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Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system☆

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ABSTRACT

Poorly water-soluble drug candidates often emerge from contemporary drug discovery programs, and present formulators with considerable technical challenges. The absorption of such compounds when presented in the crystalline state to the gastrointestinal tract is typically dissolution rate-limited, and the drugs are typically BCS class II or class IV compounds. Class IV compounds, which have low membrane permeability as well as poor aqueous solubility, are often poor candidates for development, unless the dose is expected to be low. The rate and extent of absorption of class II compounds is highly dependent on the performance of the formulated product. These drugs can be successfully formulated for oral administration, but care needs to be taken with formulation design to ensure consistent bioavailability. Essentially the options available involve either reduction of particle size (of crystalline drug) or formulation of the drug in solution, as an amorphous system or lipid formulation. The performance of amorphous or lipid formulations is dependent on their interaction with the contents of the gastrointestinal tract, therefore, a formulation exercise should involve the use of techniques which can predict the influence of gut physiology. A major consideration is the fate of metastable supersaturated solutions of drug, which are formed typically after dispersion of the formulation and its exposure to gastrointestinal digestion. A better understanding of the factors which affect drug crystallization is required, and the introduction of standardised predictive *in vitro* tests would be valuable. Although many bioavailability studies have been performed with poorly water-soluble drugs, thus far this research field has lacked a systematic approach. The use of a lipid formulation classification system combined with appropriate *in vitro* tests will help to establish a database for *in vitro–in vivo* correlation studies.

1. Introduction: opportunities and challenges

There is general consensus in the pharmaceutical industry that poorly water-soluble drug candidates are becoming more prevalent (Lipinski et al., 1997; Lipinski, 2000). If a drug candidate has reasonable membrane permeability then often the rate-limiting process of absorption is the drug dissolution step. This is characteristic of compounds which can be categorised as biopharmaceutical classification system (BCS) class II (Yu et al., 2002). Formulation plays a major role in determining

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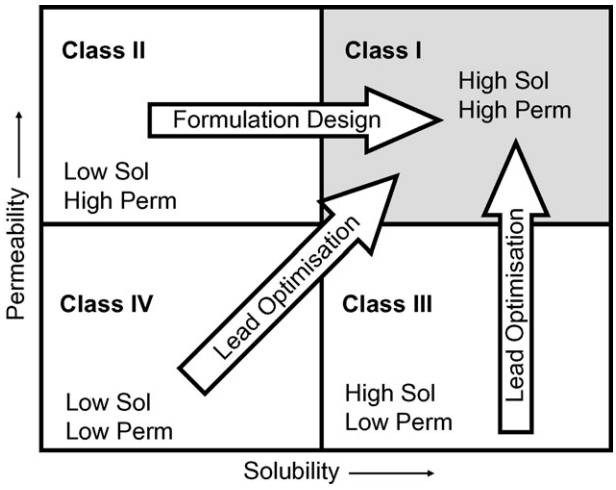

Fig. 1 – A typical representation of the biopharmaceutical classification system indicating that absorption of a class II drug can be markedly improved by attention to the formulation. If a class II drug can be maintained in a solubilized state in the lumen of the gut one can achieve an absorption profile more like that of a class I drug. Formulation strategies can do little to improve the absorption of classes I and III drugs which are limited by poor membrane permeability. These are candidates for improvement at the chemical level (i.e. lead optimisation).

the rate and extent of absorption of such drugs from the gastrointestinal tract. When water-solubility is less than 1 μg/ml, which is often the case for contemporary drug candidates, the bioavailability from conventional tablet formulations may be unacceptable. There are a number of formulation strategies that could be used to improve the bioavailability of class II drugs, either by increasing the dissolution rate or by presenting the drug in solution and maintaining the drug in solution in the intestinal lumen. Hydrophobic drugs which have poor membrane permeability as well as poor solubility, are categorised as BCS class IV drugs. Formulation may improve the bioavailability of class IV drugs but they are likely to be compromised by their poor membrane permeability. The most powerful approach to improvement of class IV drugs is to return to the lead optimisation phase of discovery and select a drug candidate with more appropriate physiochemical properties (Fig. 1).

The choice of formulation is often of critical importance to establishing a successful product for oral administration of a class II drug. If bioavailability of the drug is recognised to be formulation-dependent at an early stage it is desirable to have a strategy for maximising absorption as soon as possible. If poor formulations are used in early animal efficacy studies, the prediction of the likely human dose can be overestimated, possible compromising future development of the candidate drug. Use of a poor formulation in early toxicity studies can lead to an underestimation of the toxicity due to limited exposure resulting from low bioavailability.

In general terms the options for formulation of poorly water-soluble drugs include crystalline solid formulations, amorphous formulations and lipid formulations. The dissolution rate of drug from crystalline formulations can be increased by reducing the particle size and increasing the surface area for dissolution. Lipid formulations include simple solutions, self-emulsifying drug delivery systems (SEDDS), and systems which contain very little oil and disperse to form micellar solutions (Pouton, 2000). Amorphous formulations include 'solid solutions' which can be formed using a variety of technologies including spray drying and melt extrusion (Serajuddin, 1999; Sethia and Squillante, 2003; Kaushal et al., 2004). Amorphous formulations may include surfactants and polymers providing surface-activity during dispersion. Inclusion of surfactants may be useful to prevent a hydrophobic barrier forming on contact with water, or agglomeration of re-crystallized drug particles after dispersion. There is some overlap between the design of solid SEDDS and amorphous 'solid dispersions'. The long-term stability of the formulation is a critical issue in the design of such formulations. If the drug is not genuinely in solution, which is normally the case, then it must be immobilized in a metastable amorphous state long enough to give an adequate shelf-life. The recent adoption of melt-extrusion technology is an interesting development which makes possible continuous production without the need for organic solvents (Leuner and Dressman, 2000; Breitenbach, 2002). Table 1 provides a brief indication of the main formulation options and advantages and disadvantages of each approach.

Micronization using an air-jet mill has been used for many years to reduce the particle size of drug crystals. The equipment required for this approach is freely available and companies have complete freedom to operate with conventional milling equipment. This approach typically reduces particle size to 2–5 μm. The additional surface area may not deliver sufficient increase in dissolution rate to allow complete absorption during the 3–4 h of small intestinal transit. This led to the development of nanocrystal technology now owned by Elan, which makes use of unusually tough ball-milling media in aqueous suspension (Merisko-Liversidge et al., 1996, 2003). This technology can reduce crystalline particle size to 100–250 nm, providing a considerable increase in surface area and dissolution rate. The nanosuspension product is particularly valuable for early animal work. A secondary process, such as spray drying, is required to prepare the product for inclusion in a solid dosage form, and a strategy is required to reduce the likelihood of agglomeration of nanocrystals after disintegration of the dosage form. Nanocrystal technology is now firmly established with two products on the market and others in the pipeline. There are other alternatives to formation of nanocrystals by wet ball-milling. Dense gas technologies, typically using CO_2, have been used to produce nanoparticles. Harvesting the particles in a form which allows them to be processed down-stream will be the key to success with dense gas technology.

What distinguishes crystalline formulations from amorphous or lipid formulations is that crystalline drug is in a stable state in the formulated product (although it may not be the most thermodynamically stable polymorph), and will remain in a physically stable state throughout the dissolution phase in the gut lumen. In contrast amorphous products are clearly metastable and lipid systems potentially could be metastable within the formulated product. In addition when these prod-

Table 1 – Options for formulation of poorly water-soluble drugs

Technology	Potential advantage	Potential disadvantage
Conventional micronization	Known technology, freedom to operate, solid dosage form	Insufficient improvement in dissolution rate
Nanocrystals obtained by ball-milling	Established products on the market, experienced technology provider (Elan), solid dosage form possible	Available only under license, secondary process required to avoid aggregation of nanocrystals
Nanocrystals obtained by dense gas technology	Alternative nanocrystal processing method, still room to develop new IP	Unproven technology, secondary process required to avoid aggregation of nanocrystals
'Solid solutions'—drug immobilized in polymer	Freedom to operate, new extrusion technology offers solvent-free continuous process	Physical stability of product questionable—drug or polymer may crystallize
Self-dispersing 'solid solutions' with surfactants	Steric hindrance to aggregation built into product, amenable to extrusion	Physical stability of product questionable—drug or polymer may crystallize
Lipid solutions (LFCS Type I lipid systems)	Freedom to operate, safe and effective for lipophilic actives, drug is presented in solution avoiding the dissolution step	Limited to highly lipophilic or very potent drugs, requires encapsulation
Self-emulsifying drug delivery systems (SEDDS) and SMEDDS (LCFS Type II or Type III lipid systems)	Prior art available, dispersion leads to rapid absorption and reduced variability, absorption not dependent on digestion	Surfactant may be poorly tolerated in chronic use, soft gel or hard gel capsule can be used in principle but seal must be effective
Solid or semi-solid SEDDS	Could be prepared as a free flowing powder or compressed into tablet form	Surfactant may be poorly tolerated in chronic use, reduced problem of capsule leakage, physical stability of product questionable—drug or polymer may crystallize
Surfactant-cosolvent systems (LFCS Type IV 'lipid' systems)	Relatively high solvent capacity for typical APIs	Surfactant may be poorly tolerated in chronic use, significant threat of drug precipitation on dilution

ucts disperse in an aqueous phase such as the stomach contents, the drug may be present as a supersaturated solution, at least until it can be solubilized within the contents of the intestinal lumen. There is a risk of drug precipitation which is undesirable. Understanding the physiological and physicochemical phenomena which control the fate of the drug after administration presents both challenges and opportunities. The scientific literature is limited in this area and needs to be developed. From a practical viewpoint the industry needs standard, predictive *in vitro* tests which will help formulators choose the optimum formulation for each drug.

2. Dispersion, digestion and metastability

Many amorphous solid solutions or lipid systems contain a considerable proportion of water-soluble excipients, such as water-soluble polymers (e.g. PVP, PEG), low molecular weight cosolvents (ethanol, propylene glycol, PEG 400, transcutol, etc.) or hydrophilic surfactants (polysorbate 80, ethoxylated triglycerides, etc.). The polymers and cosolvents are particularly prone to causing drug precipitation on dilution because the relationship between drug solubility and cosolvent concentration commonly approximates to a logarithmic relationship. If the formulation comprises drug dissolved in a pure cosolvent, the vast majority of the drug will precipitate very quickly in the stomach. In contrast the relationship between drug solubility and concentration of hydrophilic surfactants is linear above the critical micelle concentration. Surfactants are less prone to lose solvent capacity on dilution in water. In practice formulations usually contain a mixture of polymers, cosolvents and surfactants. The proportion of these components will determine the likelihood of drug precipitation, and this can be very sensitive to minor modifications. Unless the product is enteric-coated, the initial dispersion process would normally take place in the stomach, in the absence of any additional solubilising capacity. There are bile salt mixed micellar systems in the intestinal lumen which will enhance solubilization of the drug, but what is difficult to establish is whether gastric emptying will occur quickly enough to allow the drug to be maintained in solution until it makes contact with mixed micelles in the intestine. Ideally the drug needs to be maintained in a metastable state in a simple aqueous dispersion for perhaps a few hours to allow enough time for gastric emptying to occur before the drug precipitates.

Fig. 2 illustrates the digestion of dietary triglyceride in the small intestine. Pancreatic lipase acts at the oil–water interface and the degradation products are solubilized by bile, secreted from the gall bladder, which is comprised of bile salt, lecithin and cholesterol (molar ratio typically 16:4:1). If a lipophilic drug arrives in the intestine in a supersaturated state it is quite possible that the drug solution can be maintained and stabilised by uptake of drug into mixed bile salt micelles.

3. Digestion and solubilization in the small intestine

The solubilization capacity of the digestive system is considerable and its presence has an effect on the dissolution and absorption of lipophilic drugs from all formulations (Embleton and Pouton, 1997). The solubilising power is greater after a fatty meal, hence, food often has a positive effect on bioavail-

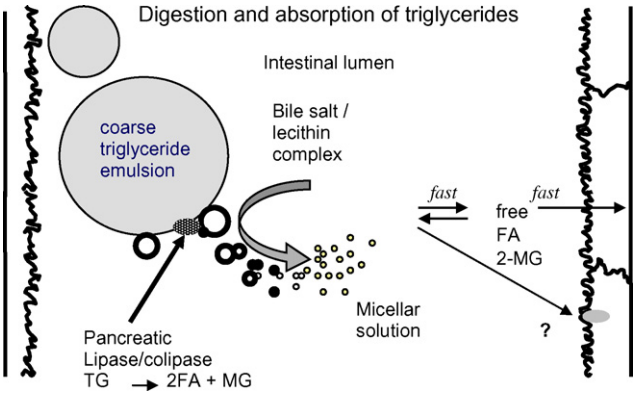

Fig. 2 – A schematic representation of the role of lipase/colipase and mixed bile salt micelles in digestion of triglycerides and solubilization of the digestion products. Each triglyceride molecule gives rise to two fatty acid molecules and a 2-monoglyceride which is solubilized in the lumen of the gut. The absorption step is less well understood and may involve partitioning into the aqueous phase as well as more direct lipophilic routes by way of mixed micellar diffusion.

ability of dissolution rate-limited BCS class II drugs. Bile salt concentrations in the gut are 3–5 mM on a fasted stomach and approximately 15 mM after food. Formulae for simulation of intestinal fluids in a fasted subject (FaSSIF) and fed subject (FeSSIF) have been used as alternative dissolution media (Dressman and Reppas, 2000), indicating that the dissolution of lipophilic drugs from conventional tablets is correlated with the availability of bile salt micelles (see, for example Fig. 3).

A well-designed amorphous or lipid formulation presents the drug as a molecular dispersion, so the corresponding issue is whether the drug can be transferred to the mixed micellar

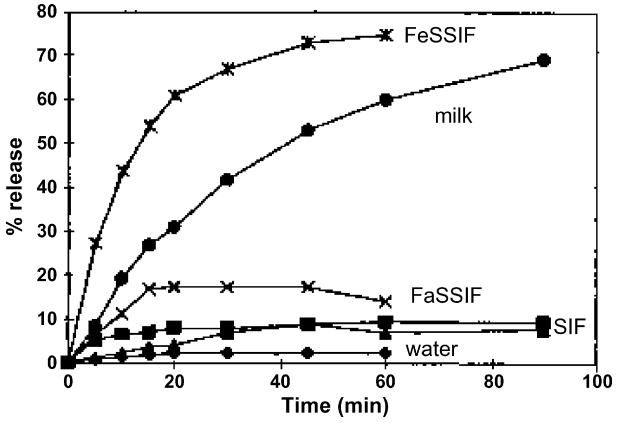

Fig. 3 – Dissolution profiles of Romazin tablets (troglitazone 200 mg) from Nicolaides et al. (1999). An example of how the rate and extent of dissolution from solid dosage forms is influenced by the likely components in the gut lumen. The presence of bile salt micelles in FaSSIF and FeSSIF increase both rate and extent. The higher concentrations of bile salt micelles in FeSSIF, representing the fed intestine, have a profound effect.

system as the formulation is diluted into the aqueous phase. The surfactant components would be expected to interact with mixed bile salt micelles, which may result in a change in their structure and solubilization capacity. There are a few publications which shed light on the structures that result from interaction of non-ionic surfactants with mixed micelles (Lim and Lawrence, 2004a,b), but a great deal more work is required in this area.

4. Amorphous formulations

Amorphous formulations, such as 'solid dispersions' may allow drugs to disperse as supersaturated solutions, at least temporarily, but eventually the drug will relax into its most thermodynamically favourable crystalline state. At present the kinetics of crystallization cannot be predicted for an individual drug, which presents the formulator with some technical problems. In some cases crystallization takes place in minutes but in others the supersaturated system may be stable for many hours. It would be useful to establish an *in vitro* protocol which would serve to predict the fate of the formulation in the gut (see below).

Amorphous formulations are rarely eutectic mixtures and therefore are usually metastable in the solid state. This aspect of formulation has been studied in more detail (Kaushal et al., 2004). A stable system results when the drug is immobilized in a rigid polymer, preferably well below the glass transition temperature of the formulation. Solvent-based methods of preparation, such as casting, or spray-drying have been used to prepare solid solutions for laboratory use. These are not attractive manufacturing methods because residual solvent presents a safety and regulatory problem. The recent introduction of melt extrusion technology is likely to overcome the problem of residual solvents. Melt extrusion can be a continuous process and equipment is available which would allow solvent-free manufacture at temperatures above the relevant T_g (Breitenbach, 2002). The inclusion of surfactants in the polymer-based formulation may help to prevent precipitation and/or protect a fine crystalline precipitate from agglomerating into much larger hydrophobic particles. Amorphous products based on hydrophilic polymers are likely to result in precipitation of drug particles, because the solvent capacity of the diluted polymer solution for a class II drug is usually very limited. However, if the particles which precipitate are submicron in diameter then the result may be analogous to presenting the drug as a nanocrystalline product.

Dense gas technology offers the opportunity to process drug and polymer in an inert solvent resulting in the formation of amorphous particles. This approach could be an alternative to melt extrusion in the future.

5. 'Lipid' formulations

'Lipid' systems have the advantage that they can present the drug as a stable liquid solution, but the term 'lipid formulation' has come to mean one of a large group of formulations which share some common features (Table 1). Lipid

Table 2 – The proposed lipid formulation classification system (LFCS) showing typical composition of various types of lipid formulations

Excipients in formulation	Content of formulation (%, w/w)				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils: triglycerides or mixed mono and diglycerides	100	40–80	40–80	<20	–
Water-insoluble surfactants (HLB < 12)	–	20–60	–	–	0–20
Water-soluble surfactants (HLB > 12)	–	–	20–40	20–50	30–80
Hydrophilic cosolvents (e.g. PEG, proylene glycol, transcutol)	–	–	0–40	20–50	0–50

systems may include triglycerides, mono and diglycerides, lipophilic surfactants, hydrophilic surfactants and cosolvents; excipients with a wide variety of physicochemical properties. A classification system was introduced in 2000 to help identify the critical performance characteristics of lipid systems (Pouton, 2000). Table 2 is an updated version of what could reasonably be called the lipid formulation classification system (LFCS). Briefly Type I formulations are oils which require to be digested, Type II formulations are water-insoluble self-emulsifying drug delivery systems (SEDDS), Type III systems are SEDDS or self-microemulsifying drug delivery systems (SMEDDS) which contain some water-soluble surfactants and/or cosolvents (Type IIIA) or a greater proportion of water-soluble components (Type IIIB).

Table 2 includes an additional category (Type IV) to represent the recent trend towards formulations which contain predominantly hydrophilic surfactants and cosolvents. Type IV formulations contain no oils and represent the most extremely hydrophilic formulations. The advantage of blending a surfactant with a cosolvent to give a Type IV formulation is that the surfactant offers much greater good solvent capacity on dilution (as a micellar solution) than the cosolvent alone. The cosolvent is useful to facilitate dispersion of the surfactant, which is likely to reduce variability and irritancy caused by high local concentrations of surfactant. A Type IV formulation is useful for drugs which are hydrophobic but not lipophilic, though it is necessary to bear in mind that Type IV formulations may not be well-tolerated if the drug is to be used on a chronic basis. An example of a Type IV formulation is the current capsule formulation of the HIV protease inhibitor amprenavir (Agenerase, GSK) (Strickley, 2004). For this clinical indication the benefit clearly outweighs the risk. The general characteristics, advantages and disadvantages of each type of lipid formulation are shown in Table 3.

The performance of lipid formulations, and the fate of the drug in the gastrointestinal tract, depend on the physical changes that occur on dispersion and dilution of the formulation, and the influence of digestion on drug solubilization. The main advantage of lipid formulation is that the drug could remain in solution throughout its period in the gastrointestinal tract. If precipitation occurs at any stage the advantage of a lipid formulation is lost. Precipitation of drug is more prevalent from lipid systems which contain more hydrophilic excipients. Care is needed with formulation because such excipients are often used to improve the solvent capacity of the formulation, to increase the dose that can be administered in a single capsule.

Fig. 4 shows a typical example of the phase changes that can occur on dilution of a Type III lipid formulation. Fig. 4 represents a simple but effective formulation comprising medium chain monoglyceride and polysorbate 80 (Mohsin et al.). The ternary phase diagram illustrates the phase changes which occur when these two excipients are combined with water. Representative formulations are shown as filled circles on the MCMG/P80 axis. The lines drawn from these points represent the pathway of dilution as each formulation undergoes self-emulsification to form an o/w emulsion or microemulsion.

The large L2 region is a transparent liquid which takes up a large mass of water during dilution without inducing any detectable phase separation. The anhydrous formulations are clearly L2 (isotropic oily solution) but as water is taken up the mixture must undergo structural changes as shown in Fig. 5. Small amounts of water will be incorporated into inverse micelles and later w/o microemulsions. But as the systems takes up almost its own mass in water it is likely that the mixture becomes 'bicontinuous'. As these changes occur the drug may migrate into water-rich or oil-rich areas or alternatively become adsorbed at the interface between oil and water

Table 3 – Characteristic features, advantages and disadvantages of the various types of 'lipid' formulations

LFCS type	Characteristics	Advantages	Disadvantages
Type I	Non-dispersing; requires digestion	GRAS status; simple; excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	SEDDS without water-soluble components	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particle size 0.25–2 μm)
Type IIIA	SEDDS/SMEDDS with water-soluble components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
Type IIIB	SMEDDS with water-soluble components and low oil content	Clear dispersion; drug absorption without digestion	Likely loss of solvent capacity on dispersion
Type IV	Oil-free formulation based on surfactants and cosolvents	Good solvent capacity for many drugs; disperses to micellar solution	Loss of solvent capacity on dispersion; may not be digestible

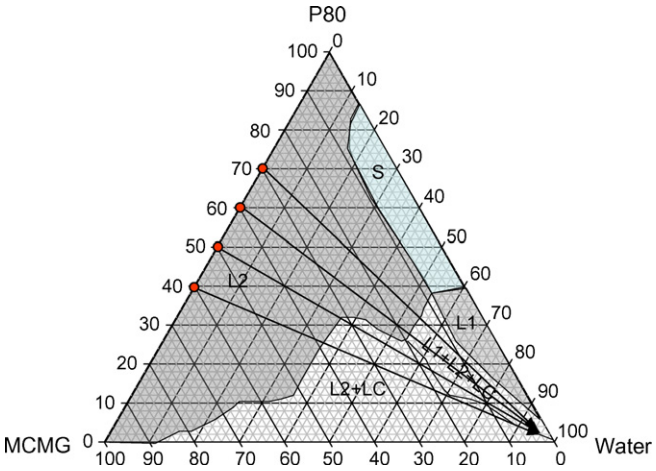

Fig. 4 – Phase behaviour of medium chain monoglyceride 98% purity (MCMG)/polysorbate 80 (P80) system on dilution with water at 20 °C. Key: L2—oil continuous phase, L1—water continuous phase, L1 + L2—two-phase (emulsion), L2 + LC—two phase mixture of oil-rich phase and liquid crystalline phase, S—surfactant-rich semi-solid gel or LC. The straight lines drawn towards the water apex show the course of dilution of anhydrous self-dispersing formulations (Mohsin et al., 2006).

regions. The fate of the drug on dilution is likely to depend on its location during dilution.

One way to investigate the possible fate of a drug is to investigate its solubility in the L2 phase during dilution. Fig. 6 shows the equilibrium solubility of a lipophilic drug in the system shown in Fig. 4. The solubility drops to approximately half its solubility in the anhydrous system when only 10% water has been added (Mohsin et al.). This might indicate that the drug should precipitate when the formulation is diluted, but its fate will depend on the kinetics of crystallization under the conditions present in the gut.

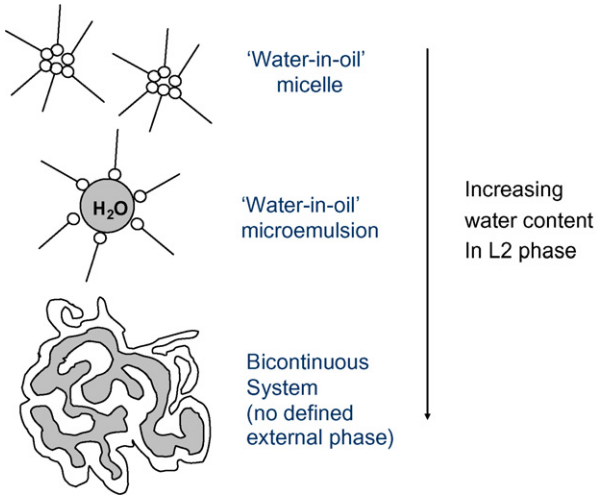

Fig. 5 – Potential structures within the 'L2' phase region. These phases could potentially be formed during dispersion of a medium chain monoglyceride/polysorbate 80 formulation.

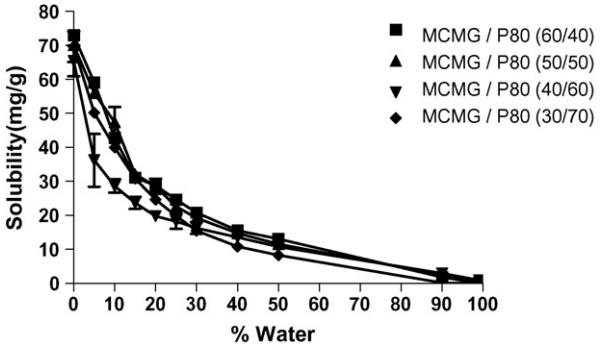

Fig. 6 – Equilibrium solubility of a lipophilic drug in medium chain monoglyceride 98% purity (MCMG)/polysorbate 80 (P80) formulations diluted with water (Mohsin et al., 2006).

6. *In vitro* testing of lipid formulations

In vitro tests can be used to help predict the effect of dilution and digestion, and it would be appropriate for standard tests to be defined for regulatory use. There are a number of useful tests available to the formulator. These include bile salt solubility tests, formulation dispersion/ drug precipitation tests, and *in vitro* digestion tests. Bile salt mixed micelle solubility is easy to determine and can give a good indication of whether the solubilising capacity of the gut will be of benefit to absorption of the drug (Naylor et al., 1995; Wiedmann et al., 2002).

The USP dissolution apparatus is suitable for the establishment of a dispersion test, but emphasis should be on precipitation rather than dissolution. Providing the lipid formulation is a good self-emulsifying system the drug will be rapidly dispersed in simulated gastric fluid in the vessel. If there is interest in an enteric-coated system then dissolution could be carried out in FeSSIF or FaSSIF as appropriate. The question is whether the drug remains in solution and for how long. The general method for assay is analogous to dissolution testing, i.e. samples need to be removed from the vessel at various times and either quickly filtered or centrifuged to remove any crystalline material before assay. Sampling should continue for approximately 24 h to determine the likelihood of precipitation during gastrointestinal transit.

In order to predict whether precipitation is likely to occur it is possible to examine the equilibrium solubility of the drug in components of the formulation after dilution, carry out corresponding dynamic dispersion/precipitation tests, and then investigate correlations between the two experiments. This procedure was carried out with dimethyl yellow (DY), a model weak base, in relation to its formulation in a variety of Type II and Type III formulations (Hasan et al.). DY was soluble in excess of 40 mg/g in various excipients sued in the formulations, including castor oil ethoxylates (Cremophors), medium chain triglyceride, mixed mono- and diglycerides, and cosolvents. Initial experiments were conducted to allow prediction of the maximum equilibrium solubility of DY in 1% (w/v) aqueous solutions of water-soluble excipients. The significance of this was that 1% solutions would be formed after a 1 in 100 dilution of 1 g formulated product. These experiments estab-

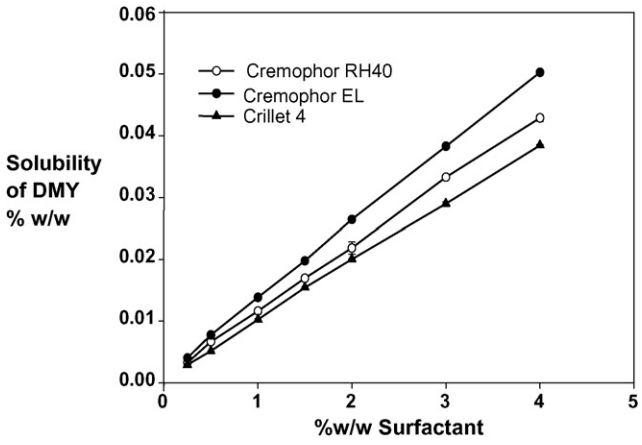

Fig. 7 – Equilibrium solubilities of dimethyl yellow (a model weak base) in aqueous surfactant solutions [Cremophor RH40 = hydrogenated castor oil 40 ethoxylate, Cremophor EL = castor oil 35 ethoxylate, Crillet 35 = polysorbate 80]. These surfactants have a similar solubilization capacity for dimethyl yellow indicating that approximately 10 mg dimethyl yellow would be solubilized in a 100 ml of a 1% solution of surfactant (Hasan et al., 2006). The significance of this is discussed in the text.

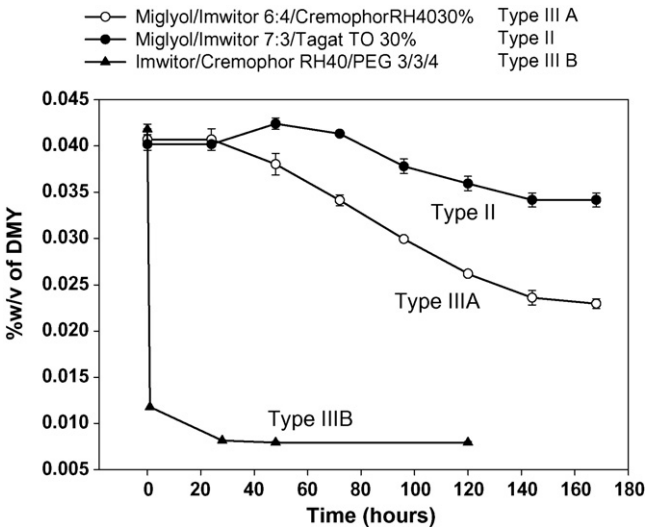

Fig. 8 – Concentration of dimethyl yellow in solution after dispersion of 1 g formulation containing 40 mg dimethyl yellow in 100 ml water. Crystallization occurred immediately after dispersion of the Type IIIB system but took up to a week after dispersion of a typical Type II system. When Type II or Type IIIA formulations were dispersed, negligible precipitation was observed during the first 24 h (Hasan et al., 2006).

lished that 100 ml of a 1% solution of pure cosolvents could only support 0.1 mg DY. Thus, if 40 mg DY was included in 1 g formulation of PEG or propylene glycol, 39.9 mg would precipitate on dilution. Fig. 7 shows the equilibrium solubility of DY in hydrophilic surfactant solutions, showing the expected linear dependence on surfactant concentration. Hundred milliliters of 1% surfactant solution was able to dissolve approximately 10 mg DY, indicating that a formulation of 40 mg DY in 1 g pure surfactant formulation could lose up to 30 mg by precipitation.

More conventional Type II and Type III lipid formulations disperse to produce o/w emulsions or microemulsions which would be expected to retain better solvent capacity for DY. Working to explore the general hypothesis that the oily components would retain solvent capacity for DY, dynamic dispersion tests were carried out on prototypical Type II, Type IIIA and Type IIIB formulations. The results of the dynamic precipitation tests are shown in Fig. 8 (Hasan et al.). When the DY remained in solution after 1 g formulation was diluted to 100 ml aqueous dispersion, the concentration in the aqueous phase was 0.04% (w/v). Fig. 8 indicates that the Type II and Type IIIA formulations retained the DY in solution for 24 h but there was a gradual precipitation of a proportion of the dose over the next few days. The Type II formulation was able to support more DY in solution than the Type IIIA formulation as the systems relaxed towards equilibrium, but both formulations would be expected to maintain DY in solution for long enough to support the drug in solution at least until the formulation is exposed to the digestive system in the small intestine. In contrast the more hydrophilic Type IIIB system was unable to support the DY in solution and approximately 75% of the dose precipitated within a few minutes. This result indicates that the Type IIIB formulation would be a poor choice for DY, and emphasises that care is needed in the formulation of lipid formulations to ensure that precipitation of the drug is minimised.

Fig. 8 indicates the value of the dispersion/precipitation test as a routine formulation tool. There is some merit in including particle size analysis as part of the dispersion/precipitation test (usually after a fixed dispersion time of perhaps 30 min), but this is not an essential requirement for formulation if the appropriate equipment is unavailable. It is easy to assess the quality of dispersion visually during the early stages of formulation. The product specification at a later stage could include the particle size of the dispersion for quality control purposes, often determined using a modern Fraunhofer diffraction instrument with capability of sizing from 0.1 to 100 μm.

The role of particle size in the performance of the formulation *in vivo* is generally less important than formulators have assumed. The main reason for this is that as soon as the dispersed formulation leaves the stomach it encounters the formidable digestive power of the small intestine. The fate of the drug after the formulation has been digested is a great deal more important than the initial particle size. Esters will be rapidly hydrolysed in the presence of pancreatic lipase and even the most commonly used surfactants (ethoxylated esters) are often rapidly hydrolysed. The physical state of the degradation products will be changed significantly by contact with the mixed bile salt micelles and the drug will partition between the various phases in the gut lumen, or could precipitate out if the total solvent capacity is reduced as a consequence of lipolysis.

Fortunately lipolysis can be carried out as an *in vitro* test using a pH-stat to maintain pH and using the lipase–colipase content of porcine pancreatin to serve as model for human

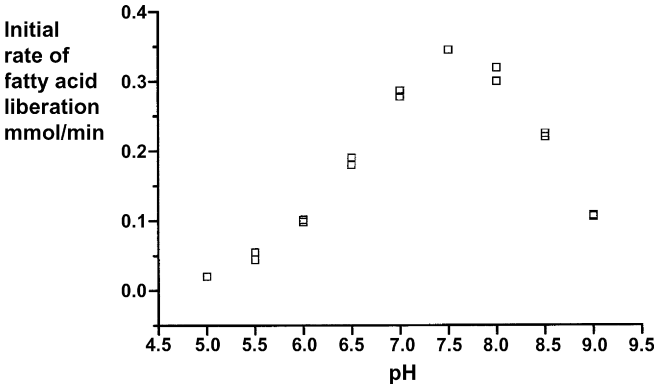

Fig. 9 – Effect of pH on the initial rate of lipolysis of medium chain triglyceride in the presence of pancreatin and simulated intestinal fluids containing bile (Solomon and Pouton, 2006).

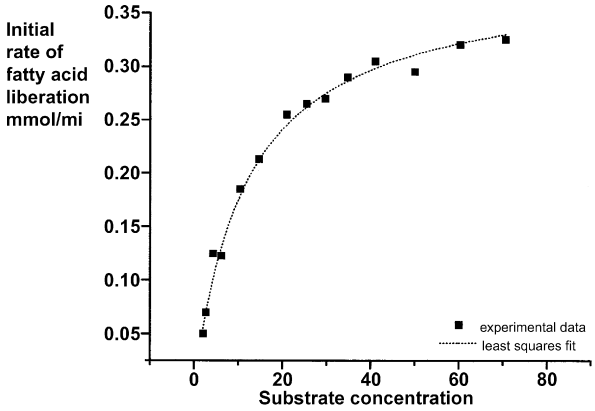

Fig. 10 – Pseudo Michaelis–Menten plot of data obtained during lipolysis of medium chain triglyceride in the presence of pancreatin and simulated intestinal fluids containing bile (Solomon and Pouton, 2006).

pancreatic juice. Bile salt-lecithin mixed micelles are added to the reaction mixture to provide a sink for solubilization of degradation products. Although lipolysis experiments have been used for many years by biochemists, the uptake of the technique by pharmaceutical scientists has been slow. Protocols for testing lipid formulations have now been documented in detail (MacGregor et al., 1997) and the technique has been used more commonly in recent years (Zangenberg et al., 2001a,b; Kaukonen et al., 2004; Porter et al., 2004). There is no standard protocol for *in vitro* lipolysis and given the number of parameters which define the conditions it would be valuable if a standard protocol was adopted in the near future. Before a standard protocol can be adopted there is a need for a more basic examination of how the parameters affect the lipolysis of various lipid formulations, and a need for a consensus to be reached by investigators from various laboratories.

One of the parameters which is required to be specified is the pH of the reaction mixture. The initial rate of lipolysis of medium chain triglycerides by porcine pancreatin is shown in Fig. 9 as a function of pH (Solomon, Pouton). The reaction proceeds quickly over the pH range 6.5–8.5, as would be expected for an intestinal enzyme, and the maximum rate occurred at pH 7.5. Most investigators have chosen to carry out experiments at pH 6.5, which is regarded as typical of the proximal small intestine. Fig. 9 suggests that the rate of hydrolysis of medium chain triglycerides proceeds at an adequate rate at pH 6.5 but it is not known how pH affects the lipolysis of more complex formulations, for example those which include surfactants. The effect of pH on the mixed micellar structures formed by fatty acids is also an issue that needs to be considered. Surprisingly, though lipolysis is an interfacial process, when the initial rate of lipolysis was plotted against substrate concentration, the plot conformed very closely to the Michaelis–Menton relationship (Fig. 10) (Solomon, Pouton). This relationship applies to enzyme reactions which are carried out in solution, and would not necessarily be expected to apply to lipolysis. In the case of the lipolysis of medium chain triglyceride this could be explained if the particle size of the triglyceride emulsion was unaffected by concentration. Each addition of lipid would then supply a quantum of surface area, analogous to a quantum of mass per unit volume which occurs when a soluble substrate is added. Fig. 10 indicates that *in vitro* lipolysis is a reliable and quantifiable technique which can certainly be adapted as a formulation and quality control tool.

In vitro lipolysis is useful for two specific purposes. Firstly the data generated from the pH-stat can be used to quantify the rate and extent of lipolysis, and is useful to establish how these parameters are affected by the formulation. Secondly the products of lipolysis can be examined after the reaction has been terminated, to determine the fate of the drug after lipolysis; whether the drug is solubilized or precipitated. The typical protocol for such an experiment is shown in Fig. 11. Lipolysis is allowed to proceed for a fixed time, the reaction is then subjected to ultracentrifugation, and the drug content in each of the phases is analysed usually by HPLC. If the drug is partially precipitated then drug will be found in the pellet. This technique has been used to great effect recently to predict the effect of formulation on the fate of a series of drugs (Kaukonen et al., 2004). There are indications that the bioavailability of some drugs, at least in fasted dogs, is sensitive to whether the drug is formulated in medium or long-chain triglycerides. Lipolysis experiments will play a vital role in the future for establishing formulations for *in vivo* studies and for establishing methods for *in vitro–in vivo* correlations.

There are specific factors which need to be considered in relation to the bioavailability of weak bases. Whatever formulation is used, weak bases may be dissolved in the acid contents of the stomach and later could precipitated when the stomach contents are emptied into the higher pH environment of the intestine. The fact that a free base is presented in solution in a lipid system does not prevent the drug partitioning into the aqueous phase of the stomach. The fate of the drug after gastric emptying will depend on how rapidly it can be solubilized by the formulation or the intestinal mixed micelles. In recent years dissolution and gastric emptying experiments have been conducted which show that precipitation depends on the rate of gastric emptying (Kostewicz et al., 2004). When seed crystals of drug are established a catastrophic precipitation can occur in the intestine which would be expected

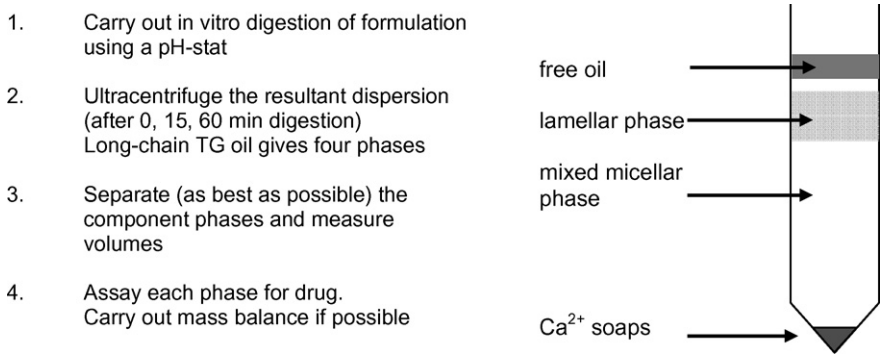

Fig. 11 – General method for *in vitro* simulation of the fate of drugs in the lumen of the intestine. Lipolysis is carried out for a fixed time and then the products are subjected to ultracentrifugation. Assay of drug in the various phases allows prediction of whether the drug will remain solubilized in the intestinal lumen after digestion of the formulation.

to have a profound effect on bioavailability. For this reason an additional *in vitro* test which simulates emptying would be valuable for formulation of weak bases.

7. Conclusions

The most significant issue to consider when formulating poorly water-soluble drugs is the threat of drug precipitation in the lumen of the gastrointestinal tract. The fate of the formulated product can be predicted using a range of *in vitro* tests to investigate the effects of dispersion, digestion, and gastric emptying on the fate of the drug. It would be useful to establish standard test protocols, particularly in the case of the lipolytic digestion test for lipid formulations, so that bioavailability data can be better understood and compared from laboratory to laboratory. The lipid formulation classification system (LFCS) provides a simple framework which can be used, in combination with appropriate *in vitro* tests, to predict how the fate of a drug is likely to be affected by formulation, and to optimise the choice of lipid formulation for a particular drug.

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