Nanostructured lipid carrier for bioavailability enhancement
Kamaldeep Kaur¹, Ujjwal Nautiyal*, Devendra Singh²

¹Himachal Institute of Pharmacy, Paonta sahib, H.P., India
² Tirupati Life Science, Paonta Sahib, H.P., India

ABSTRACT
Nanostructured lipid carriers (NLCs) are second generation drug carrier systems having solid matrix at room temperature. This carrier system is made up of physiological, biodegradable and biocompatible lipid materials and surfactants and is accepted by regulatory authorities for application in different drug delivery systems. The main objective of this review is to explore the role of NLCs system for delivering drugs by oral route and thus increasing the oral bioavailability methods. The present review article highlights the definition and types of NLCs and their importance as colloidal carriers including the production techniques and their formulation. This review article also deals with the fate of lipids used in the NLCs formulation and the NLCs toxicity.

Keywords: Bioavailability, Biocompatible, Oral route, Colloidal carriers, Toxicity

Introduction
New drug molecules are being introduced into the pharmaceutical industry everyday but only the development of new drugs alone is not sufficient to assure the progress in drug therapy. The most common problem faced is low-solubility of drug molecule which ultimately leads to low bioavailability. Therefore, there is an increasing requirement to develop a drug carrier system that overcomes these drawbacks. The carrier system should have some important characteristics such as no toxicity (acute and chronic) have a sufficient drug loading capacity and the feasibility of drug targeting and controlled release. The carrier system should also provide chemical and physical stability for the incorporated drug[1-3]. Lipid-based nanoparticle formulations may also increase the drug absorption by improving dissolution and solubility in the intestinal milieu by reducing gastric emptying rate and increase in mucosal permeability. Lipids are used to increase lymph formation and also promote lymph flow rate[4]. NLCs are composed of biocompatible solid lipid matrices and liquid lipid which have different chemical structure from the solid lipid[5]. Nanocrystals are a fast-release system that has similar effects to those of solid dispersion and cyclodextrin inclusion[6].

Bioavailability
Bioavailability is a subcategory of absorption and is the fraction of an administered dose of unchanged drug that reaches the systematic circulation one of the principal pharmacokinetic properties of drugs. By definition, when a medication is administered intravenously, its bioavailability is 100%. However, when a medication is administered via other routes (such as orally), its bioavailability generally decreases (due to incomplete absorption and first-pass metabolism) or may vary from patient to patient. Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration[7].

Objectives of bioavailability studies
Bioavailability studies are important in the…
1. Primary stages of development of a suitable dosage form for a new drug entity.
2. Determination of influence of recipients, patient related factors & possible interaction with other drugs on the efficiency of absorption.
3. Development of new formulations of the existing drugs.
4. Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage & stability on drug absorption[8].
Types of Bioavailability
1. Absolute bioavailability
2. Relative bioavailability

1. Absolute bioavailability: Absolute bioavailability is determined by comparing the blood (plasma) concentration-time-curves (usually as area under the curve) of a compound after application (usually orally) of that compound to that after intravenous application of the identical compound (e.g. tablet vs. i.v. or capsule vs. i.v. or from a food vs. i.v.). So, i.v. application is used as the reference form of application.

2. Relative bioavailability: Relative bioavailability is determined by comparing the plasma concentration-time-curves (usually as area under the curve) after administration of two different formulations of the same compound (e.g. capsule vs. tablet vs. dissolved in water etc). So, i.v. application is used as the reference form of application.

Importance to improve the Bioavailability
1. Poor aqueous solubility and slow dissolution rate in biological fluids.
2. Poor stability of the dissolved drug at the physiological pH.
3. Poor permeation through biomembrane due to inadequate partition coefficient.

Figure 1: Schematic diagram of mechanisms of intestinal drug transport from lipid-based formulations

NOVEL LIPID CARRIERS
The traditional lipid based systems are not efficient enough to solve the solubility problems associated with the lipophilic drugs and also these systems are associated with large limitations such as low stability, poor patient compliance etc. Thus there was a need for new carrier systems which led to the development of novel lipid based carriers. Some of these carriers utilized for solubility enhancement include:
1. Microemulsion and Nanoemulsion
2. Self Emulsifying Drug Delivery System
3. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers
4. Liposomes

1. Microemulsion & Nanoemulsion

In 1959, Schulman et al. visualized the existence of small emulsion-like structures by electron microscopy and subsequently coined the term 'microemulsion'. Microemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with a droplet size usually in the range of 20-200 nm. These homogeneous systems are all fluids of low viscosity. Microemulsions as drug delivery vehicle show favourable properties like thermodynamic stability (long shelf-life), easy formation (zero interfacial tension and almost spontaneous formation), optical isotropy, high surface area (high solubilisation capacity), very small droplet size and surfactant-induced permeability enhancement. The small droplets also provide better adherence to membranes and...
transport drug molecules in a controlled fashion. Further reduction in droplet size of the internal phase of the microemulsion led to the development of nanoemulsion. Nanoemulsions are thermodynamically stable, transparent (or translucent) dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size less than 100 nm.(12).

2. Self Emulsifying Drug Delivery System (SEDDS)
Although the nanosized or submicronic emulsions improve the GI absorption of hydrophobic drugs, the use of these emulsions in oral delivery is limited, owing to various limitations. These include- poor palatability due to their lipidic composition or consumption of a higher volume to achieve the necessary therapeutic concentration for certain drugs which have limited solubility in all the oils with pharmaceutical acceptability for eg. Carbamazepine, Quercetin. This severely limits patient compliance. Also, as these emulsions have high water content, they cannot be delivered through soft gelatin, hard gelatin or hydroxypropylmethylcellulose capsules and the water content of these emulsions may promote hydrolysis and/or precipitation of certain drugs on long-term storage, which could affect their utility in oral delivery(11). This led to the development of Self emulsifying drug delivery system. Self-emulsifying formulations comprise of isotropic mixtures of natural or synthetic oils with lipophilic or hydrophilic surfactants and co-solvent(s) which spontaneously emulsify when exposed to the fluids of the GIT to form oil-in-water emulsions, microemulsions or nanoemulsions. Self emulsifying formulations also provide the advantage of increased drug loading capacity when compared with lipid solutions, as the solubility of poorly water-soluble drugs with intermediate partition coefficients(13).

3. Solid Lipid Nanoparticle (SLN) & Nanostructured Lipid Carriers (NLCs)
Solid lipid nanoparticles (SLN) are produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipids. The lipid particle matrix remains solid at both room and body temperature allowing drug release over prolonged period of time. SLN are composed of 0.1 to 30% (w/w) of solid lipid dispersed in an aqueous medium having mean particle size in the submicron range of about 40nm to 1000nm. They are biodegradable, non-toxic and stable against coalescence, hydrolysis and particle growth. SLN are produced by high-pressure homogenization of the solid matrix and drug with an aqueous solution of the surfactants. The drug may be incorporated into the SLNs in different ways i.e. into a homogeneous matrix or into shells or as a lipid-coated core. Some common problems associated with SLN included drug expulsion, low loading capacity, risk of gelation and drug leakage during storage caused by lipid polymorphism. The nanostructured lipid carriers (NLCs) are regarded as the second-generation of lipid nanoparticles and have been developed to overcome the limitations associated with SLN. They are produced by controlled mixing of solid lipids with spatially incompatible liquid lipids which leads to special nanostructure with improved properties for drug loading, modulation of the drug release profile and stable drug incorporation during storage. Depending on the method of preparation and the composition of lipid blend, NLCs with different structures are obtained, i.e., the imperfect, amorphous and multiple components. Admixture of liquid lipids with solid lipids leads to a less ordered inner structure due to which the drug molecules are accommodated in between lipid layers and/or fatty acid chains. Thus, NLCs are considered a smarter generation of nanoparticles. The absorption-enhancing effect of orally administered nanoparticles may be attributed to the adhesion of the particles to the gut wall. The adhesion of lipid nanoparticles to the mucus can improve the residence time and contact of the drug with the underlying epithelium, thus increasing the concentration gradient. Also, the protection of the drug by the lipids from chemicals and enzymatic degradation, delay the in vivo metabolism . Due to their protective properties, SLNs and NLCs are of particular interest for peptide and protein delivery by oral route(13,14).

4. Liposomes
Liposome are vesicular systems in which lipid bilayer structures are present with aqueous volume entirely enclosed by a membrane, composed of lipid molecules. Liposomes are the most promising, broadly applicable, and highly researched of all novel delivery systems as they offer temporal control of drug release and site to the similarity between liposomal lipid bilayers and biomembranes and their relatively small size, liposomes significantly facilitate oral absorption of drugs(14,15).

The role of lipids in enhancement of bioavailability
The bioavailability of some of the drugs is increased when co-administered with food. However, many drug molecules have negligible interaction with food. BCS class I drugs are not affected by the presence or absence of food, but class II drugs have an altered absorption when co-administered with food. The reason for such enhanced bioavailability might be attributed to solubility, permeability and inhibition of efflux transporters in the presence of food. Some of the drugs which show enhanced bioavailability when administered along with food are griseofulvin,
halofantrine, danazol, troglitazone and atovaquone. A guidance document entitled “Food-Effect Bioavailability and Fed Bioequivalence” was issued by FDA in December 2002. The US FDA recommended high fat meals for food-effect studies because such fatty meals (800–1000 cal, 50%–65% fat, 25%–30% carbohydrates and 15%–20% proteins) affect GI physiology and maximize drug transfer into the systemic circulation(16). In particular, it is the lipid component of the food that plays a vital role in the absorption of lipophilic drugs, leading to enhanced oral bioavailability. This can be explained by the ability of a high fat meal to stimulate biliary and pancreatic secretions, to decrease metabolism and efflux activity, to increase intestinal wall permeability, and to a prolongation of gastrointestinal tract (GIT) residence time and transport via lymphatic system. Triglycerides and long chain fatty acids play a major role in prolonging the GIT residence time. Also, a high fat meal elevates the TG-rich lipoproteins which react with drug molecules. This association of lipoproteins with drug molecules enhances intestinal lymphatic transport and leads to changes in drug disposition and finally changes the kinetics of the pharmacological actions of poorly soluble drugs. This food effect on drug absorption leads to a serious concern about the sub-therapeutic plasma drug concentration when co-administered without food. Such food effect is also a serious problem for drugs with a narrow therapeutic index, where increased bioavailability may lead to serious untoward effects. Hence, control or monitoring of food intake is required when dosing such drugs. However, food-dependent bioavailability can be significantly reduced by formulating the drug as a lipid-based formulation, which can increase the solubility and dissolution of lipophilic drugs and facilitate the formation of solubilized species, from which absorption occurs. Hence, lipid-based formulations can be used to reduce the dose of drug while simultaneously enhancing its oral bioavailability(17,18).

**Nanostructured lipid carrier**

The lipid-based NLCs system was developed to overcome the limitations of SLNs, such as drug loading into a solid matrix and drug expulsion during storage because of polymorphic modification of the lipid particles. SLNs use only one form of lipid, ie, a solid lipid that orients the drug between the fatty acid chains of glycerides. In contrast, NLCs use a blend into the lymphatic system. The lipid nanoparticles accumulate at the injection site, and the drug is slowly released from the nanoparticles. The free drug can enter the blood circulation via pores on the walls of the capillaries. Smaller lipid nanoparticles (<0.1 µm) can easily access the lymphatic capillaries and concentrate in regional lymph nodes(19). Thus, based on these advantages, NLCs could be developed as a carrier for lymphatic drug delivery by subcutaneous administration because they have improved physicochemical properties compared with other lipid-based nanocarrier systems.

**Types of Nanostructured lipid carrier**

- **Highly imperfect matrix**
- **Multiple O/F/W**
- **Non-crystalline amorphous**

**Highly imperfect matrix**: Solid lipids and fluid lipids (oils) are blended. The difference in the organizations of the lipids and exceptional requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix structure proposing space for drug substances and amorphous clusters of pharmaceuticals.

**Multiple O/F/W type**: Drug can be accommodated in the solid, but at increased solubility in the oily components of the lipid matrix.

**Non-crystalline amorphous**: Lipids are blended in a way that stops them from crystallizing. The lipid matrix is solid, but in an amorphous state.

**Figure 2**: I – Highly imperfect matrix, II – Multiple O/F/W type, III – non-crystalline amorphous NLC.

**CARRIERS USED IN NLC**

Kaur et al

*International Journal of Recent Advances in Science and Technology, 2015; 2(1): 1-9*
SOLID LIPID
- Bees wax
- Carnauba wax
- Stearic acid
- Glyceryl monostearate
- Soya lecithin
- Phosphatidycholine
- Sabowax
- Apifil
- Compritol

LIQUID LIPID
- Castor oil
- Palm oil
- Olive oil
- Cetiol V
- Miglyol 81

Advantages of Nanostructured lipid carrier
- Control and targeted drug release.
- Improve stability of pharmaceuticals
- High and enhanced drug content (compared to other carriers).
- Feasibilities of carrying both lipophilic and hydrophilic drugs.
- Most lipids being biodegradable, SLNs have excellent biocompatibility.
- Water based technology (avoid organic solvents).
- More affordable (less expensive than polymeric/surfactant based carriers)
- Easier to validate and gain regulatory approval.

Disadvantages of Nanostructured lipid carrier
- Cause irritation action of some surfactant.
- Cause cytotoxic effects in nature of concentration (19,20).

Methods of Manufacturing of NLC

Different methods of SLN/NLC formulation are described here-

1. Homogenization techniques
   i. Hot high pressure homogenization technique
   ii. Cold high pressure homogenization technique
   iii. Melt emulsification ultrasound (ultrasonication) homogenization technique
      (High shear homogenization and/or ultrasound technique)
2. Microemulsion technique
3. Emulsification-solvent evaporation technique
4. Solvent displacement or injection technique
5. Emulsification-solvent diffusion technique
6. Phase inversion technique
7. Film ultrasonication dispersion technique
8. Multiple emulsion technique

High Pressure Homogenization Techniques
Since the fifties of the last century, high pressure homogenization is a well established technology for the production of emulsions for parenteral nutrition, such as Intralipid and Lipofundin, and it can also be adapted for scale-up production of lipid nanoparticles. The preparation of lipid nanoparticles applying the high pressure homogenization techniques has been developed and practiced extensively (22,23). Hot as well as cold homogenization processes can be used for the preparation of lipid nanoparticles. In both processes the active compound is dissolved or dispersed in the melted lipid prior to the high pressure homogenization. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (having size of few microns). Particles formed are in submicron range due to very high shear stress and cavitation forces generated in the homogenizer. Large scale production of lipid nanoparticles are possible with this technique with regulatory acceptance as production lines are very much in use for manufacturing of parenteral lipid emulsions since long period. This is the main advantage of this method as compared with other available technique but high energy conditions of temperature and pressure questioned its applicability in certain conditions.

Hot High Pressure Homogenization Technique
For hot homogenization, a pre-emulsion of the drug loaded lipid melt and the emulsifier solution is prepared with a high-shear mixing device (such as Ultra-Turrax). Pre-emulsion is then passed through a high pressure homogenization cycle at temperatures above the melting point of the lipid. Lipid nanoparticles are formed by the following cooling of the sample to room temperature or to temperatures below. The active compound-containing melted lipid is dispersed in the hot surfactant solution at the same temperature applying high-speed stirring. The obtained hot pre-emulsion is passed through a high pressure homogenizer applying number of homogenization cycles. A nanoemulsion is formed which is upon cooling yield aqueous dispersion of lipid nanoparticles. Hot HPH technique is the most frequently applied. It can be used for the entrapment of lipophilic and insoluble drugs in the lipid. Temperature sensitive compounds can also be processed by hot HPH as exposure time to high temperatures is relatively short.
However, for hydrophilic drugs this procedure is not the most appropriated one. During the homogenization of the melted lipid phase the drug will partition to the water phase resulting in a too low encapsulation rate. Figure 1 describes the schematic procedure for the preparation of lipid nanoparticles by this method.

### Cold High Pressure Homogenization Technique
In contrast to hot homogenization, the cold homogenization is carried out with the solid lipid without melting as done in hot process. Drug along with lipid in solid state is milled to form microparticles, and further dispersed in a solution containing emulsifier. The pre-suspension formed is then subjected to high pressure homogenization at or below room temperature (24). In the cold HPH technique, lipid is melted above its melting point and then subjected to high pressure homogenization at or below room temperature (24). In the cold HPH technique, lipid is melted above its melting point and drug is dissolved or dispersed in it. The system is cooled down by means of dry ice or liquid nitrogen. After solidification, the lipid mass is grounded using ball or mortar milling to yield lipid microparticles in a range between 50 and 100 µm. Then a microemulsion is formed by adding these microparticles into cold surfactant solution with stirring. This suspension is passed through a high pressure homogenizer at or below room temperature and the microparticles are broken down to nanoparticles. The cold HPH technique minimizes the thermal exposure to the drugs and active substances. Therefore, this technique may be applied for temperature sensitive compounds. Hydrophilic compounds can also be incorporated by this method which might partition from the liquid lipid phase to the water phase during the hot HPH. To further minimize the loss of hydrophilic compounds to the aqueous phase of the suspension, water can be replaced by liquids with low solubility for the drug, such as oils and polyethylene glycols of low molecular weight. Lipid particles prepared using the cold HPH technique possess a slightly higher PI and mean particle size compared to the ones obtained by hot HPH technique. Homogenization cycles can be increase to further reduce the particle size and to minimize the polydispersity.

### Emulsification-Solvent Evaporation Technique
This is a method analogous to the production of polymeric nanoparticles and microparticles by solvent evaporation in o/w emulsions (25,26) via precipitation. In the solvent emulsification-evaporation the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under condition of reduced pressure. The lipid precipitates upon evaporation of the solvent thus forming nanoparticles. Firstly, an organic phase has produced containing the lipid material dissolved in a water-immiscible organic solvent, and then the drug is dissolved or dispersed in that solution. This organic phase is emulsified in an o/w surfactant containing aqueous phase by mechanical stirring. Subsequent quick removal of solvent by evaporation from the obtained o/w emulsion under mechanical stirring or reduced pressure nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The solvent evaporation step must be quickly in order to avoid particle aggregation. This method is suitable for the incorporation of highly thermolabile drugs due to avoidance of heat during the preparation but presence of solvent residues in the final dispersion may create problems due to regulatory concern. Limited solubility of lipids in organic materials generally leads to dilute dispersions and need to concentrate by means of another process such as ultra-filtration, evaporation or lyophilization. On the other hand small particle size around 100 nm with narrow size distribution can be achieved by this method. This procedure has schematically depicted in Figure 3.

### Solvent Displacement or Injection Technique
The solvent displacement technique was first described for the preparation of liposomes and polymeric nanoparticles from pre-formed polymers. Recently, this technique has also been used to prepare lipid nanoparticles (26,27). Precipitation of lipid dissolved in solution is the basis of this process. In this method, a solution of the lipid in a water-miscible solvent or a water-miscible solvent mixture is rapidly injected into an aqueous phase with or without surfactant. In this process, an o/w emulsion has been formed by injecting organic phase into the aqueous phase under magnetic stirring. The oil phase is a semi-polar water-miscible solvent, such as ethanol, acetone or methanol, lipid material is dissolved in it and then the active compound is dissolved or dispersed in this phase. Aqueous phase consists of surfactant. In this procedure solvent displacement of diffusion takes place and lipid precipitate has obtained. Solvent removal is necessary and can be performed by distillation. The lipid nanoparticles are formed after total evaporation of the water miscible organic solvent. Particle size is dependent on the preparation conditions such as amount to be injected, concentration of lipid and emulsifier. This method offers clear advantages over the existing methods such as the use of organic solvents which is pharmaceutically accepted, high pressure homogenization not required, ease in handling and less time consuming without technically sophisticated equipment. Disadvantages clearly evident is use of organic solvent although they are pharmaceutically accepted excipients frequently used in formulations(28).
Characterization of prepared NLCs

**FT-IR Spectroscopy**

FT-IR helps to confirm the identity of the drug and to detect the interaction of the drug with carriers. FT-IR spectral measurement for pure Lornoxicam drug, lipid Compritol ATO 888, and physical mixtures of Lornoxicam and Compritol ATO 888 (Fig. 1, 2, 3) were taken at ambient temperature. All the spectra acquired were scanned between 400 and 4000 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. (IRAffinity1, Shimadzu (S.No.A21374801815), Japan.)

**DSC Analysis**

Differential scanning calorimetry (DSC) analysis was performed on a DSC60 detector (Shimadzu Co., Japan). Approximately 5 mg of sample was weighed into an aluminium pan and sealed hermetically. DSC scan was recorded from 30 to 300 °C at a heating rate of 10 °C/min under a nitrogen purge, using an empty Scattering particle size analyzer (Nanotrac particle size analyzer). The range of the analyzer is 0.02 nm to 2.8 µm.

**Particle size analysis**

The size distribution along the volume mean diameter of the nanoparticle was measured by Dynamic Light Scattering particle size analyzer (Nanotrac particle size analyzer). The range of the analyzer is 0.02 nm to 2.8 µm.

**Zeta potential**

Zeta potential was measured by using Zetatrac. It is easily measured because charge of a potential will move as the suspension is placed between the two electrodes that have D.C. voltage across them and the velocity will be proportional to the zeta potential of the particle. The technical term for this is electrophoresis. (Zetatrac, Korea.)

**Entrapment efficiency**

From the prepared NLC formulation 1ml of the dispersion was dissolved in the (1:1) mixture of 10 ml of 7.4 PBS buffer and ethanol. The mixture was centrifuged at 15,000 rpm for 40 min at 25°C to separate free drug in the supernatant. Concentration of Lornoxicam in the supernatant was determined by UV-visible spectrophotometer at 376 nm after suitable dilution. Entrapment efficiency was calculated using following equation. (Sigma 3K 30 Sartorious, Refrigerated Centrifuge.) (ShimadzuUV-1700 PharmaSpec

**FTIR studies**

FT-IR spectroscopy was carried out to study the compatibility of pure drug Lornoxicam with the lipid Compritol ATO 888 used in the formulation of nanostructured lipid carrier. The pure Lornoxicam has characteristic IR peaks at 3433.29 cm$^{-1}$ (aromatic N-H stretch), 1645.28 cm$^{-1}$ (aromatic C=O stretch), 1085.92 cm$^{-1}$ (S=O stretch), 3066.82 cm$^{-1}$ (aromatic C-H stretch), 1537.27 cm$^{-1}$ (aromatic C=C stretch), 1327.03 cm$^{-1}$ (C=N stretch) and 630.72 cm$^{-1}$ (C-Cl stretch) as depicted in (Fig. 1). Compritol ATO 888 has a characteristic IR absorption frequency at 1732.08 cm$^{-1}$ (C=O stretch) and 3064.89 cm$^{-1}$ (C-H stretch), depicted in (Fig. 2). The IR spectra of the physical mixture of both exhibited all the characteristic peaks of Lornoxicam and Compritol ATO 888 as depicted in Therefore, it shows compatibility of Lornoxicam with Compritol ATO 888

**Conclusion**

As particle size of NLC is less than 400 nm, it can retain in periodontal cavity for longer duration. Amount of lipid in it has profound effect on entrapment efficiency. High entrapment efficiency was attributed to the imperfections in the crystal lattice, leading to increased drugloading. The larger particles showed slow in vitro release whereas the small particles exhibited a faster release. The Zeta Potential value predicted good particle stability because the repulsive forces prevent aggregation with aging. The developed technology platform can be extended for many other potential actives required to elicit prolonged action. It is further suggested that by changing the ratio of solid lipid and liquid lipid, release profile can be modified.

**REFERENCES**


28. Varshosaz J, Eskandari S, Tabakhian M. Pharm Dev Technol 2009; 00: 000–000 Received ahead of print, courtesy Varshosaz J.


Source of Support: Nil
Conflict of Interest: None