir	Agenerase; GSK	2000	HIV, 1200 mg (8 capsules *bid*)	Soft gelatin capsule
α-Calcidiol	One-Alpha; Leo	2000	Calcium regulator, 0.5–1 µg *qd*	Soft gelatin capsule
Bexarotene	Tagretin; Ligand	2001	Antineoplastic, 300–750 mg (4–10 capsules *bid*)	Soft gelatin capsule
Calcitriol	Rocatrol; Roche	1996	Calcium regulator, 0.25–0.5 µg *qd*	Soft gelatin capsule
Ciprofloxacin	Cipro; Bayer		Antibiotic, 15 mg/kg *bid* bis max 500 mg/dose	Microcapsules
Clofazamine	Lamprene; Alliance	1998	Leprosy, max 300 mg (3 capsules *qd*)	Soft gelatin capsule (suspension)
Clomethiazole edilisate	Heminevrin; AZ	2002	Sedative, 192 mg, 1–4 capsules *prn*	Soft gelatin capsule
Cyclosporin A	a. Neoral; Novartis b. Sandimmun; Novartis	1995	Immunosuppressant, 2–10 mg/kg/day, *bid* (1–7 capsules or 1–7 ml)	Soft gelatin capsules; oral solution (generic version as hard gelatin capsule: Gengraf, Abbott)
Doyercalciferol	Hectorol; Bone Care		2° Hyper-parathyroidism, 10–20 µg, thrice weekly (4–8 capsules)	Soft gelatin capsule
Dronabinol	Marinol; Roxane, Unimed		Anorexia, Nausea; 2.5–10 mg *bid*	Soft gelatin capsule
Dustasteride	Avodart; GSK	2003	Benign prostate hyperplasia, 0.5 mg *qid*	Soft gelatin capsule
Efivarenz	Sustiva; BMS	1999	HIV, 600 mg *qd*	Oral solution
Ethylicosapentate	Epadel; Mochida	1990	Hyperlipidemia, 600 mg *tid* (2 capsules)	Soft gelatin capsule
Fenofibrate	Fenogal; Genus	2002	Hyperlipidemia, 200 mg *qd*	Hard gelatin capsule
Ibudilast	Ketas; Kyorin	1989	Asthma, cerebrovascular disorders, 10 mg *bid* or *tid*	ER granules in a hard gelatin capsule
Indomethacin farnesil	Infree; Eisai	1991	Anti-inflammatory and analgesia, 200 mg *bid*	Hard and soft gelatin capsules available

Isotretinoin	Accutane; Roche	1983	Acne, 0.5–1 mg/kg/day admin in 2 doses	Soft gelatin capsule
Lopinavir and Ritonavir	Kaletra; Abbott	2001	HIV, 400 mg/ 100 mg *bid* (5 ml)	Oral solution
Menanetrenone	Glakay; Eisai	1995	Osteoporosis, 45 mg *tid* (3 capsules)	Soft gelatin capsule
Morphine sulfate	MXL; Napp	1996	Analgesic, 30–200 mg *qd*	Hard gelatin capsule containing multiparticulate
Progesterone	Prometrium; Solvay		Hormone replacement, 200–400 mg *qd* (2–4 capsules)	Soft gelatin capsule containing a micronized suspension
Ritonavir	Norvir; Abbott	1999	HIV, 600 mg *bid* (6 capsules)	Soft gelatin capsule
Sirolimus	Rapamune; Wyeth-Ayerst	2001	Immunosuppressant, 6 mg loading dose (6 ml), then 2 mg *qd*	Oral solution
Testosterone undecanoate	Restandol; Organon	1981	Hormone replacement, 40–160 mg *qd* (1–4 capsules)	Soft gelatin capsule
Teprenone	Selbex; Eisai	1984	Acute gastritis, 150 mg *tid* (3 capsules)	Granules/powder in a hard capsule; Granules
Tipranavir	Aptivus; Boehringer-Ingelheim		HIV, 500 mg *bid* (2 capsules)	Soft gelatin capsule
Tocopherol nicotinate	Juvela N; Eisai	1984	Hypertension, hyperlipidemia, 200 mg *tid*	Soft gelatin capsule
Tolteradine tartrate	Detrol LA; Pharmacia & Upjohn	2001	Overactive bladder, 2–4 mg *qd*	ER hard gelatin capsules
Tretinoin	Vesanoid; Roche	2001	Antineoplastic, 45 mg/m^2 *bid* (8 capsules)	Soft gelatin capsule
Valproic acid	a. Depakene; Abbott b. Convulex; Pharmacia	1991	Epilepsy, 10–60 mg/kg/day (3–15 capsules)	Soft gelatin capsule

Source: Adapted from Strickley [2].

Table 1.2 Lipid formulation classification system (LFCS) according to Pouton et al. [3, 4][a].

Formulation Type	Type I	Type II	Type IIIa	Type IIIb	Type IV
Oils: triglycerides or mono/diglycerides	100	40–80	40–80	<20	None
Lipophilic emulsifier	none	20–60	none	none	0–20
Hydrophilic emulsifier	none	None	20–40	20–50	30–80
Hydrophilic cosolvent	none	None	0–40	20–50	0–50

[a] All values are given as percentages.

the dosage form to disperse in the gastrointestinal (GI) fluids and any associated potential disadvantages of the formulation [5]. These properties are discussed in Table 1.3.

For formulations based primarily on digestible lipids (Type I, II, and IIIa formulations) it is of course advisable to conduct experiments which account for digestion of the vehicle as a means of releasing the drug. This is probably true for administration in the fasted as well as the fed state, as even in the fasted state considerable amounts of lipases are present in the gastric and duodenal fluids [6, 7].

Table 1.3 Properties of lipid-based formulations based on the lipid formulation classification system (LFCS).

	Size of dispersed lipid particles	Intraluminal drug precipitation	Lipid digestion for drug absorption	Advantages of this type of formulation	Disadvantages of this type of formulation
Type I	µm-mm	Limited	Necessary	Safe, simple, compatibility with various types of capsules	Useful only for highly lipophilic molecules
Type II	nm-µm	Limited	Important	Usually solubilization capacity is maintained intralumenally	Dispersion may be problematic
Type IIIA	100–250 nm	Cannot be excluded	Important	Almost perfect dispersion	Solubilization capacity may be decreased as digestions advances
Type IIIB	50–100 nm	Possible	Not very important	Almost perfect dispersion	Solubilization capacity may be decreased as digestion advances
Type IV	~ 50 nm (micellar structures)	Likely	Not relevant	Perfect (micellar) dispersion	Solubilization capacity may be decreased upon dispersion

Source: Based on a similar table previously published by Pouton and Porter [5].

For formulations which rely at least partly on lipolysis of the vehicle for release, further mechanisms of absorption may also need to be taken into account. Classically, a drug needs to be released from the formulation and appear in solution in the micellar phase of intestinal contents in order to be available for absorption. However, for lipid formulations, this paradigm may need to be reconsidered. It has been demonstrated that, depending on drug load, flux directly from coarse lipid droplets/particles (intermediate lipolysis products) across the gut membrane may represent a significant alternative way of getting drug absorbed from lipid dosage forms [8]. This would mean that for formulations of this type, two pathways may have to be considered: the classic pathway whereby the drug is released from the dosage form and is then available to be taken up across the mucosa, and the lipid-formulation specific pathway, whereby partitioning into the membrane could also occur directly from intermediate products of lipolysis.

For those formulations primarily consisting of surfactants (Type IIIb and IV formulations), digestion is less likely to be the primary factor in determining the availability of drug for absorption. Indeed, dispersion and dissolution of the excipients in the GI fluids may cause the solubility of the drug to change from the solubility in the formulation to the solubility in the GI milieu and, if a supersaturation results, the drug may precipitate. If the surfactants themselves are susceptible to decomposition by the digestive enzymes, as has been shown to be the case for Cremophor EL® (polyoxyl 35 castor oil) [9], Labrasol® ((PEG-8 caprylocaproyl macrogolglycerides), and Gelucire® 44/14 (PEG-32 lauroyl macrogolglycerides) [10], digestion as well as dispersion may affect the ability of the GI fluids to hold the drug in solution and in this case both factors need to be accounted for to evaluate potential precipitation. Of course, if the drug can be held in solution for long enough to enable absorption to occur, the fraction absorbed will be favorably affected. In fact, this is usually the intent of formulating the drug in a lipid-based formulation. The time frame needed for absorption will naturally also be a factor here, so the better the permeability of the gut mucosa to the drug, the less time it will have to stay in solution to be absorbed.

In summary, for lipid-based formulations, the interplay between dispersion, digestion, and precipitation on the one hand and the permeability of the gut membrane to the drug on the other hand will determine the overall fraction of drug absorbed. Given the kinetic nature of these processes, measuring the rates rather than the equilibrium values associated with these processes may well be more important to predicting how well a drug will be absorbed after administration of a lipid-based formulation.

1.2 Levels of Release Testing for Lipid-Based Dosage Forms

1.2.1 Dilution

Good self-emulsifying properties of lipid-based formulations can guarantee a rapid and fine dispersion when the formulation is mixed with an aqueous environment under even gentle hydrodynamics. In turn, a fine dispersion will expose a greater surface area of drug to the GI environment and facilitate release and absorption of the drug. Thus as an initial screening tool, a simple dilution of the lipid formulation prototype in various

media offers a useful check on how well the formulation could perform *in vivo*, as was demonstrated by Khoo et al. [11]. As in the great majority of cases the first contact of the formulation will be with the gastric fluids, this is a useful place to start with the composition of the screening medium. With respect to dilution factors, one can calculate that if no water is administered with the dosage form, it will typically encounter a maximum of just a few ml of gastric fluids, whereas if a glass of water is co-ingested with the formulation, the initial contact volume could be as high as 250 ml in the fasted state. Depending on the meal, if the dosage form is ingested postprandially, the contact volume could be 500 ml or even more. Therefore it seems appropriate to perform several dilutions of the prototype formulation, ranging from as little as 1 : 5 up to 1 : 200 or even more. To visualize the ability of the formulation to self-emulsify, a lipid-soluble dye such as Sudan Red can be pre-dissolved in the prototype formulation. The formulation and the dilution medium are warmed to 37 °C, the formulation is diluted, initial observations are made and then the dilution is placed on an orbital shaker for up to 24 hours at 37 °C. Although this time interval is obviously much longer than the residence time in the stomach, it may help to differentiate between those emulsions which are very stable and those which are only metastable. Observations can also be taken at intermediate time points, e.g. after two and four hours. With respect to media composition, worst case behavior is usually observed when plain water is used as the medium. Better self-emulsification often occurs under more physiological conditions, e.g. in Fasted State Simulating Gastric Fluid (FaSSGF) [12], which has a pH value of 1.6. As some patients may have elevated gastric pH, it may also be prudent to run dilutions in FaSSGF adjusted to pH 5. If administration in the fed state is anticipated, dilutions can additionally be run in media representing the stomach in the fed state, e.g. Fed State Simulating Gastric Fluid (FeSSGF) [13, 14].

For screening purposes, a simple scoring system can be used:

a) 3 points for fine emulsions that do not show any phase separation or coalescence after 24 hours,
b) 2 points for fine emulsions that do not show any phase separation or coalescence within 4 hours,
c) 1 point for fine emulsions that do not show any phase separation or coalescence within 2 hours, and
d) 0 points for emulsions that are coarse or which show phase separation or coalescence within 2 hours.

Figure 1.1 shows typical results for a lipid formulation with excellent dilution characteristics (A) and one with very poor dilution characteristics (B). Formulation A maintains a homogeneous appearance over the entire 24-hour observation period. By contrast, Formulation B is already showing obvious phase separation after just two hours, as seen from the orange band close to the surface of the dispersion, with the effect more pronounced in FaSSGF than in FeSSGF.

Such experiments can also be run with the target drug dissolved in the formulation, to screen for potential precipitation upon dilution. Comparison of the droplet size and time to coalescence/phase separation with and without the target drug may also aid in selection of an optimal lipid formulation.

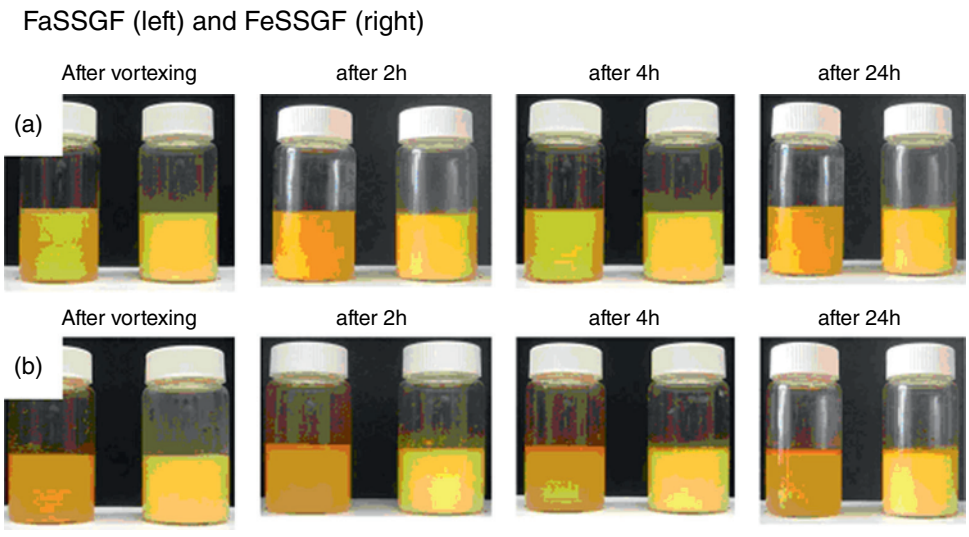

Figure 1.1 Typical dilution results of a lipid formulation with excellent dispersibility (a) and poor dispersibility (b) in Fasted State Simulating Gastric Fluid (FaSSGF) and Fed State Simulating Gastric Fluid (FeSSGF). Dilution ratio 1 : 200 (formulation: medium). The orange color originates from the use of Sudan red as a dye marker.

1.2.2 Dispersion and Drug Release

Drug release tests are an important part of preclinical development, belong to the routine quality control of dosage forms and can also be useful in predicting the *in vivo* performance of the drug product. Lipid-based dosage forms require consideration of the hydrodynamics of the apparatus used to perform the release test as well as the composition of the medium, if they are to be successfully employed for the prediction of *in vivo* performance. The importance of the hydrodynamics varies with the formulation type (see Table 1.3). Whereas good mixing conditions may be required to keep a Type I formulation adequately dispersed in the release medium, Type IV formulations should easily disperse even under mild agitation and thus not require any special hydrodynamics. For Type II and III formulations, the hydrodynamic requirements may depend quite heavily on the particular excipients used to prepare them, as will be further discussed in the case examples.

For formulations which disperse easily in aqueous media (see Dilution experiments), the paddle method, i.e. apparatus 2 of the United States Pharmacopeia (USP), Japanese Pharmacopoeia (JP), and European Pharmacopoeia (EP), may be adequate in terms of hydrodynamics, although it is likely that the rate of release will depend on the stirring rate. For other formulations, the reciprocating cylinder ("BioDis" apparatus, i.e. the USP and EP type 3 tester) may give more reproducible results and more complete drug release. Typical hydrodynamic conditions for the reciprocating cylinder are in the range 10–20 dpm, for the paddle 50–100 rpm.

Figure 1.2 shows results for release of the drug from two Type IIIa lipid-based formulations in the paddle (100 rpm) and reciprocating cylinder (20 dpm) apparatus, using

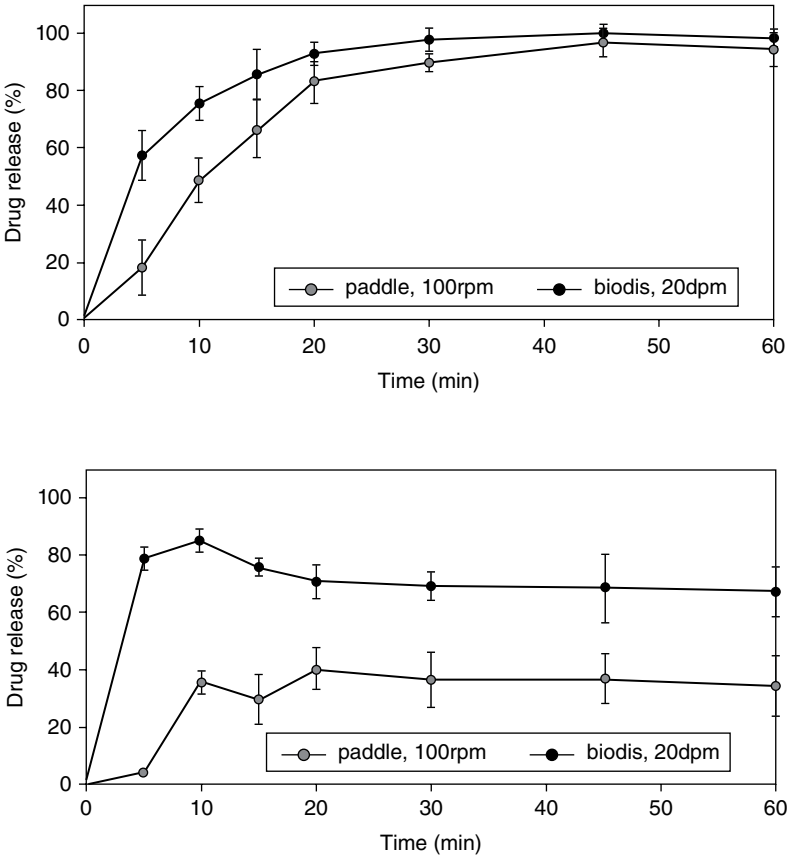

Figure 1.2 Release of fenofibrate from a lipid formulation with good dispersibility (Prototype 1, upper panel) or poor dispersibility (Prototype 2, lower panel) in SGFsp using the paddle or BioDis apparatus.

Simulated Gastric Fluid (without pepsin) (SGFsp) from the USP as the dissolution medium. Whereas Prototype 1 (a Type IIIa system, consisting of ethyl oleate and polysorbate 80 in a 7 : 3 ratio containing fenofibrate 75 mg/1 g formulation, which scored 3 points in the dilution experiments, independent of medium) shows consistent results in both apparatus, Prototype 2 (a Type IIIa system consisting of oleic acid and polysorbate 80 in a 7 : 3 ratio containing fenofibrate 57 mg/1 g formulation, which scored three points in FeSSGF, but only scored one point in the dilution experiments in SGFsp or in FaSSGF) released less than half the fenofibrate in the paddle apparatus but showed much more complete release in the reciprocating cylinder apparatus. These results illustrate the utility of characterizing the self-emulsifying characteristics of the formulation before selecting the release test conditions.

As with the dilution experiments, it is sensible to focus, at least initially, on drug release under gastric conditions. The worst case scenario for the choice of medium is water, since this medium represents neither the low pH encountered in most children

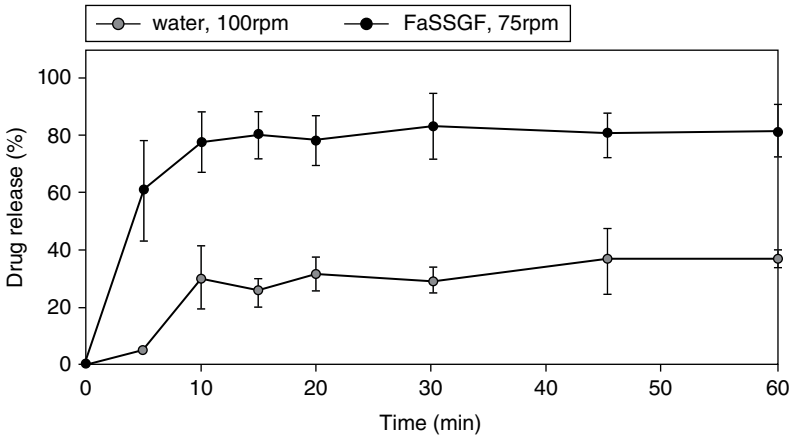

Figure 1.3 Comparison of fenofibrate release from a lipid formulation with poor dispersibility (Prototype 2) in water and Fasted State Simulating Gastric Fluid (FaSSGF) using the paddle apparatus.

and adults, nor the solubilizing components that can serve to help keep any released drug in solution.

To illustrate the use of "worst case" conditions, Mohsin et al. [15] assessed fenofibrate precipitation from four prototype formulations (Type II, IIIA, IIIB, and IV) upon dilution with water. Although the Type II and Type IIIa formulation were able to keep nearly 100% of drug in solution for 96 hours, 15–85% of drug crashed out from the Type IIIb and IV formulations within a few hours. However, as these test parameters don't adequately reflect physiological conditions, the results should be interpreted more from a technical perspective as a means to obtain maximum discrimination among formulations, rather than as a forecast of *in vivo* performance.

To obtain more physiologically representative results, biorelevant media designed to represent the stomach in the fasted and fed state are recommended. Figure 1.3 compares release from Prototype 2 in water and in FaSSGF in the paddle apparatus. It is clear from the results that FaSSGF facilitates release to a much greater extent than water, even though the agitation rate was lower.

If a prognosis about the performance in the fed state is also desired, release in FeSSGF* can be compared with the release in FaSSGF, as shown in Figure 1.4 for Prototype 2. When the paddle apparatus was used for this poorly dispersible formulation, the release in FeSSGF* appeared to be much slower than in FaSSGF (Figure 1.4 upper panel). However, under the stronger hydrodynamics of the reciprocating cylinder, the release in FeSSGF* was shown to be faster and more complete than in FaSSGF (Figure 1.4 lower panel). These results also highlight the need to consider not only the dissolution medium but also the hydrodynamics in order to predict the *in vivo* performance of a lipid formulation.

Since the analysis of drugs in FeSSGF* (milk-based) can be quite challenging (due to the need to precipitate the milk proteins), a modified version of the fed state gastric medium has been developed: Fed State Simulating Gastric Emulsion (FeSSGEm). This medium, the composition of which is shown in Table 1.4, has now superseded FeSSGF* as the preferred fed state gastric medium and will be referred to henceforth as FeSSGF.

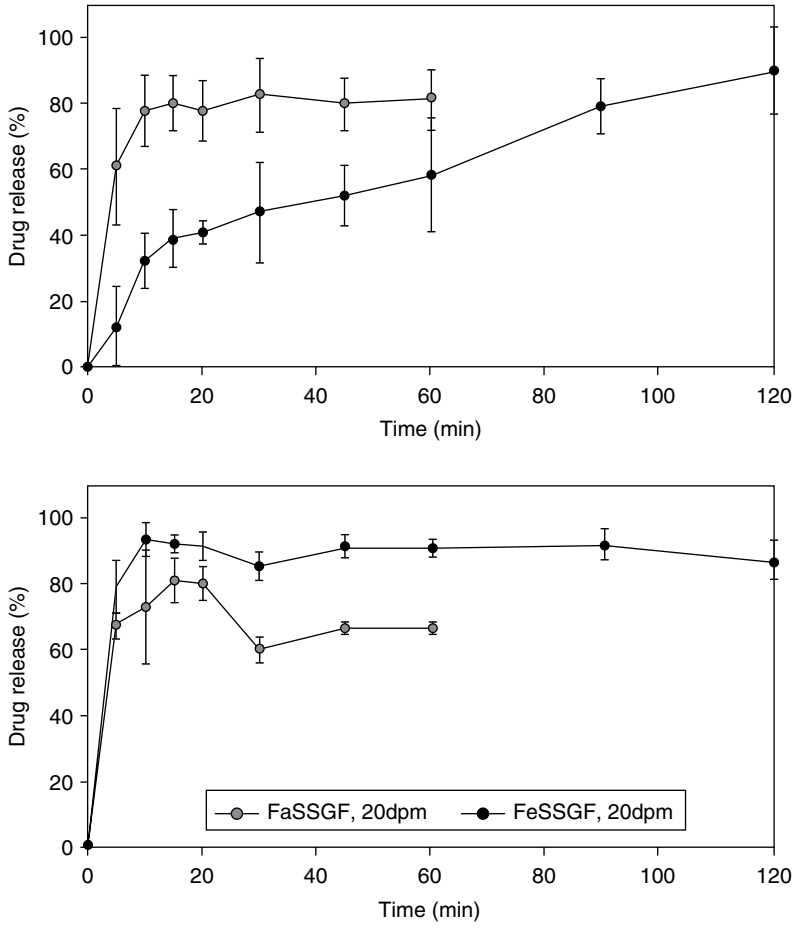

Figure 1.4 Release of fenofibrate from Prototype 2 in Fasted State Simulating Gastric Fluid (FaSSGF) and Fed State Simulating Gastric Fluid (FeSSGF*) using the paddle apparatus at 75 rpm (upper panel) and using the BioDis apparatus at 20 dpm (lower panel).

The emulsion version of FeSSGF avoids the problems associated with removing the milk proteins from samples prior to analysis for the drug, while retaining the same fat content and physicochemical properties important to drug release. So unless the drug solubility is affected strongly by binding to casein or partitioning behavior is very specific to the type of oil in the medium, one can expect similar solubility and dissolution behavior in the emulsion version of FeSSGF as in the milk-based FeSSGF*.

Figure 1.5 illustrates the release of fenofibrate from Prototype 2, indicating that in this case, results are quite similar between the two media. Of interest is that not only are the results close to each other in the two media, but the variability in the data has been reduced by using the emulsion version of FeSSGF. The reduction in the variability is probably due to the better reproducibility in the sample preparation for analysis – not only is there no need for protein precipitation, but it is also possible to use finer filters

Table 1.4 Composition and physicochemical values for Fed State Simulating Gastric Fluid (FeSSGF) (milk-based)[a] and Fed State Simulating Gastric Fluid (FeSSGF) (emulsion-based, formerly Fed State Simulating Gastric Emulsion [FeSSGEm]).

Composition	FeSSGF*[a] (milk-based)	FeSSGF (emulsion-based, formerly FeSSGEm)
Buffer		
Sodium chloride (mM)	237.02	181.7
Acetic acid (mM)	17.12	18.31
Sodium hydroxide (mM)	—	—
Sodium acetate (mM)	29.75	32.98
Demineralized water		qs
Dilution Ratio		
Ratio UHT-Milk/Buffer	1 : 1	—
Ratio Lipofundin® MCT 20/Buffer	—	8.75 : 91.25
Hydrochloric acid/sodium hydroxide	qs pH 5.0	qs pH 5.0
Physicochemical properties		
pH		
Initial	5.0	5.0
After 8 h storage ambient	5.0	5.0
After 8 h storage 37 °C	5.0	5.0
Buffer capacity (mmol/l/ΔpH)		
Initial	26.1 (0.4)	25.0 (2.0)
After 8 h storage ambient	25.5 (0.6)	25.4 (0.8)
After 8 h storage 37 °C	25.1 (0.5)	26.1 (0.6)
Osmolality (mOsm/kg)		
Initial	400.3 (3.8)	402.0 (1.0)
After 8 h storage ambient	400.3 (4.5)	405.3 (0.6)
After 8 h storage 37 °C	408.7 (2.9)	408.0 (1.0)

[a] Data from Jantratid et al. [13]; Standard deviations in brackets.

(0.45 µm compared to 2.7 µm) with the emulsion-based medium. A further bonus is that the stability of the emulsion-based version is better than that of the milk-based version, as it can be kept for up to 72 hours at room temperature before use (data not shown). Thus, in addition to its use for lipid formulations, it may represent a useful medium to represent fed state conditions in the stomach for a variety of formulation types.

1.2.3 Digestion

Lipolysis, the enzymatic hydrolysis of lipids, is initiated in the stomach by gastric lipase. Currently, it is assumed that 5–40% of fat digestion occurs via this mechanism, with the remainder taking place in the small intestine by the action of pancreatic lipases [16].

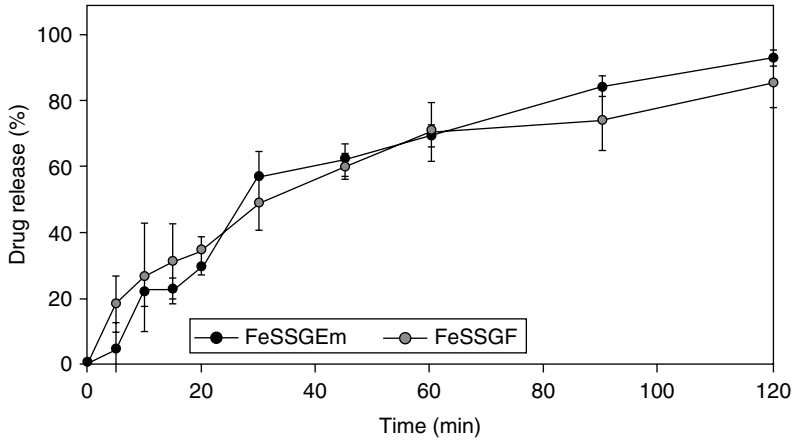

Figure 1.5 Release of fenofibrate from Prototype 2 in emulsion-based and milk-based FeSSGF using the BioDis at 20 dpm.

Complete lipolysis by pancreatic lipases, which requires assistance from co-lipase and bile components, breaks triglycerides down into two fatty acid molecules and one monoglyceride, all of which have solubilizing properties. For simple drug formulations in Type I, II, and IIIa systems, it is assumed that lipolysis is vital to drug release and subsequent absorption. *In vitro* lipid digestion models have been proposed in order to investigate the dynamic formation of colloidal particles composed of bile salts, phospholipids, and lipid digestion products, and their role in drug dissolution [17, 18].

On the other hand, it has been suggested that for formulations of Type IIIb and IV, lipolysis of the excipients can lead to precipitation of the drug and hence hinder absorption [4].

Although significant lipolysis occurs in the fed stomach, in the fasted state, the limited residence time and the highly acidic conditions decrease the importance of gastric lipase and the greater contribution to lipolysis is in the small intestine. As a result, the digestion characteristics of lipid formulations are usually investigated under conditions simulating the intestinal environment [19]. Several relevant lipolysis setups have been developed [20–23]. Most current methods are based on pH-stat experiments, in which the released free fatty acids are titrated by hydroxyl ion to maintain a constant pH level in the medium. Typical compositions of the lipolysis medium are shown in Table 1.5. In Figure 1.6 the lipid digestion model of Porter et al. is illustrated graphically. An attempt to standardize the *in vitro* digestion tests was initiated in 2012 [24].

An issue with these models is that, unlike in the small intestine, the lipolysis products are not absorbed, and can subsequently inhibit any further lipolysis. To overcome this problem, the lipolysis products are sequestered by calcium ions, which are added continuously in the Copenhagen model or are present in a large excess in the Monash model. In both cases, the calcium concentrations exceed the physiological values of about 3 mM in pancreatic juice, bile and intestinal secretions by far, whereas the LFCS consortium model better reflects conditions of the fasted small intestine from this aspect. Another issue with these models is that, to promote lipase activity, the pH is held typically at pH 7.5, a value which far exceeds the pH in the upper small intestine in

Table 1.5 Typical composition of media and test conditions in lipolysis models.

Composition and test conditions	Copenhagen model [21, 22]	Monash model [20]	LFCS-Consortium model [23]
pH	6.5	7.5	6.5
Buffer system	2 mM trismaleate, 150 mM NaCl	50 mM trismaleate/ 150 mM NaCl	2 mM trismaleate, 150 mM NaCl
Lipase	Porcine pancreatin (USP × 3) 800 U/ml	Porcine pancreatin (USP × 8), 10 000 tributyrin U/ml	Porcine pancreatin (USP × 8), 10 000 tributyrin U/ml
Bile salts	Porcine bile extract (5 mM bile salts)	20 mM sodium taurodeoxycholate	3 mM sodium taurodeoxycholate
Phospholipids	1 mM phosphatidylcholine	Lecithin (60% phosphatidylcholine; corr. 5 mM)	0.75 mM phosphatidylcholine
Media volume (initial)	300 ml	10 ml	40 ml
Calcium conc.	Constant addition of 0.5 M $CaCl_2$; 0.045 mM/min	5 mM $CaCl_2$	1.4 mM $CaCl_2$
Remarks	Dynamic lipolysis model ensures control of lipolysis rate by gradual addition of calcium	Classical pH-stat lipolysis model	Classical pH-stat lipolysis model based on an extensive study in order to establish a standardized lipolysis model

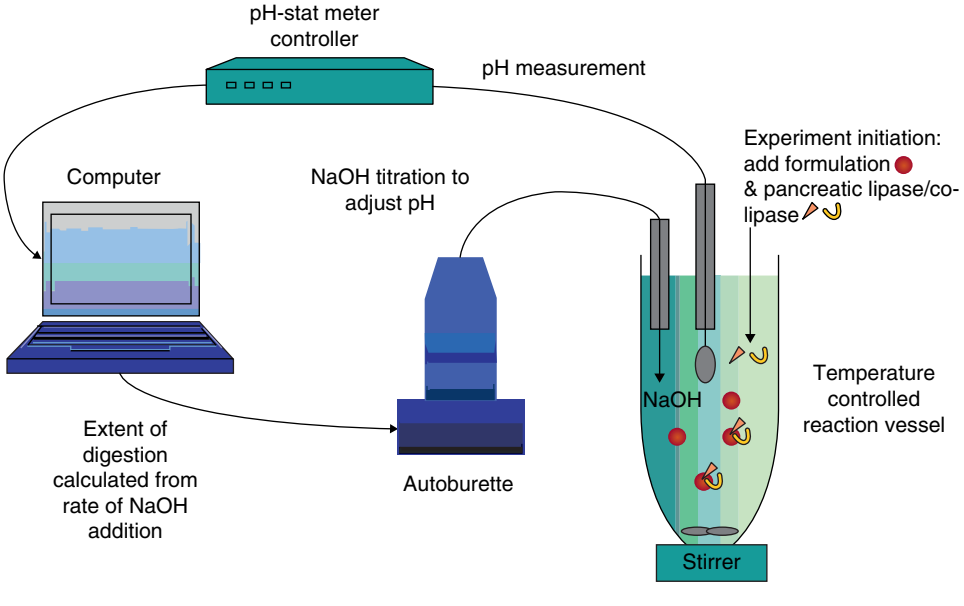

Figure 1.6 A typical *in vitro* lipid digestion model applied for the *in vitro* assessment of lipid formulations. *Source:* Reproduced with permission from [19].

the fed state. For these reasons, although such experiments can be used to determine whether a formulation is subject to lipolysis effects on drug release or not, the quantitative contribution of lipolysis to drug release *in vivo* may remain unclear from such experiments.

For the above reasons, an alternative method for screening for lipolysis effects on drug release was recommended by Jantratid et al. [13]. The recommended method had the aims of (i) using a more physiologically relevant set of test conditions, (ii) being able to screen for lipolysis effects in the fasted as well as the fed state and (iii) being simpler to use than the pH-stat methods. An additional advantage lay in the ability to run the tests using classical dissolution equipment. In detail, as discussed by Jantratid et al., digestion of one dose of lipid formulation would require 1–2 USP Units/ml lipase activity. In the fed small intestine this value is exceeded by far, with lipase levels ranging from about 500 to 1000 USP Units/ml [13], but also in the fasted state lipolytic activity can be assumed to be sufficient [25–28]. Thus, release tests in FaSSIF-V2 and FeSSIF-V2 including 100 USP Units/ml porcine pancreatin per medium (for 1 l of biorelevant medium this corresponds approximately to 20 ml of supernatant of a porcine pancreatin suspension consisting of 6.25 g pancreatin in 30 ml demineralized water [13] and 5 mM $CaCl_2$) may be suitable for the assessment of lipolysis and food effects on drug release. Additionally, comparing release profiles obtained under both prandial conditions with *and* without pancreatic activity would provide a way of investigating the impact of lipolysis on drug release.

1.2.4 Assessing Direct Uptake from the Vehicle

It has been suggested that luminal coarse lipid droplets/particles that are formed after administration of triglyceride solution of danazol to adults in the fed state can significantly contribute to the flux of danazol across the intestinal mucosa [8]. The study was performed by using the Caco-2 cell model and, therefore, due to cell toxicity issues, relevant experiments had to be performed by using diluted human intestinal aspirates and diluted micellar phases of aspirates. Interestingly, similar conclusions have been drawn using PAMPA, a technique that was applied without prior dilution of the luminal samples [29]. In both cases, danazol permeability from the aspirates was reduced compared to that from micellar phases, but the overall fluxes were similar. These two studies suggest that, after administration of a triglyceride solution of danazol, high danazol concentrations in coarse lipid particles are able to compensate for the reduced permeability in terms of drug flux. In addition, these data call for further investigations on the direct uptake of the drug from the partly digested lipid vehicles intralumenally.

1.3 Case Examples

In addition to the data discussed in the preceding sections, various release test systems that have been designed or adapted to test the release from and behavior of lipid-based dosage forms are used in the following case examples to illustrate the diversity of questions arising with the performance of lipid-based dosage forms and how these have been addressed with test systems over the last years.

1.3.1 Compendial or Fed State Biorelevant Media to Evaluate Fenofibrate Lipid-based Formulations?

The usefulness of fed state simulating media over simple aqueous was evaluated using Fenogal®/Lidose® Gelules (SMB, Germany) and the paddle apparatus. Fenogal®/Lidose® Gelules is a capsule lipid formulation of fenofibrate (200 mg/cap) that contains Gelucire 44/14, HPMC and PEG 20000. In all cases 500 ml of release medium was used and the paddle was rotated at 75 rpm. FeSSGF* and FeSSIF-V2 in absence and in presence of pancreatin (Copenhagen model) were used as release media. Pancreatin was added in FeSSIF-V2 so as to result in lipase activity of 800 USP Units/ml in the final medium. Each sample was filtered via 0.45 µm or, in FeSSGF*, via 2.7 µm filters of regenerated cellulose and the fenofibrate content was determined using a high-performance liquid chromatography (HPLC) method that was based on Mohsin et al. [15]. The results shown in Figure 1.7 indicate that release in simple aqueous media is very limited. Under fed state simulating conditions release is higher, whereas, as expected from the type of lipid excipients in this product, the presence of pancreatin had minimal impact on the release profile in FeSSIF-V2. Release data were included in a Stella® model, assuming that fenofibrate is absorbed without permeability restrictions from the small intestine (e.g. [30]), absorption lasts for a total of 12 hours post-dosing, and fenofibrate is rapidly transformed to fenofibric acid in plasma. Pharmacokinetic parameters were estimated with WinNonlin® (WinNonlin Professional, version 5.2, Pharsight Corporation) using published plasma data of fenofibric acid collected after single-dose administration of one Fenogal®/Lidose® capsule to 24 healthy adults in the fed state (achieved by administering a 500 ml meal, total of 1000 Kcal, [31]. Based on the results shown in Figure 1.8, *in vitro* data in FeSSGF* and FeSSIF-V2 (with or without pancreatin) led to adequate prediction of average plasma concentrations of fenofibric acid achieved after single-dose administration of one Fenogal®/Lidose® capsule to healthy individuals in the fed state.

1.3.2 Paddle or Biodis Method for Testing a Lipid-based Formulation?

A lipid-based, soft gelatin capsule (SGC) formulation was developed by F. Hoffmann La Roche for an experimental compound, RO-50X (a poorly soluble weak acid), to decrease dusting during the manufacturing process. In this Type II formulation, some of the drug was in solution but some was present as a suspension. The formulation, which contained 2.5 mg of RO-50X was administered as a single dose to dogs in the fed state in a pharmacokinetic study. Jantratid et al. [32] compared biorelevant dissolution test methods with standard compendial methods, using both the USP apparatus 2 (paddle method) and the USP apparatus 3 (BioDis method), in terms of their ability to predict the plasma profiles observed. Only a very low drug release was observed with the paddle method and this was attributed to the poor dispersibility of the lipids in the dissolution media with the paddle hydrodynamics. By contrast, the reciprocating motion of the BioDis method combined with the shearing action of the sieve at the bottom of the cylinder facilitated release of the drug by keeping the formulation emulsified in the medium. Results are shown in Figure 1.9 for dissolution in the fed state intestinal media in the paddle apparatus (upper panel) and the BioDis apparatus (lower panel).

As can be seen from the data in Figure 1.9, although dissolution of the pure drug reached about 15–25% in the paddle, release from the SGC formulation in the paddle

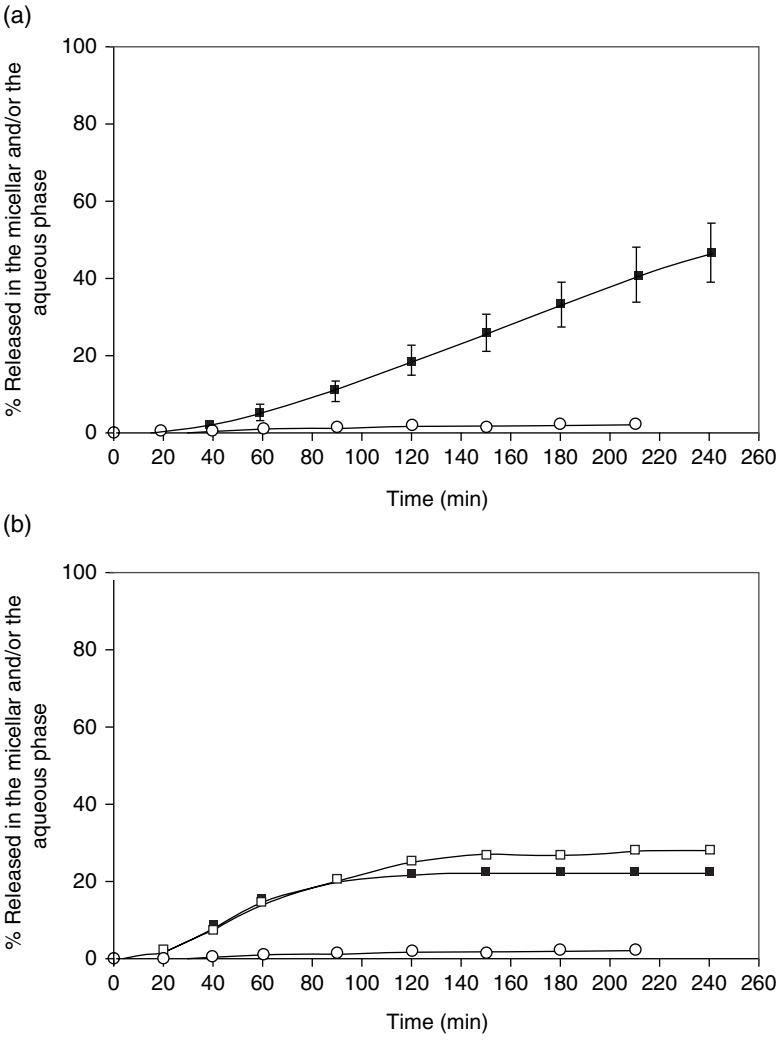

Figure 1.7 (a) % of Fenogal®/Lidose® Gelules released in the micellar and/or the aqueous phase of 500 mL water (○) and FeSSGF* (■); (b) % of Fenogal®/Lidose® Gelules released in the micellar and/or the aqueous phase of 500 ml SIFsp (○), FeSSIF-V2 (■), and FeSSIF-V2 containing 800 units/ml pancreatin (□).

was very poor compared to the BioDis. This was attributed to the poor dispersion of the dosage form in the medium in the paddle, as illustrated in Figure 1.10.

Subsequently, an *in vitro–in vivo* correlation (*IVIVC*) analysis according to USP Level A and curve comparison of fraction drug dissolved vs. absorbed using the Weibull distribution were used to evaluate the *in vitro* methods in terms of their ability to fit the *in vivo* plasma profiles. The best *IVIVC* was obtained using a dissolution medium representing fed gastric conditions in combination with the BioDis method, as shown in Figure 1.11. The authors concluded that for these types of lipid formulations, the BioDis

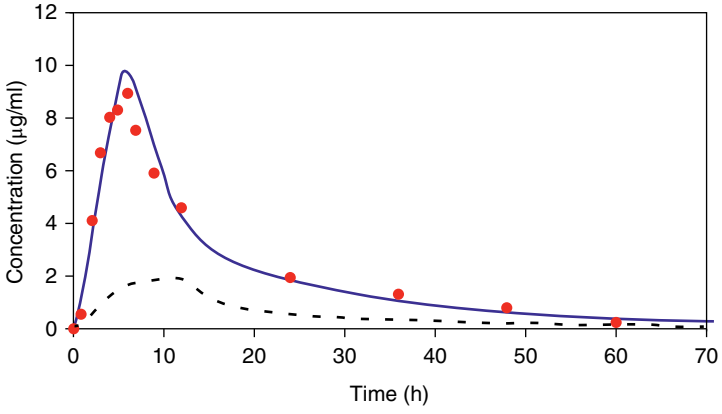

Figure 1.8 Mean observed fenofibric acid levels after single-dose administration of Fenogal/Lidose Gelules (200 mg fenofibrate/cap) to healthy adults in the fed state (●) [31] and predicted profiles that were constructed based on *in vitro* release data (Figure 1.7) collected in biorelevant media (————) and in simple aqueous media (- - - -).

apparatus combined with the use of biorelevant media (in this case FeSSGF and FeSSIF) produced the best forecast of *in vivo* results.

1.3.3 Does Nifedipine Precipitate after Administration as a Soft Gelatin Capsule?

Thelen et al. [33] investigated *in vitro* the potential precipitation of nifedipine, after the administration of nifedipine immediate-release (IR) liquid-filled soft gelatin capsules (Adalat® 10 mg). The composition of the capsules comprised glycerol, purified water, saccharin sodium, peppermint oil and macrogol (PEG) 400. Release characteristics in FaSSGF were investigated by using the paddle and the mini-paddle apparatus. Release of nifedipine from a 10 mg capsule in 250 and in 500 ml FaSSGF was rapidly completed within 15 minutes. By contrast, the release profile of 20 mg capsule in 250 ml FaSSGF exhibited a peak at about 75% released within 15 minutes and declined thereafter for 60 minutes, reaching a final plateau at about 40% released. The *in vitro* data were successfully used in subsequent PBPK modeling [33].

1.3.4 Screening of Indomethacin Lipid-Based Formulations

Arnold et al. [34] investigated the release profile of indomethacin (25 mg/cap) using three different Type III lipid formulations: semisolid Formulation 1 (Gelucire 44/14 76%, Transcutol HP 19%, Labrafil M-2125 CS 5%), liquid Formulation 2 (Cremophor RH 40 34%, Imwitor 742 25.5%, Miglyol 812 25.5%, Ethanol 15%), and liquid Formulation 3 (Solutol HS 15 34%, Imwitor 742 25.5%, Miglyol 812 25.5%, Ethanol 15%). The *in vitro* tests were performed in buffers, FaSSGF and fasted state simulated intestinal fluid (FaSSIF) using the paddle method, the flow-through test (USP apparatus 4), and lipolysis test. Based on the results of the different *in vitro* tests, the lipid formulations displayed

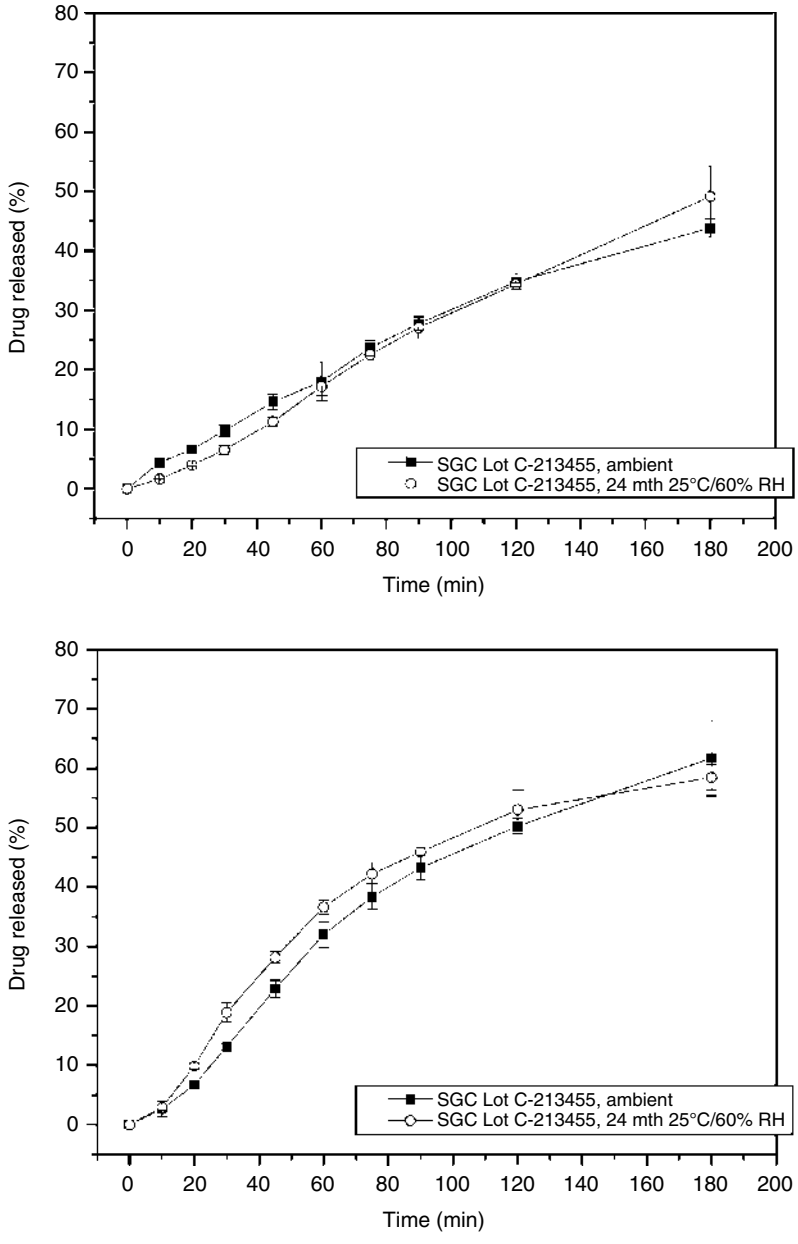

Figure 1.9 Release of RO-50X from a lipid-based formulation in FeSSIF using the paddle apparatus (upper panel) and the BioDis apparatus (lower panel).

higher solubilized drug concentrations than the pure drug in a capsule. In dispersion/precipitation tests in 0.1 N HCL using the paddle apparatus, precipitation of indomethacin was observed for the two liquid lipid formulations. In FaSSGF, indomethacin was initially released/solubilized but then precipitation was observed for all three lipid formulations. In 2010 Arnold et al. [34] suggested that simple dilution tests in aqueous

Figure 1.10 Appearance of the lipid formulation of RO-50X in the paddle apparatus after dissolution of the capsule shell. The lipid phase has been colored with Sudan red for visualization.

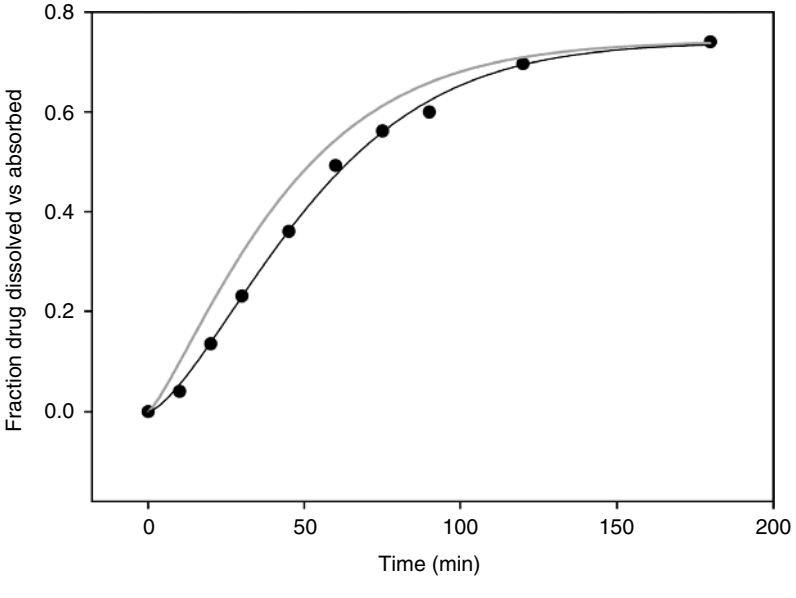

Figure 1.11 Level A *IVIVC* for a lipid-based formulation of RO-50X, based on dissolution results in the Biodis apparatus using biorelevant media. The solid gray line is the simulation using the dissolution results, and the black circles are the *in vivo* data from dogs. *Source:* Reproduced with permission from [32].

buffer systems would be useful for characterizing lipid-based formulations according to their type of self-emulsification. It is also interesting to check the possibility of drug precipitation and the particle size of dispersion. The ability to keep the drug solubilized in combination with a small final particle size may indicate a viable lipid-based formulation. Dilution tests may therefore help to screen initial formulation candidates.

1.3.5 Effect of Supersaturation on *In Vivo* Performance

Over the last years there has been increased interest on characterization of the morphological form of the drug which precipitates during *in vitro* lipolysis tests, to enable better understanding of *in vivo* performance of lipid-based formulations [34–36].

As an example, Thomas et al. [37] investigated the *in vitro* performance using dynamic lipolysis (Copenhagen model) of two supersaturated self-nanoemulsifying drug delivery systems (SNEDDS) and a corresponding conventional SNEDDS containing the poorly soluble drug simvastatin. Briefly, in the lipolysis test an almost proportional increase of drug concentration in the aqueous phase of the lipolysis medium depending on the employed drug load was observed, resulting in the following ranking of drug concentrations: 1 g of SuperSNEDDS with 200% drug load, >2 g SNEDDS with 75% drug load, >1 g SuperSNEDDS with 150% drug load, >1 g of SNEDDS with 75% drug load relative to the drug solubility in the formulation. The SuperSNEDDS with 200% drug load led to a pronounced supersaturation of simvastatin within the first 30 minutes, but then crashed rapidly out. A characterization of this precipitate by polarized light microscope and x-ray powder diffraction on the one hand and a significantly faster *in vitro* redissolution of the precipitate pellet comparing to crystalline drug on the other hand indicated an amorphous solid state of the drug.

In the next step, the bioavailability of these formulations was investigated in dogs (one capsule SNEDDS 75% vs. two capsules SNEDDS 75% vs. one capsule SuperSNEDDS 150%). The relative bioavailabilities of the conventional SNEDDS were comparable, whereas the superSNEDDS showed a much higher bioavailability (180% relative to the one capsule SNEDDS).

The authors see two potential explanations for this difference between the application of two capsules SNEDDS 75% drug load and one capsule SuperSNEDDS 150% drug load despite the equivalent dosing. One is attributed to an immediate drug release of SuperSNEDDS leading to a saturation of intestinal metabolic enzymes and therefore reduction of pre-systematic metabolism of simvastatin. The other hypothesis to explain the higher bioavailability of the SuperSNEDDS connects back to the *in vitro* lipolysis results: *in vitro* the drug precipitates out of the SuperSNEDDS in an amorphous form. If this also occurs *in vivo*, it could result in a continuous re-dissolution of drug throughout the intestine, also in distal regions, where a reduced pre-systematic metabolism is expected.

1.4 Conclusions and Future Directions

Lipid formulations represent a diverse array of compositions and therefore there is obviously no “one size fits all” approach to designing release tests for this group of formulations. Rather, one needs to take into account the composition of the vehicle and

assess to what extent digestion will play a role in release and to what extent the formulation might be expected to release the drug into the micellar phase. The recent suggestion (see section 2.4) that drugs might be directly absorbed from contact between the dosage form and the gut wall, instead of having to go through the aqueous phase, opens up a new way of thinking about how lipid-based dosage forms work and also about how they should be appropriately tested *in vitro*. Although it has been generally accepted that digestion is needed to model the *in vivo* release from Type I and II dosage forms (see Table 1.3), this paradigm might be replaced to some extent by a partitioning mechanism if indeed the drug can be directly absorbed from the vehicle. In addition, it needs to be considered whether the drug is entirely dissolved in the dosage form, or whether it is partly suspended, as this seems to have some bearing on which type of test to perform. The case example of 3.2, in which a BioDis method sufficed to model the *in vivo* performance, without having to describe digestion, even though the drug was formulated in a Type II formulation, illustrates this point. It also brings out the need to adequately simulate the *in vivo* hydrodynamics if a Level A correlation is to be established – and no doubt this is a key point for many other lipid-based formulations as well as this particular example.

As yet, from a pharmacokinetic perspective the question of the importance of digestion to drug release from lipid-based dosage forms thus remains unresolved. Perhaps the best way forward is to compare release with and without lipolysis for a range of formulations and determine for each Type of lipid-based formulation whether or not digestion makes a difference to the prediction of *in vivo* profiles. If such an analysis were to be carried out, a decision tree recommending the release test to be performed depending on the composition of the formulation could be developed. This, in turn, would streamline the development of lipid-based formulations for oral use.

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