Introduction

New chemical entities (NCEs) in modern discovery libraries and development programs have an ever-increasing number of unconventional physical and chemical properties. In particular, up to 70% of discovery compounds and 40% of pipeline candidates are insoluble in water. [1] These promising molecules present unique formulation and development challenges and often suffer from poor bioavailability. [1] Therefore, conventional formulation systems cannot be employed.[2] Techniques available for scientists to increase the bioavailability of poorly soluble drugs are either increasing their solubility in biological media of choice, or increasing their dissolution kinetics.

The use of advanced lipid-based drug delivery systems (LBDDS) is a strategy to design pharmaceutical dosage forms with improved therapeutic benefits[3] [4]. A list of commercially available lipid-based formulations has been published by Strickley in Hauss’ book [2], and another recent study published by Chakraborty [5] has demonstrated that LBDDS show better results than traditional oral formulations [6] [7] [8]. Good examples of successful bioavailability enhancements are cyclosporine A (Neoral®), HIV protease inhibitor ritonavir (Norvir®) or saquinavir (Fortovase®).

This article will provide a background and review the recent advances and updates on Lipid-based Drug Delivery Systems with a primarily focus on those designed for their projected use in the oral route. Regulatory perspectives regarding their applications will also be discussed.

Wide application of LBDDS

The use of lipids in drug delivery is by no means a new phenomenon. “Old” lipid dosage forms such as suppositories, creams or emulsions have been on the market for a long time, and some of them in use
since Egyptian times. However, over the last decade, approaches in new designs of lipid carriers have considerably evolved for the delivery of poorly soluble drugs.

If we consider the number of articles referenced in Pubmed and published on Lipid-based Drug Delivery Systems in the last 10 years, we can infer that these new types of formulations have gained increasing interest.

Evolution of literature issued in Pubmed during the last 10 years

LBDDS can be used to deliver various types of drugs from new chemical entities to more recent new developments for proteins and peptides, nucleic acids (DNA, siRNA), cellular site-specific delivery.[9] [10] [11]

Lipid-based Dosage forms are administered through many routes: oral, parenteral, ocular, intranasal, dermal/transdermal, vaginal.[12] [13]. There have been recent developments in pulmonary drug delivery. In the inhalation field, excipients considered as GRAS (generally recognized as safe) can comprise materials that are endogenous to the lungs and locally present in large quantities, such as phosphatidylcholine. [14]. However, oral remains the preferred route because it is non-invasive, less expensive, and is less prone for side effects, such as injection-site reactions. It is also the easiest and the most convenient method of drug delivery for chronic therapies. Nevertheless, at a very early stage of development, formulation strategies based on a rational and systematic approach need to be developed to avoid erratic and poor in vitro/in vivo correlations, and thus increase the chances of success in formulation development. Some useful guidelines have been published by several authors [15] [16] [17] [18].

Advantages of Lipids

Lipids are a large class of materials that includes fatty acids, glycerides, phospholipids, sphingolipids, waxes and sterols to name a few [19] They may be insoluble in water, amphiphilic, and often identified by their fatty acid composition, melting point, Hydrophilic-Lipophilic Balance (HLB), and solubility in organic solvents. Vegetable oils and their derivatives are the primary source for the manufacture of hundreds of lipid-based excipients intended for the development of solid, semi-solid or liquid lipid-based formulations. [20] Gibson provides a detailed list of the most used excipients for oral administration [2].

A normal diet includes a daily intake of 60-80g of lipid, mostly composed of triglycerides. A normal adult’s digestive system is powerful enough to hydrolyze around 100 -140g of lipid every day. Due to
their resemblance to in vivo components, lipids used in LBDDS are well tolerated in the organism, and less cytotoxic. Their presence in the gastrointestinal (GI) tract mimics the fed state, which in turn stimulates the secretion of bile salts. [5]

Two parameters are of interest if we consider oral drug delivery: the solubilisation of the drug in GI tract digestive phase and the absorption phase through the intestinal barrier followed by circulatory uptake of the drug to allow its therapeutic action.

The poor aqueous solubility of a drug leads to poor solubilisation in gastrointestinal fluids, low and/or variable bioavailability and poor in vitro/in vivo correlation[21]. Lipids and lipophilic excipients can have a significant positive effect on the absorption of poorly soluble drugs after oral delivery. There are three mechanisms by which lipids and lipophilic excipients can improve the bioavailability after oral administration:

- alteration of the composition and character of gastro-intestinal milieu; the stimulation of bile salts secretion causes emulsification of the poorly soluble drug in the gastrointestinal fluid and thus enhances its in vivo solubility
- interaction with enterocyte-based transport and influence on drug uptake and efflux,
- recruitment of lymphatic drug transport, avoiding hepatic first pass drug metabolism.[17]

In 2006, Pouton proposed the Lipid Formulation Classification System (LFCS) to help predict the fate of a formulated drug based on the criteria of dispersion and digestion, and to optimize the choice of formulation for a specific drug.[22] Due to their stability at varying pH and moisture levels, lipids provide adequate protection from gastric or environmental conditions for certain sensitive actives. They also provide a hydrophobic environment to delay the release of the loaded drug. This property has been widely used in the design of sustained release beads, tablets, suspensions, implants or microcapsules. Their hydrophobic properties may also be used for masking the bitter or generally bad taste of certain drugs by hot melt coating [23], or inclusion in solid lipid pellets. Moreover, lipids offer advantages for manufacturing. Drugs with low melting points or poor compression properties, or low dosage forms are all difficult to process using conventional approaches, especially when the drug is liquid at ambient temperature. Lipid-based formulations can be filled into hard or soft capsules[24].

**Design of Lipid-based DDS**

Several LBDDS have been developed over the years, most of them for oral delivery. Hauss has given a comprehensive summary of the development, characterization and use of oral lipid based formulations from both physicochemical and biopharmaceutical perspectives.[25] Fricker has provided/described good examples of different types of lipid-based formulations using phospholipids. [26]. Approaches in the design can be separated in 2 categories: lipid-based formulations, and lipid carriers as particulate systems (liposomes, solid lipid nanoparticles, lipid implants, lipid microtubules and microcylinders, lipid microbubbles, lipospheres, microspheres, pellets, nanostructure lipid carriers…).[5, 26-30]

Formulations can be classified as:

- **Liquid lipid-based formulations**
  - Emulsions or microemulsions (oil/water; water/oil, bicontinuous structures)
An emulsion is a blend of two immiscible phases wherein a surfactant is added to stabilize the dispersed droplets. The proper choice of surfactants and manufacturing conditions is important to stabilize the mixture. A microemulsion is a thermodynamically stable system composed of at least water, oil and surfactant/co-surfactant producing a transparent and thermodynamically stable system whose droplet size is 10-140 nm [31]. Drugs will partition between the aqueous and hydrophobic phases depending on their lipophilicity. Oral, ocular pulmonary, nasal vaginal and intravenous routes are the main routes of administration.

- **Self-Emulsifying Drug Delivery Systems (SEDDS)** and related systems SEDDS can be binary systems made of oil and surfactant. Their dispersions have a turbid appearance because lipid droplet sizes are between 200nm to 5µm. Self-Micro-Emulsifying DDS (SMEDDS) or Self-Nano-Emulsifying DDS (SNEDDS) is an isotropic mixture of lipid, surfactant, co-surfactant and sometimes co-solvents and drug substance that can spontaneously form fine oil-in-water microemulsions (particle size less than 100nm) under mild agitation following dilution with an aqueous phase.

The choice between a SEDDS or a SMEDDS often depends of the intrinsic drug properties, and its solubility and dissolution profile during *in vitro* screening with a number of excipients[32] [33],[34] SMEDDS have demonstrated to have a high solubilisation capacity, and excellent stability. They can substantially provide reproducible and increased oral bioavailability as they enhance the permeation across the intestinal membrane and eliminate food effects.[35]

- **Solid-in-oil (S/O) suspension** is an alternative LBDDS: Piao *et al.* reported that Diclofenac sodium, a non-steroid anti-inflammatory drug (NSAID), can be orally and successfully delivered in S/O suspension without serious GI injuries and side effect.[4]

**Solid lipid-based formulations**

Recently, increasing research has focused on this area. Techniques to obtain diverse solid or semi-solid lipid-based formulations have been described by several authors Jannin *et al.* [36] Canon *et al.* [37]

A solid state microemulsion of cyclosporine was prepared by coating a microemulsion with enteric coating material. Solid SEDDS can be used for several dosage forms (dry emulsions, self-emulsifying capsules, implants, sustained/controlled release tablets or pellets, beads, microspheres, nanoparticles, suppositories and can provide flexible solutions for oral and parenteral administration[32].

**Lipid as particulate drug carriers**

- **Liposomes**

  Liposomes are vesicles comprising a phospholipid bilayer surrounding an aqueous compartment. In the lipid domain of the bilayer membrane, lipophilic drug can be included. Despite the poor stability of vesicles in the GI tract, studies indicate the potential of liposomes to offer a dynamic technology for enhancing the oral bioavailability of poorly soluble drugs, including peptides and proteins. Several studies indicate that blood glucose levels can be decreased by orally administered liposomal insulin. They can be suitable for oral vaccination systems.[26]. The therapeutic index of vincristine can be significantly enhanced through the use of liposomal delivery system in the treatment of human carcinoma [38]. Liposomal
formulations are often regarded as safe due to the GRAS status of the phospholipid constituents. They are mostly used for parenteral applications or injectable dosage forms (e.g. Doxil®).

- **Solid Lipid Nanoparticles (SLNs),**
  SLNs are particulate systems with particle diameters ranging 50-1000nm. They are derived from oil-in-water emulsions, by replacing the liquid oil by a solid lipid. They present several advantages: the lipid matrix is generally made from physiologically well-tolerated lipid components, which decreases the toxicity; they combine physical integrity of particle shape and physical protection capacities of sensitive compounds. They have a stability of around 3 years and can easily be manufactured at industrial scales[39]. SLNs have been reported to increase the bioavailability of several drugs after oral administration, such as piribedil, cyclosporine A and vinpocetin. [40]

Solid lipid particulate systems such as SLNs, lipid micro particles and lipospheres have been sought as alternative carriers for therapeutic peptides, proteins and antigens. Formulation as SLNs confers improved protein stability, avoids proteolysis, as well as providing sustained release of the incorporated molecules. Well-known peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles and are currently under investigation.[10]

- **Squalene**
  Squalene is a natural lipid belonging to the terpenoid family, and a precursor of cholesterol biosynthesis. Because of its significant dietary benefits, biocompatibility, inertness, and other advantageous properties, squalene is extensively used as an excipient in pharmaceutical formulations for disease management and therapy. In addition, squalene acts as a protective agent, has been shown to decrease chemotherapy-induced side-effects and exhibits chemopreventive activity. Although it is a weak inhibitor of tumor cell proliferation, it contributes either directly or indirectly to the treatment of cancer due to its potentiating effect. In addition, squalene enhances the immune response to various associated antigens, and it is therefore being investigated for vaccine delivery applications. Since this triterpene is well absorbed orally, it has been used to improve the oral delivery of therapeutic molecules. All of these properties have made squalene a potential excipient for pharmaceutical applications, especially for the delivery of vaccines, drugs, genes, and other biological substances. [41]

**Challenges**

**Lipid oxidation**

One of the challenges faced by lipids is their sensitivity to oxidation, especially for unsaturated triglycerides and fatty acids. It occurs during storage or processing, and leads to a loss in product quality. When lipids are exposed to environmental factors such as light, air or temperature, autoxidation may occur, and can produce change of texture, color, rancid flavor or, generally, loss of quality and even the generation of toxic compounds with health risks for patients. Other degradation pathways are catalyzed by lipoxygenases enzymes. Trace of metals (e.g iron, copper, cobalt) can have a significant impact in promoting oxidation. Autoxidation seems to be a key and complex mechanism in lipid oxidation. It mainly generates
hydroperoxides and volatile compounds, generally through a three-phase process (initiation, propagation and termination).

- The initiation phase involves homolytic breakdown of hydrogen in \( \alpha \)-position relative to the LH fatty acid chain double bond. The reaction can, however, be initiated via external physical agents such as heat, ionizing radiation or a photonic impact in the ultraviolet spectrum, and also by chemical agents such as metal ions, free radicals and metalloproteins. This results in the formation of (L●). free radicals, i.e., chemical species with an unpaired electron. These are highly unstable, short-lived intermediates that stabilize by abstracting a hydrogen from another chemical species. The oxidation process remains slow during this phase.

- Propagation: In aerobic environments, the (L●). radical centered on the carbon molecule and formed during the initiation phase reacts very quickly with triplet oxygen to generate different radical species, including (L●OO) peroxyradicals. The peroxyradical then abstracts a hydrogen atom from another unsaturated lipid molecule to form hydroperoxide (primary oxidation compound) and another L● radical, thus replenishing reaction. This is a rapidly peaking irreversible reaction. Hydroperoxide formation from unsaturated fatty acids is generally accompanied by stabilization of the radical state via double-bond rearrangement (electron delocalization), which gives rise to conjugated dienes and trienes.

- Termination: The oxidation process then continues with the transformation of hydroperoxides into secondary nonradical oxidation compounds. The main hydroperoxide decomposition mechanism involves scission of the double bond adjacent to the hydroperoxyl group, leading to the formation of hydrocarbons, aldehydes, alcohols and volatile ketones. Other nonvolatile secondary compounds are also formed, including nonvolatile aldehydes, oxidized triacylglycerols and their polymers. [42]

Nitrogen flushing can be a means of preventing oxidation in closed systems such as capsules. [43] To avoid metal-based catalysis, the use of chelators (EDTA or citric acid) is an alternative. The use of antioxidants can prevent oxidation reaction by different mechanisms that have been described by several authors and reported by Karabulut [44].

As an example, alpha-tocopherol is a primary antioxidant responsible of terminating free-radical chain reactions by donating hydrogen or electrons to free radicals, and converting them to more stable products. The pathways mainly used to inhibit oxidation with antioxidants are singlet oxygen deactivation (ascorbic acid), free radical scavenging (ascorbyl palmitate) and chain-breaking reactions (β-Carotene).

Blends of antioxidants can be used to combine the effects.

To assess the effects of antioxidants on oxidative stability, several analytical methods can be used, such as peroxide value for primary oxidation value and p-anisidine value for secondary oxidation products, scanning calorimetry[45], and thermogravimetry [46]. Cyclic voltammetry is a rapid method used for identifying excipients in which the drug is more sensitive to oxidation, and for screening antioxidants. [47]

**Regulatory aspects**
From a regulatory point of view, quality and safety issues related to preclinical and clinical studies are the main difficulties likely to be encountered in launching a lipid-based dosage form on the market, and above all the demonstration of the therapeutic efficacy. The overall drug stability and absence of immunological reactions to the oils or lipid excipients has to be demonstrated. Sufficient details explaining the use of lipid excipients and the types of dosage form, the drug release mechanism and their manufacture should be provided to convince the regulatory authorities of their acceptability. [48] Safety assessment and the potential influence of biopharmaceutical factors on the drug or lipid excipients need to be explored. It may be difficult to predict in vivo performances of a lipid dosage form based on in vitro results obtained with conventional dissolution methods in view of the convoluted GI processing of lipid formulations. More mechanistic studies should be conducted to facilitate a better understanding of the pharmaceutical characteristics of lipid formulations and interactions between lipid excipients, drug and physiological environment. The lack of predictability for product quality and performance may be due to the nature of empirical and iterative processes traditionally employed. [49]

With the aim of rationalizing the design of lipid formulation, and to better understand the fate of a drug after oral administration in a lipid-based formulation, a Consortium, composed of academics and industrial scientists, has been created (www.lfcsconsortium.org). The Consortium sponsors and conducts research to develop in vitro methods to assess the performance of LBDDS during dispersion and digestion, which are critical parameters. The primary objective is to develop guidelines that rationalize and accelerate the development of drug candidates through the identification of key performance criteria, and the validation and eventual publication of universal standard tests and operating procedures. In order to establish approved guidelines, appropriate dialogue with pharmaceutical regulatory bodies (FDA, EMEA) is also foreseen.

Conclusion

Lipid-based drug delivery systems, a physiologically well-tolerated class of formulations, provide a vast array of possibilities to formulate and potentially increase the bioavailability of an ever-growing number of poorly soluble drugs. In recent years, progressive elucidation of the various mechanisms through which lipids can increase bioavailability – a unique combination of solubilisation, dispersion or encapsulation, and stimulation of the digestive process, potential inhibition of receptor-mediated efflux or pre-systemic metabolism, or (even if poorly understood), passage through the lymphatic system – have all contributed, and will continue to contribute to a greater number of products in preclinical and clinical development, and in the market. Investigational initiatives such as the Lipid Formulation Classification System Consortium will further advance our understanding of key criteria dictating the performance of LBDDS, and generate universally acceptable methods to evaluate the same. However, the progress of these innovative systems remains more challenging than that of traditional dosage forms, in particular due to the as yet limited toxicological data – even if some excipient suppliers have taken on the task of generating such data –, and the lack of widespread awareness at the regulatory level – even if, again, collective efforts progressively address the issue.
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