Review Article

Nanostructured lipid carriers: An emerging platform for improving oral bioavailability of lipophilic drugs

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Abstract

Nowadays exploration of novel lipid-based formulations is akin to a magnet for researchers worldwide for improving the *in vivo* performance of highly lipophilic drugs. Over the last few years, new compositions of lipids have been developed, and the probable bioavailability enhancement has been investigated. We reviewed the most recent data dealing with backlogs of conventional lipid-based formulations such as physical instability, limited drug loading capacities, drug expulsion during storage along with all the possible hindrances resulting in poor absorption of highly lipophilic drugs such as P-glycoprotein efflux, extensive metabolism by cytochrome P450 etc. In tandem with these aspects, an exclusive formulation approach has been discussed in detail in this paper. Therefore, this review focuses on resolving the concerned ambiguity with successful oral administration of highly lipophilic drugs through designing novel lipidic formulations (nanostructured lipid carriers [NLC]) that constitute a blend of solid and liquid lipids. The article highlights the potential role of such formulation in normalizing the *in vivo* fate of poorly soluble drugs. Finally, the present manuscript discusses the dominance of NLC over other lipid-based formulations and provides a perspective of how they defeat and overcome the barriers that lead to the poor bioavailability of hydrophobic drugs.

Key words: Bioavailability, cytochrome P450, drug loading, *in vivo* performance, lipid-based formulations, lipophilic, nanostructured lipid carriers, P-glycoprotein efflux, solid-lipid nanoparticles

INTRODUCTION

The oral route is the route of preference for the administration of drugs, but the efficient oral delivery of lipophilic drugs with unsteady metabolism is a challenging chore.[1] As per reports in the literature, the majority of drugs exhibit inconsistent gastrointestinal absorption, thereby resulting in unsatisfactory therapeutic efficacy, the reason for which can be attributed to their inadequate solubility *in vivo*.[2] Besides poor solubilization of a drug in the gastrointestinal tract (GIT), there are multiple other factors, which decrease the degree of absorption of poorly soluble drugs. Several lipophilic drugs are candidates for efflux transporters such as P-glycoprotein (P-gp) (anticancer drugs, antiretroviral drugs etc.,) and are also frequently predisposed to metabolism through cytochrome P450 (CYP) enzymes which leads to their significant first pass elimination and poor *in vivo* prospect.[3-5] At times, these factors are the chief reasons behind insufficient oral bioavailability of hydrophobic drugs. Therefore, there is a substantial call for an ideal nanocarrier system, which takes into account all these aspects and subsequently plugs the loopholes for the booming deliverance of lipophilic drugs. In this regard, lipid-based nanocarriers are a promising formulation approach as they have the potential of fixing these predicaments by improving and normalizing the absorption of such drugs. In this review, various barriers to the absorption of lipophilic drugs are discussed in detail and role of novel lipid nanocarriers known as nanostructured

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lipid carriers (NLC) in overcoming such hurdles and thereby improving their bioavailability is elucidated.

A hybrid is often considered to synergize the desirable attributes of sources from, which it is derived and results in a better prospective. Here, the term hybrid signifies the combination of solid and liquid lipids. In this review, solid-liquid lipid hybrid (SLLH) also known as NLC formulation is investigated in detail and the characteristic features of this hybrid formulation are compared with that of the parent formulations that is, liquid emulsion (LE) and solid lipid nanoparticles (SLN) which are constituted solely of liquid and solid lipids, respectively. Furthermore, this paper briefly explains the mechanisms by which NLC affect the oral absorption of drugs with poor aqueous solubility and provides a perspective on the potential of NLC in enhancing the bioavailability of such drugs. In conclusion, the paper explicates how NLC, which is a hybrid of spatially incompatible solid and liquid lipids, is a superior formulation approach than the matrices having either solid or liquid lipids as core composition for improving oral bioavailability of lipophilic drugs.

BARRIERS IN ORAL DELIVERY OF LIPOPHILIC DRUGS

Effective oral delivery of drugs is extremely influenced by poor aqueous solubility and intrinsic dissolution rate (mass of the drug dissolved per time unit and area).[1] Certainly, dissolution is the rate-limiting step in the absorption of hydrophobic drugs. Usually, such drugs are codified as Class II or IV as per biopharmaceutical classification system (BCS),[6] and they exhibit low oral bioavailability, which is attributed to their poor water solubility. Besides this, low oral bioavailability of such drugs could be attributed to other factors such as hepatic first pass metabolism, drug efflux by P-gp, intra-enterocyte metabolism and chemical and enzymatic degradation.

When a drug candidate with poor aqueous solubility enters GIT, a series of events limit its absorption. First, the limited capacity of the emulsification process by biliary secretions in the upper part of GIT, which plays a significant role in the solubilization of poorly soluble drugs. This process assists the solubilization of the lipophilic drug in the aqueous intestinal milieu by facilitating the formation of minute micelles in which the lipophilic molecule is solubilized and subsequently reaches the absorptive membrane of the enterocyte.[7,8] However, the capability of this process is very limited and variable. Second, unstirred water layer (UWL) is the major hydrophilic barrier for the absorption of hydrophobic drugs that separates the brush border (apical) membrane of enterocytes from the bulk fluid phase of the small intestine lumen.[9] In order to reach the brush border membrane drug molecules in the bulk phase of the intestinal lumen must cross the UWL. This corresponds to a major diffusional barrier for lipids and lipophilic molecules as their solubility in aqueous media is very low. Moreover, the thickness of the UWL in the human jejunum was found to be over 500 μm.[10] Owing to its thickness and hydrophilicity, the UWL happens to be a major permeability barrier to the absorption of lipophilic compounds.

In spite of the above barriers, when a drug molecule enters the enterocyte, it encounters biochemical barriers that affect its absorption. The enterocyte CYP 3A4 (CYP3A4) enzymes, located in the endoplasmic reticulum of the enterocyte are responsible for most of drug metabolism in the intestinal wall.[11] Various studies have shown it to be a major barrier to the absorption of lipophilic drugs.

Importantly, drug efflux transporters such as P-gp are also accountable for poor oral bioavailability of various drugs (e.g., digoxin, paclitaxel, doxorubicin, atorvastatin etc.,). Though some transporters located in the apical wall of the enterocyte facilitate absorption, others act as efflux transporters.[12] The most comprehensively studied transporters are the apical P-gp efflux pumps, which trim down the magnitude of drug absorption by transporting the drug from the enterocyte back to the intestinal lumen.[13,14] There is a link between the activity of the metabolic CYP3A4 enzymes and the P-gp system as they work in concert to prevent the access of lipophilic drugs in the systemic circulation.[15-17] A drug molecule that is able to get away from the intra-enterocyte metabolism and the P-gp efflux systems is transferred to the liver before reaching the systemic circulation, where it is exposed to various metabolic enzymes. This first pass hepatic metabolism is another significant barrier to the absorption of lipophilic drugs. Oral bioavailability of drugs such as anti-hypertensive and cardiovascular agents (β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors), and anti-diabetic agents is considerably low owing to such extensive first pass (hepatic) metabolism.[1]

LIPID-BASED FORMULATIONS

Currently, great importance has been given to the use of formulations that comprise the combinations of natural lipids with surfactants, cosurfactants, and cosolvents.[18] These lipid-based systems with forthcoming advancements demonstrate potential option for the delivery of drugs which have poor aqueous solubility. These lipidic formulations can be broadly categorized into two groups namely LEs and SLNs. The LE systems could be lipid solution, emulsions, microemulsions, self-emulsifying drug delivery system, self-micro emulsifying drug delivery system, or micellar systems. SLN are novel lipid-based formulations which are constituted exclusively of biodegradable lipids such as highly purified triglycerides, monoglycerides (MGs), hard fats, complex glyceride mixtures or even waxes, which are solid at physiological temperature.[19] In spite of the fact that these entire lipid-based systems obviate the dissolution step upon oral administration, they differ from each other in the context of surfactant and other ingredients, as well as the particle size of the dispersed phase.[20]

The highly lipophilic drugs exhibit dissolution limited absorption, which consequently results in reduced permeation of

such drugs across gastrointestinal epithelium membrane which is a major issue of concern. To address this issue, lipid-based carriers such as LE and SLN present these hydrophobic drugs in a dissolved form which improves the oral absorption by evading the dissolution step. Furthermore, lipid digestion in the formulation increases the dispersion of drug which promotes its absorption.[20] For notable enhancement of oral absorption, these lipid-based delivery systems need to maximize the rate and extent of drug dissolution and combat all the barriers such as P-gp efflux and presystemic clearance, which lead to poor oral bioavailability. Interestingly, the accomplishment of the advanced form of lipid-based delivery system progress from the suitable selection of the matrix composition and rational design for the specific drug candidate.[21] In a way, pertinent lipid selection which has a significant influence on the specific barriers to absorption of particular drug candidates becomes a key factor in constructing the base of the novel lipid-based formulation. In light of these aspects, NLC are being explored for their ability as a formulation approach in directing the *in vivo* fate of lipophilic drugs.

Different lipid-based carrier systems

It is known that SLNs and LEs are colloidal systems constituted of solid and liquid lipids core, respectively. These systems exhibit several advantages for delivery of lipophilic drugs, such as the use of biocompatible lipids, large-scale production, and protection of drugs from degradation, improved bioavailability, and controlled-release characteristics.[22] However, there are certain drawbacks which gives a setback to these formulation systems such as restricted drug loading capacities, the expulsion of drug from the formulation, and high surfactant concentration.

In LE system, very few formulations have been commercialized owing to their poor drug loading capacity, usage level of excipients, e.g., surfactants and cosolvents, and the possibility of drug precipitation upon aqueous dilution *in vivo* which can cause failure in bioavailability improvement and can neutralize the competitive benefit of this dosage form. Due to toxic effects of the surfactants and cosolvents at high doses, the per day and per dose uptake level is very restricted in LE.[20]

Furthermore, the expulsion of the drug during storage has been reported to be one of the major drawbacks with the lipid-based formulation. It happens because of the transformation of lipids to an ideal version and this augmentation in the perfection of the crystal leaves hardly any space to lodge drug molecules thus leading to drug expulsion. The similar trend crops up after preparing LE and SLN. Thus, shaping up of lipids into highly ordered lipid crystals confines the drug loading capacity of the prepared formulation.[23] It is interesting to note that identical chemical nature of lipid is the root cause, which results in formation of perfect lipid crystals[24] and this is supposedly the rationale behind drug expulsion and poor drug loading potential of LE and SLN.

Recently, novel lipid nanocarriers, NLC, have gained huge notice for oral delivery of lipophilic drugs. Their exclusivity lies in their unique matrix composition, which contains a mixture of incompatible liquid lipids and solid lipids in appropriate and permissible proportions.[25-27]

The presence of solid cum liquid lipid in the NLC leads to greater drug encapsulation and loading and long-term colloidal stability unlike SLN and LE as shown in Figure 1.[25-27] The Figure 1 depicts the characteristic composition of nanostructured lipid carriers (spatially incompatible solid and liquid lipids), solid lipid nanoparticles (solid lipid), and liquid emulsion (liquid lipid) describing clearly the reason for higher drug loading capacity of solid and liquid imperfect lipid matrix (a) and reduced drug payload and expulsion in highly packed solid (b) and oily (c) lipid core matrix. O = Drug. The is a modified version of Figure 1 of reference 29 [29]). The precondition for good drug accommodation is larger distances between fatty acid (FA) chains of the glycerides and common imperfections in the crystal which can be achieved by using glycerides composed of very different FAs (e.g., in length of carbon chain, mixture of saturated and unsaturated acids) in NLC matrix. Thus, in NLC, by mixing solid lipids with chemically very different liquid lipids (oils), expulsion of drug can be prevented because of usual matrix structural defect (in contrast to a liquid lipid matrix of LE, or a solid lipid matrix of SLN).[22]

The future prospect of NLC for all applications as for SLN and LE is budding gradually.[22] Though, areas of meticulous research in NLC are oral and topical delivery.[23] Currently, much work is going on in this regard and in future NLC are likely to be commercialized at bigger level owing to their desirable features and invincible potential.

As a consequence of their special nanostructures consisting of spatially incompatible solid and liquid lipids, NLC are considered as the smarter, latest generation of lipid nanoparticles possessing improved properties in contrast to other lipid-based formulations, as given in Figure 2.[23] For profound perception of this system of SLLH, the sections 3.2 discusses in detail the characteristic features of NLC and provides a perspective of the dominance of NLC over LE and SLN.

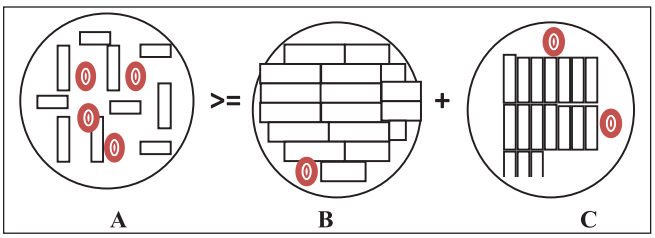

Figure 1: The characteristic composition of nanostructured lipid carriers (spatially incompatible solid and liquid lipids), solid lipid nanoparticles (solid lipid), and liquid emulsion (liquid lipid) describing clearly the reason for higher drug loading capacity of solid and liquid imperfect lipid matrix (a) and reduced drug payload and expulsion in highly packed solid (b) and oily (c) lipid core matrix. O = Drug

Advantages of nanostructured lipid carriers over other lipid-based formulation

The following sections explicate various mechanisms and theories that let NLC system overcome general limitations with conventional lipid formulations and prove them to be a better formulation design. It is known that NLC are a new type of lipid nanoparticles which offer the advantages of improved drug loading capacity and release properties along with stable drug incorporation during storage.[23,28] The possible explanations for these desirable attributes of NLC are discussed in detail in subsections below.

Higher drug loading and entrapment potential

In SLN, it is observed that the drug amount soluble in the lipid melt before particle production is higher than in the final SLN and such higher drug concentration in the melt might result in immediate drug expulsion during the cooling process. In contrast, in NLC the solid matrix of the lipid nanoparticle contains a nano-oil section in which drug solubility is higher, thus increasing the total drug loading capacity.[23,29] Thus, in NLC, liquid lipids content affects the entrapment efficiency to a great extent because they cause several crystal defects in solid lipid and cause imperfections in highly ordered crystal matrix providing sufficient space for large amount of drug to lodge successfully.[30]

Modulation of drug release pattern

NLC exhibit a biphasic drug release pattern that is, initial burst release of drug followed by a sustained release at a constant rate. The liquid lipid located in the outer layers of the nanoparticles forms drug-enriched casing which leads to burst release of the drug at the initial stage. Unlike SLN, these oil-enriched outer layers possess substantially higher solubility for lipophilic drugs;[31] therefore, a higher amount of drug could be easily loaded, as well as released by the drug diffusion or the matrix erosion.[32] The initial faster drug release phase is followed by slow release from the solid lipid core. Interestingly, in NLC it is feasible to improvise the release profiles as a function of the lipid matrix composition like by varying the amount of liquid lipid content with respect to solid lipid.[33]

Long-term stability of incorporated drug during storage

The conception of NLC that is, lipid matrices which are solid, but not crystalline is derived from the fact that crystallization process itself causes the expulsion of the drug. By using special mixtures of solid lipids and liquid lipids, the particles become solid after cooling but do not crystallize.[22] In addition to affecting the particle size, entrapment efficiency and *in vitro* drug release characteristics, the liquid lipids built-in the solid lipids in NLC[25] also resolve the hitch of changes mainly crystallinity and polymorphism that may occur upon long-term storage. As crystallization happens because of supersaturation, the presence of liquid lipid is likely to hold on to sub saturation condition of the solid lipid, hence mitigating crystallization.[33]

Minimum level of surfactant with maximum drug loading potential

NLC are easily stabilized with a minimum possible concentration of surfactants along with best results of stability, entrapment, and release. Sometimes, even 0.5-1% of the surfactant is sufficient for developing stable NLC of lipophilic drugs. In addition, full range of excipients is available being of accepted state in contrast to LE where there is a very little scope to play with excipients. This attribute makes NLC far more preferred formulation approach than LE where high and limited use of surfactants is an issue of concern.

Drug candidates for nanostructured lipid carriers formulation

In general, the criterion utilized to classify the drugs includes BCS, which implies that aqueous solubility and membrane permeability are two major factors limiting drug absorption.[6] Considering the biopharmaceutical obstacles in oral absorption of lipophilic drugs such as UWL, P-gp efflux, intra-enterocyte, and hepatic metabolism, BCS alone is not a satisfactory tool for selecting drug candidates for advanced lipid-based formulations. There is a great need of a modified classification system, which also takes into account attributes such as drug metabolism, disposition, and the role of transporters as they affect the absorption process to a very large extent.

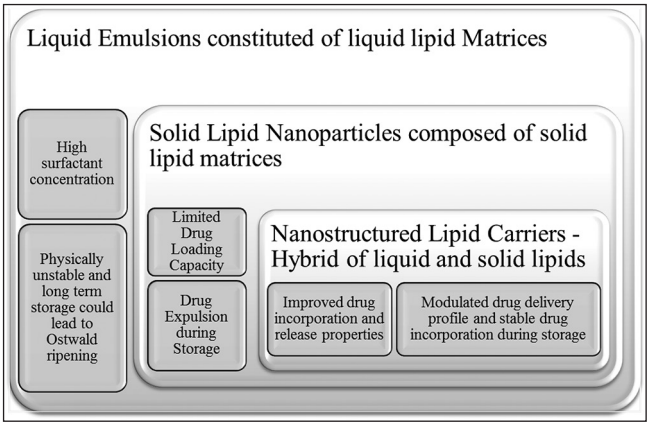

Figure 2: Classification of lipid-based drug delivery system depicting potential advantages of solid-liquid lipid hybrid matrices in contrast to only solid and only liquid lipid formulations

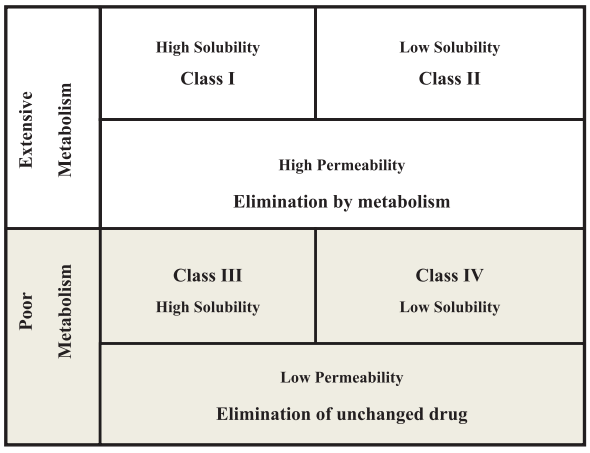

Figure 3: Depiction of a biopharmaceutics drug disposition classification system based on metabolism and elimination of drug.

A review of the drugs, done by Wu and Benet,[34] classified the drugs in Classes I-IV of BCS such that drugs in Classes I and II were metabolized and eliminated, in contrast, Classes III and IV drugs were eliminated unchanged as shown in Figure 3. Figure 3 shows biopharmaceutics drug disposition classification system based on metabolism and elimination of drug which is a modification of Figure 2 of reference 37.[37] This serves as a core criterion for the customized classification system, namely the biopharmaceutics drug disposition classification system (BDDCS). According to this system, the extent of metabolism (or major route of drug elimination) substitutes membrane permeability as classification condition. Importantly, this system takes into account the knowledge of efflux transporters and presystemic metabolism. Lipophilic and poorly water soluble drugs, which are classified as Class II or IV have been known to be potential substrates for intestinal efflux transporters such as P-gp[34-36] and are also known to be metabolized by intestinal CYP enzymes. Consequently, BDDCS could play an essential role in identifying suitable drug candidates which are expected to benefit from NLC formulations. As per this classification, absorption of Class II drugs could be greatly enhanced possibly by selection of those lipids in the formulations, which influence metabolism and/or efflux. The understanding of a particular transporter(s) in the disposition of a specific drug will guide appropriate lipidic excipient selection, with intent of modulating this effect and improving bioavailability. Hence, BDDCS helps in choosing the appropriate drug candidate for apt lipid carrier and maximizes the benefits from coadministration of suitable lipids in NLC.[37]

ROLE OF NANOSTRUCTURED LIPID CARRIERS IN IMPROVING *IN VIVO* PERFORMANCE OF LIPOPHILIC DRUGS

In the earlier sections of this paper, it is justified that NLC are better formulation system than other lipid-based carriers, but how NLC influence the *in vivo* fate of lipophilic drugs is the crux element of this discussion.

Barriers overcome by nanostructured lipid carriers in oral delivery of lipophilic drugs

The main obstacles for efficient absorption of lipophilic drugs and the anticipated mechanisms by which NLC surmount these hurdles are discussed in detail in the following section and pictorially depicted in Figure 4.

Restricted capacity of process of biliary secretion emulsification

Since, poor solubilization is a notable absorption barrier for lipophilic drugs, thus it would be favorable undoubtedly to enhance the biliary secretion course by means of incorporated lipid excipients of the formulation, which would fuel this emulsification dependent absorption cascade in upper part of GIT. The composition of NLC is such that solid and liquid lipids are similar to a fat-rich diet which not only induce the bile

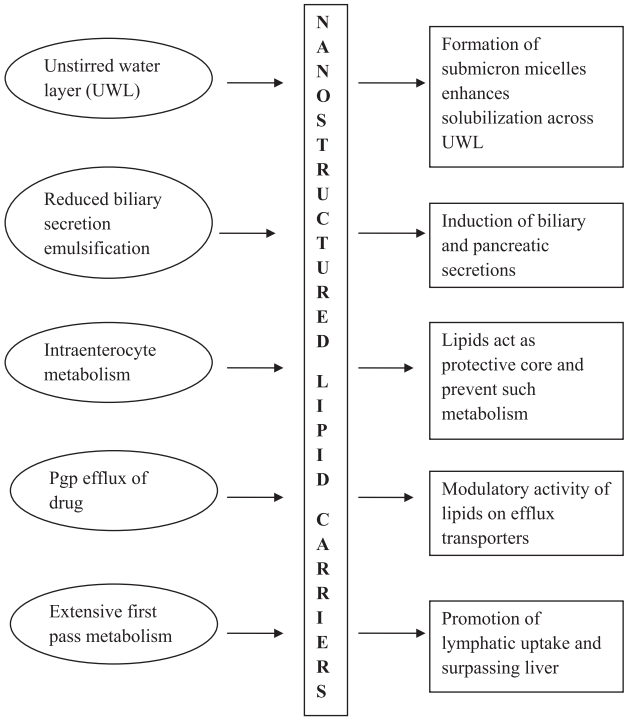

Figure 4: Overview of how nanostructured lipid carriers overcome various barriers for the efficient absorption of poorly water soluble drugs

secretion in the small intestine but also mix with it to form mixed micelles. These micelles would enhance the luminal solubility of the lipid digestion products, and also facilitate their passage across the UWL thereby providing a concentration gradient for absorption.[38,39]

In various research studies, analysis of the composition of NLC of drug candidates (vinpocetine, simvastatin and lovastatin) [Table 1] suggested that enhancement in their bioavailability is attributed to the solid and liquid lipid, which were similar to fat food. These lipids could induce bile secretion in the small intestinal and the drug loaded NLC were associated with bile salts to form mixed micelles which helped the intact NLC to get into the lymphatic vessels and avoid the liver first pass metabolism. The uptake and lymphatic transport of intact NLC played a dominant role in the promoted absorption.[30,33,41]

Unstirred water layer

Free FA, MG and lipophilic molecules, such as lipophilic drugs diffuse very slowly across the UWL, but their micellar solubilization greatly enhances their solubility in the UWL.[45,46] Thus, micellar solubilization plays a key role in improving the solubility of hydrophobic molecules in UWL. As discussed earlier, NLC matrices favor the process of micellar solubilization and make a huge contribution in enhancing the absorption of drugs across the intestinal enterocytes. Furthermore, the particle size of the NLC formulations is extremely small, which improves the surface area of the NLC thereby improving the dissolution of a hydrophobic drug like in UWL of the intestinal epithelial cells.[47]

As shown in various studies, the hybrid lipid composition and reduced particle size of the drug loaded NLC formulations (tamoxifen, saquinavir (SQV), etoposides, vinpocetine, simvastatin and lovastatin) which ranges between 50 and 200 nm, enable these hydrophobic drugs to get across UWL of the intestinal epithelial cells [Table 1]. Such smaller sized lipid nanoparticles also have an efficient gastrointestinal tissue uptake by different routes, like by intracellular pathways.[40]

Intra-enterocyte metabolism

It has been reported that lipids present in the NLC formulation could protect the drug from enzymatic degradation.[41] The choice of a suitable lipids and surfactant mixture of suitable concentration confer metabolic stability to the drug in NLC.[29]

In different studies of drugs loaded NLC (etoposide, lovastatin) [Table 1] one of the major reasons of enhanced bioavailability is that the drug embedded into a lipid matrix reduces its exposure to bacterium, as well as enzymatic degradation during absorption process and thus offers a long time contact with the intestinal wall *in vivo*.

Efflux transporters such as P-glycoprotein

Of significance is the drug integrated NLC formulations with specific lipid excipients, which can modulate P-gp-mediated efflux activity and have the potential to alter the pharmacokinetics of the administered compound to a great extent.[48-51] A substantial investigation has shown that certain lipids and surfactants utilized in NLC are capable of inhibiting P-gp-mediated drug efflux by the gut wall.[52-56] These include surfactants like Cremophor EL and Solutol HS 15, which can modulate efflux pump activity,[57] Pluronic P85 block copolymer has been shown to inhibit P-gp[58] Reportedly, widely used lipid excipients such as Peceol and Gelucire 44/14 have also been shown to influence drug transport by P-gp. However, the mechanism by which these excipients inhibit P-gp activity is at present unidentified, but various theories suggest that alteration of the integrity of the cell membrane, blocking binding sites competitively, noncompetitively, or allosterically, interfering with ATP hydrolysis and creating a futile cycle of ATP hydrolysis could be the possible mechanisms for inhibition of P-gp efflux.[59-61]

In a study, NLC formulations were assessed for their potential to increase the drug bioavailability using SQV (a P-gp substrate) as a model drug. Results suggested that NLC enhanced SQV permeability up to 3.5-fold. With modification of critical physicochemical parameters of the formulation such as concentration of surfactant (poloxamer 188 and tween 80) P-gp drug efflux was overcome and transcytosis mechanism of the nanoparticles was altered as proved in a mechanistic study of NLC transport across intestinal *in vitro* models [Table 1]. Study findings were encouraging for the delivery of P-gp substrates by means of their NLC formulation through the oral route.[42]

Extensive hepatic metabolism

The transport of NLC through the intestinal lymphatic through the thoracic lymph duct to the systemic circulation at the junction of the jugular and left subclavian vein, bypasses the liver. It

Table 1: Drug candidates prepared as NLC which are formulated using hybrid of solid and liquid lipids

Drug candidate	Drug Selection criterion besides poor water solubility	Liquid lipid and Solid lipid	Surfactants	Method of preparation	In *vivo* model/*In vitro* cell culture study	References
Etoposide	Substrate of P-gp	Soyabean oil and monostearin	Soy lecithin and PEG-40	Emulsification and low temperature solidification	Male Sprague-Dawley rats	[40]
Vinpocetine	Extensive hepatic metabolism	Miglyol and monostearin and compritol 888	Poloxamer 888 and lecithin	High pressure homogenization	Male Wistar rats	[30]
Simvastatin	Extensively metabolized in the intestinal gut and liver by CYP3A4	Oleic acid and glyceryl monostearate	Poloxamer 407	Solvent injection method	Balb/c mice	[33]
Lovastatin	Significant first pass metabolism and short half life	Squalene and precirol	Myverol and pluronic F68	High shear homogenization	Male Sprague-Dawley rats	[41]
Saquinavir	Substrate of P-gp	Miglyol and precirol ATO5	Tween 80 and poloxamer 188	High pressure homogenization	Caco-2 and FAE monolayers	[42]
Tamoxifen	Extensive first pass metabolism	Labrafil WL 2609BS and glyceryl monostearate		Solvent diffusion	*In vivo* testing in female Sprague-Dawley rats/ assessment of *in vitro* anticancer activity in MCF-7 and ZR-75-1	[43]
CMT-3 (4-dedimethylam inosancyline)	Suitable candidate for lymphatic transport (besides poor water solubility)	Oleic acid, stearic acid, and glyceryl monostearate	Cremophor EL	High pressure homogenization	HeLa human cervical cancer cell line	[44]

NLC: Nanostructured lipid carriers, P-gp: P-glycoprotein, PEG: Polyethylene glycol, CYP3A4: Cytochrome P450 3A4, FAE: Follicle-associated epithelium

is reported that long chain lipids utilized in NLC stimulate lymphatic transport, whereas medium chain lipids integrated in NLC are poor stimulators of lymphatic convey.[62,63]

Reportedly, the uptake and lymphatic transport of intact colloidal nanosized NLC supposedly played a dominant role in promoting absorption of drugs such as tamoxifen, vinpocetine, simvastatin, and lovastatin which are extensively metabolized in liver [Table 1].

Lymphatic uptake factor

Highly lipophilic drugs, when incorporated into NLC, prefer to reach the systemic blood circulation via the intestinal lymphatic system rather than through direct absorption into the portal blood.[64,65] This substitute absorptive pathway has been a noteworthy player in enhancing the overall bioavailability of a number of lipophilic drugs when formulated as SLLH matrices.[21] Solid and liquid lipids present in formulation adjust the pathway of drug transport to the systemic circulation instead of portal vein which trims down the possibilities of first-pass drug metabolism as intestinal lymph travels directly to systemic circulation thereby surpassing liver. Therefore, many lipophilic drugs with poor solubility can be successfully targeted through lymphatic route by means of NLC formulations with enhanced bioavailability.[18]

The lipophilic drugs after oral administration are absorbed and then diffuse across the intestinal enterocytes, where they combine with enterocyte lipoproteins. This is followed by the secretion of the chylomicron-associated drug from the enterocyte to the lymphatic circulation, instead of the portal circulation, thereby bypassing the liver.[66] Therefore, for drugs which are extensively metabolized on the first pass through the liver, their transport via the lymphatic route can provide them rescue and significantly enhance their oral bioavailability.[67,68]

STUDY OF THE EFFECT OF SOLID-LIPID HYBRID MATRICES ON BIOAVAILABILITY OF LIPOPHILIC DRUGS

Drug candidates for oral NLC system are selected on the basis of BDDCS classification. Therefore, besides drug solubility and permeability other factors that are to be given due consideration include drug metabolism, disposition and affinity toward efflux transporters mainly P-gp. NLC of poorly water soluble and highly lipophilic drugs such as etoposide, vinpocetine, lovastatin, and simvastatin have been prepared by research groups worldwide using suitable liquid and solid lipid excipients. An extensive review of such studies has been carried out and is briefly summarized in Table 1.

From these studies, it has been observed that drugs can be loaded with high efficiency in NLC's which are also comparatively more stable during storage as compared to LE and SLN. Importantly, there was manifolds increase in bioavailability of these above stated highly lipophilic drugs when formulated as NLC confirmed via *in vivo* testing. *In vitro* cell cultures, studies showed a highly efficient drug transport through target cells (Caco-2 and follicle-associated epithelium monolayers for SQV formulated as NLC).[42] An assessment of anticancer activity of drug loaded NLC in cell cultures resulted in great inhibition of proliferation of cancerous cells (HeLa human cervical cancer cell for CMT-3 incorporated in NLC and MCF-7 and ZR-75-1 for tamoxifen integrated NLC)[43,44] as given in Table 1. Besides this, a sustained and targeted therapeutic action was also achieved.

There is a large amount of *in vivo* data showing the increase in absorption of poorly water-soluble and highly lipophilic drugs when formulated as NLC. Oral bioavailability of hydrophobic drugs such as etoposide, SQV, lovastatin, simvastatin, vinpocetine, fenofibrate, etc. have been greatly enhanced by means of their NLC formulation. From the studies conducted so far, it can be concluded that NLC are potential delivery system for successful targeting of highly lipophilic and poorly water soluble drugs having significant first pass metabolism and affinity toward P-gp efflux, with improved bioavailability. The increase in bioavailability is mainly attributed to the presence of a mixture of solid and liquid lipids. Various mechanisms by which these lipids would have enhanced the phenomenon of absorption are depicted in Figure 4.

Different mechanisms have been documented for the improved absorption of the NLC from the intestine that include direct uptake through the gastrointestinal tract (through lymphatic route), inducing pancreatic and biliary secretion, promoting formation of micelles and decreased metabolic degradation by enzymes present in gut wall (CYP3A4). Furthermore, the particle size of the NLC formulations is extremely small, and this reduced particle size improves the surface area of the NLC.[47] This small size allows the efficient uptake in the intestine particularly in the lymphoidal section of the tissue thus by passing the first pass metabolism. Another factor that facilitates absorption of NLC in the intestinal milieu is their high dispersibility. Besides this, the NLC can also adhere onto the gut wall prolonging the residence time, and consequently the absorption.[33] Another marked elucidation for the phenomenon of enhancement in bioavailability of drugs is that due to incorporation of excipients/surfactants such as poloxamer and gelucire., NLC are capable of minimizing the drug efflux by P-gp and this strategy has been utilized by various researchers for the development of oral lipid nanoparticles of drugs which are substrates of P-gp.[69]

However, the amplified bioavailability of these lipophilic drugs is mainly credited to the fact that solid and liquid lipids in NLC affect the absorption of poorly soluble drugs by varying the characteristics of intestinal milieu, facilitation of intestinal lymphatic drug transport and significant input in enterocyte based transport processes.[18,70,71] However, there is a lack of sufficient evident data generated through confirmatory studies (on *in vitro* or *in vivo* models) based on which these mechanisms underlying such markedly improved bioavailability could be validated for further development.

Therefore, the drug integrated NLC should be exploited for known biochemical processes in the GIT to decipher the accurate mechanisms of their *in vivo* proceedings. Advanced and composite human intestinal cell culture models articulating a wide range of enzymes and transporters which direct the transport and metabolism of lipophilic drugs are requisite for performing mechanistic studies at the cellular level. In-depth assessment of the functional activity of various transporters and enzymes in intestinal membranes, which govern the ultimate destination of poorly soluble drugs is highly required. Highly sophisticated and complex *in vitro* and *in vivo* models are needed to be employed to accomplish the task of comprehensive experimentation for investigating the mechanisms of absorption enhancement.[72]

Through this review, two important aspects of NLC have been explored, firstly NLC as a formulation approach and secondly the *in vivo* providence of this formulation. Though one aspect deals with various attributes of SLLH that makes it a superior lipid-based formulation than LE and SLN, the other one elucidates how SLLH defy all the possible barriers of oral absorption of poorly water-soluble drugs. In the end, a survey of recent NLC of drugs establish the impregnable role of NLC formulation in enhancing the bioavailability of lipophilic drugs.

In this review, the major difficulties encountered with the conventional lipid-based system are recognized. Among them, one of the major issues is that the drug is expelled out of formulation on cooling, which disrupts the stability of the lipidic formulation. The main benefit with NLC matrix is that drug has adequate space to lodge itself in the imperfections of solid and liquid lipids and as a result matrices are not jam packed, and, therefore, do not lead to expulsion of drugs. Thus, the problem of the expulsion of drugs associated with lipid-based formulations is sorted satisfactorily in NLC owing to their special nanostructures. In addition, the other difficulties accompanied with LE and SLN regarding crystallization, long-term stability, etc. are also well addressed by NLC as the liquid lipid embedded solid-liquid resolves all these ambiguities. Besides this, one of the main advantageous features of NLC is the highly minimized concentration of surfactants incorporated in them. In general, the minimum possible quantity of surfactants is desirable in the formulations to avoid toxicological concerns and with NLC; it is possible to stabilize the formulations with an extremely low concentration of surfactants.

Of interest, the review explains how NLC interplay with various barriers of oral absorption of lipophilic drugs like UWL, P-gp efflux, intra-enterocyte and hepatic metabolism. Lipids and surfactants used in NLC possess distinguished and desirable characteristic features which can enhance the possibility of NLC to combat all possible limitations of oral absorption. The role of lipids in enhancing biliary secretions, modulating CYP enzyme and P-gp activity and stimulating lymphatic uptake has already got noticed, and considerable research is being done currently to further investigate their contribution. In a nutshell, NLC represent a novel formulation strategy for improving *in vivo* proceedings of lipophilic drugs by successfully overcoming the hurdles to efficient oral absorption such as poor solubilization because of limited capacity of process of biliary secretion emulsification, UWL, efflux transporters such as P-gp, significant hepatic metabolism and intra-enterocyte metabolism.

The promising results obtained using NLC's in animal models suggest that the formulations should be extensively tested in humans subjects, which would in turn strengthen the fact that NLC's significantly enhance bioavailability of lipophilic drugs.

In vitro model studies, preclinical and clinical data on a wider range of drug candidates with differing physicochemical properties would help elucidate the mechanisms of action of the NLC formulations. In addition, it would also provide valuable information leading to the intelligent and biocompatible selection of suitable lipid components for improved oral bioavailability of lipophilic drugs. Moreover, it would facilitate a strategic design of NLC, which be considered as "personalized NLC" which stepwise consider different aspects of formulation development starting with selection of specific drug candidate followed by identification of particular cause of its poor absorption and then accordingly selecting components of formulation, which can modulate that cause (efflux transporter, intra-enterocyte enzyme activity etc.,). Such formulations can then be successfully brought to industrial and marketable levels.

Further exploration of the potential of NLC formulations in improving the absorption of highly lipophilic drugs in conjunction with thorough understanding of the effect of formulation components on efflux transporter, metabolic enzymes and lymphatic uptake, would help in completely addressing all the negative aspects of oral delivery of lipophilic drugs. Thus, a complete and better understanding of the drug integrated NLC formulations in tandem with biopharmaceutical complexities is required to drive a successful execution of this hybrid system so as to maximize the therapeutic efficacies of lipophilic drugs after their oral delivery.

CONCLUSIONS

NLC is a potential approach for improving the bioavailability of highly lipophilic drugs with poor aqueous solubility, extensive first pass metabolism, affinity for P-gp efflux transporters, and susceptibility to intra-enterocyte metabolism. In a way, the high caliber of NLC system shadows the petition of other lipid-based dosage form. Disrupted matrices of NLC consequently lead to higher drug loading, higher drug entrapment, modulated drug release and ultimately enhanced drug absorption as compared with other lipid-based formulations (SLN and LE) having uniform matrices of lipids. These special features of NLC are exclusively attributed to their unique composition, which is constituted of a blend of incompatible solid and liquid lipids.

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Conflicts of interest

There are no conflicts of interest.

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