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Review

# Stable drug encapsulation in micelles and microemulsions

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**Abstract**

Oral absorption of hydrophobic drugs can be significantly improved using lipid-based non-particulate drug delivery systems, which avoid the dissolution step. Micellar and microemulsion systems, being the most dispersed of all, appear the most promising. While these systems show high drug entrapment and release under sink conditions, the improvement in oral drug bioavailability is often unpredictable. The formulation and drug-related biopharmaceutical aspects of these systems that govern oral absorption have been widely studied. Among these, preventing drug precipitation upon aqueous dilution could play a predominant role in many cases. Predictive ability and quick methods for assessment of such problems could be very useful to the formulators in selecting lead formulations. This review will attempt to summarize the research work that could be useful in developing these tools.


*Keywords:* Bioavailability; Hydrophobic drugs; Micelles; Microemulsions; Precipitation; SEDDS; SMEDDS; Self-emulsifying; Solubilization; Self-microemulsifying

## Contents



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## 1. Introduction

Oral liquid dosage forms are often required of new molecules, especially at the discovery and pre-clinical stages of drug development, and of existing molecules as a part of product life-cycle management. When permitted by the aqueous solubility and stability of the drug substance, a simple solution in water is preferred, e.g., Prozac® oral solution. More often, however, drug solubility (in relation to its required concentration) and stability are the limiting factors. Hydrophobic drugs may be formulated as emulsions and suspensions, e.g., Megace ES® suspension and Diprivan® emulsion. Drugs that show rapid degradation in aqueous media can be formulated as either powder for suspension, e.g., Augmentin®, Amoxil®, and Zegerid®; powder for solution, e.g., Zerit®; oily solution, e.g., Aquasol E® (Vitamin E) soft gelatin capsules; or oily suspension, e.g., Accutane® soft gelatin capsules. Hydrolysis-sensitive hydrophobic drugs may also be formulated as oily concentrates called self-emulsifying drug delivery systems (SEDDS) that form an emulsion upon addition of water or an aqueous solution with mild agitation, e.g., Sandimmune® oral solution.

Emulsions and suspensions allow the drug to be administered as a dispersed oil solution or as suspended particles, respectively. These dosage forms, however, have particulate nature and show phase separation upon storage due to their thermodynamic instability. In contrast, micelles and microemulsions do not show the physical instability in terms of agglomeration or separation of the dispersed phase. These systems also have lower dispersed phase size ($\leq$200 nm) than emulsions, giving them transparency. Also, these dosage forms allow the drug to be formulated as both ready-to-use aqueous solutions and as non-aqueous concentrates. The concentrate may be a solution, reverse micellar solution, or a microemulsion, which is diluted with water immediately before administration, or administered as it is and gets diluted with gastric fluids *in vivo*. In cases where they form transparent microemulsions upon dilution, the concentrates are known as the self-microemulsifying drug delivery systems (SMEDDS). SEDDS, SMEDDS, and micellar systems offer further advantage over conventional emulsions in the significantly reduced energy requirement for their preparation, such that simple mixing is enough for their formation. SEDDS and SMEDDS may also be administered as concentrates, e.g., in a soft gelatin capsule, and expected to form solubilized drug containing micelles or microemulsions *in vivo* upon dilution in stomach.

The use of SEDDS, SMEDDS, and micellar systems is limited by their drug loading capacity and the usage level of excipients. Surfactants and cosolvents can be toxic at high doses and may be limited in their daily and per-dose uptake levels. Formulators aim to develop systems with maximum drug loading capacity while using minimum possible amounts of surfactants and cosolvents. These limitations lead formulators to a limited range of compositions.

In addition, micelles and microemulsions can be metastable with respect to drug solubility and show drug precipitation upon dilution or crystallization over a period of storage. *In vivo* drug precipitation upon dilution in stomach can lead to failure in bioavailability enhancement and compromise the competitive advantage of this dosage form. *In vitro* drug crystallization in a micellar solution or microemulsion could be very slow and dependent on temperature and handling of the formulation. The ready-to-use formulations are expected to have a shelf life of at least 2 years, while concentrates (SEDDS and SMEDDS) are expected to be physically and chemically stable after reconstitution for the duration of the therapy or until administration.

Examples of commercialized SMEDDS formulations include cyclosporine (Neoral®), ritonavir (Norvir®), and saquinavir (Fortovase®) (Cooney et al., 1998, Porter and Charman, 2001). Very few SEDDS and SMEDDS formulations have been commercialized because of limitations in the usage level of excipients, e.g., surfactants and cosolvents, and the unpredictable improvement of oral bioavailability due to possibility of drug precipitation upon aqueous dilution *in vivo*. Predictive ability and quick methods for assessment of such problems could be very useful to the formulators in selecting lead formulations. This review will attempt to summarize the research work that could be useful in developing these tools.

### 1.1. Solutions, emulsions, microemulsions, and micelles

Simple aqueous drug solutions involve hydrogen-bonding and dipole interactions of drug molecules with the surrounding water. Hydrophobic drugs have low solubility because of lower capacity for these interactions. In such cases, the solute–solvent interactions can be qualitatively as well as quantitatively changed to improve the drug solubility. For example, pH can be adjusted with buffers to increase ionization of a weakly acidic or a weakly basic drug, resulting in higher ion-dipole solute–solvent interactions. Cosolvent addition reduces the dielectric constant of water and facilitates hydrophobic interactions of drug molecules with the solvent system. Solubility may also be increased by drug complexation with a hydrophilic compound, e.g., hydroxypropyl-β-cyclodextrin (HPBCD). Hydrophobic and/or specific ionic interactions lead to drug entrapment in HPBCD, which, in turn, is soluble in water. In addition, incorporation of amphiphilic surfactants in

aqueous solutions can solubilize hydrophobic drugs by different mechanisms.

Surfactants have both hydrophilic and lipophilic properties and are characterized by their hydrophile–lipophile balance (HLB) values. Surfactants with an HLB value >10 are predominantly hydrophilic and favor the formation of o/w emulsions, while surfactants with HLB values <10 are hydrophobic and form w/o emulsions. High HLB surfactants are used to form aqueous solutions or dispersions of hydrophobic drug molecules.

Surfactants in solution below their critical micellization concentration (CMC) improve drug solubility by providing regions for hydrophobic drug interactions in solution. Above the CMC, surfactants self-aggregate in defined orientation to form micelles with a hydrophobic core and a hydrophilic surface. The hydrophobic core enhances the entrapment of drug, thus increasing its solubility. In the presence of a significant amount of oil, surfactants concentrate on the oil/water interface forming emulsions, wherein the drug is solubilized in the internal oil phase. When the oil content is low, minute oil-entrapped surfactant globules are produced, which are known as swollen-micelles or microemulsions. Drug may be solubilized in the oily core and/or on the interface of these structures. The predominant location of drug solubilization depends on its hydrophobicity and interactions with the surfactant and/or cosurfactant. Microemulsions differ from micelles in the presence of oil and from emulsions in the amount of the dispersed phase. Furthermore, microemulsions often require a cosolvent and/or cosurfactant to facilitate their formation.

Both microemulsions and micelles are useful for preparing aqueous solutions of hydrophobic drugs. Several recent reviews have summarized physical and biopharmaceutical aspects of these systems (Constantinides, 1995; Flanagan and Singh, 2006; Gursoy and Benita, 2004; Pouton, 2000; Pouton, 1997; Lawrence and Rees, 2000; Humberstone and Charman, 1997). The physical nature of these systems, mechanism of drug entrapment, as well as the physicochemical interactions of constituents determine their drug solubilization capacity and physical stability during storage and upon dilution.

### *1.2. Components of micelles and microemulsions*

Pharmaceutical microemulsions are typically composed of oil and surfactant in water, and often also include a cosurfactant and/or a cosolvent. SMEDDS contain the non-aqueous components of microemulsions and readily disperse upon dilution in aqueous phase with mild agitation to form microemulsions. SMEDDS are often preferred over microemulsion formulations for hydrolytically sensitive drugs and their low volume enables packing into soft gelatin capsules for oral administration.

The surfactant used in microemulsion formation could be ionic or nonionic, which determines the stabilizing interactions of the hydrophilic end of the surfactant with the aqueous phase. Thus, while a nonionic surfactant is stabilized by dipole and hydrogen bond interactions with the hydration layer of water on its hydrophilic surface, an ionic surfactant is additionally stabilized by the electrical double layer. Thus, the effect of salt concentration on the stability of an emulsion or a microemulsion is more profound in the case of ionic surfactant than nonionic surfactants. Additionally, for pharmaceutical applications, ionic surfactants are not preferred due to toxicological concerns.

Microemulsions often include a cosurfactant. A cosurfactant is an amphiphilic molecule that substantially accumulates with the surfactant at the interfacial layer. Usually a very low HLB cosurfactant is used with a high HLB surfactant to modify the overall HLB of the system. Unlike surfactant, the cosurfactant may not be capable of forming self-associated structures like micelles on its own. Several kinds of molecules including nonionic surfactants and alcohols can function as cosurfactants in a given system. The quantity of a cosurfactant in a system is usually less than that of the surfactant and it often serves to modify the overall HLB value of the system.

Cosolvents are often included in microemulsion formulations to increase drug solubility by cosolvency and to stabilize the dispersed phase. In addition to making the environment more hydrophobic by reducing the dielectric constant of water, cosolvents increase the amount of molecularly dispersed surfactant in the aqueous phase. Availability of free surfactant aids in drug solubilization by creating pockets of hydrophobic regions within the aqueous phase. Examples of surfactants, cosurfactants, and cosolvents that have been used in commercial lipid-based products are listed in Table 1. In addition, various surfactants and cosurfactants have been listed with their HLB values, chemical classification, and commercial names in US patent application PCT/US00/32255. Also, Strickley has summarized the solubilizing excipients used in commercial formulations (Strickley, 2004).

Structurally, the dispersed phase of microemulsions consists of microstructures of oil-entrapped pockets stabilized by surfactant/cosurfactant accumulation on the oil/water boundary, similar to conventional emulsions. In addition, surfactant molecules self-associate to form micelles in the bulk phase. These structures coexist in equilibrium, with their relative abundance determined by the proportions of different components. In addition, the size and shape of oil molecules relative to the hydrophobic region of the surfactant determine the extent of oil entrapment in the surfactant layer.

Microemulsion formation is a function of composition of the system. The composition ranges with respect to the number of phases that exist in a system are graphically demonstrated as a phase diagram. A ternary phase diagram, with three corners of a triangle representing three components of a system, describes phase regions. A pseudo-ternary phase diagram is used for systems of more than three components, when the ratio of at least two of the components is kept constant and represented by one of the axis of the triangle. A hypothetical phase diagram of a three component system is presented in Fig. 1, representing oil, water, and emulsifier as the three phases of the triangle (Prince, 1975). At different concentrations of each component, macroemulsions or emulsions, micelles, or inverted micellar structures are formed. The $L_1$ and $L_2$ phases in these diagrams correspond to the normal and inverted micelles, and corresponding types of microemulsions, respectively. The microemulsions transition into each other with variation in composition through

Table 1
Examples of surfactants, cosurfactants, and cosolvents used in commercial lipid-based formulations

| Excipient name (commercial name) | Examples of commercial products in which it has been used |
|---|---|
| Surfactants/cosurfactants | |
| Polysorbate 20 (Tween 20) | Targretin soft gelatin capsule |
| Polysorbate 80 (Tween 80) | Gengraf hard gelatin capsule |
| Sorbitan monooleate (Span 80) | Gengraf hard gelatin capsule |
| Polyoxyl-35-castor oil (Cremophor EL) | Gengraf hard gelatin capsule, Ritonavir soft gelatin capsule |
| Polyoxyl-40-hydrogenated castor oil (Cremophor RH40) | Neoral soft gelatin capsule, Ritonavir oral solution |
| Polyoxyethylated glycerides (Labrafil M 2125Cs) | Sandimmune soft gelatin capsules |
| Polyoxyethylated oleic glycerides (Labrafil M 1944Cs) | Sandimmune oral solution |
| D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) | Agenerase soft gelatin capsule, Agenerase oral solution |
| Cosolvents | |
| Ethanol | Neoral soft gelatin capsule, Neoral oral solution, Gengraf hard gelatin capsule, Sandimmune soft gelatin capsule, Sandimmune oral solution |
| Glycerin | Neoral soft gelatin capsule, Sandimmune soft gelatin capsule |
| Propylene glycol | Neoral soft gelatin capsule, Neoral oral solution, Lamprene soft gelatin capsule, Agenerase soft gelatin capsule, Agenerase oral solution, Gengraf hard gelatin capsule |
| Polyethylene glycol | Targretin soft gelatin capsule, Gengraf hard gelatin capsule, Agenerase soft gelatin capsule, Agenerase oral solution |
| Lipid ingredients | |
| Corn oil mono-, di-, tri-glycerides | Neoral soft gelatin capsule, Neoral oral solution |
| DL-α-Tocopherol | Neoral oral solution, Fortovase soft gelatin capsule |
| Fractionated triglyceride of coconut oil (medium-chain triglyceride) | Rocaltrol soft gelatin capsule, Hectorol soft gelatin capsule |
| Fractionated triglyceride of palm seed oil (medium chain triglyceride) | Rocaltrol oral solution |
| Mixture of mono- and di-glycerides of caprylic/capric acid | Avodart soft gelatin capsule |
| Medium chain mono- and di-glycerides | Fortovase soft gelatin capsule |
| Corn oil | Sandimmune soft gelatin capsule, Depakene capsule |
| Olive oil | Sandimmune oral solution |
| Oleic acid | Ritonavir soft gelatin capsule, Norvir soft gelatin capsule |
| Sesame oil | Marinol soft gelatin capsule |
| Hydrogenated soybean oil | Accutane soft gelatin capsule, Vesanoid soft gelatin capsule |
| Hydrogenated vegetable oils | Accutane soft gelatin capsule, Vesanoid soft gelatin capsule |
| Soybean oil | Accutane soft gelatin capsule |
| Peanut oil | Prometrium soft gelatin capsule |
| Beeswax | Vesanoid soft gelatin capsule |

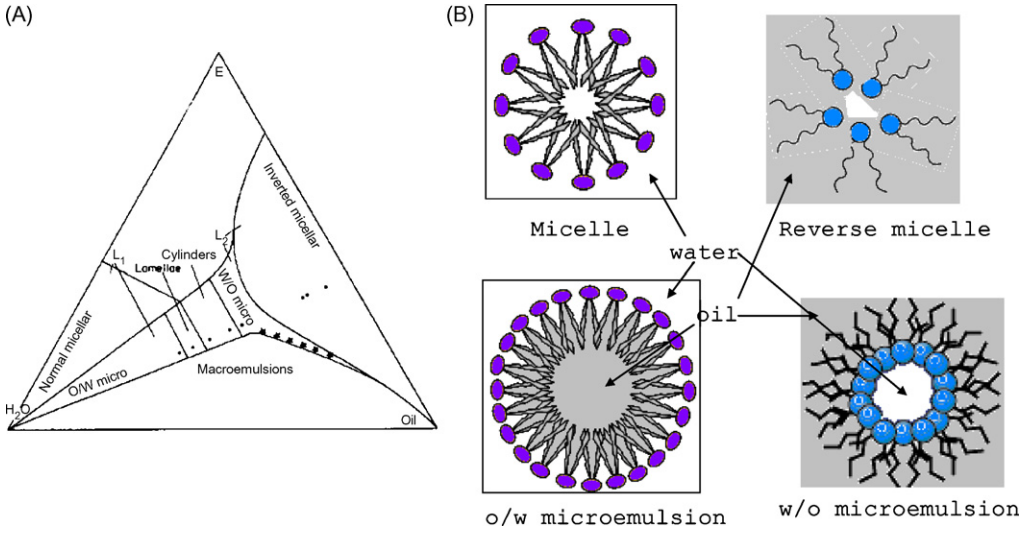


Fig. 1. (A) A hypothetical ternary phase diagram representing three components of the system (water, emulsifier (E), and oil) as three axis of an equilateral triangle. Different compositions of the formulation result in the formation of different phase structures: normal micellar solution, inverted micellar solution, macroemulsions or emulsions, o/w microemulsions, w/o microemulsions, and various transition phases represented by cylinders and lamellae structures. The conventionally designated $L_1$ phase consists of micelles and o/w microemulsions while the $L_2$ phase consists of inverted micelles and w/o microemulsions (Prince, 1975). (B) Schematic representation of the dispersed phase structure of micelles, reverse micelles, o/w microemulsions, and w/o microemulsions.

intermediate liquid crystalline phases, which are viscoelastic gels composed of hexagonal array of water cylinders adjacent to the w/o phase and a lamellar phase of swollen bimolecular leaflets adjacent to the o/w phase (Prince, 1975). These phases are characterized by the presence of birefringence, as opposed to microemulsion regions which are optically isotropic. Incorporation of cosurfactant and/or cosolvent increases the one-phase region. Construction of phase diagrams enables determination of aqueous dilutability and range of compositions that form a monophasic region.

### 1.3. Characterization of microemulsions

Characterization of reverse micelles, SMEDDS, and microemulsions involves the physical and chemical tests related to oral liquid dosage forms, e.g., assay, uniformity of content, stability of the active (impurities), appearance, pH, viscosity, density, conductivity, surface tension, size and zeta potential of the dispersed phase, etc. with respect to the effect of the composition on physical parameters (Podlogar et al., 2004). Additionally, differential scanning calorimetry (DSC) provides information on the interactions of different components and polarization microscopy using crossed polarizers is employed to confirm isotropicity of the formulation (Neubert et al., 2005). Size of the dispersed phase in o/w microemulsions has been measured by photon correlation spectroscopy (PCS) and total-intensity light scattering (TILS) techniques (Malcolmson et al., 2002). The use of scattering techniques, e.g., static light scattering (SLS), dynamic light scattering (DLS), and small-angle neutron scattering (SANS), for dispersed phase size measurement requires correction for non-ideality of the hard sphere model arising from interparticle interactions in concentrated microemulsions (Shukla et al., 2002; Shukla et al., 2003). Structural features of microemulsions have been studied using self-diffusion nuclear magnetic resonance (SD NMR) (Spernath et al., 2003; Johannessen et al., 2004) and small-angle X-ray scattering (SAXS) (Garti et al., 2006).

During the development of these systems, pseudo-ternary phase diagrams are constructed by titrating a reverse micelle mix with one of the components and observing visually for transparency and through crosspolarizers for optical isotropy (Moreno et al., 2003). Maintenance of monophasic characteristics and drug solubility is tested upon dilution with water. Phase stability of formed microemulsions is evaluated by accelerated tests such as centrifugation or freeze thaw cycles (Brime et al., 2002). Partitioning behavior of drug in the dispersed phase of these systems has been studied by electrokinetic chromatography (EKC) for both micelles (Ishihama et al., 1994) and microemulsions (Huie, 2006), and by gel permeation chromatography (GPC) in micelles (Scherlund et al., 2000). The log of capacity factor obtained by EKC of hydrophobic compounds in microemulsions correlated well with their octanol water partition coefficients (log $P$) (Mrestani et al., 1998). In addition, this dosage form is tested to evaluate the tendency for drug precipitation or crystallization by physical observation upon undisturbed storage at room temperature and refrigerated conditions, and upon dilution with water to form o/w microemulsions, which can be done by dropwise addition, static serial dilution, or dynamic injection (Li et al., 1998). Modified *in vitro* tests can be used for more accurate assessment of tendency for drug precipitation (Gao et al., 2004; Gao et al., 2003). Solubilization capacity of the drug is measured by saturation solubility evaluation in different components and component mixtures (Aramaki et al., 2001).

Drugs can be incorporated in microemulsions by the phase inversion temperature (PIT) method (Brime et al., 2002) and in SMEDDS by dissolving the drug in the hydrophilic or the hydrophobic component(s). The PIT method involves mixing drug solution with microemulsions and applying heat to form transparent drug loaded systems. In addition, drug release rate studies may be carried out, when desired, in Franz diffusion cell across the donor and acceptor compartments separated by a semipermeable membrane (Peltola et al., 2003; Spiclin et al., 2003) or using US Pharmacopeial methods for dissolution testing (Porter and Charman, 2001).

### 1.4. Drug entrapment and structure

Location of the solubilized drug in microemulsion systems depends on the hydrophobicity and structure of the solute. Enhanced drug solubility in microemulsion and micellar systems usually arises from the solubilization at the interface. The interface-associated solute, in turn, may affect the size and shape of the microemulsion droplets. For example, incorporation of hydrophobic amino acids in di-2-ethylhexyl sulfosuccinate (AOT) reverse micelles (Leodidis and Hatton, 1990a; Leodidis and Hatton, 1990b; Leodidis and Hatton, 1991a; Leodidis and Hatton, 1991b) and w/o microemulsions (Yano et al., 2000) leads to their association at the interface, and they may act as cosurfactants. Upon comparing the solubilization of glycine, L-histidine, and L-phenylalanine in AOT stabilized water-in-isooctane microemulsions, Yano et al. observed that hydrophilic amino acid glycine was solubilized primarily in the dispersed aqueous phase while hydrophobic amino acids, L-histidine and L-phenylalanine, migrated to the AOT interface layer (Yano et al., 2000). Furedi-Milhofer et al. obtained similar results with the solubilization of aspartame in water/isooctane/AOT microemulsions (Furedi-Milhofer et al., 2003). Aspartame was solubilized at the interface and resulted in a sharp reduction of surface tension depending on aspartame concentration, indicating its role as a cosurfactant.

The maximum amount of solubilized hydrophobic drug is dependent on the curvature of the interface. Surfactant layer on the interface has a positive curvature towards the dispersed phase, which is determined both by the relative volume of dispersed phase and the spontaneous curvature of surfactant molecules. Entrapment of drug molecules in the interface is facilitated, leading to higher drug loading capacity, if the spontaneous curvature is lower than the actual curvature. Higher spontaneous curvature, on the other hand, leads to lower drug loading capacity at the interface.

Partitioning of the drug into the interface was quantified by the interfacial partition coefficient by Leodidis and Hatton

(Leodidis and Hatton, 1990a). Using phase equilibrium analyses on the solubilization of amino acids in AOT reverse micelles, the authors showed that interfacial partition coefficient of the solute depended weakly on surfactant concentration and did not depend on solute concentration and aggregate geometry. It depended strongly on the factors that affect surface pressure or bending moment of the surface film, e.g., solvent type and external electrolyte type and concentration. Also, Testard and Zemb showed a general linear relationship between induced curvature variation and solute content of the interfacial film for a hydrophobic solute using nonionic surfactant based o/w microemulsions (Testard and Zemb, 1999).

These studies indicate that hydrophobic solute is solubilized at the interface of reverse micellar and microemulsion systems and its solubility is affected by system variables that affect the curvature of the interfacial film. Moreover, the presence of the solute itself affects the system, depending on the nature of the solute and the surfactant. The phenomenon of drug solubilization at the interface affects not only drug loading capacity but also drug precipitation upon dilution. For example, for a drug whose solubilization capacity at the interface has been increased with the use of a cosurfactant, dilution with aqueous phase leading to cosurfactant migration away from the interface can lead to dramatic reduction in drug loading capacity, causing precipitation.

### *1.5. Microemulsions for protein and peptide delivery*

Improvement in the oral bioavailability of hydrophobic cyclic peptides, like cyclosporine A, using SEDDS and SMEDDS is discussed in Section 3.1 and Section 4.3. SMEDDS systems have also shown promise in improving the oral bioavailability of hydrophilic linear peptides and proteins. For example, Cilek et al. tested the oral absorption of recombinant human insulin dissolved in the aqueous phase of w/o microemulsions composed of Labrafil®, lecithin, ethanol, and water in streptozotocin-induced diabetic male Wistar rats. The authors demonstrated significant improvement in oral pharmacological availability compared with insulin solution, although it was ~0.1% compared with sub-cutaneous administration (Cilek et al., 2005). On the other hand, Kraeling and Ritschel found that the oral pharmacological availability of insulin microemulsions as compared to intravenous insulin in beagle dogs was 2.1%, which further increased to 6.4% with the encapsulation of gelled microemulsions in hard gelatin capsules along with the protease inhibitor aprotinin and coating of the capsules for colonic release (Kraeling and Ritschel, 1992). Improved oral delivery of insulin from microemulsion system was also demonstrated by others (Cho and Flynn, 1989).

Improved oral bioavailability from the w/o microemulsion system was also shown for the linear water-soluble nonapeptide leuprolide acetate (Zheng and Fulu, 2006) and dipeptide *N*-acetylglucosaminyl-*N*-acetylmuramic acid (Lyons et al., 2000). Also, intra-gastric administration of w/o microemulsion of epidermal growth factor was more effective in healing acute gastric ulcers in rats as compared to both intra-peritoneal and intragastric aqueous solution administration (Celebi et al., 2002). The beneficial effects of microemulsions in these applications were attributed to the prevention of degradation in the gastrointestinal environment and the permeability enhancing effect of the lipid components.

Microemulsion systems have also been claimed to improve storage stability of proteins. For example, Owen and Yiv (US Patent #5,633,226) disclose improved chemical stability of horse radish peroxidase after storage in w/o microemulsions as compared to aqueous solution. In addition, w/o microemulsion-based media have been utilized for immobilization of water soluble enzymes, such as lipase, in the internal, dispersed aqueous phase for biocatalytic conversion of water-insoluble substrates in the outer non-aqueous layer (Schuleit and Luisi, 2001; Madamwar and Thakar, 2004). In a similar application of enhancing enzyme mediated catalysis of non-aqueous substrates, water soluble protein myoglobin was cross-linked to poly(L-lysine), which was in turn covalently attached to oxidized cathode, in an o/w microemulsion environment such that the protein was present in the water-rich external environment, while the reactant, styrene, was present in the internal oil-rich environment. Catalysis of epoxidation of styrene by myoglobin in this system was higher than aqueous solution, which increased further in the presence of bicontinuous microemulsion system (Vaze et al., 2004).

In all these applications hydrophilic peptides or proteins were dissolved in the aqueous phase at or below their solubility levels. This review, however, will focus on solubilization of hydrophobic molecules in SMEDDS and diluted o/w microemulsions while preventing physical instability of drug separation by crystallization on storage or precipitation upon aqueous dilution, with particular relevance to oral administration.

## 2. Drug loading capacity in micelles and microemulsions

Pharmaceutical micellar and microemulsion systems are usually formulated as oil + surfactant ± cosurfactant/cosolvent mixtures that exist as reverse micelles or w/o type microemulsions. These systems are diluted with water *in vivo* or before administration. Solubilization or drug loading capacity in these systems refers to the drug concentration achievable in reverse micelles and the ability of these systems to undergo aqueous dilution as monophasic systems.

Drug precipitation from a self-emulsifying drug delivery system is a consequence of concentration exceeding the equilibrium solubilization capacity. Consequently, systems formulated to have drug solubilization capacity much higher than the required concentration would be expected to show the least propensity for precipitation *in vivo*. Drug loading or solubilization capacity in the system also determines the minimum volume per unit dose that can be formulated. Thus, an understanding of factors influencing drug loading capacity while maintaining the capability of the system to undergo monophasic dilution with water and minimizing the tendency for drug precipitation or crystallization in diluted systems is essential to the design of stable and appropriately low-volume systems for drug delivery applications.

### 2.1. Solubilization capacity in reverse micelles

Micellar and microemulsion systems are often able to solubilize higher amount of drug than its individual components. For example, Spernath et al. reported that the solubility of lycopene, a hydrophobic carotenoid obtained from tomatoes, in the reverse micelles of (*R*)-(+)-limonene (limonene) and polysorbate 60 (Tween 60®) (4:6) was 2500 ppm, about three times higher than in either individual component (700 ppm in (*R*)-(+)-limonene and 800 ppm in Tween 60®) (Spernath et al., 2002). Higher solubilization capacity in reverse micellar systems was also noted for phytosterol, whose solubility was 150,000 ppm in the reverse micelles of limonene and Tween 60® (4:6), about six times higher than in either individual component (25,000 ppm in each) (Spernath et al., 2003). This higher capacity for solubilization was attributable to the interfacial locus of drug solubilization, which has higher solubilization capacity than the core. Higher solubilization capacity at the interface is a function of drug–surfactant interactions leading to drug association at the interface. These interactions depend on the hydrophobicity, functional groups, and shape of both the drug and the surfactant/cosurfactant. The shape influences sub-molecular proximity or fit of interacting molecules to maximize interactions. Thus, different excipients and different grades of similar excipients can show markedly different solubilization capacity for a given drug.

The solubilization capacity progressively decreases upon aqueous dilution, as the micellar system passes through swollen w/o reverse micelles, to bicontinuous phase, to o/w microemulsion system. This reduction in solubilization capacity is thought to be caused by the change in the locus of drug solubilization associated with microstructural transitions during aqueous dilution (Spernath et al., 2003). In addition, migration of water miscible cosurfactant away from the interface upon aqueous dilution could lead to reduced drug solubilization capacity at the interface. Evaluation of drug solubilization capacity at different dilution levels allows the formulator to define the appropriate dilution range for a given formulation with minimum likelihood of drug precipitation.

### 2.2. Dilutability as monophasic systems

An approach to improve the dilutability of drug containing surfactant/oil reverse micelles with aqueous phase is to expand the monophasic/isotropic region through a wide range of compositions. When the expanded isotropic region covers aqueous dilutability through a range of compositions with different water content, called ‘dilution line’, the systems so formed have been called dilutable U-type microemulsions. An example of the role of surfactant in determining the monophasic region and dilution line are represented in Fig. 2 (Spernath et al., 2006). The dilution line N73 in Fig. 2A represents 7:3 composition of the ethyl laurate/acetic acid (1:3) and phosphatidyl choline (PC)/Tween 80®/propylene glycol (PG) (1:3:10) axis in reverse micelles (in the absence of water). Upon progressive addition of water, the system progresses to the third axis of the phase diagram along the dilution line N73 through the monophasic region (Fig. 2A). Therefore, both the composition of the formulation and the area of the monophasic region are important to ensuring successful aqueous dilution without ‘breaking’ the microemulsions.

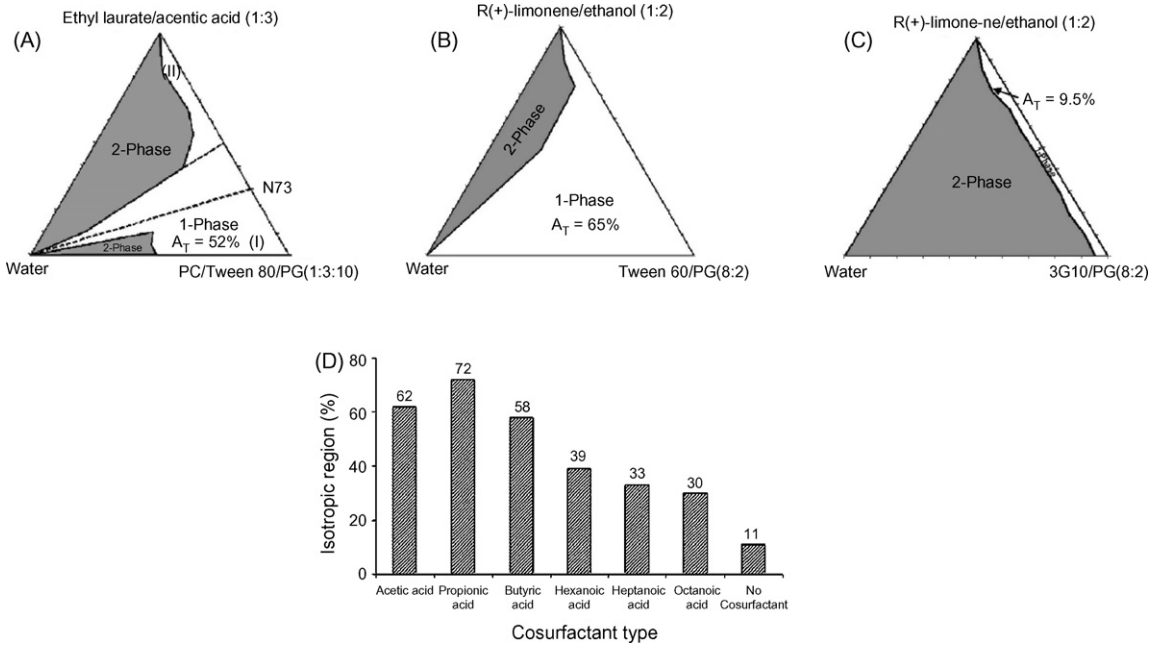


Fig. 2. Phase diagram of a 6-component system and factors influencing monophasic region. (A) demonstrates 1-phase and 2-phase regions of a ethyl laurate/acetic acid (1:3) system stabilized with mixed surfactants PC/HECO40/PG (1:3:10) and an aqueous dilution line N73 from the non-aqueous reverse micelles to the water axis. $A_T$ represents the percentage of monophasic region. (B) and (C) represent the influence of using Tween 60® (B) versus triglycerol monooleaste (C) on the monophasic region. (D) represents the variation in the percentage of isotropic or monophasic region with the use of different chain length acid surfactants (Spernath et al., 2006).

The role of HLB of the surfactant in determining the area of monophasic region is illustrated in an extreme case in Fig. 2B and C. The isotropic or single phase region of 5-component system composed of limonene, water, ethanol, propylene glycol, and Tween 60® (Fig. 2B) reduced significantly when the hydrophilic surfactant, Tween 60® (HLB 14.9), was replaced with a hydrophobic surfactant, triglycerol monooleate (HLB 6.2) (Fig. 2C) (Spernath et al., 2006). Aqueous dilution of reverse micelles of the latter system would invariably result in 'breaking' of the microemulsion system into two phases.

Certain formulation approaches can lead to increase in the monophasic region. Addition of polyols, e.g., glycerin and propylene glycol; short-chain alcohols, e.g., ethanol; and organic acids, e.g., propionic acid, increase the monophasic region of o/w microemulsions (Garti et al., 2001). These additives act as cosolvents, by promoting solubility of the drug in the bulk phase, and/or cosurfactants, by affecting interfacial structure and promoting drug solubility at the interface.

Aqueous dilutability of w/o reverse micellar or microemulsion systems proceeds through a series of structural changes from w/o to bicontinuous to o/w system, which concurrently involves changes in drug solubilization capacity. Factors affecting water solubilization capacity of w/o microemulsions before their breakdown into bicontinuous structures were reported by Hou and Shah (Hou and Shah, 1987). Addition of water to a w/o microemulsion system could result in water incorporation in the dispersed phase. The growth of microemulsion droplets without coalescence during this process is limited by either the radius of curvature of the interface or the attractive interactions among droplets (Hou and Shah, 1987). For the systems where solubilization capacity for water is limited by the curvature of the interfacial layer, reduction in spontaneous curvature by modification of the interface or the continuous phase can result in increased solubilization. For systems where solubilization capacity is limited by the critical droplet radius, reduction in attractive forces among droplets would increase the solubilization capacity of water (Hou and Shah, 1987). These principles provide useful insights to the analogous scenario of solubilization of hydrophobic solute in the dispersed phase of o/w microemulsions. Thus, incorporating components that increase the spontaneous curvature and/or increase solute–interface interactions can be useful in increasing drug solubilization while maintaining monophasic characteristics of the system.

By partitioning into the interface, short-chain alcohols and acids alter the molecular structure of the interface and decrease the spontaneous curvature, thus leading to higher solubilization capacity for the dispersed phase. In reverse micelles, when the system is rich in oil and poor in surfactants, the surfactant mixture has a tendency to partition mainly into the oil phase and its level at the interface is below the concentration that is needed to form a large area of w/o microemulsions. Ethanol, however, has a tendency to penetrate the interface at low surfactant content to form mixed films (Spernath et al., 2006). Thus, ethanol enlarges the isotropic region by increasing the flexibility of the surfactant film.

Use of organic acids as a cosurfactant also leads to significant increase in the isotropic region of microemulsion formation depending on the type of acid used. As shown in Fig. 2D, propionic acid was the most efficient in increasing the area of the isotropic region in systems stabilized with PC, polyoxyethylene-40-hydrogenated castor oil (HECO40 or Cremophor RH40®), and PG in 1:3:10 weight ratio. The area of isotropic region progressively decreased with increasing carbon chain length of organic acid (Spernath et al., 2006). This behavior is similar to that observed with alcohols and is postulated to proceed through similar mechanisms (Garti et al., 2001; Hou and Shah, 1987).

### 2.3. Solubilization capacity in diluted microemulsions

Drug solubilization capacity in microemulsions vis-à-vis corresponding micelles and the oil used for solubilization was evaluated by Malcolmson et al. (1998). The authors used 2% o/w microemulsions and micelles of nonionic surfactant polyoxyethylene-10-oleyl ether (Brij 96) to solubilize the hydrophobic drug testosterone propionate (log $P$ 4.78) and studied the role of the type of oil on drug solubility in microemulsions. As shown in Table 2, drug solubility was higher in microemulsions than corresponding micelles and the oil, which was attributed to drug solubilization in the interfacial surfactant monolayer.

The type of oil significantly influenced drug solubility in microemulsions. This was due to oil penetration in the surfactant monolayer, causing a dilution of the polyoxyethylene region of the surfactant that lies close to the hydrophobic region and contributes to drug solubility. Variations in the oil molecular volume, polarity, size, and shape led to variations in its penetration of the surfactant monolayer and influence on drug solubilization. The authors concluded that the ability of an o/w microemulsion to increase drug solubility over the equivalent micelle depends on both the solubility of drug in the dispersed phase, influence of oil on the nature of microemulsion droplet, and the site of drug solubilization within the surfactant aggregate. The use of large molecular volume polar oils, e.g., caprylic acid triglycerides (Miglyol 812®), was recommended to maximize drug solubilization in microemulsions.

The role of surfactant type and percent aqueous phase composition on the solubilization capacity in diluted o/w microemulsions was reported by Spernath et al. (2002). Solubilization of lycopene in microemulsions stabilized by different surfactants in 25% limonene/ethanol/Tween 60® (1:1:3 and 1:1:8) and 75% water containing o/w microemulsions was a function of the HLB of surfactants (Fig. 3A). Maximum lycopene solubilization was observed using Tween 60® (HLB 14.9), which reduced dramatically when more hydrophilic surfactants, e.g., Tween 40® and Tween 20® (HLB 16.7) were used (Spernath et al., 2002). This indicated a suitable range of HLB of surfactant or system to maximize drug solubilization. This range could be drug specific, but is usually 10–16.

Solubilization capacity of lycopene was also dependent on the aqueous phase dilution of a 1:1:3 mixture of limonene, ethanol and Tween 60® (Fig. 3B). Four different regions were identified in terms of lycopene solubilization capacity along the aqueous dilution line. The solubilization capacity decreases dramatically upon increasing aqueous phase content of the system from 0

Table 2
Solubility of testosterone propionate in micelles, various oils, and corresponding microemulsions at two different surfactant (Brij 96) concentrations

| Oil type | Solubility in oil (%w/w) | Drug contribution from oil content to the solubility in microemulsions | Solubility in micelles/microemulsions (%w/v) at surfactant level of | |
|---|---|---|---|---|
| | | | 15% | 20% |
| Micelles | – | 0.000 | 0.365 | 0.430 |
| Tributyrin | 8.78 | 0.176 | 0.553 | 0.641 |
| Miglyol 812 | 6.20 | 0.124 | 1.150 | 1.300 |
| Soybean oil | 3.42 | 0.068 | 0.531 | 0.656 |
| Ethyl butyrate | 18.64 | 0.373 | 0.471 | 0.486 |
| Ethyl caprylate | 12.17 | 0.243 | 0.489 | 0.599 |
| Ethyl oleate | 5.79 | 0.116 | 0.497 | 0.641 |
| Heptane | 0.92 | 0.018 | 0.354 | 0.486 |
| 1-Heptene | 4.28 | 0.086 | 0.402 | 0.424 |
| Hexadecane | 1.70 | 0.034 | 0.431 | 0.520 |
| 1-Hexadecene | 1.74 | 0.035 | 0.389 | 0.573 |

Abbreviations: DMTG: dimethoxytetraethylene glycol. *Note*: Table modified from Malcomson et al. to report only mean values. Solubility in water 0.009% (w/w).

to 20% (region I), remains almost unchanged from 20 to 50% (region II), increases again from 50 to 67% (region III), and then reduces upon further dilution (region IV).

Solubilization capacity of lycopene was related to the structural transitions taking place during aqueous dilution of the reverse micelle system. Structural transitions in the system were studied by self-diffusion nuclear magnetic resonance (SD NMR) to calculate diffusion coefficients of water and limonene in systems with and without lycopene, as a function of aqueous dilution. The decrease in drug solubilization capacity in region I was related to increasing interactions between the surfactant and water molecules, with a gradual swelling of reverse micelles, leaving less surfactant available for interaction with the solute. Region II was associated with gradual transformation of the system into a biocontinuous phase structure, while the interfacial area remains almost unchanged. Over region III, the system changed from a bicontinuous to an o/w microstructure, which was strengthened in region IV (Spernath et al., 2002). These results indicate that the amount of aqueous phase dilution influences solute solubilization capacity upon dilution of the reverse micelles to o/w microemulsions, which is related to the structural state of the system. Assuming fasted state gastric fluid volume of ∼50 mL, SMEDDS that show highest solubilization capacity at this dilution would, therefore, be expected to have the least tendency for drug precipitation *in vivo*.

## 3. Drug precipitation and solute crystallization

Drug precipitation upon oral administration and *in vivo* dilution of a SEDDS or SMEDDS formulation is a rapid process that involves solute exclusion from the solution whose solubilization capacity for the drug has suddenly reduced. In addition to the drug and formulation variables, this process is affected by conditions in the gastrointestinal tract and the fate of lipids upon coming in contact with gastrointestinal fluids. Approaches to minimize and models to mimic *in vivo* drug precipitation could be helpful in improving bioavailability from these systems.

In contrast, *in vitro* drug crystallization from diluted micelles and microemulsions involves formation of solute crystals over prolonged undisturbed storage. This process is usually slow, temperature dependent, and influenced by such factors governing crystallization as saturation solubility of the drug in the system. A system with lower drug solubility will show higher propensity for crystallization, and vice versa. A comparison of tendency of several formulations to crystallize over time can be observed upon undisturbed storage of samples under refrigerated conditions, which accelerates solute crystallization, or by using modified *in vitro* tests (Gao et al., 2004; Gao et al., 2003). Therefore, modeling *in vitro* drug crystallization can help develop ready-to-use oral and parenteral microemulsion dosage forms of drugs.

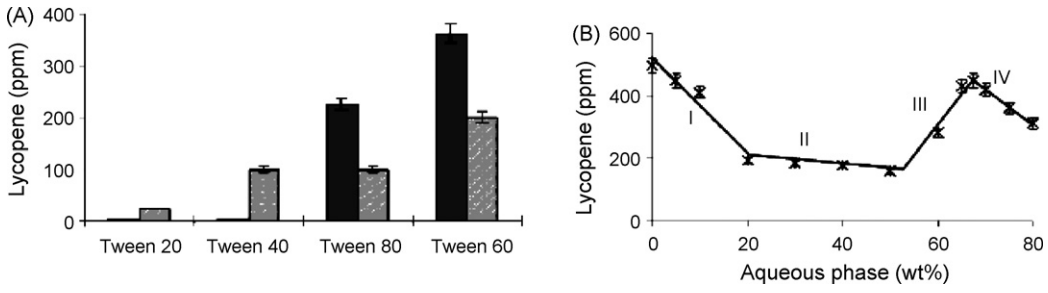


Fig. 3. Solubilization capacity in microemulsions as a function of surfactant type and aqueous dilution. (A) represents the solubilization capacity of lycopene in microemulsions of composition (1, solid bars) (*R*)-(+)-limonene/ethanol/Tween 60® (1:1:3) and 75% aqueous phase and (2, hatched bars) limonene/ethanol/Tween 60® (1:1:8) and 75% aqueous phase. (B) represents lycopene solubilization as a function of aqueous weight percent in the microemulsions in relation to the structural transition regions of the microemulsion (Spernath et al., 2002).

### 3.1. In vivo drug precipitation

Lipid solutions often achieve higher oral absorption than corresponding solid dosage forms of hydrophobic drugs (Shen and Zhong, 2006), particularly class II (low solubility, high permeability) compounds as per the biopharmaceutics classification system (Lindenberg et al., 2004). However, improvement of bioavailability upon presenting a hydrophobic drug in the solution or emulsion form can be compromised if the drug precipitates from the dosage form *in vivo*. In several cases, avoidance of drug precipitation could be the predominant factor governing improvement of oral bioavailability from lipid vehicles than the size of the dispersed phase. The SEDDS, SMEDDS, and micellar systems have different levels of drug dispersion. The dispersion size, upon *in vivo* dilution and bile-surfactants induced emulsification, of SMEDDS is expected to be smaller than that of SEDDS, which, in turn, would be smaller than that of a lipid-solution of drug. The influence of dispersion size on bioavailability has been observed for several molecules, e.g., Vitamin E (Julianto et al., 2000), cyclosporine (Trull et al., 1995), and halofantrine (Khoo et al., 1998); while it is limited for some others, e.g., atovaquone (Sek et al., 2006), danazol (Porter et al., 2004), and ontazolast (Hauss et al., 1998) (Table 3).

For example, the self-emulsifying formulations had equivalent bioavailability to corresponding lipid-solution formulations for atovaquone (log *P* 5.31) (Sek et al., 2006) and danazol (log *P* 4.53) (Porter et al., 2004) in dogs, and for ontazolast (log *P* 4.00) (Hauss et al., 1998) in rats. The bioavailability of all these formulations was higher than the corresponding aqueous suspensions. These studies suggest that the role of dispersion size in improving oral bioavailability could be limited depending on the drug, the animal species, or other overriding factors.

Presentation of a hydrophobic drug in a dissolved form improves oral absorption as compared to a corresponding solid or suspension dosage form by avoiding the dissolution step. In all cases, lack of *in vivo* precipitation plays a predominant role in improving oral bioavailability of hydrophobic compounds. The assessment and minimization of the tendency for precipitation of drugs, both *in vivo* and *in vitro*, upon aqueous dilution of dosage forms is important to their utilization in improving the oral bioavailability of hydrophobic drugs.

### 3.2. Prediction of in vivo drug precipitation

Development of a lipid formulation of a hydrophobic compound presents overabundance of choices of vehicles (de Smidt et al., 2004) and the development strategies are mostly empirical (Dahan and Hoffman, 2006). Formulation choices can be compared with respect to their tendency towards drug precipitation *in vivo* by such empirical tests as dilutability in water *in vitro* and the rate of drug crystallization.

The tendency for *in vivo* drug precipitation in a formulation is often also evident in absorption simulation experiments. For example, Dahan and Hoffman used an *in vitro* lipolysis model to perform *in vitro in vivo* correlation (IVIVC) between lipolysis of solubilized lipophilic solute, vitamin $D_3$, and oral bioavailability (Dahan and Hoffman, 2006). The dynamic *in vitro* lipolysis model (Sek et al., 2002) incorporates the use of temperature, enzymes, and pH control to simulate *in vivo* conditions, followed by ultracentrifugation, and separation of the formulation into three phases: an aqueous phase containing bile salts, fatty acids, and monoglycerides along with dissolved drug (which is considered available for absorption), a lipid phase containing undigested diglycerides and triglycerides, and a sediment containing undissolved fatty acids (Dahan and Hoffman, 2006).

Fig. 4A represents the distribution of vitamin D3 molecules across the aqueous and sediment phase using long-chain triglycerides (LCT) and medium chain triglycerides (MCT) in the formulation. Upon 5-fold reduction of the amount of lipid in the formulation, drug precipitation was evident with increasing percentage of drug in the sediment (Fig. 4B). This experiment shows that *in vitro* simulation studies could be extrapolated to evaluate the *in vivo* drug precipitation tendency of the formulation.

### 3.3. Avoiding in vivo drug precipitation

Increasing the solubilization capacity of the formulation significantly over the desired drug concentration could help avoid *in vivo* drug precipitation. Formulations that can be diluted with water *in vitro* without drug precipitation are likely to be more stable under *in vivo* conditions than those that are not dilutable. These aspects are discussed in Section 2.

Another approach in this direction is to promote the formation of supersaturated drug solution *in vivo* by incorporation of hydrophilic polymeric ingredients in the formulation that act as precipitation inhibitors. The supersaturated drug solutions will eventually precipitate due to the thermodynamic instability of the system, but if the precipitation is delayed long enough *in vivo* to cover the drug absorption time, bioavailability from these systems can be improved. Several common pharmaceutical excipients act as precipitation inhibitors, e.g., methyl cellulose (MC), hydroxypropyle methylcellulse (HPMC), HPMC phthalate (HPMCP), sodium carboxymethyl cellulose (Na CMC), and polyvinylpyrrolidone (PVP) (Hasegawa et al., 1988; Raghavan et al., 2001a; Raghavan et al., 2000; Raghavan et al., 2001b; Simonelli et al., 1970). For example, Gao et al. demonstrated the improved oral bioavailability of an experimental hydrophobic drug, PNU-91325, with the use of 20 mg/g HPMC in the formulation using both cosolvent and SEDDS formulation approaches. The bioavailability improvement with the incorporation of HPMC in a PEG 400 cosolvent-based formulation was >4-fold, while it was ~2-fold for supersaturable SEDDS formulation using Cremophor EL® compared with a micelle formulation using Tween 80® (Gao et al., 2004). In application to SMEDDS formulation, inclusion of HPMC was demonstrated to increase the bioavailability of paclitaxel more than 9-fold in rats (Gao et al., 2003).

### 3.4. Mechanism of solute crystallization

The efficiency of a system to solubilize drug is commonly interpreted in terms of the amount of drug dissolved over a short period of time with reasonable degree of agitation. Whether nucleation and crystallization would subsequently occur in such

Table 3
Relative bioavailability of lipid-based formulations of hydrophobic drugs

| Drug name (log *P* value) | Species tested | Test product | | Reference product | | Increase in AUC |
|---|---|---|---|---|---|---|
| | | Formulation | AUC (Mean ± S.D.) | Formulation | AUC (Mean ± S.D.) | |
| Vitamin E (log *P* 9.96) | Humans | Tween 80, Span 80, and Vitamin E dissolved in palm oil in the proportion 4:2:4 to form SEDDS | $AUC_{0-\infty} = 210.7 \pm 63.0$ h μg/mL | Natopherol® soft gelatin capsules (solution in soybean oil) | $AUC_{0-\infty} = 94.6 \pm 80.0$ h μg/mL | ~2-fold |
| Cyclosporine (log *P* 4.29) | Humans | SMEDDS, Neoral® soft gelatin capsules | | SEDDS, Sandimmune® soft gelatin capsules | | ~6.5-fold |
| Halofantrine (log *P* 9.20) | Dogs | SEDDS, MCT | $AUC_{0-\infty} = 5313 \pm 1956$ h ng/mL | SMEDDS, MCT | $AUC_{0-\infty} = 5426 \pm 2481$ h ng/mL | None |
| | | SMEDDS, LCT | $AUC_{0-\infty} = 6973 \pm 2388$ h ng/mL | | | ~1.3 fold |
| Atovaquone (log *P* 5.31) | Dogs | Solution in lipids + ethanol | $AUC_{0-73h} = 31.8 \pm 9.3$ h μg/mL | Aqueous suspension | $AUC_{0-73h} = 9.4 \pm 1.0$ h μg/mL | ~3.4-fold |
| | | SMEDDS, lipids + Cremophor EL® + ethanol | $AUC_{0-73h} = 31.8 \pm 8.4$ h μg/mL | | | ~3.4-fold |
| | | SMEDDS, lipids + Pluronic 121® + ethanol | $AUC_{0-73h} = 33.7 \pm 13.0$ h μg/mL | | | ~3.4-fold |
| Danazol (log *P* 4.53) | Dogs | SMEDDS, LCT | $AUC_{0-10h} = 270.5 \pm 38.5$ h ng/mL | Micronized powder | $AUC_{0-10h} = 35.3 \pm 5.2$ h ng/mL | ~7-fold |
| | | SMEDDS, MCT | $AUC_{0-10h} = 47.7 \pm 29.5$ h ng/Ml | | | ~1.3-fold |
| | | Lipid solution, LCT | $AUC_{0-10h} = 340.2 \pm 64.4$ h ng/mL | | | ~9-fold |
| Ontazolast (log *P* 4.00) | Rats | SEDDS, 1:1 mix of Gelucire 44/14® and Peceol® | $AUC_{0-8h} = 752 \pm 236$ h ng/mL | Aqueous suspension, Tween 80® + HPMC | $AUC_{0-8h} = 65 \pm 15$ h ng/mL | ~11-fold |
| | | SEDDS, 8:2 mix of Gelucire 44/14® and Peceol® | $AUC_{0-8h} = 877 \pm 104$ h ng/mL | | | ~13-fold |
| | | SEDDS, Peceol® | $AUC_{0-8h} = 528 \pm 68$ h ng/mL | | | ~8-fold |
| | | Emulsion, soybean oil + Tween 80® | $AUC_{0-8h} = 1003 \pm 270$ h ng/mL | | | ~15-fold |
| Atorvastatin (log *P* 6.26) | Dogs | SMEDDS, Labrafil®, Cremophor RH40®, propylene glycol | $AUC_{0-24h} = 2613.0 \pm 367.6$ h ng/mL | Lipitor® Tablets 10 mg | $AUC_{0-24h} = 1738.0 \pm 207.9$ h ng/mL | ~1.5-fold |
| | | SMEDDS, Estol®, Cremophor RH40®, propylene glycol | $AUC_{0-24h} = 2568.3 \pm 408.0$ h ng/mL | Lipitor® Tablets 10 mg | $AUC_{0-24h} = 1738.0 \pm 207.9$ h. ng/mL | ~1.5-fold |
| | | SMEDDS, Labrafac®, Cremophor RH40®, propylene glycol | $AUC_{0-24h} = 2520.81 \pm 308.4$ h ng/mL | Lipitor® Tablets 10 mg | $AUC_{0-24h} = 1738.0 \pm 207.9$ h ng/mL | ~1.5-fold |

Abbreviations: LCT, long-chain triglycerides; MCT, medium chain triglycerides.

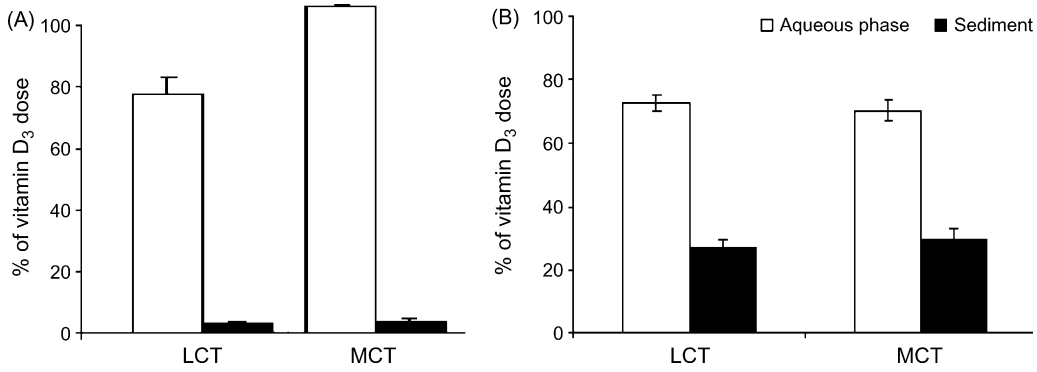


Fig. 4. Distribution of Vitamin $D_3$ molecules across the aqueous phase and the sediment of the dynamic *in vitro* lipolysis medium using high (A) or 5-times lower (B) lipid load of its long-chain triglyceride (LCT) or medium-chain triglyceride (MCT) solution. Modified from Dahan and Hoffman (Dahan and Hoffman, 2006).

a system depends on relative levels of drug solubilized vis-à-vis its saturation concentration in the system. Above saturation concentration, the rate of nucleation would depend on actual solute concentration in the system and other factors, e.g., seed crystals, leading to either immediate or delayed drug precipitation.

Principles governing solute precipitation with progressively increasing concentration in solution were elaborated by LaMer and Dinegar in the study of formation of monodisperse colloids (LaMer and Dinegar, 1950). In the classical LaMer diagram, solute concentration progressively increases in solution beyond saturation concentration until it reaches a threshold for nucleation (the concentration that would lead to immediate, heterogeneous nucleation and solute precipitation). Thereafter, crystal growth occurs on the formed nuclei leading to reduction of solution concentration until the saturation concentration is reached (Fig. 5). Nucleation can occur heterogeneously on impurity centers or homogeneously through spontaneous nucleation. The former leads to fewer, larger crystals than the latter (Beattie, 1989).

This principle could be extrapolated to the hypothetical scenario of drug concentration in micellar and microemulsion systems as illustrated in Fig. 6. This figure represents drug concentration (*y*-axis) in a reverse micelle upon progressive dilution with water (*x*-axis) to form an o/w microemulsion. Saturation drug concentration in the system upon dilution is non-linear (Garti et al., 2006; Spernath et al., 2002; Spernath et al., 2003).

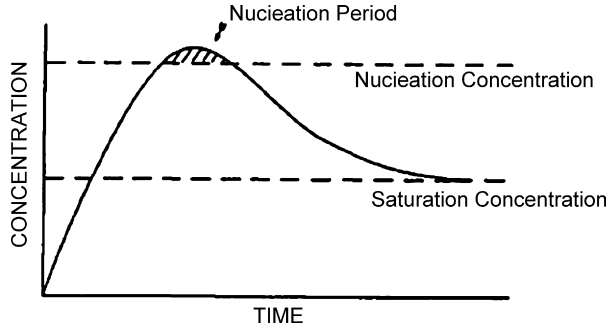


Fig. 5. LaMer diagram representing the time dependence of concentration required for monodispersity. This figure illustrates the supersaturation region of drug solubility between the saturation and the concentration that would lead to immediate, heterogeneous nucleation in the case of monodisperse colloids (LaMer and Dinegar, 1950).

Assuming the saturation concentration of drug in the system with dilution follow the double lines as marked, reduction in drug concentration with dilution in the formulation would lead to tendency for precipitation along either of lines 1, 2, or 3 depending upon the starting drug concentration in the system. Based on the amount by which drug concentration in the system exceeds the saturation concentration and the length of dilution line along which it exceeds, dilution along line 1 would be expected to lead to faster drug precipitation than line 2, while a system diluted along line 3 would be expected to maintain the drug in the solubilized state throughout.

Formulation modifications tend to influence the saturation drug concentration in the SMEDDS as well as upon dilution. Thus, in addition to formulation approaches to minimize and inhibit drug precipitation, starting drug concentration plays a crucial role in determining the window of permissible drug concentrations upon dilution that do not lead to precipitation.

### 3.5. Preventing drug crystallization

High solubilization capacity of reverse micelles, however, is of limited use in improving oral bioavailability if aqueous phase dilution were to cause migration of the solubilized drug molecule from interface to the outer aqueous phase, followed by

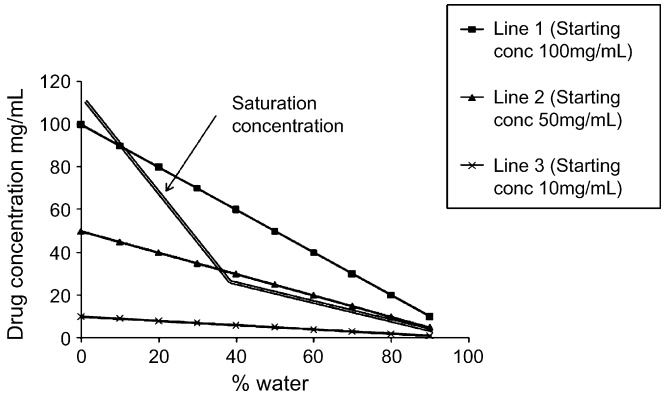


Fig. 6. A hypothetical set of scenarios for SEDDS, SMEDDS, and micellar systems depicting different possibilities for drug supersaturation upon aqueous dilution. With the defined saturation drug concentrations at each composition of the system over the dilution curve, different starting drug concentrations would lead to different outcomes in drug precipitation upon dilution.

drug precipitation, and uncontrolled absorption (Spernath et al., 2006). It is important, therefore, to develop systems that maintain high drug solubilization upon aqueous dilution of reverse micelles.

The problem of drug crystallizing out of solution upon aqueous dilution of systems that form micelles, emulsions, and microemulsions has been widely discussed in several patent documents, which also discuss ways to address this issue. Drug crystallization of aqueous oil/surfactant solutions of the hydrophobic drug fenofibrate (log *P* 5.58) was assessed by simple physical observation of appearance of crystals immediately upon addition of water (US 2004/0005339 A1). The authors proposed the use of a water-miscible solubilizer that allows complete drug dissolution and prevents or minimizes drug crystallization in the formulation upon coming in contact with an aqueous environment. Liang et al. (US 7,022,337 B2) extended the observation for possible crystallization up to 24 h. The use of solubilizers such as *N*-alkyl derivatives of 2-pyrrolidone, ethylene glycol monoether, $C_{8–12}$ fatty acid esters of polyethylene glycol helped maintain drug in solution upon dilution with water.

Another approach that has been proposed to prevent the precipitation of drug upon aqueous dilution is to balance the HLB value of surfactants used in the formulation. Preferentially water-soluble surfactants have an HLB value of greater than 10, while surfactants that have higher solubility in oil have a value of less than 10. Chacra-Vernet et al. describe in US patent application 2004/0052824 A1 that the risk of recrystallization of drug is the greatest when using hydrophilic SEDDS, i.e., which contain a hydrophilic surfactant and co-surfactant with having HLB values greater than 12. Although these formulations do help to solubilize hydrophobic drugs, they may not lead to the desired improvement in bioavailability. To prevent crystallization of the drug upon aqueous dilution, these authors proposed the use of small quantities of lipophilic phase with very low HLB values, and the essential presence of a cosurfactant which is also a good solvent for the drug.

The tendency for solute crystallization is amply demonstrated in studies that have deliberately sought to achieve new crystal forms of molecules by using microemulsions. For example, Furedi-Milhofer et al. prepared new polymorphs of aspartame by crystallization from microemulsions (Furedi-Milhofer et al., 1999). The authors produced water/isooctane microemulsions of the artificial sweetener aspartame using diisooctyl sulfosuccinate as a surfactant. Amount of surfactant and temperature were the primary factors determining the amount of aspartame which could be solubilized. Aspartame was primarily located at the water/oil interface and acted as a cosurfactant. Crystallization of aspartame was achieved by slow cooling of the microemulsion to 5 °C. For drugs solubilized in the w/o microemulsions, nucleation could occur in either the dispersed water droplets or at the interface. The type of crystals formed depends on the location of the drug in the system. Crystallization at the interface leads to the formation of long crystals, while crystallization initiated in the dispersed phase results in short crystals.

For pharmaceutical applications, preventing the crystallization is the desired goal. The tendency for crystallization is reflected in the crystallization temperature or time to crystallization at a given temperature. In the o/w microemulsions solubilizing a hydrophobic solute, the primary location of drug in the system would influence the preferred site of nucleation. In cases where drug resides at the interface along with surfactant (and sometimes also cosurfactant) molecules, molecular packing and structure of the amphiphilic surfactant and drug at the interface would play a role in facilitating or inhibiting nucleation. For example, resemblance of molecular structure of the emulsifier to that of the crystallizing solute, which affects proximity and packing of solute molecules, could increase nucleation and the rate of crystallization (Davey et al., 1996). Therefore, choice of a surfactant with reference to its molecular structure resemblance to that of the hydrophobic solute could influence the rate of drug crystallization from a microemulsion.

### *3.6. Combined use of solubilization approaches*

A combination of pH control with the use of micellization, cosolvency, or complexation is the first choice approach to increase the solubility of hydrophobic drugs. Theoretical treatment of the increase in solubility observed with a combination of pH and other approaches has involved segregation of the contribution of the ionized and the unionized species to solubilization (Li et al., 1999a). The increase in solubility achieved with a combination of cosolvent (ethanol) or micellization (polysorbate 20) with pH modulation was demonstrated by Li et al. using flavopiridol as a model compound, which is weakly basic with an apparent p$K_a$ of 5.68 and intrinsic solubility of 0.025 mg/mL (Li et al., 1999b). Flavopiridol solubility increased linearly with the increase in surfactant content of solution, with a slope that increased with the reduction in pH. In contrast, increasing the proportion of cosolvent led to logarithmic increase in flavopiridol solubility at all pH conditions, with the greatest increase at acidic pH. These approaches may be incorporated in microemulsion formulation to increase the saturation concentration and solubilization capacity of the system.

Aqueous solubility of a nonelectrolyte is also influenced by both the type and concentration of the electrolyte present in solution. The reduction in solubility of a hydrophobic drug in the presence of a salt or electrolyte is a function of salt concentration, as described by the Setschenow equation (Ni et al., 2000). This "salting-out" effect of electrolytes is also dependent on the molar volume, aqueous solubility, and the log *P* of the solute (Shukla et al., 2003). Presence of electrolytes and salts also affects the critical micellar concentration (CMC) of surfactants and the structure of micelles and microemulsions. These considerations should be taken into account with the use of ionized pharmaceutical excipients in these formulations.

## 4. Other factors influencing bioavailability

In addition to drug precipitation in the gastrointestinal tract, drug bioavailability from self-emulsifying formulations is influenced by biopharmaceutical properties of the lipid, e.g., lipolysis; and the drug, e.g., lymphatic transport, enteric metabolism, and efflux. Lipid-based formulations can influence the bioavailability of hydrophobic drugs through several mech-

anisms, e.g., stimulation of pancreatic and biliary secretions, prolongation of gastrointestinal residence time, stimulation of lymphatic transport, increased intestinal wall permeability, and reduced metabolism and efflux pump activity.

### 4.1. Lymphatic transport and lipolysis

Lipid digestion in the formulation increases the dispersion of the drug, which promotes its absorption. Lipolysis rate of medium chain triglycerides (MCT) is higher than long-chain triglycerides (LCT), which has been shown to influence the bioavailability of hydrophobic drugs from lipid-based dosage forms. Bioavailability from a lipid-based formulation can be reduced by the use of lipolysis inhibiting surfactants, e.g., polyoxyethylene-10-oleoyl ether (Brij 96®), polyoxyle-35-castor oil (Cremophor EL®), Cremophor RH40®, and polysorbate 80 (Crillet 4®) (US patents 5,645,856 and 6,096,338) in cases where lipolysis is important to drug absorption. Rate of lipolysis of various lipids and formulations can be compared *in vitro*. The effect of lipids on lymphatic drug transport, however, can overwhelm the difference in their rate of lipolysis.

Dahan and Hoffman evaluated the impact of using short ($C_2$, triacetin), medium ($C_{8–10}$, glyceryl tricaprylate/caprate (Captex 355®)), and long-chain ($C_{18}$, peanut oil) triglycerides (SCT, MCT, and LCT, respectively) on hydrophobic drug absorption as a function of lymphatic transport of the drug molecule and lipolysis of the formulation (Dahan and Hoffman, 2006). They selected progesterone (log *P* 4.0) and vitamin $D_3$ (log *P* 9.1) as hydrophobic drugs, of which only the latter has significant lymphatic transport. Bioavailability of progesterone from the formulations followed the trend MCT > LCT > SCT which strongly correlated with *in vitro* lipolysis data of these formulations, while that of vitamin $D_3$ was LCT > MCT > SCT and did not correlate with the lipolysis data (MCT > LCT > SCT). These results were explained as a stimulation of lipid turnover in enterocytes by LCT, which led to increased lymphatic transport pathway capacity (Dahan and Hoffman, 2006). Increased lymphatic transport can also reduce hepatic metabolism of drugs that have significant first pass effect. Thus, to maximize bioavailability of a hydrophobic drug from the lipidic formulation, the choice of excipients should also take into consideration biopharmaceutical properties of the drug.

### 4.2. Inhibition of drug efflux

Absorbed drug molecules entering the enterocyte are exposed to metabolizing enzymes, e.g., cytochrome P-450 3A4 (CYP3A4), or can be secreted back into the gastrointestinal lumen by P-glycoprotein (P-gp) efflux pumps on the enterocyte membrane. The impact of formulation ingredients on the biopharmaceutical properties of drugs is also illustrated by the inhibition of drug efflux pumps by certain formulation ingredients. For example, common pharmaceutical excipients like polyethylene glycol, Tween 80®, and Cremophor EL®, have been shown to inhibit P-gp activity (Hugger et al., 2002). Their inclusion in the formulation, therefore, can be expected to increase the bioavailability for drugs which are known substrates of P-gp efflux pumps.

### 4.3. Dispersion size of emulsions

Presenting the drug in the dissolved form using lipid-based formulations provides significant improvement of oral absorption as compared to an oral solid or suspension dosage form. This advantage can be further improved in several cases by reducing the dispersion size of the dosage form. The reduction in dispersion size of cyclosporine A (log *P* 4.29) SEDDS formulation, Sandimmune®, to its SMEDDS formulation, Neoral®, improved its bioavailability by ~6.5-fold (Trull et al., 1995) (Table 1).

Similarly, Julianto et al. (2000) observed that the self-emulsifying formulation of Vitamin E (log *P* 9.96) had ~3-fold higher extent of absorption than its solution in soybean oil (Natopherol® soft gelatin capsules). The SEDDS formulation consisted of Tween 80®, sorbitan monooleate (Span 80®), and Vitamin E dissolved in palm oil in the proportion 4:2:4. These results indicated that, in addition to bile mediated emulsification and absorption mechanism, formulation-induced *in vivo* emulsification was useful in enhancing drug absorption. Similar results were shown by Yap and Yuen for tocotrienols, which belong to the Vitamin E family (Yap and Yuen, 2004). Thus, given other things being equal, SMEDDS formulation is expected to have higher bioavailability than the SEDDS formulation because of lower dispersed phase size.

## 5. Conclusions

Lipid-based systems are a promising choice for the delivery of hydrophobic molecules. These systems could be lipid solution, emulsions, microemulsions, SEDDS, SMEDDS, or micellar systems. These systems avoid the dissolution step upon oral administration and differ from one another with respect to the size of the dispersed phase and the content of surfactant and other ingredients. They help improve the bioavailability of hydrophobic drugs through several mechanisms, e.g., facilitation of *in vivo* dispersion through the added surfactant, lipolysis of constituent lipids, increased lymphatic transport, etc. Micellar and microemulsion systems, being the most dispersed of all, appear the most promising.

The use of lipid-based delivery systems has become increasingly popular for pre-clinical studies since most of the new molecular entities are highly hydrophobic. Several studies have reviewed the formation of these systems, the role of composition on phase diagram, and drug release and bioavailability from these systems. While improved drug entrapment and release is observed in almost all cases, improvement in bioavailability is often unpredictable. Several studies have focused on formulation and drug-related biopharmaceutical aspects that are important in governing oral bioavailability. These factors include precipitation of drug *in vivo*, digestability of lipids in the formulation, overall HLB of surfactant mix in the system, intestinal efflux pumps and metabolizing enzymes, contribution of lymphatic transport of drug to its absorption, etc. The design of SEDDS, SMEDDS, and micellar systems presents a plethora of choices

that appear equivalent on surface and are usually selected empirically. Incorporation of these formulation and biopharmaceutical considerations into the design of these systems will help improve their *in vivo* performance.

Among factors that influence the bioavailability of drugs from these systems, lack of drug precipitation upon aqueous dilution plays the predominant role in many cases. While several factors need to be incorporated into the design of SEDDS, SMEDDS, and micellar drug delivery systems, as discussed in Section 5 above, due attention needs to be given to the propensity of these systems for precipitation *in vivo* upon oral administration. While this aspect has been recognized by several studies and empirical rationale for minimizing the tendency of drug for precipitation from the system have been developed, there remains a need to have predictive ability and objective parameters for assessing this risk.

Some key features of these systems can be useful in addressing these needs. For example, solubilization capacity of the system can be increased much above the required drug concentration, so that it remains below the saturation and nucleation concentration of the drug in the system and upon dilution. The aspects that affect solubilization capacity and saturation concentration as both undiluted reverse micelles and diluted microemulsions, as well as dilutability as a single phase system, have been reviewed. Some *in vitro* models can be extrapolated to predict the relative tendency of formulations for *in vivo* drug precipitation. The use of some polymeric hydrophilic excipients in the formulation can help prevent or delay drug precipitation by the formation of a supersaturated state upon aqueous dilution.

These studies provide the background and basis on which models to predict, and approaches to prevent, *in vivo* drug precipitation may be developed. These efforts will help improve the outcome of formulation efforts towards improving the bioavailability of hydrophobic drugs.

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