Research Article

FORMULATION AND EVALUATION OF SILICA LIPID HYBRID MICROPARTICLES CONTAINING FUROSEMIDE

D. Radhesh Kumar, Mohammad Habeeb, Mangalarapu Shravya, K.V. Ramana Reddy*

Department of Pharmaceutics, Sree Dattha Institute of Pharmacy (SDIP), Nagarjuna Sagar Road, Sheriguda, Ibrahimpatnam, Hyderabad, Telangana, India

*Corresponding Author Email: kvrreddy.sdip@gmail.com

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ABSTRACT

The study of the silica-lipid hybrid (SLH) microparticles is to enhance bioavailability and efficacy of poorly water-soluble drugs after oral administration. Lipid-based delivery systems recognized as a potential strategy for improving the oral absorption. Clinical applications of any lipid-based formulations are limited by the lack of clear guidelines of incomplete understanding of absorption mechanism and invitro, invivo formulation performance. Withstanding these problems, the availability of 2-4% oral lipid based products in the pharmaceutical market worldwide has supported in addressing low bioavailability of poorly soluble drugs. A novel silica-lipid hybrid microparticulate system is used for enhancing the oral absorption of low solubility and low permeability of (BCS Class IV) drugs, that has been developed for the drug, furosemide. It describes the systematic invitro characterization of dissolution and lipolysis properties. Silica-lipid hybrid microparticles include the drug solubilizing effect of dispersed lipids and stabilizing effect of hydrophilic silica particles to increase drug solubilization, which leads to enhanced oral bioavailability. Furosemide is mainly used in the treatment of hypertension and congestive heart failure. The role of lipids and hydrophilic silica based carrier highlighted in enhancing solubility and permeability, and hence the oral bioavailability of poorly soluble drugs. The formulation composed of poorly soluble drug (furosemide) and dispersion of oil phase (Soya bean oil) in lecithin (Phospholipoid 90H), surfactant and vortexed until no crystals of drug is observed and are subsequently adsorbed by Aerosol 380 (hydrophilic fumed silica) and stabilized after being transformed to powder state. Saturation solubility studies were performed in different oils and surfactants with increased concentration of drug revealed increased solubility of furosemide. In vitro dissolution studies conducted under stimulated gastric medium revealed 2-4 fold increase in dissolution efficiencies for furosemide formulated as SLH microparticles, when compared to that of pure drug (furosemide) and a marketed formulation. Ex vivo studies showed enhanced lipid digestibility, which improved drug permeability. Solid-state characterization of SLH microparticles by XRPD (X-ray powder diffraction) and FTIR (Fourier transform infrared spectroscopy) analysis confirmed non-crystalline nature and less compatibility of furosemide in silica-lipid hybrid microparticles.

Keywords: Furosemide, Silica Lipid Hybrid, microparticles, Soya bean oil, Aerosol 380, FTIR.

INTRODUCTION

Of all the drug delivery systems oral route is the most convenient and non-invasive method of drug administration which receives the highest degree of patient compliance. For a drug substance that to be well absorbed following oral administration, it has to: (i) be sufficiently soluble in the gastrointestinal fluids and (ii) easily permeate across the GI membrane without undergoing significant degradation or elimination mediated by the GI enzymes and enterocyte transporters.¹ According to recent estimates, nearly 30% of the oral immediate-release drug products and 40-70% of the newly discovered chemical entities are poorly soluble in water.²⁻³ Drugs with poor aqueous solubility and dissolution properties are not suitable for oral delivery using conventional tablet formulations as it produces low and variable bioavailability, which leads to erratic biological effects.

In order to improve the solubility-limited bioavailability, lipid-based formulations have emerged as an effective and versatile solubilization technology.⁴ Successful therapeutic applications of oral lipid-based formulations, that accounted for 2-4% of the pharmaceutical market worldwide. It suggests the need for more intensive and systematic studies on lipid-based systems to foster the growth of viable formulations of poorly water-soluble drugs.

This research aims to develop a novel formulation (i.e. silica-lipid hybrid microparticles) for improving the absorption of poorly water-

soluble drug furosemide based on appropriately selected lipid excipients.

FORMULATION OF POORLY WATER-SOLUBLE DRUGS Biopharmaceutical Classification of Drug Substances

As described in Biopharmaceutics Classification Scheme (BCS), based on their aqueous solubility and intestinal permeability properties, drug substances are categorized into four classes i.e. BCS Class I to IV as summarized in Table 1.

This classification scheme is to determine the importance of solubility and permeability on drug absorption. The factors that govern the rate and extent of drug absorption include,⁵(i) Solubility: a drug substance is considered “highly soluble” when the highest dose strength is soluble in ≤ 250 ml of aqueous media over a pH range of 1 to 7.5;

(ii) Permeability: a drug substance is considered “highly permeable” when the extent of drug absorption in humans is $\geq 90\%$ of an administered dose;

(iii) Dissolution rate: a drug product is considered to be “rapidly dissolving” when $> 85\%$ of the labeled amount of drug substance dissolves within 30 min using the United States Pharmacopoeial (USP) Apparatus I or II in a volume of less than 900 ml of buffer solution.

Biopharmaceutical Classifications of Drug Substances

(A) FDA Biopharmaceutics Classification Scheme (BCS)

(B) Biopharmaceutics Drug Disposition Classification System (BDDCS)¹

BCS scheme implemented by the United States Food and Drug Administration (USFDA) as an industrial guidance to justify bio waiver through bioequivalence testing of immediate-release solid dosage forms for highly soluble–highly permeable drugs. Poorly water-soluble drug compounds (BCS Class II and IV) are those with a dose/solubility ratio of > 250 ml or with an aqueous solubility of < 100 $\mu\text{g/ml}$.⁶ ‘Highly permeable’ drugs are the compounds; with an oral dose that exhibit $\geq 90\%$ absorption. The enterocyte transporter-enzyme functions (uptake or efflux effects) are taken into consideration in addition to lipophilicity properties. Considering the BCS, Log P is the logarithm of octanol-water partition coefficient, which is widely used as a quantitative descriptor for lipophilicity. Biopharmaceutics Drug Disposition Classification System (BDDCS) (Table 2) indicates the overall exposure of drugs following oral administration, the parameter permeability is replaced with ‘first-pass metabolism’.

ORAL FORMULATION STRATEGIES FOR POORLY WATER-SOLUBLE DRUGS

(A) Particle Size Reduction/ Micronisation

According to the Noyes-Whitney principle, increased rate of drug dissolution is due to an increased wetted surface area or reduced particle size. This has exemplified for poorly water-soluble drug models such as indomethacin, fenofibrate⁷, ibuprofen, itraconazole, and ketoconazole. (Table 3)

(B) Solid Solutions/ Dispersions as Supersaturating Drug Delivery Systems

Solid dispersions are produced by the dispersion of drugs in a hydrophilic or water soluble matrix in a solid state. These are eutectic dispersions, in which drugs are presented in solid solution or microcrystalline state, where the fraction of drugs are dispersed in the matrix.⁸

Improvement in drug dissolution by solid dispersion attain in two ways:

(i) The inclusion of a polymer-based stabilizer such as: (e.g. PEG, HPMC) is an effective method to maintain the supersaturation state or to delay precipitation (i.e. ‘crystal growth’) of drugs in the GI fluids.

(ii) Typically, a surface-active stabiliser (e.g. SLS, PEG-polysorbate 80 mixture) is included in a solid dispersion system to prevent the formation of a drug-rich hydrophobic barrier on the surface of the dissolving matrix.⁴ This system presents the drugs in high energy, thermodynamically unstable forms (metastable or amorphous) and that provides accelerated solubility and dissolution in GIT. It also enhances the intestinal uptake of drug molecules down the concentration gradient.

(C) Surfactant/Co-Solvent System

These are used alone or in combination to increase the solubilization power of aqueous medium.

(i) These are amphiphilic agents which improves drug solubility and dissolution by lowering the surface tension or by forming micelles at concentrations exceeding their critical micelle concentrations (normally in the range of 0.05–0.1% for most surfactants)⁸

E.g. sodium lauryl sulphate (anionic), polysorbates, polyoxyethylated castor oil (non-ionic).

(ii) The commercialization of products is mainly based on non-ionic surfactants, as it increases the acceptability for oral administration

and the risk benefit ratio helps in using of surfactants in chronic products.

E.g. HIV antiretroviral drugs, including amprenavir, lopinavir/ritonavir tablet, and ritonavir, (polyoxyethylated castor oil, Cremophor).⁴

(iii) Pharmaceutically employed cosolvents are water-miscible organic solvents which can confer high solubility to hydrophobic solutes via specific molecular interactions (such as hydrogen bonding).⁸

E.g. ethanol, glycerin, polyethylene glycol (PEG) and propylene glycol, nifedipine.⁹

(D) Cyclodextrins

Cyclodextrins (CDs) are starch-derived cyclic oligosaccharides with a hydrophilic exterior and hydrophobic interior, which may contain six (α -CD), seven (β -CD), eight (γ -CD) or more α -D-glucopyranose units.¹⁰

The major mechanism which includes:

(i) CDs with the formation of non-covalent inclusion complexes increase the apparent solubility and dissolution of drugs.

(ii) CDs may also form non inclusion-based complexes and act as a surfactant, supersaturation stabilizer and precipitation inhibitor in the aqueous environment.

(iii) Drug release from covalently bound CD-drug conjugates is induced by enzymatic degradation and is useful for colon targeted drug delivery. Dissociation of drug molecules from CD-drug complex is triggered by aqueous dilution, replacement by dietary lipids or close affinity of drug molecules towards biological membranes (oral mucosal).

Commercialized oral CD-based products include: e.g. a α -CD-based cefotiam-hexetil HCl tablet, a β -CD-based nicotine sublingual tablet, a 2-hydroxypropyl- β -CD-based itraconazole solution.

(iv) For targeted delivery, increased complexation efficacy and prolonged therapeutic effect of poorly water soluble drugs, the combined use of CDs and surfactants, liposomes have been investigated.

(E) Crystal Habit Modification

(i) A solid drug substance may exist in one or more crystalline form (i.e. polymorphs) which exhibits different physicochemical properties such as melting point and stability, has impact on solubility and dissolution rate.⁹

(ii) The important consideration while selecting a polymorph for formulation is to balance between manufacturability, solubility and stability of a compound. So, the potency is maintained over shelf-life period.

(iii) With the supersaturation or recrystallisation of drugs from different solvents, metastable crystals are formed in the presence or absence of surfactants and polymers.⁹

(iv) Manipulation of the solid state by decreasing crystallinity of the drug substances through formation of solid dispersions is one of the methods used for promoting drug dissolution. The solid dispersion technique has often proved to be the most successful in improving the dissolution and bioavailability of poorly water soluble active pharmaceutical ingredients because it is simple, economic and advantageous technique. Spray drying is one of such techniques of preparing solid dispersion and is widely used as an alternative to milling to reduce particle size.¹¹

(F) Prodrugs

(i) Prodrugs are biologically inactive compounds that undergoes transformation into the active parent drug via., chemical or enzymatic reactions.

(ii) It involves chemical modification of the pharmacophore by adding a polar (either ionisable or non-ionisable) functional group. It

should always be balance between the aqueous solubility and the membrane permeability to ensure adequate intraluminal solubilisation and intestinal uptake.¹²

It is a suitable approach for drugs requiring high doses of administration and those with poor aqueous and lipid soluble drugs. E.g. fosamprenavir calcium. This prodrug formulation showed higher solubility than the parent drug (i.e. ~10 times increased solubility)

(iii) Besides solubility enhancement, prodrug masks the local GI irritation by the non-ionisable, sulfoxide prodrug of sulindac sulfide, which is a non-steroidal anti-inflammatory drug (NSAID).¹³

(G) Salt Formation

(i) Most of the drug pharmacopoeial monographs demonstrates a solubility of >10 mg/ml are hydrochloride, sulphate, maleate and citrate salts of basic drugs (e.g. chlorpromazine HCl and morphine sulphate); and potassium, calcium and sodium salts of acidic drugs (e.g. dicloxacillin sodium and losartan potassium).

(ii) If a drug molecule is ionisable within GI pH range of 1–9 and includes chemical conversion of parent unionized drug into crystalline salt form and exhibits a strong pH dependency of the distribution coefficient (log D) is the most fundamental approach for improving the solubility and dissolution rate of drug.⁵

(H) Lipid-Based Formulations

Among the four classes of drugs, BCS Class IV drugs are likely to benefit more especially by lipid dosage forms due to their miscibility in lipids and significance of food effects in enhancing solubilization and absorption *in vivo*.⁴

(i) Based on the composition of lipids and surfactants, these are broadly classified into Lipid Formulation Classification System (LFCS) Type I (oil based), Type II (oil and lipophilic surfactant-based), Type III (oil and hydrophilic surfactant-based) and Type IV (surfactant and cosolvent-based) formulations.

(ii) Lipid-based formulations, are those which increases the drug bioavailability by presenting the drug in a molecularly dissolved state during GI transit, as well as initiating a series of physiological responses (e.g. increased biliary secretions) and biochemical changes (e.g. increased intestinal permeability) in the GI tract which favours drug absorption.¹⁴

LIPID-BASED CARRIERS FOR ORAL DELIVERY OF POORLY WATER-SOLUBLE DRUGS

Lipid excipients include: solutions, suspensions, emulsions (liquid or dry form), micellar systems, solid lipid particles, liquid crystalline phase carriers, liposomes and various self-emulsifying systems. Applications of these formulations mainly depend on nature of processing in the GI lumen, physicochemical properties of lipid excipients and their compatibility with the drug molecules.

Types of Lipid Excipients

Commercially available lipid excipients are categorised into the following classes based on manufacturing methods and chemical compositions (Table 4)

(A) Fatty Acids

These are monocarboxylic acid derivatives of saturated or unsaturated aliphatic hydrocarbons.

(i) Saturated FA with ≤ 8 carbons in the chain length are flowable liquids, where as those with ≥ 10 carbons are semi-solids at room temperature.

(ii) Fatty acids are used as a solubiliser in pharmaceutical formulations.

(iii) Instability of lipids can be minimized resulted from oxidative reactions which include:

(a) Purification to remove the impurities.

(b) Addition of antioxidant.

(c) Hydrogenation to decrease the number of carbon-carbon double bonds.

(d) Maintaining a pH of 6.5 during processing and in final product.

(e) Protection from light and excessive heat.

(iv) Lipids containing unsaturated FA are much prone to oxidation, which is catalyzed by impurities such as metal ions, peroxides, and photochemical sensitizers (e.g. chlorophyll and riboflavin); which is identified by the unpleasant tastes and odours of the 'rancid' lipids.

(v) Due to adequate solvent capacity, appreciable self-emulsifying efficiency in aqueous media and high fluidity, natural MCT oils (e.g. Miglyol and Capmul) have gained much importance in formulating drugs of intermediate lipophilicity ($\log P < 5$) in comparison to LCT.

(vi) Miglyol 812 (TG) is a mixture of saturated caprylic/capric (C8/C12) triglycerides, due to its resistance against oxidative decomposition, it is preferred for developing a stable formulation.¹⁵

(vii) Capmul MCM shows unique amphiphilic character, in which it maintains a hydrophile-lipophile balance (HLB) of 5-6 and is often used as solubiliser, emulsifier.

(viii) Oleic acid potentiates lymphatic uptake of lipophilic drugs through the formation of chylomicrons (80–1000 nm) in the enterocytes.¹⁶

(B) Natural Oils and Fats

Lipids are the mixtures of naturally occurring triglycerides (TG) which contains FA of different chain length and degree of unsaturation.

Depending upon hydrocarbon chain length of fatty acid, oils and fats can be classified as:

(a) short-chain triglycerides SCT (< 5 carbons),

(b) medium-chain triglycerides, MCT (6–12 carbons)

(c) long-chain triglycerides, LCT (> 12 carbons)

They can be used as solubilizers in oral formulation products.

(C) Emulsifiers

(i) Lecithins are heterogenous materials which are extracted from biological sources (e.g. soybean, eggs, sunflower seeds) due to their high surface active properties and biodegradability, these are extensively used in stabilizing oil-water systems.¹⁷

(ii) Saturated lecithins showed higher risk of haemolysis that used in intravenous emulsions when compared with unsaturated lecithins.

(iii) Due to least haemolytic risk, Soybean lecithin is selected for the current oral formulation development in which the lecithin component undergoes enzymatic digestion prior to intestinal absorption.

(iv) The hydrophile-lipophile balance (HLB) is a measure of the relative hydrophilicity and lipophilicity of amphiphilic molecules. compounds with HLB values < 12 are hydrophobic, whereas those with values > 12 are hydrophilic.

(D) Semi-Synthetic Mono-, di-, and Triglycerides

(i) Semi-synthetic mono-, di-, and triglycerides (MG, DG, and TG) are fractioned glycerides when compared to naturally occurring oils and fats, these have less variability and specified range of acceptability in their compositions.

(ii) Due to specific compositions and improved uniformity, it attains greater pharmaceutical applications, including emulsifiers, suspending, wetting agents and solubilising agents. E.g. Fractionated coconut oil (e.g. Miglyol 810 and Captex 300), contains $\geq 95\%$ MCT and suitably used as an oily excipient in soft gelatin encapsulated products.

(E) Semi-Synthetic PEG Derivatives of Glycerides and Fatty Acids

(i) These are chemically modified mixtures of MG, DG and TG with fatty acid esters of PEG are used as emulsifier in self-emulsifying systems, fluid or thermo-softening semi-solid solubilizing vehicles, surfactants and wetting agents.

(ii) Thermo-softening semisolids require melting at (26–70 °C) is then filled into hard gelatin capsules before they can be used as a solubilizing or suspending agent.

(iii) The fluid excipients with the addition of colloidal silicon dioxide modified to form thixotropic formulations useful for preparing lipid suspensions of solid drugs.

(F) Polyglyceryl Fatty Acid Esters

(i) These acts as crystallization inhibitors, solubilizers, vehicles, emulsifiers and surfactants.

(ii) These are made by linking a chain of glycerol molecules via., ether linkages, followed by esterification with one or more FA molecules.

(iii) Hydrophilicity mainly depends on number of free hydroxyl groups and the polyglycerol chain length.

(G) Stabilizers/ Solid Carriers

(i) A solid carrier is used as an excipient which is incorporated into an emulsion system for encapsulating lipids and conversion into dry state.

(ii) Water soluble carriers (e.g. gelatine, sugars, glycine, povidone, and HPMC), water-insoluble carriers (e.g. magnesium aluminosilicate, Neusilin and colloidal silica) are mostly used.

(iii) Colloidal silica is commonly used as pharmaceutical/food excipient approved by the Therapeutic Goods Administration without specific restriction on the daily oral dose.

(iv) In the current research work, hydrophilic fumed silica (Aerosil 380) is used as an insoluble nanoparticulate stabilizer and solid carrier for lipid encapsulation.

(H) Cholesterol and Phospholipids

(i) Phospholipids can be subdivided into negatively charged lipids (e.g. phosphatidic acid and phosphatidyl glycerol), Zwitterions (e.g. phosphatidyl choline and phosphatidyl ethanolamine) or a mixture of both (e.g. L- α - lecithin and egg lecithin).

(ii) The use of charged phospholipids may confer stability to the emulsion and liposomal systems by preventing droplet aggregation and fusion.

(iii) Lipids are classified into two groups: (Table 5)

Polar lipids (which interact with water) and non-polar lipids (which do not interact with water).

GASTROINTESTINAL PROCESSING OF LIPIDS AND DRUG TRANSPORT

(i) Digestion of the lipid excipients mediated by the GI enzymes has a crucial impact on the intra luminal release and solubilization state of the compound.

(ii) It is essential to understand the fundamental process of lipid digestion and the possible drug transport pathways across the enterocytes.

(iii) Digestion of lipids is mainly catalysed by the water-soluble enzymes, lipases, producing the lipid monomers of FA, MG, and to a lesser extent, glycerols (Table 6). Other enzymes which are pancreatic carboxyl esterase, a non-specific catalyst for non-emulsified substrates, and phospholipase A2, which is responsible mainly for the digestion of phospholipids (PL).^{16,14}

Gastric Processing of Lipids Physiological Aspects

(i) The digestion of lipids (up to 30%) is initiated in the stomach by gastric lipase and lingual lipase. The ingested lipids are prone to emulsification facilitated by the gastric shear forces, the presence of membrane-derived PL and the generated amphiphilic lipolysis products.

(ii) The FA products potentially re-organize into peripheral particles which entrap the gastric lipase that typically acts at the lipid-water interface. However, the FA-entrapped gastric lipase found to retain its enzymatic activity following gastric emptying.

Impact on Drug Delivery

(i) Encapsulation of poorly water-soluble drugs in lipids has several advantages.

(a) Drug compounds are dispersed in lipid phases. This inherently eliminate the need for pre-absorptive drug dissolution in the GI fluids, and the intraluminal processing of lipids.¹⁹ (Figure 1)

(b) Presence of lipids in the intestines induces a dose-dependent feedback inhibition of gastric emptying, which result in increase the drug solubilization degree in stomach.

(A) Gastric lipolysis catalysed by gastric lipase, which is gradually inhibited by the accumulated digestion products (mainly DG and FA) at the lipid droplet surfaces (scanning electron micrographs adapted from.²⁰

(B) Duodenal lipolysis of lipid droplets emptied from the stomach catalyzed by pancreatic lipase-colipase complexes, which results in the formation of a highly solubilizing lipophilic microenvironment that subsequently facilitates drug transport across the unstirred water layer and enterocytes (photomicrographs adapted from.¹⁹

(ii) The slowdown in gastric emptying has been suggested to be a means of controlling and prolonging the transit time of drugs at the absorption site, thereby increasing the drug bioavailability.²¹

Intestinal Processing of Lipids Physiological Aspects

(i) Upon entry into the duodenum, the partially digested lipid substrates (i.e. a mixture of TG, DG, MG and FA) stimulate the secretion of bile salts (BS) and biliary-derived lipids (PL and cholesterol) from the gall-bladder, as well as the release of lipase enzymes from the pancreas.

(ii) The endogenous BS/PL components further reduce the droplet sizes and enhance the colloidal stability of the lipid droplets.¹⁴

(iii) Pancreatic lipase plays a major role in completing the hydrolysis of insoluble lipid substrates in a basic environment.

(iv) For each TG molecule hydrolysed, one molecule of 2-MG and two molecules of FA (with traces of 1-MG and glycerols) are produced.

(v) Lipolysis of TG and DG produces the less hydrophobic lipid monomers (FA and MG) which accumulate at the droplet surface as the liquid crystalline vesicles, which rapidly re-organise to form lipid-BS/PL mixed micelles.

Impact on Drug Delivery

- (i) As lipid droplets digestion progresses, the liquid crystalline lamellae and the colloidal species formed serve as the key lipophilic reservoirs to keep the drug molecules solubilised in the bulk aqueous phase, and ultimately reach the intestinal absorptive cells (Figure 1B).
- (ii) Solubilisation in BS micelles increases the aqueous concentration of FA and MG. Such saturation state in turn creates a concentration gradient that drives diffusion of the micellar structures across the UWL, and induces intestinal uptake of the lipolysis products.¹⁴

Intestinal Uptake Physiological Aspects

- (i) The apical brush border membrane of the enterocytes is packed with microvilli (~100 nm), where the spaces between the microvilli range from 5–20 nm. The absorption of lipolysis products has long been thought to be a concentration gradient-driven process in which the FA and MG monomers are absorbed predominantly via passive diffusion across the enterocytes.¹⁴
- (ii) Besides the uptake transporter systems, embedded in the apical membrane are the multidrug efflux proteins (particularly P-glycoprotein, P-gp) and the metabolising enzymes (primarily the cytochrome P450 3A enzymes, CYP3A). These act to control the uptake and elimination of a wide range of lipophilic and amphiphatic xenobiotics.

Impact on Drug Delivery

- (i) Using excised intestinal segments and Caco-2 cell lines,^{22, 23} It has been suggested that the digested lipids and BS components enhances drug permeation by:
  - (a) Increasing the intestinal membrane fluidity
  - (b) Disrupting the integrity of the tight junctions
- (ii) The inhibitory effects of various lipid and surfactant excipients on the intestinal P-gp efflux and CYP3A metabolizing systems, which eventually leads to an enhanced drug bioavailability.

TYPES OF LIPID-BASED FORMULATIONS AND FACTORS AFFECTING DRUG ABSORPTION

Some potential mechanisms by which lipid-based vehicles enhance the oral absorption of poorly water-soluble drugs,

- (i) Presence of drugs stable in the molecularly dispersed/ dissolved state.
- (ii) Increased GI residence time.
- (iii) Improved intestinal solubilizing capacity.
- (iv) Down regulation of the intestinal efflux and metabolism functions.
- (v) Intestinal lymphatic transport, which minimizes the first-pass metabolism effect.

Lipid solutions and Suspensions

- (i) Both lipid solutions and suspensions are relatively simple approaches shown to enhance oral bioavailability of many lipophilic drugs,
- (ii) Lipid solutions are oily vehicles which contain the drug substance in a fully solubilised form, whereas lipid suspensions are two-phase systems in which the finely divided drug substance is dispersed in the lipid matrix.²⁻³
- (iii) For e.g., cinnarizine when administered as a lipid (oleic acid) solution, has been reported to produce approximately 3- and 4-fold higher maximum plasma concentration (C_{max}) and bioavailability (BA), respectively, in comparison with the conventional tablet formulation.²⁴

Lipid Emulsions/ Microemulsion

- (i) The presence of lipid vehicles in the emulsified form has proved to be effective in increasing the rate of drug release and/or the rate of lipid digestion via an increased total surface area.¹⁴
- (ii) Microemulsions used for oral delivery of lipophilic drugs are thermodynamically stable, isotropically clear dispersions of oil droplets stabilised by surfactants/ emulsifiers or co-surfactants in an aqueous continuous phase.^{2-3, 25}

Dry Emulsions

- (i) The transformation of liquid emulsions into the solid state by spray-drying, this was followed by lyophilization and more recently, by solvent evaporation.
- (ii) The use of dry emulsions as a solid dosage form has gained much importance due to elimination of some drawbacks associated with liquid emulsions, such as physical instability, relatively large volume of administration as compared to solid dosage forms, strict storage conditions (e.g. refrigeration required), dosage precision problem, possible patient noncompliance, and difficulty in sterilization.¹⁴

Self-Emulsifying Drug Delivery Systems (SEDDS)

- (i) These are physically stable, isotropic mixtures of oil, surfactant/ emulsifier (and co-solvent) and drug, which forms fine o/w emulsions on exposure to an aqueous medium.^{26, 14}
- (ii) It offers several advantages in comparison with lipid solutions, such as increased drug loading capacity, increased rate and extent of absorption as well as improved reproducibility in the bioavailability.²⁶
  - a. The hydrophile-lipophile balance (HLB) system is a measure of the overall hydrophilicity/ hydrophobicity of a surfactant or emulsifier; compounds with HLB < 12 is considered lipophilic, whereas those with HLB > 12 is regarded hydrophilic
  - b. The loss of solvent capacity is likely to increase the risk of drug precipitation during dispersion in an aqueous medium
  - (iii) Additionally, the formation of small droplets may promote rapid stomach emptying and wide dispersion throughout the GI lumen, thereby minimizing local exposure of the GI mucosa to surfactants at high concentrations and reducing the irritation.²⁻³

ROLE OF SILICA-BASED MATERIALS IN ORAL DELIVERY OF POORLY WATER SOLUBLE DRUGS

1. A few mechanisms have been proposed through which silica-based materials improve the oral bioavailability of poorly water-soluble drugs:
  - (i) Preservation of drug molecules in the amorphous or molecularly dispersed form and increased drug wettability in aqueous media, which leads to enhanced dissolution or release kinetics.²⁷
  - (ii) Solubilization of drugs at a super saturation state in the GI fluids, which creates a driving force which favors drug absorption across the intestinal membrane.
  - (iii) Silica-based drug delivery systems can be categorised into three types:
    - (A) mesoporous silica xerogels
    - (B) Ordered mesoporous silica-based materials
    - (C) mesoporous hollow silica spheres.
- (a) Silica-based xerogels are amorphous, porous materials in which the drug molecules are encapsulated inside the silica matrix using sol-gel technology. This has been investigated for some poorly water-soluble drugs such as cisplatin, nifedipine and ibuprofen.
- (b) Ordered mesoporous silica based materials shows more well-defined surface properties, and their porous interiors are well suitable for encapsulating different molecules of drugs such as itraconazole, fenofibrate.²⁷ and carbamazepine²⁸, in which the

encapsulated drug molecules are released mainly via a diffusion-controlled mechanism.

(c) Mesoporous hollow silica spheres facilitates a higher drug loading capacity through the hollow core structure. E.g., ibuprofen. Some clinical studies conducted by have demonstrated an enhanced bioavailability effect for a poorly water-soluble antibiotic, amoxicillin trihydrate, resulting from a tablet formulation containing fat-silica matrix; enhancement effect was found to increase with higher silica content in the tablet formulation.²⁸ The synergistic role of lipid and silica excipients enhanced the oral absorption of poorly water-soluble drugs.

MATERIALS AND METHODS

Analytical Method to Estimate Furosemide

Accurately weighed amount of furosemide (25 mg) was transferred to 25 ml volumetric flask and is dissolved in 10 ml of methanol and volume made up to mark with methanol which gives 1000 µg/ml (primary stock). From primary stock, 1 ml was taken and diluted to 10 ml with stimulated gastric fluid (SGF) which gives 100 µg/ml (secondary stock) and from the above stock solution different concentrations of solutions were prepared i.e. 4, 6, 8, 10, 12, 14, 16 µg/ml. Absorbance values of all these were measured by using UV spectrophotometer at λ max 276 nm and calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. Similarly calibration curves were plotted in other solvents such as pH 5.8 phosphate buffer and pH 7.4 phosphate buffer, respectively at the λ max values of corresponding solvents.

Saturation Solubility Studies

Saturation solubility analysis is the quantitative determination of the purity of a substance through the application of precise solubility measurements. Saturation solubility studies of pure drug (furosemide) was done in various oils and surfactants such as Transcutol P, Cremophore, Captex 355, Captex 200, Capmul MCM, soya bean oil, Miglyol 812, Tween 80. To each of these oils and surfactants, excess amount of drug was added and filled in vials. The solutions were equilibrated under continuous shaking on rotary shaker for 48 h. And after 48 h samples were centrifuged, the supernatant was suitably diluted and filtered through Whatman filter paper to obtain a clear solution and estimated for furosemide concentration using UV spectrophotometry at 276 nm after appropriate dilution. Three determinations were carried out for each sample to calculate the solubility of furosemide.

Formulation Methodology

SLH microparticles were prepared with two different oils such as soya bean oil and miglyol 812. The method followed in the preparation of SLH microparticles is schematically represented in the following figure 2 and is described below.

SLH microparticle formulations of F1-F5 and F11-F15 were prepared by taking known amount of drug (20 mg), lipid (Phospholipoid 90H) (5 mg), different oil:surfactant (soya bean oil/miglyol 812:Tween 80) ratio of 2:1,4:1,6:1,8:1 and 10:1, respectively.

Formulations F6-F10 and F16-F20 were prepared by keeping the oil:surfactant ratio constant at 10:1, and by increasing the lipid concentration from 5 to 10 mg, compositions of various formulations are given in the (Table 8 & 9)

EVALUATION OF SLH MICROPARTICLES

Micromeritic Properties

Angle of Repose

Angle of repose of powder blend was determined by using funnel method. Accurately weighed powder blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of heap of the powder. The powder blend was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan \theta = h/r$$

Where h and r are the height and radius of the powder cone, θ is the angle of repose.

The relationship between angle of repose and flow properties were given in Table 10.

Determination of Bulk Density and Tapped Density

An accurately weighed amount of powder blend (W) was carefully poured into the graduated cylinder and its initial volume (V_0) was measured. The cylinder was closed with lid and was placed into the tap density tester (USP), which was set for 100 taps. After 100 tappings the final volume of powder blend (V_f) was measured and operation was continued till the two consecutive readings were equal.

The bulk density and the tapped density were calculated using the following formula.

$$\begin{aligned}\text{Bulk density} &= W/V_0 \\ \text{Tapped density} &= W/V_f\end{aligned}$$

where, W = Weight of the powder; V_0 = Initial volume; V_f = final volume

Carr's Index/ Carr's Consolidation Index/ Compressibility Index (CI)

It is an important measure that can be obtained from the bulk and tapped densities. In theory, the less compressible a material the more flowable. The relationship between CI and flow properties were shown in Table 11.

$$CI = (TD - BD) \times 100 / TD$$

Where, TD is the tapped density and BD is the bulk density.

Hausner's Ratio

Hausner's ratio is the ratio of tapped density to bulk density. Hausner found that this ratio was related to interparticle friction and, as such, could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's index.

Assay of SLH Microparticles

SLH microcapsules of soya bean oil and miglyol 812 were determined for assay. An accurately weighed portion of the powder equivalent to about 100 mg was transferred to a 100 ml volumetric flask containing SGF phosphate buffer. It was kept on rotary shaker for 1hr. After this time period, the solutions were centrifuged, filtered through membrane filter 0.45 µm and 1 ml of filtrate was diluted to 10 ml with buffer and absorbance was measured against blank at 276 nm by UV Spectrophotometry. Each content

determination was performed in triplicate and the average and standard deviations were calculated.

In vitro Dissolution Study

Dissolution studies of pure furosemide, SLH microparticles and marketed formulation were performed by using the US Pharmacopeia (USP) XXIV type II apparatus (Electrolab, Mumbai, India) at the paddle rotation speed of 50 rpm in 900 mL of SGF as dissolution media at $37 \pm 0.5^\circ\text{C}$. A sample equivalent to 20 mg furosemide of the prepared systems was placed in dissolution medium. During the release studies, samples of 5 ml were collected after 5, 10, 20, 25, 30, 45, 60, 90 and 120 min using a syringe and were replaced with the same volume of phosphate buffer. The samples were subsequently filtered using $0.45\ \mu\text{m}$ pore size membrane filters. Then Filtrate (1 ml) was diluted to 10ml with phosphate buffer and absorbance was measured against blank at 276 nm using a UV Spectrophotometry. Each content determination was performed in triplicate and the average and standard deviations were calculated.

Diffusion Study

Comparative diffusion study for pure drug and selected formulation of SLH microparticles was conducted using gelatin membrane by Franz diffusion. SLH microparticles with equivalent to 20 mg of drug dissolved in 2 ml of buffer and it was then introduced into the donor compartment. The receptor compartment consists of 18 ml of 7.4pH phosphate buffer. Samples were collected at predetermined time intervals and replenished with equal volume of medium and the samples were filtered through membrane filter $0.45\ \mu\text{m}$ and quantified by using UV spectrophotometry at a wavelength of 276 nm.

Ex vivo Permeation Studies

Goat intestinal membrane was collected from slaughter house. It was flushed with Krebs-Ringer solution to remove the mucus and adhered intestinal contents. One end of the intestine segment was tied and the formulation equivalent to 20 mg of drug was dispersed in 2 ml of buffer and it was then introduced into the intestinal lumen and was tightly closed. The tissue was placed in an organ bath with continuous aeration and maintained at a temperature of 37°C . The receptor compartment consists of 200 ml of 7.4pH phosphate buffer. Comparative studies of pure drug, selected batch of SLH microparticles, marketed formulation were done. Samples were collected at predetermined time intervals and replenished with equal volume of medium and the samples were filtered through membrane filter $0.45\ \mu\text{m}$ and quantified by using UV spectrophotometry at a wavelength of 276 nm.

Solid State Characterization

Solid state study was performed for drug (furosemide) and for selected batch of SLH microparticles.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectra were obtained using a IR prestige 21 Shimadzu model FTIR spectrometer which was employed to characterize the possible interactions between the drug and the carrier in the solid state. The samples are prepared by the KBr pellet method and the spectrum was recorded in the range of $4000\text{--}400\ \text{cm}^{-1}$.

X- Ray Diffraction

SLH microparticles were analyzed using an X'Pert PRO MPD diffractometer (PANalytical, Almelo, the Netherlands) with a copper anode (Cu $K\alpha$ radiation, $\lambda = 0.15406\ \text{nm}$, 45 kV, 40 mA). The diffraction pattern was measured with a step size of 0.017° and a dwell time of 45 s at each step between 3 and $50\ 2\theta$ at ambient temperature.

RESULTS AND DISCUSSION

Analytical Method to Estimate Furosemide

Calibration curve of furosemide were plotted in different solvents of methanol, SGF, 5.8 pH phosphate buffer and 7.4 pH phosphate buffer, which are shown in Figure 3 to 6. R^2 values and slope values were calculated and are given in Table 12.

Saturation Solubility Studies

Saturation solubility studies of furosemide pure drug was done in various oils and surfactants such as Transcutol P, Cremophore, Captex 355, Captex 200, Capmul MCM, soya bean oil, Miglyol 812, Tween 80. The results obtained are shown in bar graph, which is shown in Figure 7 and Table 13. It was found that furosemide is having high solubility in Miglyol 812, Tween 80 and soya bean oil. Hence these solvents were selected for the formulation. Miglyol 812 and soya bean oil are selected as solvents and Tween 80 as surfactant.

Micromeritic Evaluation

SLH microparticles was characterized with respect to angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio and drug content. The results obtained are given in Table 14 and 15. It was observed that the powder blends of all the formulations were shown to have excellent to good flow characteristics. Angle of repose was found to be in the range of 24 ± 0.03 to 29 ± 0.02 , bulk density was found to be in the range of 0.32 ± 0.04 to 0.47 ± 0.13 , tapped density was found to be in the range of 0.29 ± 0.18 to 0.46 ± 0.03 , Carr's index was found to be in the range of 9.04 ± 0.14 to 16.45 ± 0.09 , Hausner's ratio was found to be in the range of 0.91 ± 0.06 to 1.12 ± 0.04 . It was observed that all the formulations have passed the test. Drug content was found to be in the range of 84.3 ± 0.47 to 99.7 .

In vitro Drug Release Studies

Comparative in vitro drug release studies of SLH microparticles of soya bean oil and Miglyol 812 are done.

In vitro Drug Release of SLH Microparticles Containing Soya Bean Oil

SLH microparticle formulations were subjected to dissolution studies at SGF to determine the release profile of SLH microparticles. The results of release studies of formulation are shown in Table 16. A graph was plotted by taking % drug release on Y-axis and time on X-axis, which is shown in Figure 8.

It was found that F5 formulation has shown faster drug release than the remaining formulations. As $84.5 \pm 0.7\%$ of drug was released within 5 minutes and $99.3 \pm 1.8\%$ of drug was released within 30 minutes. Hence, it was chosen as the best ratio of oil to surfactant, and the remaining formulations F6 to F10 were prepared by keeping this ratio at constant level and increasing the lipid concentration up to 10 mg. It was found that the formulations F6 to F8 have shown good drug release characteristics similar to F5 irrespective of lipid concentration. However, the drug release was found to be gradually

reduced in F9 and F10 formulations. This might be due to the higher concentration of lipid. Hence F5 and F8 formulations were finally selected for the permeability studies.

In vitro Drug Release of SLH Microparticles Containing Miglyol 812

SLH microparticle formulations were subjected to dissolution studies at SGF to determine the release profile of SLH microparticles. The results of release studies of formulation are shown in Table 17. A graph was plotted by taking % drug release on Y-axis and time on X-axis, as shown in Figure 9. It was found that F15 formulation has shown faster drug release than the remaining formulations. As $85.4 \pm 1.6\%$ of drug was released within 5 minutes and $99.2 \pm 1.2\%$ of drug was released within 30 minutes. Hence, it was chosen as the best ratio of oil to surfactant, and the remaining formulations F16 to F20 were prepared by keeping this ratio at constant level and increasing the lipid concentration up to 10 mg. It was found that the formulations F16 to F17 have shown good drug release characteristics similar to F15 irrespective of lipid concentration. However, the drug release was found to be gradually reduced in F18 to F20 formulations. This might be due to the higher concentration of lipid. Hence F15 and F17 formulations were finally selected for the permeability studies.

However, among the SLH microparticles of soya bean oil and Miglyol 812, SLH microparticles containing Miglyol 812 (F15) was found to be the best formulation for the in vitro drug release studies, as 100% of drug was released within 10 minutes.

Comparative In vitro Drug Release Profile

SLH microparticle formulations were subjected to dissolution studies at SGF to determine the release profile of SLH microparticles. The results of release studies of formulation are shown in Table 18. A graph was plotted by taking % drug release on Y-axis and time on X-axis as shown in Figure 10. Comparative in vitro drug release studies were carried out for pure drug, marketed formulation and best selected formulation. It was found that F15 formulation has shown faster drug release than the pure and marketed formulation. As $85.4 \pm 1.6\%$ of drug was released within 5 minutes and $100.5 \pm 1.8\%$ of drug was released within 30 minutes. Whereas for pure drug, $11.5 \pm 1.8\%$ of drug was released within 5 minutes and $100.4 \pm 1.9\%$ of drug was released within 120 minutes. Whereas for marketed formulation, $17.5 \pm 1.4\%$ of drug was released within 5 minutes and $100.4 \pm 1.6\%$ of drug was released within 90 minutes. Hence F15 formulation was finally selected as best formulation when compared with pure drug and marketed formulation.

Comparative Diffusion Study

SLH microparticle formulations were subjected to diffusion studies at 7.4 pH phosphate buffer to determine the release profile of SLH microparticles. The results of release studies of formulation are shown in Table 19. A graph was plotted by taking % drug release on Y-axis and time on X-axis as shown in Figure 11. Comparative diffusion studies were carried out for pure drug and best-selected formulations. It was found that F17 formulation has shown faster diffusion than pure drug and other remaining formulations. For F17 formulation, as $26.4 \pm 1.2\%$ of drug was diffused within 15 minutes and $83.7 \pm 2.1\%$ of drug was diffused within 120 minutes. Whereas for pure drug, $6.4 \pm 0.9\%$ of drug was diffused within 15 minutes and $48.4 \pm 2.0\%$ of drug was diffused within 120 minutes. Whereas for remaining other selected formulations, for F5 $10.8 \pm 1.4\%$ of drug was diffused within 15 minutes and $70.2 \pm 1.2\%$ of drug was diffused within 120 minutes. For F8 formulation, $17.4 \pm 1.6\%$ of drug was diffused within 15 minutes and $79.4 \pm 0.6\%$ of drug was diffused within 120 minutes. For F15 formulation, $16.9 \pm 0.6\%$ of drug was

diffused within 15 minutes and $71.2 \pm 1.7\%$ of drug was diffused within 120 minutes. Hence F17 formulation was finally selected as best formulation when compared with pure drug and other selected formulations.

Comparative Ex vivo Permeation Studies

SLH microparticle formulations were subjected to ex vivo permeation studies at 7.4 pH phosphate buffer to determine the release profile of SLH microparticles. The results of diffusion studies of formulation are shown in Table 20. A graph was plotted by taking % drug release on Y-axis and time on X-axis as shown in the Figure 12. Comparative ex vivo permeation studies were carried out for pure drug, marketed formulation and best-selected formulation. It was found that F17 formulation has shown faster diffusion than pure drug and marketed formulation. For F17 formulation, as $27.2 \pm 1.6\%$ of drug was diffused within 10 minutes and $86.3 \pm 0.9\%$ of drug was diffused within 90 minutes. Whereas for pure drug, $7.4 \pm 0.9\%$ of drug was diffused within 10 minutes and $49.8 \pm 2.0\%$ of drug was diffused within 90 minutes. Whereas, for marketed formulation $15.8 \pm 2.0\%$ of drug was diffused within 10 minutes and $67.2 \pm 0.9\%$ of drug was diffused within 90 minutes. Hence F17 formulation was finally selected as best formulation when compared with pure drug and marketed formulation.

Solid State Characterization

X-Ray Diffraction

Polymorphic changes of the drug are important factors that may affect the dissolution rate and bioavailability. On the other hand, the importance of polymorphism on the therapeutic effectiveness of a drug and the pharmaceutical implication of the presence of metastable crystalline forms in the bulk powder are well recognized.

Transformation from crystalline state to amorphous state favours the faster dissolution of drug there by improved solubility and dissolution rate as later contains high internal energy and improved thermodynamic properties compared to pure crystalline drug.

Powder X-Ray Diffraction scans of pure drug and optimized formulation were conducted to understand the crystallinity of pure drug and any loss or modification of pure drug crystallinity after its formulation into SLH microparticles.

The X-ray diffractograms of pure furosemide (Figure 13) showed sharp distinct characteristic peaks at 2-Theta diffraction angles of 22° and 25° due to its crystalline nature.

The X-ray diffractograms of optimized SLH microparticles formulation (Figure 14) showed only broad peak at 2-Theta of 20° due to its amorphous nature and no peak was seen at 2-Theta of 22° thus the results of X-ray diffractograms of furosemide and optimized formulation showed that there was reduction of peak at 22° of 2-theta scales in optimized formulation because of lower concentration of drug in the formulation or dissolution of some fraction of drug in oil and surfactant. Whereas presence of certain pure drug peak in the formulation is due to the fact that after saturation of absorption process adsorption occurs on the surface of carrier. It can be concluded that no significant interaction between drug and excipients.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR studies were done for pure drug and optimized formulation to determine compatibility between pure drug and excipients. If the drug and excipients interact, the peaks corresponding to the functional groups in the drug FTIR will shift to different wave numbers compared to spectra of the pure drug and pure excipients.

The FTIR spectra of pure drug furosemide and optimized formulation were illustrated in figure. 15, figure16 and characteristic peaks observed were listed in table 21 and the results showed that no interaction is seen between drug and excipients.

The characteristic peaks observed for pure drug are present in the SLH microparticles formulation. The peaks seen in pure drug are at N-H stretching at a wavelength of 3446.54, S = O at 1141.46, acid C=O stretching at a wavelength of 1671.57 and C-OH bending at a

wavelength of 1492.97. The same peaks were seen in optimized formulation as a result the formulation was compatible with the excipients. Thus results of FTIR studies suggest that a reduction in intensity of the characteristic absorption bands of furosemide were observed in SLH microparticles formulations, which might be attributed to the hydrogen bonding interaction between the furosemide and of the carrier. It can be concluded from the results that there was no interaction between drug and excipients.

Table 1: BCS Classification

Class I High solubility, High permeability E.g. verapamil, acyclovir, propranolol	Class II Low solubility, High permeability E.g. cyclosporin, ketoprofen, mebendazole
Class III High solubility, Low permeability E.g. ranitidine, frusemide, acyclovir	Class IV Low solubility, Low permeability E.g. ciprofloxacin, mebendazole, frusemide

Table 2: Biopharmaceutics drug disposition classification system

Class I High solubility, Extensive metabolism E.g. verapamil, propranolol	Class II Low solubility, Extensive metabolism E.g. cyclosporine, ketoprofen, mebendazole
Class III High solubility, Poor metabolism E.g. ranitidine, acyclovir	Class IV Low solubility, Poor metabolism E.g. ciprofloxacin, frusemide

Table 3: Comparison of various formulation technologies for poorly water-soluble drugs⁸

Technology	Potential advantages	Potential disadvantages
Physical modifications		
Micronisation (conventional)	Known technology; freedom to operate; easily adaptable to pharmaceutical manufacturing set-ups; relatively inexpensive and reproducible production; solid dosage form	Insufficient improvement in dissolution rate; no effect on the saturation solubility of drugs (these limitations also apply to other micronisation techniques)
Dense gas technology	Alternative nanocrystal processing method.	Unproven technology for industrial use; secondary process required to avoid aggregation of nanocrystals
Fluid pearl- or ball-milling	Established products on the market; experienced technology provider (Elan Corporation); versatility to produce solid or liquid dosage forms	Available only under license; secondary process required to avoid aggregation of nanocrystals
Crystal habit Modification	Relatively inexpensive; versatility to produce solid or liquid dosage forms	Risk of polymorph instability; possible need of incorporating stabilisers to inhibit crystal growth/ change
Surfactant and cosolvent system	Relatively high solvent capacity for hydrophobic compounds	Possible poor tolerance to excipient-related side effects in chronic use; tendency of drug precipitation on excessive dilution

Table 4: Currently marketed lipid excipients include²⁻³

Class of lipid excipients	Examples of excipients used in marketed lipid based oral products	Solubilizer	Emulsifier	Surfactant	Others
Fatty acids	Oleic acid, soya fatty acids	Yes	No	No	No
Natural oils and fats	Medium Chain Triglycerides: Coconut oil, palm seed oil	Yes	No	No	No
	Long Chain Triglycerides: Olive oil, soya bean oil	Yes	No	No	No
Semi synthetic MG,DG,TG	Medium chain glyceryl mono & dicaprylate	Yes	No	Yes	Co-emulsifier
	MCT Glyceryl tricaprylate/caprate (miglyol 810)	Yes	No	No	Vehicle for capsule formulation
	Long chain MG Glyceryl mono oleate (capmul GMO)	Yes	No	Yes	Wetting agent, vehicle for capsule formulation
Semi-synthetic PEG derivatives of glycerides and fatty acid	Medium chain glycerides	Yes	Yes	No	Vehicle
	Long chain glycerides	Yes	No	No	Vehicle for soft gels, co-emulsifier, cosurfactant
	PEG 1500 Glyceryl laurate (Gelucire 44/14)	Yes	No	Yes	Semi-solid matrix. Capsule vehicle.
Cholesterol and fatty lipids	Egg lecithin, soyabean lecithin	Yes	Yes	Yes	(in mixed micelles, emulsions, liposomes)
Polyglycerol fatty acid esters	Polyglycerol-3-dioleate (pluril oleique CC 497)	Yes	Yes	Yes	Vehicle for capsule
	Polyglycerol-10-decaoleate (caprol 10 G100)	Yes	Yes	Yes	Lubricant, crystallization inhibitor.

Table 5: Lipid classification based on their interactions with water^{2-3, 18}

Class of lipids	Physical appearance	Schematic physical appearance	Examples
Non-polar lipids	Surface: will not spread to form a monolayer Bulk: insoluble		Cholesteryl esters, paraffin oil, carotene, hydrocarbons
Polar lipids			
I (Non-swelling)	Surface: Stable monolayer Bulk: insoluble		TG, DG, cholesterol, fat soluble vitamins (A,D,E,K), long chain FA
II (Swelling)	Surface: stable monolayer Bulk: insoluble		MG, phospholipids
IIIA (Swelling amphiphiles with lyotropic mesomorphism)	Surface: unstable monolayer Bulk: liquid crystalline intermediates Micelles		Detergents, sodium and potassium salts of long chain FA
IIIB (Swelling amphiphiles without lyotropic mesomorphism)	Surface: unstable monolayer Bulk: micelles		Bile salts, saponins

Table 6: Comparison of the properties of gastric lipase and pancreatic lipase

Properties	Gastric lipase	Pancreatic lipase
Origin	Gastric mucosa	Pancreas
Maximum catalytic pH	3.0 - 6.0	6.5 - 9.0
Measured medium pH	1.5 - 4.0	4.0 - 6.8
% TG Hydrolysis	10 - 20% (in stomach) 10 % (in small intestine)	60 - 90% (in small intestine)
Typical reactions	TG = DG + FA	TG = DG + FA DG = 2-MG + FA 2-MG = 1-MG + Glycerol + FA
Cofactor	NIL	Colipase

Table 7: Lipid formulation classification system (LFCS) based on the types and compositions of lipid and surfactant excipients as proposed by pouton⁴

Formulation type, properties	Type I	Type II	Type IIIA	Type IIIB	Type IV
Description	Simple lipid solution or suspension	SEDSS	SEDSS/SMEDDS	SMEDDS	Surfactant/ cosolvent blend
Typical compositions					
TG/mixed glycerides	100%	40-80%	40-80%	< 20%	--
Hydrophilic cosolvents	--	--	0-40%	50-100%	0-50%
Surfactants	--	20-60% HLB <12	20-40% HLB >12	20-50% HLB >12	0-20% HLB <12 30-8-% HLB >12
Particle size of dispersion	Coarse	100-250 nm	100-250 nm	50-100 nm	<100 nm
Significance of aqueous dispersion	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity	Loss of solvent capacity
Significance of digestability	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required and not likely to occur	Not required and not likely to occur

Table 8: Formulation of SLH microparticles containing soya bean as oil

FORMULA	Drug (mg)	Phospholipid 90 H (Lipid)	Soya bean oil	Oil:surfactant ratio	Surfactant Tween 80	Carrier Aerosil 380
F1	20	5	20	2:1	10	40
F2	20	5	40	4:1	10	50
F3	20	5	60	6:1	10	75
F4	20	5	80	8:1	10	95
F5	20	5	100	10:1	10	125
F6	20	6	100	10:1	10	125
F7	20	7	100	10:1	10	125
F8	20	8	100	10:1	10	125
F9	20	9	100	10:1	10	125
F10	20	10	100	10:1	10	125

Table 9: Formulation of SLH microparticles containing miglyol 812 as oil

FORMULA	Drug(mg)	Phospholipid 90 H (Lipid)	Miglyol 812	Oil:surfactant ratio	Surfactant Tween 80	Carrier Aerosil 380
F11	20	5	20	2:1	10	50
F12	20	5	40	4:1	10	75
F13	20	5	60	6:1	10	100
F14	20	5	80	8:1	10	150
F15	20	5	100	10:1	10	150
F16	20	6	100	10:1	10	150
F17	20	7	100	10:1	10	150
F18	20	8	100	10:1	10	150
F19	20	9	100	10:1	10	150
F20	20	10	100	10:1	10	150

Table 10: Relationship between angle of repose and flow properties

Angle of Repose	Properties
< 25	Excellent flow
25-30	Good flow
30-40	Passable flow
> 40	Poor flow

Table 11: Relationship between Carr's index and flow properties

Carr's Index	Properties
5-12	Free flowing
13-16	Good
18-21	Fair
23-35	Poor
33-38	Very poor
>40	Extremely poor

Table 12: R² values and slope values of furosemide in different solvents

Solvent	R ²	Slope
SGF	0.9981	0.0712
5.8 phosphate buffer	0.9986	0.0654
7.4 Phosphate buffer	0.9904	0.0552
Methanol	0.9971	0.0665

Table 13: Saturation solubility studies of furosemide in various oils and surfactants

Solvents	Solubility (mg/mL) (Mean ± SD)
Transcutol P	52.3 ± 0.06
Cremophore	48.6 ± 0.24
Captex 355	82.7 ± 0.25
Captex 200	64.3 ± 0.21
Capmul MCM	86.4 ± 1.92
Soya bean oil	128.4 ± 1.51
Miglyol 812	146.4 ± 1.91
Tween 80	148.3 ± 1.64

Table 14: Micromeritic properties of SLH microparticles containing soya bean oil

Formulation	Bulk density*(g/ml)	Tapped density* (g/ml)	Carr's index*(%)	Hausner's ratio*	Angle of repose* (°)	Drug content* (%)
F1	0.46±0.07	0.36±0.04	13.02±0.06	0.91 ± 0.01	25 ± 0.12	84.3±0.47
F2	0.39±0.06	0.30±0.17	9.08 ± 0.12	0.95 ± 0.08	27 ± 0.06	89.6±1.36
F3	0.47±0.13	0.29±0.18	14.50 ± 0.04	0.92 ± 0.02	28 ± 0.04	96.3±0.75
F4	0.45±0.17	0.41±0.07	16.45 ± 0.09	0.92 ± 0.06	27 ± 0.12	94.8±1.34
F5	0.34±0.08	0.30±0.14	9.21 ± 0.07	0.91 ± 0.06	24 ± 0.02	99.5±1.03
F6	0.46±0.09	0.37±0.08	15.33 ± 0.18	0.93 ± 0.07	28 ± 0.17	97.7±1.49
F7	0.42±0.12	0.32±0.07	9.04 ± 0.14	0.96 ± 0.02	26 ± 0.13	96.6±0.29
F8	0.34±0.18	0.36±0.04	16.40 ± 0.08	0.91 ± 0.06	25 ± 0.09	97.4±0.43
F9	0.32±0.04	0.39±0.06	12.39 ± 0.06	1.12 ± 0.04	26 ± 0.14	89.7±1.33
F10	0.36±0.08	0.38±0.03	11.51 ± 0.08	0.93 ± 0.02	27 ± 0.09	94.2±0.48

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 15: Micromeritic properties of SLH microparticles containing miglyol 812

Formulation	Bulk density*(g/ml)	Tapped density* (g/ml)	Carr's index*(%)	Hausner's ratio*	Angle of repose* (°)	Drug content* (%)
F11	0.34±0.04	0.32±0.07	10.02±0.05	0.95 ± 0.02	26 ± 0.20	86.3±1.55
F12	0.46±0.03	0.41±0.04	13.08±0.06	0.91 ± 0.07	25 ± 0.07	91.6±1.43
F13	0.45±0.07	0.46±0.03	16.50±0.01	0.93 ± 0.03	28 ± 0.05	93.3±0.65
F14	0.47±0.03	0.42±0.03	14.45±0.02	0.92 ± 0.05	27 ± 0.08	94.8±1.86
F15	0.33±0.02	0.30±0.04	9.21 ± 0.04	0.91 ± 0.06	24 ± 0.03	99.7±1.07
F16	0.42±0.06	0.37±0.03	15.33±0.08	0.94 ± 0.08	28 ± 0.07	95.7±1.34
F17	0.46±0.03	0.41±0.04	10.18±0.02	0.93 ± 0.02	26 ± 0.16	87.6±0.39
F18	0.34±0.04	0.29±0.03	12.40±0.03	0.91 ± 0.08	29 ± 0.02	96.4±0.75
F19	0.36±0.07	0.30±0.03	16.39±0.02	0.95 ± 0.01	27 ± 0.07	89.7±1.36
F20	0.39±0.05	0.34±0.04	9.51 ± 0.06	0.99 ± 0.01	25 ± 0.12	95.2±0.47

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 16: In vitro drug release studies of SLH microparticles containing soya bean oil

Formulation	% Drug Release within						
	0 min	5min	10min	15min	20min	25min	30min
F1	0	32.5±1.2	38.4±1.7	44.5±0.8	53.5±2.4	58.4±1.3	66.4±2.1
F2	0	46.5±0.9	49.7±1.4	56.3±1.8	64.1±0.7	66.3±1.4	75.1±1.8
F3	0	61.2±1.3	69.5±1.9	73.4±2.6	78.1±1.9	82.6±0.8	86.4±1.6
F4	0	76.9±1.1	83.5±1.8	86.2±0.7	91.6±2.1	95.8±1.6	97.4±0.6
F5	0	84.5±0.7	96.2±1.6	100.8±0.9	99.8±1.7	99.6±1.4	99.3±1.8
F6	0	84.3±0.5	90.4±1.3	100.6±2.1	99.5±0.9	99.8±1.4	99.6±1.8
F7	0	79.6±1.2	85.4±1.7	99.8±0.8	100.6±1.9	99.8±0.9	99.2±0.5
F8	0	74.2±1.3	80.6±1.9	99.7±0.9	99.2±0.5	100.2±1.8	99.4±1.4
F9	0	63.7±0.8	70.4±2.1	81.3±0.9	86.4±0.6	92.6±1.4	99.8±1.8
F10	0	45.2±2.1	65.7±1.7	78.4±1.3	85.1±1.9	94.8±1.5	96.2±0.7

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 17: In vitro drug release studies of SLH microparticles containing miglyol 812

Formulation	% Drug Release within						
	0 min	5min	10min	15min	20min	25min	30min
F11	0	43.6±0.9	55.2±1.7	67.4±1.4	79.9±2.1	86.4±2.4	92.1±1.8
F12	0	66.8±1.7	82.7±1.4	87.3±0.8	92.4±1.4	97.2±0.6	100.2±0.9
F13	0	81.4±1.8	83.7±0.7	91.4±1.3	96.5±1.2	100.7±1.7	99.2±0.9
F14	0	84.3±0.6	92.1±1.5	97.6±1.7	100.8±1.9	99.3±0.9	99.7±0.6
F15	0	85.4±1.6	100.7±0.9	99.2±1.2	99.8±1.8	99.6±0.7	99.2±1.2
F16	0	84.9±1.8	100.6±1.5	99.4±0.9	99.8±0.7	99.2±1.1	99.4±1.7
F17	0	82.3±1.6	100.4±0.9	99.2±1.8	99.8±0.7	99.6±0.9	99.2±1.4
F18	0	81.7±0.9	95.4±1.2	100.8±1.8	99.6±1.4	99.4±0.6	99.7±0.9
F19	0	76.1±2.1	79.3±0.7	82.6±1.8	96.1±1.3	99.4±2.4	100.2±0.7
F20	0	72.7±1.8	76.5±0.6	80.2±1.3	88.4±0.7	90.8±1.9	93.7±1.4

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 18: Comparative In vitro drug release studies of pure drug, selected SLH microparticles (F15), marketed formulation

Time (min)	Pure Drug	SLH Microparticles (F15)	Marketed Product
0	0	0	0
5	11.5 ± 1.8	85.4 ± 1.6	17.5 ± 1.4
10	16.4 ± 0.7	100.7 ± 0.9	26.4 ± 1.2
15	22.4 ± 1.2	100.2 ± 1.2	39.6 ± 0.7
30	37.5 ± 2.1	100.8 ± 1.8	52.3 ± 1.9
45	44.4 ± 1.7		64.6 ± 2.1
60	52.7 ± 0.6		86.5 ± 0.8
90	86.3 ± 2.4		100.4 ± 1.6
120	100.4 ± 1.9		99.2 ± 0.9

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 19: Comparative diffusion studies of pure drug and selected SLH microparticles

Time (Min)	Pure	F5	F8	F15	F17
0	0	0	0	0	0
15	6.4 ± 0.9	10.8 ± 1.4	17.4 ± 1.6	16.9 ± 0.6	26.4 ± 1.2
30	12.9 ± 1.2	17.5 ± 0.8	25.8 ± 0.9	21.4 ± 1.5	37.3 ± 1.7
45	26.1 ± 1.5	28.1 ± 1.9	41.4 ± 2.1	38.4 ± 1.2	48.2 ± 0.6
60	32.5 ± 0.5	37.4 ± 1.6	49.1 ± 1.7	46.7 ± 1.4	56.1 ± 2.1
90	42.3 ± 1.8	54.2 ± 0.9	66.3 ± 1.4	59.3 ± 0.9	68.9 ± 0.9
120	48.4 ± 2.0	70.2 ± 1.2	79.4 ± 0.6	71.2 ± 1.7	83.7 ± 2.1

*Each value represents Mean±standard deviation (SD) of 3 observations.

Table 20: Comparative Ex vivo permeation studies of pure drug, marketed formulation and selected SLH microparticles

Time (min)	Pure Drug	F17	Marketed Product
0	0	0	0
10	7.4 ± 0.9	27.2 ± 1.6	15.8 ± 2.0
15	16.8 ± 1.4	39.4 ± 0.9	22.6 ± 1.6
30	26.7 ± 1.6	57.9 ± 2.4	37.2 ± 1.2
45	38.4 ± 1.2	75.8 ± 1.7	45.7 ± 1.6
60	46.1 ± 1.6	81.2 ± 1.3	58.4 ± 1.4
90	49.8 ± 2.0	86.3 ± 0.9	67.2 ± 0.9

*Each value represents Mean±standard deviation (SD) of 3 observations

Table 21: Fourier transform infrared spectroscopy with characteristic peaks

Functional Group	Wavelength(cm^{-1})		Inference
	Pure drug	Optimized formulation	
N-H stretching 1° - amine	3398.66	3446.54	No interaction
S = O	1141.46	1064.72	No interaction
acid C = O stretching	1671.57	1738.87	No interaction
C-OH bending	1492.97	1459.69	No interaction

Figure 1 (A, B): Gastrointestinal digestion of triglyceride substrates and fate of the encapsulated drug molecules

- A) Gastric lipolysis catalysed by gastric lipase, which is gradually inhibited by the accumulated digestion products (mainly DG and FA) at the lipid droplet surfaces (scanning electron micrographs adapted from.²⁰)
- B) Duodenal lipolysis of lipid droplets emptied from the stomach catalyzed by pancreatic lipase-colipase complexes, which results in the formation of a highly solubilizing lipophilic microenvironment that subsequently facilitates drug transport across the unstirred water layer and enterocytes (photomicrographs adapted from.¹⁹)

Figure 2: Methodology of SLH microparticles

Figure 3: Calibration curve of furosemide in SGF

Figure 4: Calibration curve of furosemide in 5.8 phosphate buffer

Figure 5: Calibration curve of furosemide in 7.4 phosphate buffer

Figure 6: Calibration curve of furosemide in methanol

Figure 7: Saturation solubility studies of furosemide in various oils and surfactant

Figure 8: In vitro drug release profile of SLH microparticles containing soya bean oil

Figure 9: In vitro drug release profile of SLH microparticles containing miglyol 812

Figure 10: In vitro drug release profile of SLH microparticles containing miglyol 812

Figure 11: Comparative diffusion studies of pure drug and selected SLH microparticles

Figure 12: Comparative Ex vivo permeation studies of pure drug, selected SLH microparticles and marketed formulation

Figure 13: X-ray diffractograms of furosemide

Figure 14: X-ray diffractograms of optimized SLH microparticles formulation

Figure 15: FTIR spectra of furosemide

Figure 16: FTIR spectra of optimized SLH microparticles formulation

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