2. LITERATURE REVIEW

2.1 Oral Lipid based Formulations

"These new compounds, like rocks, never dissolve in water." …… Rong (Ron) Liu [1]

Approximately 41% of new drug candidates failed due to poor biopharmaceutical behavior, mainly due to poor water-solubility, less luminal solubility and slow dissolution rate. These undesirable properties of these compounds often limit their development as useful medicines [2]. To facilitate effective absorption following oral administration, drug candidates must be both stable and soluble in the gastrointestinal (GI) fluids and possess reasonable gastrointestinal permeability. Lipid-based drug delivery systems (LBDDS) is one of the numerous ways that have been investigated to increase the oral bioavailability of poorly water-soluble molecules [3]. LBDDS range from simple solutions of drug in oil to emulsions and self-microemulsifying drug delivery systems (SMEDDS) containing lipids, surfactants and co-solvents and vesicular systems such as niosomes. LBDDS are generally filled into hard or soft gelatin capsules to allow for oral administration. Traditionally, major attention was focused on the composition and self-emulsification properties of LBDDS as the critical determinants of utility. However, their optimization and their *in vivo* fate were majorly neglected [4]. In particular, formulation which is not optimized for size or entrapment efficiency, in many cases, fails to perform superior in *in vivo* conditions due to drug precipitation and may decrease bioavailability [5]. Hence, here in this research work we have focused on optimization of LBDDS and their *in vivo* fate after oral administration.

2.2 Factors affecting oral bioavailability and LBDDS role to increase bioavailability

To reach the molecule from gut to blood, it must be absorbed from the gastrointestinal tract and avoid first pass metabolism effect through the enterocytes and liver. To get absorbed the prime requirement is to get solubilized in luminal fluid [6]. Hence, in totality, the factors affecting bioavailability are:

1. Solubility
2. Transport across GI tract
3. Pre-systemic metabolism

1. Solubility

Molecular dispersion of a drug is a prerequisite for its absorption across biological membranes as partitioning into and across the enterocytes won't happen. Dissolution and solubility in GIT are the rate determining steps for drug absorption of poorly water-soluble molecules [7]. Noyes-Whitney equation indicates that rate of dissolution is directly proportional to drug solubility. As such, low solubility often also dictates slow dissolution, and together low solubility and slow dissolution provide a significant barrier to effective absorption [8].

The thermodynamic theory governing drug solubilization shows that increased solubility is favored by reduced intermolecular forces in solid state and enhanced solute–solvent interactions in the bulk solution [9]. In turn these properties are typically predicated by differences in melting point and lipophilicity. Thus, compounds having high melting and high log P are poorly water soluble, whereas those having low melting point, low (or negative) log P compounds are typically water-soluble [10]. Solubility is also indicated by changes in molecular weight and planarity, since both have an impact on crystal packing and therefore melting point. However, lipophilicity is a major determinant factor of both solubility and permeability and in general, drugs must be hydrophilic enough to be solubilized in the aqueous environment of the GI tract, and at the same time possess sufficient lipophilic character to partition into and ultimately cross, the GI membrane.

The nature of the GI fluids and their associated solubilization capacity can be regarded as the combined the enhancements in solubility resulting from the presence of endogenous solubilizing components, and the enhancements in solubility resulting from the presence of exogenous components (that is, formulation-derived) [11]. It is undeniable that certain exogenous components might be expected to lead the changes in the nature of the GI fluids and enhance drug solubilization. Typical examples of such components include surfactants, co-solvents and complexation agents. However, formulation- or food-derived lipids can influence GI solubilization through an increase in solubilization capacity attributable to the lipid itself and through stimulation of physiological processes, which lead to the enhanced secretion of endogenous biliary-derived solubilizing components such as bile salts and phospholipids [12]. The solubilization capacity of the GI tract is therefore determined by the interaction of exogenous lipids with the GI environment [13]. The physiological changes that the lipid

component stimulates and the combined involvement of both exogenous and endogenous components in the colloidal species that support enhanced drug solubilization.

2. Transport across GI tract

Drug transport across the intestinal epithelial layer can occur via passive diffusion or active transport [14]. Passage across the enterocytes by diffusion is restricted to small, lipophilic molecules whereas transcytosis is for particles less than 200 nm in diameter. Passage through the paracellular pathway is impossible if the tight junctions are intact [15]. However, numerous research reports the appearance of particles in the circulation after oral dosing of LBDDS which goes through the roller-coaster ride in GIT [13].

These indicates that the uptake might have occurred via Peyer's patches, which are specialized areas of the gut-associated immune system [16]. The epithelium overlying the follicle is called the follicle-associated epithelium (FAE). This FAE contains specialized cells termed as M cells, which have unique structural features, with sparse irregular microvilli on the apical side.

Another pathway of absorption is transcellular route, via the intestinal transport system. This is the major mechanism of intestinal lymphatic delivery of lipophilic compounds following co-administration of lipid-based vehicles [17].

The stability of the drug in the GIT and transport across the enterocyte and liver, are also key determinants of oral bioavailability. Drug stability may be affected by chemical degradation, for example as a result of exposure to the acidic pH within the stomach, by luminal instability such as breakdown caused by digestive enzymes, or by more classical first pass metabolic events mediated by enzymes present in the enterocyte or liver [18].

3. Pre-systemic metabolism

Cytochrome P 450 (CYPs) are one of the metabolizing enzymes that plays an important role in oxidative metabolism reactions. Subfamily CYP3A is located in enterocytes and hepatocytes. CYP3A within enterocytes of intestine is the major reason behind gut wall mediated metabolism [19]. The fraction of an orally administered dose that reaches the systemic circulation can be reduced by both extra-hepatic (intestinal) and hepatic first pass metabolism. However, orally administered drugs encounter intestinal drug-metabolizing enzymes before

reaching the liver and as such, the intestinal enterocyte sees the highest concentrations of drug (and formulation excipients). Saturation of enterocyte based first pass metabolism is therefore a significant contributor to non-linear changes to drug exposure stemming from changes in first pas metabolism [20].

Many CYP3A substrates are also substrates of the membrane efflux pump, P-glycoprotein (P-gp). P-gp efflux provide a potential limitation to drug absorption directly and can further influence the pre-systemic metabolism, and subsequent fraction of dose absorbed, by promoting the recycling of drugs between enterocytes and the gut lumen, thereby increasing drug exposure to intestinal CYP enzymes [21].

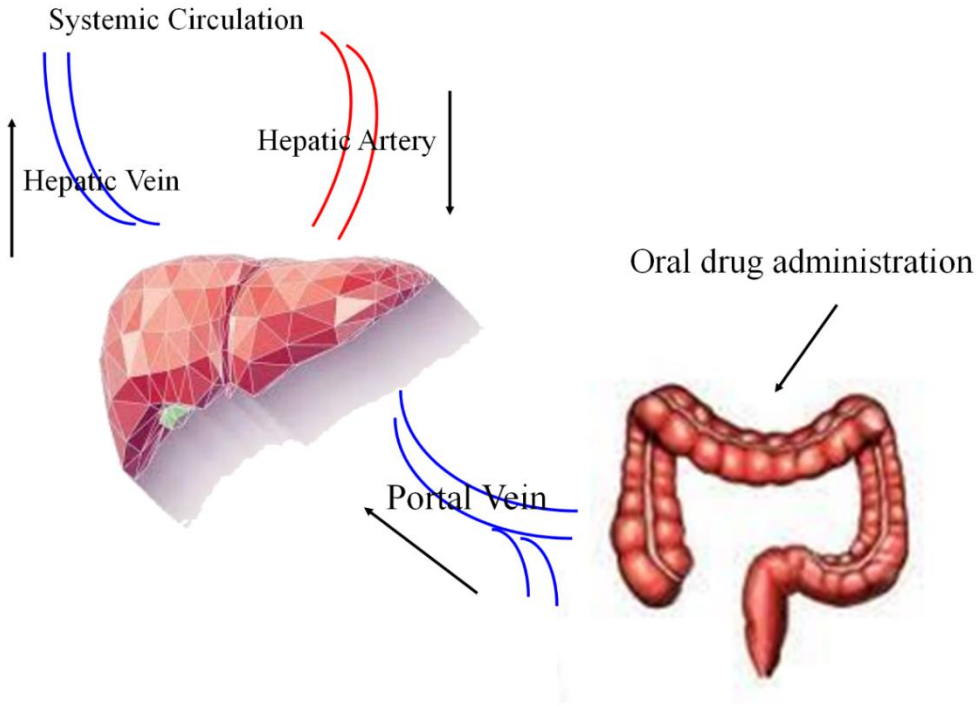

Figure 2.1 First Pass (Pre-systemic) Metabolism

As shown in figure 2.1, any oral medication will go through the portal circulation which lead to first pass metabolism [22]. However, LBDDS by virtue of increasing solubility of poorly water-soluble drug candidate, give a way for absorption via lymphatic pathway. This route can be of importance if it provides an improved transport sink away from the intestine.

2.3 Digestion and absorption of lipids

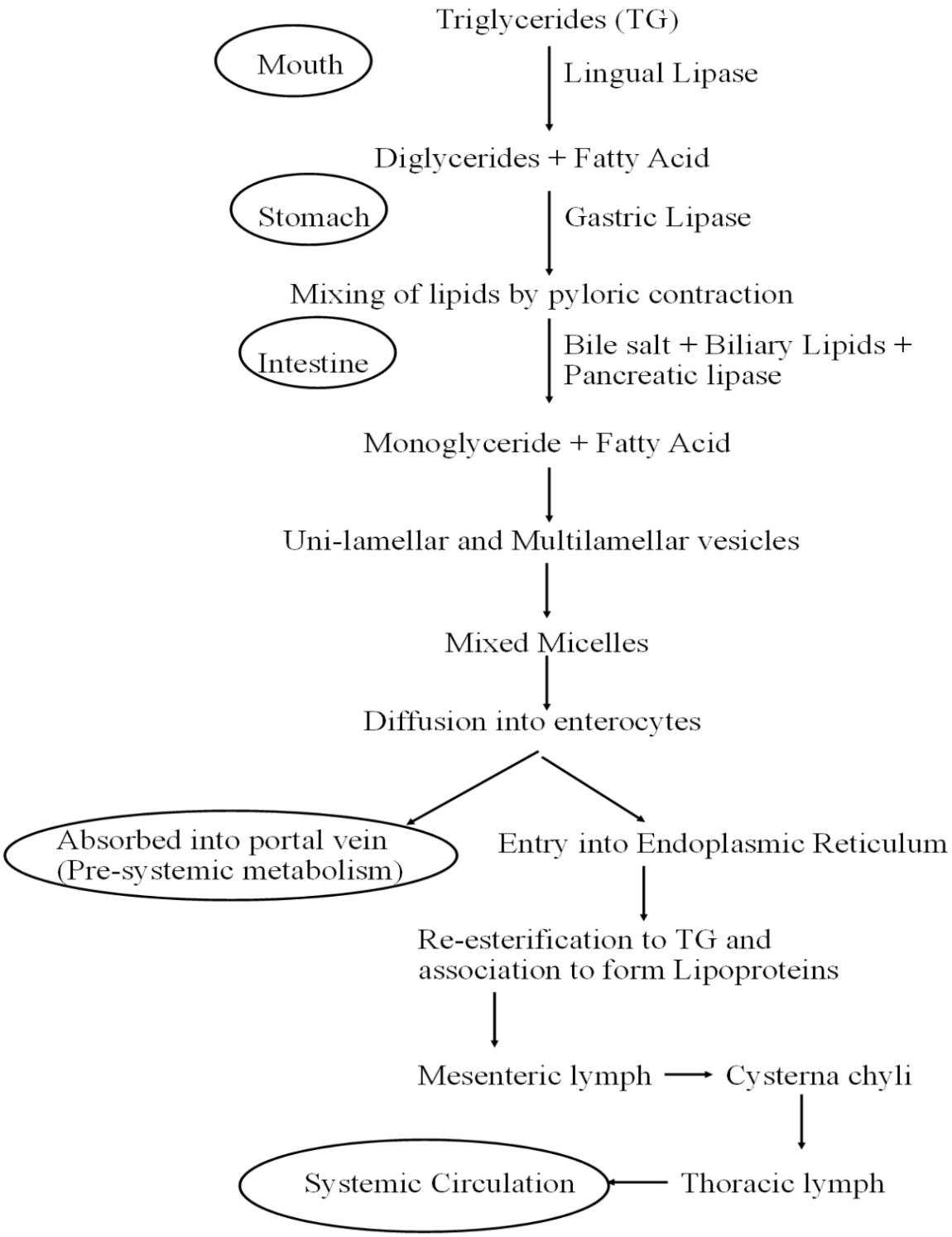

Figure 2.2 Digestion and absorption pathway of Dietary Lipid

The triglycerides (TG) of diet undergoes hydrolysis by the action of lingual and gastric lipase to form monoglycerides and free fatty acids. Entry of cholesterol, and lipid's byproducts in duodenum stimulates release of bile salt and biliary lipids from gall bladder. Due to surfactant like action of bile salts, it forms mixed micelles. These greatly enhances luminal solubility of cholesterol and lipid byproducts and provides concentration gradient for diffusion of cholesterol and lipid byproducts into enterocytes at intestinal absorption site.

Fatty acids are transported to enterocytes by fatty acid binding protein and fatty acid transporters. These carrier molecules transports lipids from enterocytes depending on their chain length. Short chain lipid enters to portal circulation whereas long chain lipids enters endoplasmic reticulum and undergo re-esterification to form triglycerides either by mono-acyl glycerol pathway or phosphatidic pathway. These formed triglycerides assemble into lipoproteins and fuses with basolateral cell membrane of enterocytes and get released into systemic circulation via thoracic pathway [23].

As shown in figure 2.2, the natural absorption pathway of lipids is the main mechanism of action of LBDDS. However, the interaction of lipid-based colloidal formulations with GI system and associated digestive process is not completely understood and appears to be more complex than mere solubility enhancement. The other possible mechanisms are:

1. Solubilization of the drug
2. Reduction of P-glycoprotein-mediated efflux
3. Mitigation of hepatic first pass metabolism through enhanced lymphatic transport
4. Prolongation of gastrointestinal (GI) transit time
5. Protection from degradation in the GI tract.

2.4 Self Microemulsifying Drug Delivery System (SMEDDS)

SMEDDS is defined as isotropic mixture of oil and surfactants/co-surfactants that form fine O/W microemulsion of less than 200 nm size upon dilution in aqueous media. This thermodynamically stable microemulsions are attracting attention for oral delivery of poorly water-soluble drugs due to advantages in terms of improving the absorption of drugs, increased solubility, higher drug loading capacity and suitability for large-scale production.

The major advantages of SMEDDS are:

1. Increased oral bioavailability → reduction in dose
2. Selective targeting toward specific absorption window in GIT i.e. small intestine → promotes lymphatic absorption
3. Protection of drug from the hostile environment in the GIT
4. Reduced variability due to food effects
5. Protection of sensitive drugs
6. High drug payload
7. Thermodynamically stable
8. Require minimum energy for formation
9. Ease of manufacturing and scale-up

Microemulsions formed after dilution of SMEDDS in aqueous media are in submicron size which gives transparent appearance to diluted SMEDDS [24]. The microemulsion also known as swollen micelles or solubilized oil is formed when the interfacial tension at the oil/water interface is at very low level. Practically, microemulsion won't form spontaneously due to the presence of kinetic energy barriers. Hence, it is necessary to agitate or heat it in order to form a microemulsion from surfactant/co-surfactant, oil, and water. In a microemulsion, free energy of the colloidal dispersion (o/w) is less than the free energy (ΔG) of the separate phases i.e. oil and water, which means that a microemulsion is thermodynamically stable. Table 2.1 enlists marketed formulations of SMEDDS.

Table 2.1 Marketed formulation of SMEDDS

Trade name	Molecule	Target Disease	Marketed by
Vesanoid®	Tretinoin	Acne	Roche laboratories, USA
Accutane®	Isotretinoin	Acne	Roche laboratories, USA
Aptivus®	Tipranavir	HIV	Boehringer Ingelheim, Germany
Gengraf®	Cyclosporine A	After organ transplant	Abbott Laboratories, USA
Neoral®	Cyclosporine A	After organ transplant	Novartis, Switzerland
Sandimmune®	Cyclosporine A	After organ transplant	Novartis, Switzerland
Norvir®	Ritonavir	HIV	Abbott laboratories, USA
Fortovase®	Saquinavir	HIV	Hoffmann-LaRoche inc., Switzerland
Agenerase®	Amprenavir	HIV	Glaxo Smithkline, USA
Convulex®	Valproic acid	Epilepsy	Pharmacia, USA
Lipirex®	Fenofibrate	hypercholesterolemia	Genus, United Kingdom
Rocaltrol®	Calcitriol	Vitamin D3 deficiency	Roche laboratories, USA
Sustiva®	Efavirenz	HIV	Bristol-Myers Squibb, USA
Lamprene®	Clofazamine	Leprosy	Novartis, Switzerland

2.5 Composition of SMEDDS

SMEDDS mainly consists of Oil (lipophilic phase), Surfactant and Co-surfactants.

A. Oil (Lipophilic Phase)

Both medium and long chain triglycerides (MCT and LCT), with different degrees of unsaturation, natural oils and modified oils have been widely used as lipophilic phase for SMEDDS development. Natural oils are unarguably best as one of the component for SMEDDS but poor solubility of drug molecules and difficulty in self-emulsification markedly reduce their use for SMEDDS development. Whereas, MCT, LCT and modified (hydrogenated) vegetable

oils are widely used for SMEDDS as they easily overcome issues prevailing with natural oils. Different oils used for SMEDDS are listed in table 2.2.

Table 2.2 Oils (Lipophilic phase) used in SMEDDS

Natural oil (LCT)	Soyabean oil, Castor oil, Sunflower oil, Olive oil, Sesame oil, Palm oil, Peanut oil, Corn oil, Cottonseed oil
MCT	Capryli acid, capric acid, fractionated coconut oil, Traicetin, Captex 300, Labrafac Capmul MCM, Capmul MCM C8
Long chain Monoglyceride (LCM)	Glyceryl mono-oleate (Peceol), Glyceryl mono-linoleate (Maisine 35)
Propylene glycol (PG) fatty acid ester	PG dicaprylate (Miglyol 840) PG diacrylate ester (Sefsol 218) PG diester of caprylic/capric acid (Labrafac PG) PG monolaurate (Lauroglycol FCC, Lauroglycol 90, Capmul PG 12)
Fatty acid ester	Ethyl oleate, ethyl butyrate, isopropyl myristate, isopropyl palmitate
Fatty acid	Oleic acid, Caprylic acid

B. Surfactants

The most preferred surfactant is non-ionic surfactant for SMEDDS as they are reported to be less toxic. In that also, those with high HLB are advocated for SMEDDS. Generally, polysorbates series (TWEEN) and ethoxylated castor oil (Cremophor EL) is the widely used surfactant. The high HLB and their hydrophilic nature is of utmost importance for immediate formation of o/w microemulsions in the aqueous environment which provides good self-emulsification.

Commonly used surfactants for SMEDDS are:

1. Poly-oxy-ethylene sorbitan monolaurate – Tween 20
2. Poly-oxy-ethylene sorbitan monopalmitate – Tween 40

3. Poly-oxy-ethylene sorbitan monostearate – Tween 60
4. Poly-oxy-ethylene sorbitan monooleate – Tween 80
5. Poly-oxy-ethylene glycerol trioleate – Tagot TO
6. Poly-oxy-ethylene 40 hydrogenated castor oil – Cremophor RH 40
7. Poly-oxy-ethylene 35 castor oil – Cremophor EL
8. Poly-oxy-ethylene 10 oleyl ether – Brij 96
9. Poly-oxy-ethylene 23 Lauryl ether – Brij 35
10. Alpha tocopherol TPGS

C. Co-surfactants

Co-surfactants (co-solvents) increases solvent capacity of surfactants and helps in the self-dispersion of SMEDDS which contain high payload of drug in oil, thus enhancing the solubilization process for drugs in water. Generally, solvents are used as co-surfactants such as ethanol or Transcutol HP. Other than these, PEG 200, PEG 400, propylene glycol is also used.

2.6 Pseudo-ternary phase diagram

Phase diagrams are triangle diagram, which shows number of phases and their respective % weight. A pseudo-ternary diagram is constructed to determine the optimum ratio of the individual components. In a ternary diagram only 3 components are there on each axis. Whereas, in SMEDDS, we have mixture of surfactant and co-surfactant (Smix). Hence, at one axis we put fixed ratio of Smix. Hence, it is termed as pseudo-ternary diagram [25]. This requires the pre-determination of a fixed ratio between surfactants and co-surfactants considering their surface-active properties.

When oil, surfactant, co-surfactant and water are mixed, formation of microemulsion is one of the possible association structure that can be formed. Hence, pseudo-ternary phase diagram is often constructed to find different zones, including the microemulsion zone (Figure 2.3). At high water content, O/W type of microemulsion is formed, while at lower water concentration, as the situation is reversed it forms W/O microemulsion. In each phase, a surfactant rich film separates oil and water droplets. In systems when amount of oil and water are nearly equivalent, it forms bi-continuous structure. Surface active agents, which forms interface between two

immiscible liquids (oil and water) helps in forming microemulsion by decreasing interfacial tension between the phases. There can be formation of different structures, such as droplet, rod-like, hexagonal, lamellar or cubic.

Proper selection of components and its amount is very important for the development of a robust self-emulsifying drug delivery system for oral delivery [26]. As shown in figure 2.3, construction of pseudo-ternary phase diagram is an important tool to assess the effect of different formulation component on *in vitro* performance. It provides scientific basis for the selection of different components and *in vitro* performance of optimized formulation can be correlated with its *in vivo* efficacy.

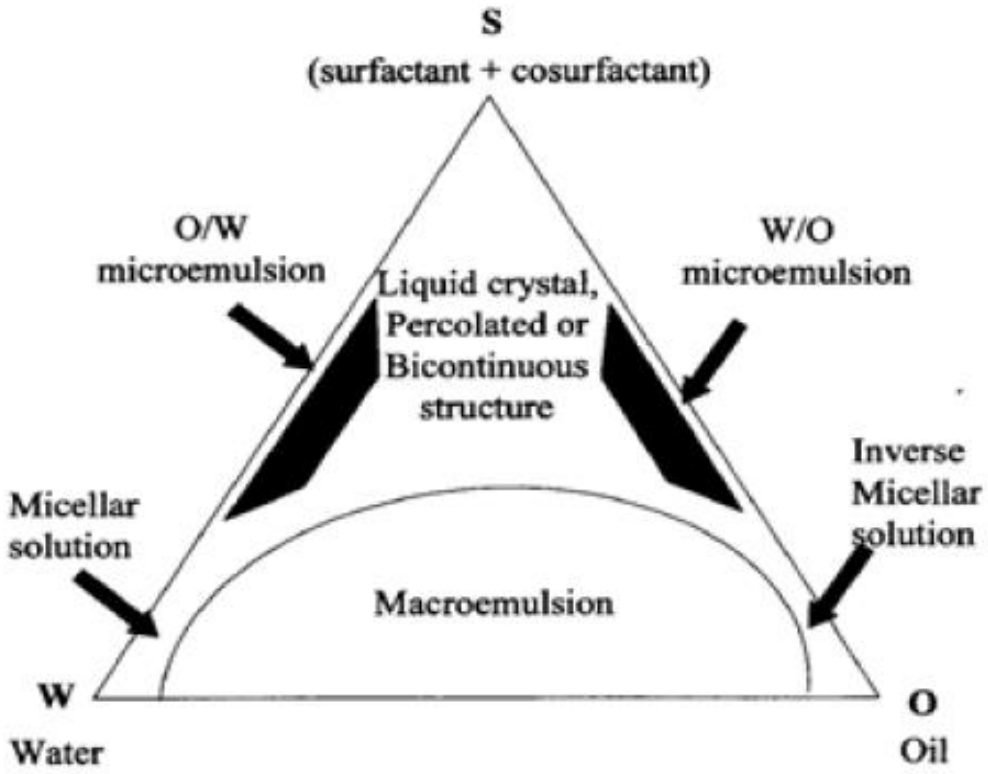

Figure 2.3 Pseudo ternary phase diagram showing zones of emulsion

2.7 Formulation development

Micro-emulsions are usually developed empirically since no adequate theory exists to predict from which materials they are formed. These isotropic systems are usually difficult to formulate than ordinary emulsions because their formation is highly specific process involving

spontaneous interactions among the constituent molecules [27]. To create the micro droplets, the microemulsion must form spontaneously during the preparation; stirring or other mechanical disintegration of a liquid system cannot create small droplets of size less than 200nm. This means that both the ratio and the nature of the emulsifiers are critical for a microemulsion system. Thus, it is essential for a systematic study of microemulsion composition, to establish phase diagrams for the system under investigation. From these, the extent of the microemulsion region can be identified and its relation to other phases established. Phase diagrams are constructed from the data gathered either by titration or by the preparation of a large number of samples of different compositions. An advantage of the titration method is that it can be used to study a large number of compositions relatively quickly.

2.8 Examples of studies related to SMEDDS for oral delivery

SMEDDS have been increasingly employed to enhance oral bioavailability of poorly water-soluble molecules and a number of examples of increased drug bioavailability following oral administration of SMEDDS are summarized in Table 2.3.

Table 2.3 Examples of studies describing the effectiveness of SMEDDS formulations for poorly water-soluble drugs

Molecule	Use	Formulation Composition	In vivo Study Design	Results	Ref
Atorvastatin	Anti-hypercholesterolemia	Three SMEDDS formulations comprising Cremophor RH40, propylene glycol and labrafil or estol or labrafac	Relative BA in dogs	BA significantly increased using SMEDDS formulations	[28]
Baicalein	Anti-inflammatory	Caprylic capric triglyceride, Cremophor RH40, Transcutol P	Relative BA in rats	200.7% increase in relative bioavailability compared to suspension	[29]
Celecoxib	Anti-inflammatory	PEG-8 caprylic/capric glycerides, mixture of Tween20 and Propylene glycol monocaprylic ester	Relative BA in human male volunteers	Relative BA increased 132% as compared to conventional capsules	[30]
Dabigatran Etexilate	Anti-coagulant	Capmul MCM C8, Cremophor EL and Transcutol HP as	Relative BA in rats	3.36-fold increase in bioavailability	[31]
Exemestane	Anticancer	Capryol 90, Cremophore ELP and Transcutol P	Relative BA in rats	2.9-fold increase in bioavailability	[32]

Fenofibrate	Anti-hypercholesterolemia	Labrafac CM10, Tween 80, PEG 400	Pharmacodynamic study in rats	Lipid lowering efficiency of SMEDDS showed higher pharmacodynamic potential than drug suspension	[33]
Halofantrine Hf	Anti-malarial	F1: Captex 355, Capmul MCM, Cremophor EL, ethanol (MCT SMEDDS) F2: Soyabean oil, maisine 35-l, Cremophor EL, ethanol (LCT SMEDDS)	Absolute BA in dogs	52.7 % (MCT SMEDDS) and 67.3% (LCT SMEDDS) absolute BA of Hf which was six- to eight-fold higher relative to Hf.HCl tablet formulation	[34]
Itraconazole	Anti-fungal	Transcutol, pluronic L64 and tocopherol acetate	Relative BA in rats	Increased BA and reduced food effect from SMEDDS	[35]
Itraconazole	Anti-fungal	Transcutol, pluronic L64 and tocopherol acetate	Relative BA under differing dietary conditions in rats	More consistent (and in some cases enhanced) BA from the SEDDS formulation across the differing dietary conditions	[35]
Leuprorelin	Anticancer	Cremophor EL, Capmul MCM, Propylene glycol and Captex 355	Relative BA in rats	17.2-fold improved oral bioavailability of leuprolide oleate SMEDDS compared to a	[36]

				leuprolide acetate control solution	
Mitotane	Anticancer	Capryol, Tween and Cremophor EL	Relative BA in rabbits	3.4-fold increase in bioavailability as compared to marketed formulation Lysodren®	[37]
Oridonin	Anticancer	Maisine 35-1 and Labrafac CC, Cremopher EL, and Transcutol P	Relative BA in rats	2.2-fold increase in bioavailability as compared to suspension	[38]
Puerarin	Alcoholism treatment	Castor oil, Cremophor® EL and 1,2-propanediol	Relative BA in dogs	2.6-fold increase in bioavailability compared with the conventional tablet	[39]
Ro-15-0778	(Not given)	polyglycolysed glycerides and peanut oil), PEG 400 solution	Relative BA in dogs	3-fold increase in bioavailability compared with the conventional tablet	[40]
Simvastatin	Anti-hypercholesterolemia	Carpryol 90, Cremophor EL, and Carbitol	Relative BA in dogs	1.5-fold increase in bioavailability compared with the conventional tablet	[41]
Tacrolimus	Eczema treatment	Two SMEDDS system containing Miglyol 840,	Relative BA in rats	Sevenfold (TPGS-SMEDDS) and eightfold (Crem-	[42]

		Transcutol P and TPGS/Cremophor EL40		SMEDDS) increase in bioavailability compared with the drug solution	
Vinpocetine	Neuro-protection Anti-oxidant	Labrafac, oleic acid, Cremophor EL, Transcutol P	Relative BA in rats	1.91-fold increase in bioavailability	[43]

2.9 Niosomes

Niosomes are well known effective drug carrier systems for drug delivery. Fundamentally, these are non-ionic surfactant vesicles [44]. Niosomes are prepared using non-ionic surfactants along with cholesterol. It is a bilayer structure, where the hydrophilic heads of surfactant remain in contact with the aqueous solvent and the hydrophobic parts are oriented away from the water giving it a vesicular shape. Because of the presence of amphiphilic moieties in the structure, drug is either entrapped inside the aqueous core or is located in the bilayer [45]. An ideal structure of niosomes is depicted in Figure 2.4.

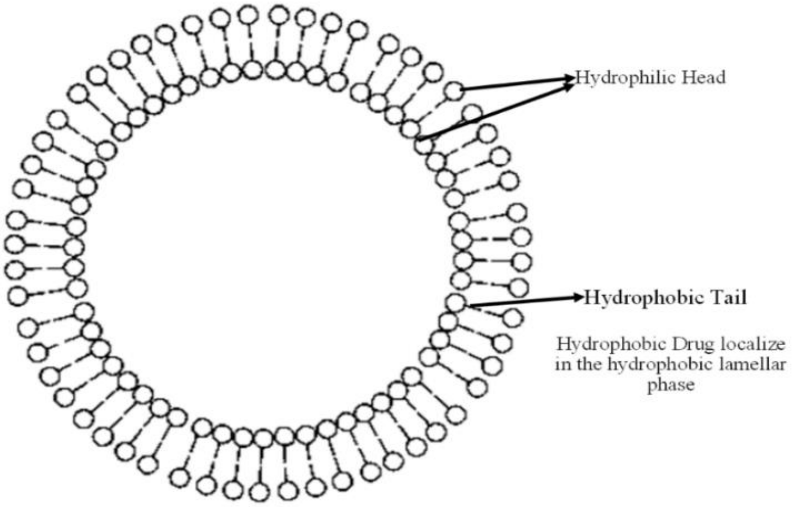

Figure 2.4 Niosomes structure showing bilayer formed by surfactant molecules

The properties of these vesicles changes with variation in composition which lead to difference in size, or lamellarity, and surface charge. The stability of niosomes is affected by the type of surfactant, the nature of the encapsulated drug, the storage temperature, presence of detergents, presence or absence of membrane spanning lipids, importantly interfacial polymerisation of surfactant monomers in situ and inclusion of charged molecule [46].

Niosomes offer better therapeutic performance of drug molecules as they are not cleared promptly from the blood and exert substantial effect on target cells. Any surfactant used for the preparation of niosomes should be biodegradable, biocompatible and most importantly non-immunogenic. Niosomes are similar to liposomes but consist of non-ionic surfactants than

phospholipids. They also sustain drug release and show high tissue accumulation and metabolic stability like liposomes but are cost effective. Intercalation of cholesterol in the bilayers of niosomes is also done to increase stability [47].

Niosomes were first made for a cosmetic product of Tretinoin (Niosome+®) by L'Oreal as antiaging and till date it is the only available marketed product. Table 2.4 shows the marketed formulation of niosomes.

Many synthetic non-ionic surfactants are reported to be used in the formulation of niosomes. Water soluble drugs get accumulated in the aqueous compartment of niosomes. Their entrapment is low and on storage, leakage of the drug is a problem. However, such problems are not countered if the drug is lipid soluble. The drug remains lipid membrane bound and such preparation can be stored in a dry form, meant to be rehydrated prior to use. Therefore, selection of the lipid soluble drug can almost result in 100% entrapment [48].

For the development of niosomes, selection of the best suited techniques amongst the available different techniques is an important criterion. Alongside, selection of surfactant also plays a major role in drug entrapment.

The major advantages of niosomes are [49]:

1. Increased oral bioavailability → reduction in dose
2. Surfactants improve solubility of drugs
3. Increased bio-compatibility due to low toxicity because of their non-ionic nature
4. Biodegradable and non-immunogenic
5. Can be used to entrap lipophilic drugs in vesicular bilayer structure and hydrophilic drugs in aqueous compartments
6. Handling and storage of surfactants requires no special precautions.

2.10 Formulation consideration for Niosomes [44,45,50,51]

1. Non-ionic Surfactants

Nonionic surfactants are most commonly used surface-active agents (SAA) for preparation of vesicles due to the excellent benefits they impart with respect to stability, toxicity and

compatibility than their counterparts i.e. ionic surfactants. Non-ionic SAA are less toxic and less irritating to cellular surfaces.

Usually HLB of 4-8 of any non-ionic SAA is considered ideal for formulation of niosomes. Any SAA with HLB value ranging from 14–17 is not suitable to produce niosomes whereas one with an HLB values between 6-8 gives niosomes with the highest entrapment efficiency. When HLB value of SAA decrease than 6, entrapment efficiency decreases.

The entrapment efficiency is affected by the phase transition temperature (Tc) of the SAA. As Span 60 is having highest Tc, it exhibits the highest entrapment efficiency.

2. Cholesterol

Cholesterol is added to the SAA in order to form a bilayered stable vesicles. It decreases leakiness and give stiffness to the bilayer. It is observed that the addition of cholesterol to SAA suppresses SAA tendency to form aggregates, which provides better stability to the lipid bilayer by promoting the gel liquid transition temperature of the vesicle.

Cholesterol forms hydrogen bonds with hydrophilic head of SAA thereby cholesterol content influences the structures of niosomes and their physical properties such as size, %entrapment efficiency, stability, drug release profile.

%entrapment efficiency plays an important role in vesicular formulations. Increasing cholesterol content improves stability but sometimes if not optimized it decreases %EE as cholesterol fits in the SAA bilayer which decreases space for drug molecules arrangement. Hence, according to the reported results, the amount of cholesterol needs to be optimized depending on the physicochemical properties of SAA and loaded drugs.

3. Surfactant and lipid (cholesterol) concentration

The concentration of surfactant and cholesterol used for niosomes development varies from 1 to 2.5% w/w. The change in amount determines the lamellarity and during hydration lamellar structure may change. Higher surfactant: cholesterol ratio may increase %entrapment but it may affect stability, as it may lead to leakage and it may increase viscosity of formulation as well. As the surfactant/lipid level increases, the amount of drug to be encapsulated also increases leading to an increase in the viscosity of the system. Hence, amount of cholesterol needs to be

optimized to get stable niosomes batch. Cholesterol improves the cohesion and mechanical strength of niosomes and their permeability. Fluidity of niosomes is changed considerably and they become rigid required for their stability. The amount of cholesterol to be added depends on the HLB value of the SAA. As the HLB value increases above 10, minimum amount of cholesterol is added in order to compensate for the larger head groups.

Cholesterol influences the physical properties and structure of niosomes possibly due to its interaction with the nonionic SAA. Surfactants with high HLB cannot form vesicles without cholesterol. This might be due to higher HLB and the smaller CPP (critical packing parameter) of the SAA.

4. Nature of Drug

The drug molecules interact with the hydrophilic head of SAA and develops charge which causes repulsion between the bilayers leading to increased vesicle size as compared to blank niosomes. Usually hydrophobic drugs show less leakage from vesicles than hydrophilic drugs Nature of drug also affects the encapsulation. Hence, combination of hydrophilic and liophilic drug in a single vesicle is possible as hydrophilic drug accumulates in the inner aqueous core of the niosomes whereas hydrophobic drug accumulates in the shell.

5. Hydration temperature

Temperature of the hydration medium plays a major role in the assembly of surfactants into vesicles. The temperature should always be above the gel to liquid phase transition temperature (Tc) of the system. Temperature affects the assembly of surfactants into vesicles.

2.11 Formulation development

There are various methods for niosomes preparation as described here in detail [44,45].

A. Lipid layer hydration

In this technique, thin film of lipids is prepared by dissolving them in organic solvents followed by evaporation of solvent under reduced pressure. The film is then hydrated with an aqueous media at a temperature slightly above the phase transition temperature of the surfactants.

Process variables and formulation variables to be validated include the mass per batch, amount of surfactant, drug: lipid ratio, cholesterol: surfactant ratio, rotation speed of rotary evaporator and the hydration process.

B. Reversed Phase Evaporation

In this method, lipid and surfactant are dissolved in a mixture of organic solvents. An aqueous phase having solubilized drug is then added to this. Followed by sonication w/o microemulsion if formed. To this, after addition of a small amount of aqueous phase, organic phase is removed under low pressure. This forms viscous niosomes suspension which if required is diluted with aqueous phase and is then ultimately heated on a water bath at higher temperature than phase transition temperature of surfactants to yield rigid niosomes.

C. Ethanol Injection

In this technique, surfactant, drug and cholesterol are dissolved in ethanol. It is then injected into aqueous phase (hydrating medium) maintained above phase transition temperature of surfactants to yield niosomes.

D. Micro fluidization

In this method, two streams (one containing drug and the other surfactant) interact at ultra-high velocity. From the interaction chamber, the solution is then passed through a cooling loop to decrease temperature. This forms rigid niosomes. If required, it is returned to reservoir chamber and is recirculate to get desired size of niosomes.

E. Sonication

In this technique, drug solution in aqueous phase is added drop wise to surfactant-cholesterol solution in organic solvent. The mixture is then sonicated using probe sonicator for a pre-determined time interval. The longer the sonication time, it forms smaller niosomes.

F. Multiple Membrane Extrusion

In an organic solvent such as, surfactant and cholesterol are dissolved and a dry thin film is formed. The film is then hydrated with aqueous drug solution. The suspension thus formed is extruded through the polycarbonate membrane to obtain smaller sized vesicles.

G. Handjani-Vila Method

In this method, homogeneous lamellar phase is produced by mixing lipid mixture with an aqueous solution containing drug. The resultant mixture is then homogenized at a controlled temperature by means of ultracentrifugation or agitation.

H. Bubble method

In this method nitrogen gas is passed through a sample of homogenized surfactants to give large unilamellar vesicles. These are then subjected to size reduction to give small unilamellar vesicles.

2.12 Examples of studies related to Niosomes

Table 2.5 shows recent research for niosomes. Table 2.6 shows clinical trials related to niosomes.

Table 2.4 Recent research related to Niosomes

Drug	Surfactant	%Entrapment efficiency	Route of administration	Use	Performance over free drug	Ref
Antioxidants (gallic acid, ascorbic acid)	Tween 60	59.40 ± 1.43	Oral	Nutraceutical application	Promote ability of reducing free radicals	[45]
Beclomethasone dipropionate	Span 60	36.37 ± 2.81	Pulmonary	Anti-asthmatic	Decrease side effect with the aid of fine particle fraction	[44]
Diallyl disulfide	Span-20,40,60,80	74.5 ± 3.2	Intra-peritoneal	Anti-bacterial and antifungal drug	Decrease immunogenicity and lower erythrocyte lysis	[52]
Morin hydrate	Span 60,80 Tween 60	98.62 ± 0.01	Intra-venous	Anti-oxidant, Anti-cancer	Decrease side effect, improve area under curve	[53]
Diclofenac sodium	Span 20,40,60,80,85 Tween 20,40,60,80	58.20 ± 1.75	-	Arthritis	Decreased Immunogenicity and side effect of NSAID	[54]
Ellagic acid	Span 60 Tween 60	38.73 ± 1.58	Transdermal	Anti-oxidant	Lower side effect with higher drug	[55]

					concentration in the dermis layer	
Fluconazole	Span 60	>91%	Oral	Antifungal	Improved effectiveness	[44]
Candesartan Cilexetil	Span 60	99.09 ± 0.04%	Oral	Hypertension	185.88% relative bioavailability reference to drug suspension	[56]
Ginkgo biloba	Tween 80 Span 80	77.5 ± 1.0	Oral	protective effects in the central nervous system, Anticancer	Increased blood concentration and brain deposition	[57]

Table 2.5 Clinical Trials of niosomes

Study	Conditions evaluated	Drug	Development stage	Ref
Pharmacokinetics of Melatonin Niosomes Oral Gel in Healthy Volunteers	Pharmacokinetics of Melatonin	Melatonin	Phase II	[58]
In Vivo Investigation of Novel Nano-vesicles of Salbutamol Sulphate	• Drug effect • Pulmonary Disease	Salbutamol Sulphate	Phase I completed	[59]

2.13 Drug Profile: ILOPERIDONE

Schizophrenia, characterized by profound disruptions in thinking, affecting language, perception and the sense of self, is a severe mental disorder which typically begins in late adolescence or early adulthood [60]. Although recent progress has been achieved in the identification of aberrant genetic events and signaling pathways, the rate of schizophrenic episodes still increases remarkably and they continue to be the cause of a disproportionate level of suicide and mortality across individuals worldwide. Urbanization has been repeatedly associated with increased incidence of schizophrenia. In this internet Wi-Fi technology era without tender touch of human there is increased cases of schizophrenia opposite to decrease in interest rate by banks. This often leads to treatment of schizophrenia costly. Oral administration of drug is one of the economic approach for the treatment. However, a crucial challenge is to deliver therapeutic agents in effective concentration to the D2 and 5HT2 receptors where it provides antagonistic action. As most of the new atypical antipsychotics are BCS class II drugs, dissolution become the rate determining step for absorption. This often leads to BID to QID administration of the molecule to maintain the minimum effective concentration in plasma, which drop – off the patient compliance in the therapy [61]. The potential way to ward off this rate limiting step of mass transfer from the solid surface to liquid phase is to hasten it by solubilizing drug in lipid or oil prior which can enhance solubilization in gut and permeation through the mucosal membrane.

Iloperidone is a psychotropic agent belonging to chemical class of piperidinylbenzisoxazole derivatives, having empirical formula of $C_{24}H_{27}N_2O_4F$ and a molecular weight of 426.5 g/mol. Iloperidone is practically insoluble in water (0.012 mg/mL), sparingly soluble in basic aqueous solution and slightly soluble in acidic aqueous solutions. The limited solubility of drug even at different pH scale leads to increased unabsorbed fraction in the gut, which in turn decrease the effectiveness of drug due to less bioavailable percentage from the total intake. Practical insolubility of Iloperidone in water and significant first-pass metabolism limits its use for conventional oral drug delivery. This necessitates enhancement of solubility to increase its effectiveness. This BCS class II drug, is a white crystalline powder, having biological half-life of 15 – 22 h. Though the relative bioavailability of the oral tablet formulation compared to oral

solution is 96%, the marketed oral dosage form shows poor absolute bioavailability (36%) [62,63]. Hence it is necessary to develop a formulation which will help to overcome the above limitations, thereby providing patient compliant formulation.

ILOPERIDONE (ILO): [64,65]

A. Chemical IUPAC name: 1-[4-[3-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]propoxy]-3-methoxyphenyl]ethenone
B. Proprietary name: Fanapt
C. Empirical Formula: $C_{24}H_{27}FN_2O_4$
D. Molecular Weight: 426.488 g/mol
E. Structure:

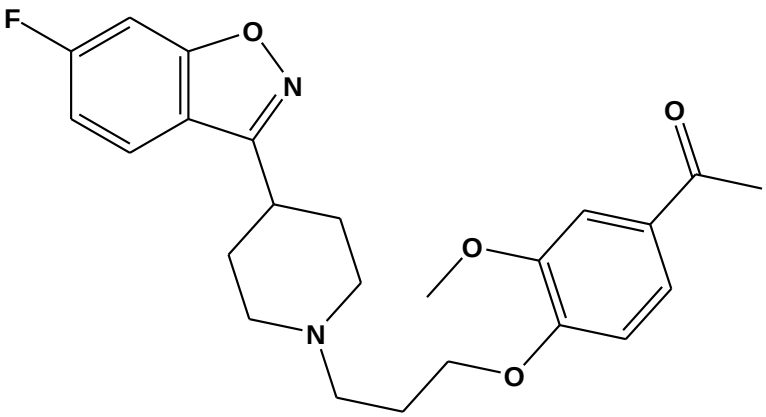

Figure 2.5 Chemical structure of Iloperidone

F. Physicochemical properties
    a. Melting point: 118 – 120 ^{0}C
    b. Solubility: Practically insoluble in water, Very slightly soluble in 0.1 N HCl; freely soluble in chloroform, ethanol, methanol, acetonitrile
    c. pKa: 7.91
    d. LogP: 4.43
    e. BCS Class: II
    f. Hydrogen Bond donor count: 0

g. Hydrogen Bond acceptor count: 7

G. Indication and Use:

ILO is an atypical antipsychotic indicated for the treatment of schizophrenia in adults.

H. Dose and dosing frequency:

The recommended target dosage of ILO is 24 mg/day. This target dosage range is achieved by twice daily dosage adjustments, alerting patients to symptoms of orthostatic hypotension, starting at a dose of 1 mg twice daily, then moving to 2 mg, 4 mg, 6 mg, 8 mg, 10 mg, and 12 mg twice daily to reach the 24 mg/day dose range.

I. Mechanism of Action:

ILO shows high affinity and maximal receptor occupancy for dopamine D2 receptors in the caudate nucleus and putamen of the brains of schizophrenic patients. The improvement in cognition is attributed to ILO's high affinity for α adrenergic receptors. ILO also binds with high affinity to serotonin 5-HT2a and dopamine 3 receptors. ILO binds with moderate affinity to dopamine D4, serotonin 5-HT6 and 5-HT7, and norepinephrine NEα1 receptors. Furthermore, iloperidone binds with weak affinity to serotonin 5-HT1A, dopamine D1, and histamine H1 receptors.

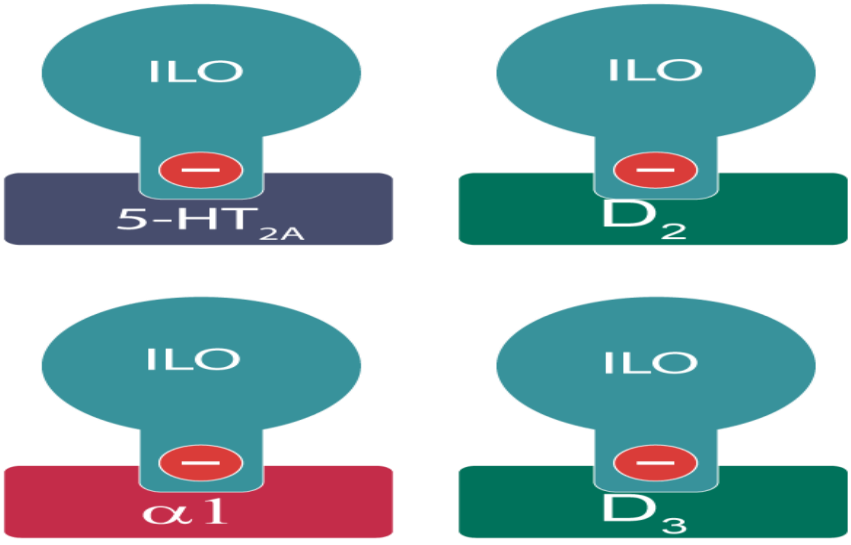

Figure 2.6 ILO's antagonistic action on receptors

J. Pharmacokinetics:

a. Absorption:

Well absorbed from the GI tract and Cmax is reached within 2-4 hours. Steady-state concentration is achieved in 3-4 days post-administration of iloperidone. Relative bioavailability of the tablet formulation compared to oral solution is 96%. Absolute bioavailability is 36%.

b. Distribution:

Volume of distribution: 1340-2800 L

Protein binding: 95%

c. Metabolism:

Hepatically metabolized by cytochrome enzymes which mediates O-dealkylation (CYP3A4), hydroxylation (CYP2D6), and decarboxylation/reduction processes.

Pre-systemic metabolism

Gut-wall CYP3A metabolism

d. Excretion:

Renal, <1% excreted unchanged

K. Adverse reactions:

Cerebrovascular Adverse Reactions in Elderly Patients with Dementia Related Psychosis: Increased incidence of cerebrovascular adverse reactions (e.g., stroke, transient ischemic attack).

QT prolongation: Prolongs QT interval and may be associated with arrhythmia and sudden death—consider using other antipsychotics first.

L. Contraindications:

In the patients who demonstrated hypersensitivity to ILO

M. Drug Interaction: Due to primary CNS effects of ILO, caution should be used when it is taken in combination with other centrally acting drugs and alcohol. Due to its -

alpha1-adrenergic receptor antagonism, iloperidone has the potential to enhance the effect of certain antihypertensive agents.

N. Marketed formulations:

Only oral tablets are available of the dose 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg, 12 mg

O. Review of research work done on Iloperidone:

Table 2.7 enlist the research undertaken for iloperidone.

Table 2.6 Review of work done on ILO

Study	Formulation	Route of administration	Conclusion	Ref
Development and Analysis of Long Acting Depot Formulation for Pharmaceutical Use	Depot	Intramuscular	Developed pamoate salt is safe and can give control release of ILO	[66]
Injectable depot formulation comprising crystals of iloperidone (US20090099232A1)	Depot	Intramuscular or Subcutaneous	Control release of ILO	[67]
Enhanced in vitro dissolution of Iloperidone using Caesalpinia Pulcherrima mucoadhesive microspheres	Microspheres	Oral	Increased solubility and dissolution rate	[68]
Quality by design approach to understand the process of optimization of iloperidone nanostructured lipid carriers for oral bioavailability enhancement	nanostructured lipid carriers (NLC)	Oral	8.3-fold increase in bioavailability of ILO compared to drug suspension	[69]
Targeted Brain Delivery of Iloperidone Nanostructured Lipid Carriers Following Intranasal Administration: In Vivo Pharmacokinetics and Brain Distribution Studies	nanostructured lipid carriers (NLC)	Nose to brain	Effective targeted delivery to brain via intranasal route	[70]

Assessment of novel iloperidone- and idebenone-loaded nanostructured lipid carriers: brain targeting efficiency and neuroprotective potential	nanostructured lipid carriers (NLC)	Nose to brain	Combination therapy effectively reduced oxidative stress	[71]

2.14 Drug Profile: VARDENAFIL HCL TRIHYDRATE

Previously the idea that erectile dysfunction (ED) or impotence might be a common topic of discussion on late night television or in the lay public press would have been unthinkable, it currently is a mainstay of the media outlets. ED is a major sexual problem affecting a major population especially geriatrics. Prevalence of ED has been reported to be 52% with complete ED ranging from 5 to 15% in men aged 40-70 yrs. However, its prevalence varies from 2% in men aged < 40 years to 86% in men aged >80 yrs [72]. ED is a medical condition that has significant impact on self-esteem, interpersonal relationships and quality of life. Incomplete erection or inability to maintain erection during sexual intercourse is known as ED [73]. The quality of men's erections deteriorates gradually over time. The stigma or embarrassment of having ED symptoms may even lead to denial of problem. Due to this man himself does not identify as erectile dysfunction sufferer and due to performance issues in marital life, cases of depression are becoming a part of snowball sampling. Advances in understanding of the physiologic basis to ED over the past decade have led to important new therapeutic approaches to treat ED. Injectable medications, pumps, surgery or oral PDE-5 inhibitors are being prescribed. However, self - injection therapy in which the drug is injected to the base of penis makes self- administration quite challenging. This has very serious side effects such as priapism, penile pain etc. For other therapies men has to endure varying degrees of discomfort. This has led to increased use of PDE-5 inhibitors as PDE-5 inhibitors provide the most physiologic assisted erection in which no device is required, sexual arousal is necessary for erection and detumescence occurs when a stimulus has been removed [74].

Vardenafil hydrochloride tri-hydrate is a potent and highly selective inhibitor of cGMP specific PDE-5, which belongs to BCS class II. The chemical class of the drug is benzenesulfonamide. Vardenafil HCl tri-hydrate has been proven to be safe and effective treatment for ED. It is the most potent and specific of the three commercially available PDE-5 inhibitors. The drug is generally well tolerated, with a favorable safety profile. However, the drug is poorly absorbed following oral administration. The absolute oral bioavailability of the drug is only 15% due to high first pass metabolism [75]. Hence it is necessary to develop a patient compliant formulation that will help to overcome the above limitations and enhance its bioavailability.

VARDENAFIL HCL TRIHYDRATE (VDN): [76,77]

A. Chemical IUPAC name: 2-[2-ethoxy-5-(4-ethylpiperazin-1-yl)sulfonylphenyl]-5-methyl-7-propyl-1H-imidazo[5,1-f][1,2,4]triazin-4-one;trihydrate;hydrochloride

B. Proprietary name: Levitra

C. Empirical Formula: $C_{23}H_{39}ClN_6O_7S$

D. Molecular Weight: 579.11 g/mol

E. Structure:

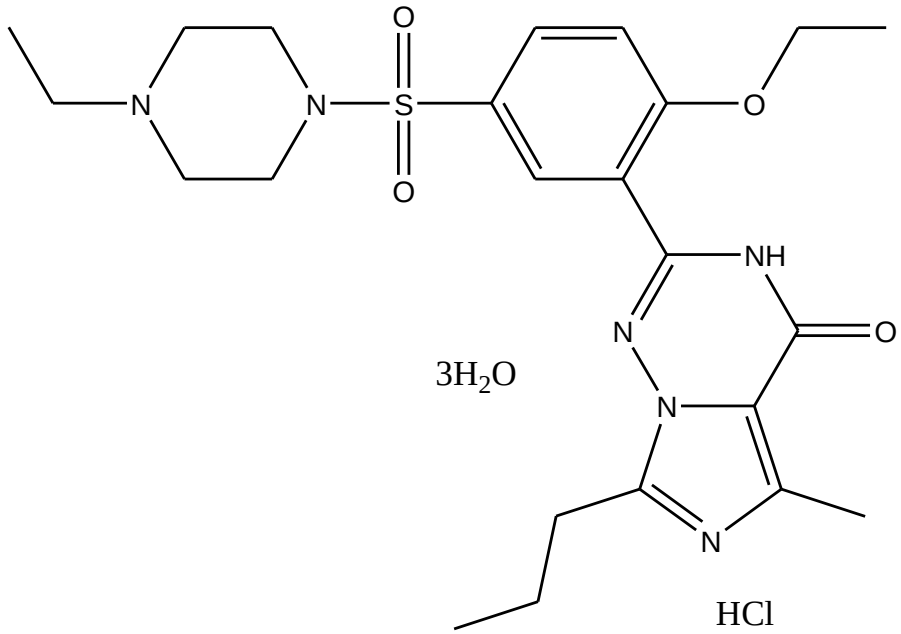

Figure 2.7 Chemical structure of Vardenafil HCl trihydrate

F. Physicochemical properties

a. Melting point: 190 – 193 ^{0}C

b. Solubility: 3.5 mg/L in water at 25 ^{0}C

c. pKa: 4.72

d. LogP: 2.79

e. BCS Class: II

f. Hydrogen Bond donor count: 5

g. Hydrogen Bond acceptor count: 10

G. Indication and Use:

VDN is used for treatment of erectile dysfunction in adult men. Erectile dysfunction is the inability to achieve or maintain a penile erection sufficient for satisfactory sexual performance. In order for VDN to be effective, sexual stimulation is required.

H. Dose and dosing frequency:

For most patients, the recommended starting dose of VDN is 10 mg, taken orally. The dose may be increased to a maximum recommended dose of 20 mg based on efficacy and side effects.

I. Mechanism of action:

Penile erection is a hemodynamic process initiated by the relaxation of smooth muscle in the corpus cavernosum and its associated arterioles. During sexual stimulation, nitric oxide is released from nerve endings and endothelial cells in the corpus cavernosum. Nitric oxide activates the enzyme guanylate cyclase resulting in increased synthesis of cyclic guanosine monophosphate (cGMP) in the smooth muscle cells of the corpus cavernosum. The cGMP in turn triggers smooth muscle relaxation, allowing increased blood flow into the penis, resulting in erection. The tissue concentration of cGMP is regulated by both the rates of synthesis and degradation via phosphodiesterases (PDEs). The most abundant PDE in the human corpus cavernosum is the cGMP-specific phosphodiesterase type 5 (PDE5); therefore, the inhibition of PDE5 enhances erectile function by increasing the amount of cGMP. Because sexual stimulation is required to initiate the local release of nitric oxide, the inhibition of PDE5 has no effect in the absence of sexual stimulation. In vitro studies have shown that vardenafil is a selective inhibitor of PDE5. The inhibitory effect of vardenafil is more selective on PDE5 than for other known phosphodiesterases (>15-fold relative to PDE6, >130-fold relative to PDE1, >300-fold relative to PDE11, and >1,000-fold relative to PDE2, 3, 4, 7, 8, 9, and 10).

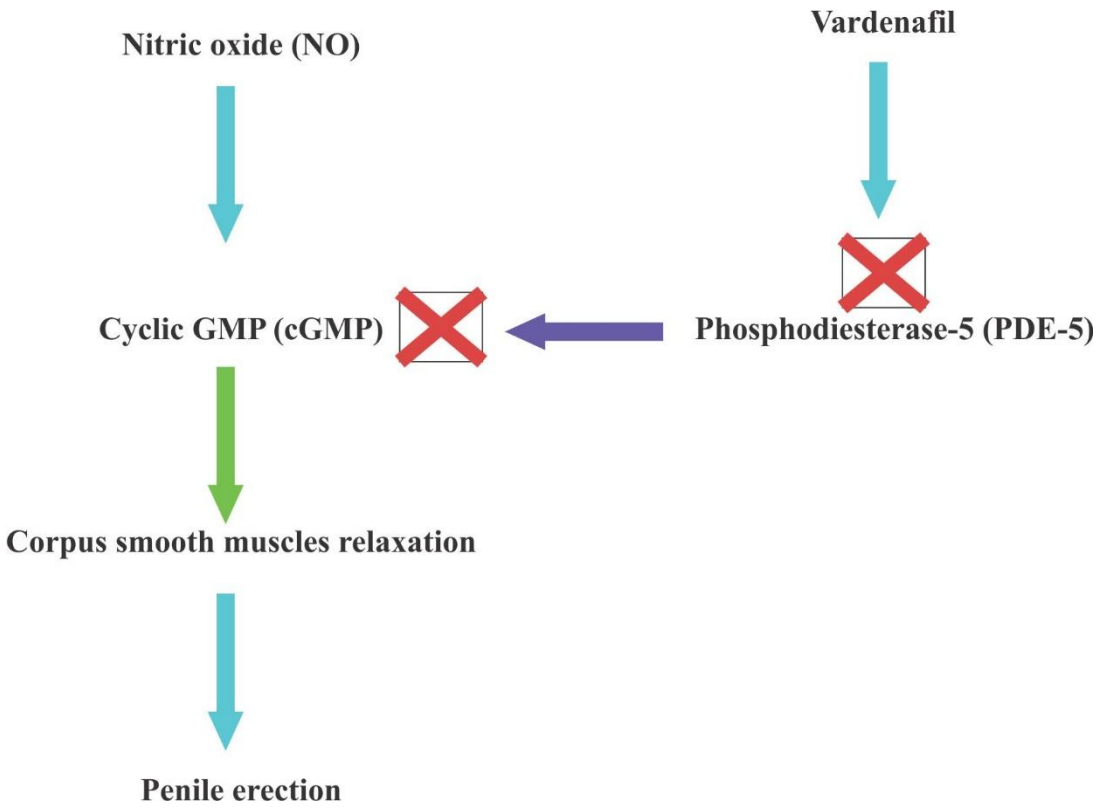

Figure 2.8 Mechanism of action of VDN

J. Pharmacokinetics:

a. Absorption:

Vardenafil is rapidly absorbed with absolute bioavailability of approximately 15%. Maximum observed plasma concentrations after a single 20 mg dose in healthy volunteers are usually reached between 30 minutes and 2 hours (median 60 minutes) after oral dosing in the fasted state.

b. Distribution:

Volume of distribution: 208 L → indicates extensive tissue distribution

Protein binding: 95%

c. Metabolism:

Vardenafil is metabolized predominantly by the hepatic enzyme CYP3A4, with contribution from the CYP3A5 and CYP2C isoforms. The major circulating metabolite, M1, results from desethylation at the piperazine moiety of

vardenafil. M1 is subject to further metabolism. The plasma concentration of M1 is approximately 26% that of the parent compound. This metabolite shows a phosphodiesterase selectivity profile similar to that of vardenafil and an in vitro inhibitory potency for PDE5 28% of that of vardenafil. Therefore, M1 accounts for approximately 7% of total pharmacologic activity.

d. Excretion:

The total body clearance of vardenafil is 56 L/h, and the terminal half-life of vardenafil and its primary metabolite (M1) is approximately 4-5 hours. After oral administration, vardenafil is excreted as metabolites predominantly in the feces (approximately 91-95% of administered oral dose) and to a lesser extent in the urine (approximately 2-6% of administered oral dose).

K. Adverse events:

Headache, Flushing, Rhinitis, Dyspepsia

Body as a whole: anaphylactic reaction (including laryngeal edema), asthenia, face edema, pain

Auditory: sudden decrease or loss of hearing, tinnitus

Cardiovascular: angina pectoris, chest pain, hypertension, hypotension, myocardial ischemia, myocardial infarction, palpitation, postural hypotension, syncope, tachycardia

Digestive: abdominal pain, abnormal liver function tests, diarrhea, dry mouth, dysphagia, esophagitis, gastritis, gastroesophageal reflux, GGTP increased, vomiting

Musculoskeletal: arthralgia, back pain, myalgia, neck pain

Nervous: hypertonia, hypesthesia, insomnia, paresthesia, somnolence, vertigo

Respiratory: dyspnea, epistaxis, pharyngitis

Skin and appendages: photosensitivity reaction, pruritus, rash, sweating

Ophthalmologic: abnormal vision, blurred vision, chromatopsia, changes in color vision, conjunctivitis (increased redness of the eye), dim vision, eye pain, glaucoma, photophobia, watery eyes

Urogenital: abnormal ejaculation, priapism (including prolonged or painful erections)

L. Contraindications:

Nitrates: Administration of VDN with nitrates and nitric oxide donors is contraindicated. Consistent with the effects of PDE5 inhibition on the nitric oxide/cyclic guanosine monophosphate pathway, PDE5 inhibitors - VDN may potentiate the hypotensive effects of nitrates.

Hypersensitivity: VDN is contraindicated for patients with a known hypersensitivity to Drug or the dosage form.

M. Drug Interaction: Studies in human liver microsomes showed that vardenafil is metabolized primarily by cytochrome P450 (CYP) isoforms 3A4/5, and to a lesser degree by CYP2C9. Therefore, inhibitors of these enzymes are expected to reduce VDN clearance.

N. Marketed formulations:

Orally disintegrating or film coated tablets are available of the dose 5 mg, 10 mg and 20 mg

O. Review of research work done on Vardenafil HCL trihydrate:

Table 2.8 enlist the research undertaken for VDN.

Table 2.7 Review of work done on VDN

Study	Formulation	Route of administration	Conclusion	Ref
Nanoethosomal transdermal delivery of vardenafil for treatment of erectile dysfunction: optimization, characterization, and in vivo evaluation	Ethosome	Transdermal	Two-fold increase of VDN bioavailability from the transdermal films compared with an oral drug suspension	[78]
Enhancing transdermal delivery of pde-5 inhibitor	Solution, gel, cream	Transdermal	Amount of vardenafil found in the blood stream increased by about 4 to 5 folds, as compared with that of the control.	[79]
A stable pharmaceutical formulation containing vardenafil hydrochloride	Tablet	Oral	No change of salt to base after stress test	[80]
Controlled-release formulations containing Vardenafil	1.Multiarticulate system (MUPS) prepared by hot melt extrusion 2.MUPS prepared by coating of polymer on beads	Oral	Sustained release of VDN over prolonged period of time	[81]

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