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Self-nanoemulsifying drug delivery systems (SNEDDS) for the oral delivery of lipophilic drugs

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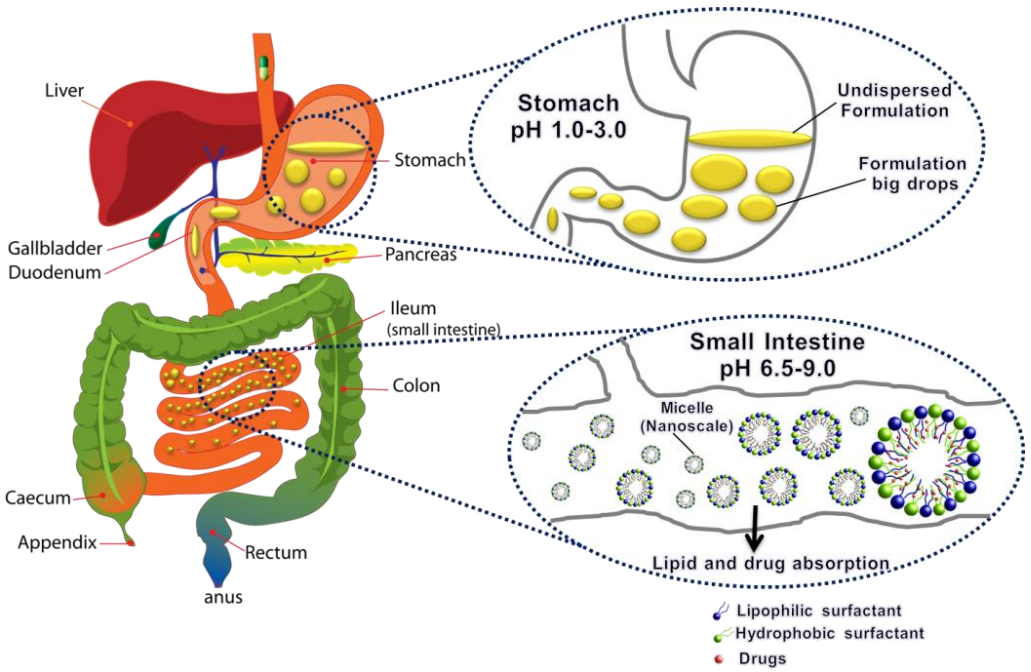

November 2015

SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS (SNEDDS)

FOR THE ORAL DELIVERY OF LIPOPHILIC DRUGS

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To those who get lost;

Believe that God help those who help themselves.

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Abstract

The increasing number of lipophilic drug candidates in development in the pharmaceutical industry calls for advanced drug delivery systems with increased bioavailability less day-to-day and food-intake-dependent. Many of these drug candidates possess poor water solubility, so that their dissolution rate in the gastrointestinal tract (GIT) limits their absorption following oral administration.

In the past few decades, various lipid-based formulations have been investigated to enhance the bioavailability of such challenging drug candidates and to increase their clinical efficacy when administered orally.

Recently, self-emulsifying drug delivery systems (SEDDS) have attracted increasing interests and, in particular, self-nanoemulsifying drug delivery systems (SNEDDS). SEDDS and SNEDDS consist in micro- or nano-emulsions of oil containing the drug that spontaneously form in aqueous media on mild agitation. Usually, they use high amounts of surfactant that may cause degradation and instability of the drugs, being moreover toxic for the gastrointestinal tract.

The aim of the present thesis was the preparation of novel self-nanoemulsifying drug delivery systems to overcome the shortages of conventional SEDDS or SNEDDS.

To reduce the amount of surfactant, we formulated first a self-nanoemulsifying drug delivery system containing high proportion of essential lemon oil, that was characterized in terms of drug solubility, formulation stability, viscosity, emulsion droplet size, ζ-potential and *in vitro* drug release.

Then, a pH-sensitive SNEDDS was developed that emulsify only at basic pHs. The goal was to protect the lipophilic drugs from the harsh acidic environment in

stomach and render it available in the enteric tract where the bioactive compound should be absorbed.

Chapter I General Introduction

1. Oral delivery systems for lipophilic drugs

Oral delivery route is the most convenient route for drug administration to achieve desired therapeutic effects and the greatest degree of patient compliance, especially for chronic condition diseases [1]. Despite some clinical oral formulations have been developed, their low oral bioavailability is still a major hurdle, leading to challenges for pharmaceutical manufacturers to design delivery systems that can provide improved pharmacokinetic profiles and therapeutic responses [2-4]. Currently, many efforts such as efflux pump inhibitors, permeation enhancers and drug nanonization, have been made to overcome the challenges of low oral bioavailability resulting from low drug solubility, poor permeation and enzymatic degradation, which limiting drug effective delivery[5].

1.1. Physicochemical properties of the drugs

1.1.1. Biopharmaceutics classification system (BCS)

The Biopharmaceutics classification system (BCS) is a guide for predicting the intestinal drug absorption provided by the U.S. Food and Drug Administration (FDA). BCS is a useful tool for decision-making in formulation development from a biopharmaceutical point of view [6].

On the basis of drug solubility and intestinal permeability, BCS categorize the drugs into four categories, as follows [7-9]:

- Class I - high permeability, high solubility (Example: metoprolol),
- Class II - high permeability, low solubility (Example: silibinin, ibuprofen),
- Class III - low permeability, high solubility (Example: cimetidine),

- Class IV - low permeability, low solubility (Example: hydrochlorothiazide, Bifonazole)

As recommended by FDA, the solubility class boundary is based on the highest dose strength of an immediately release product. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-6.8, while a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 85% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose [9]. The low permeability of Class II and Class IV drugs renders them poorly bioavailable, so reducing their potential pharmaceutical effect or requiring high dosage to achieve it [10, 11]. Biopharmaceutics classification system (BCS) and viable formulation options based on the BCS are summarized in Figure 1.1.

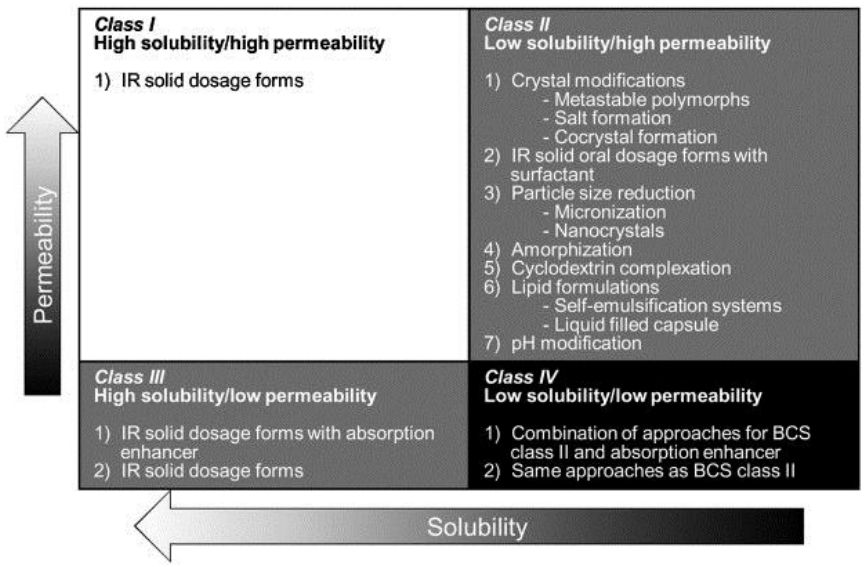

Figure 1.1 Biopharmaceutics classification system (BCS) and viable formulation options based on the BCS [12].

1.1.2. Physicochemical properties of lipophilic drugs

More than 40% of new drug candidates of recent years possess poor aqueous solubility, and approximately 40% of the marketed immediate-release (IR) oral drugs are categorized as practically insoluble [12]. The term “lipophilic drugs” roughly describes a heterogeneous group of molecules that exhibit poor solubility in water, but certainly not always, are soluble in various organic solvents [13]. Usually, the terms practically insoluble (< 0.1 mg/ml), very slightly soluble (0.1–1 mg/ml), and slightly soluble (1–10 mg/ml) are used to categorize lipophilic drug substances [14].

Partition coefficient, P, is the ratio of the concentrations of a compound in a mixture of two immiscible phases at equilibrium, which particularly are water and 1-octanol in chemical and pharmaceutical sciences [15]. P is a measure of how hydrophilic ("water-loving") or lipophilic ("water-fearing") a chemical substance is [16]. The poorly soluble drug candidates exist in two types of molecule structure, “grease ball” and “brick dust” [17]. Grease ball molecules are highly lipophilic with high log P due to no interactions with water. Brick dust molecules have melting point above 200 ℃ and low log P. Their poor solubility in water is caused by the strong intermolecular bonding and high lattice energy in solid state [18].

1.1.3. Drug stability

Drugs that are instable in the gastrointestinal tract (GIT) may undergo degradation. For instance, acid-labile drugs to be released in the small intestine must be protected with enteric coating.

Drug stability studies should address the sensitivity of dissolved drug to acids, alkalis, and oxidation as well as solid-state humidity-related, thermal, and photo-degradation which are very useful in drug delivery system design [13, 19].

As mentioned above, many drugs are unstable under certain chemical conditions, such as pH, ionic strength, or ingredient interactions. In this case, it is necessary to protect the active component from any constituents or environmental conditions that

promote chemical degradation. On the other hand, a food or drink product may also contain a number of functional ingredients that adversely interact with each other and cause physical instability [20]. In this case, it may be necessary to isolate the different active ingredients from each other to avoid undesirable physical changes in the systems [21].

An ideal oral drug delivery system must protect the drug from the degradation in the gastrointestinal tract, and deliver the bioactive compounds to the specific area where it is better absorbed. According to these reasons, plenty of efforts in oral drug delivery have been made on improving drug stability in the GIT, increasing drug solubility and further the bioavailability [22].

1.2. Advantages of oral delivery systems

Oral administration is the most widely accepted and preferred route for pharmaceuticals, due to its high convenience and better patient compliance [23]. Oral administration of drugs can avoid hospitalization, sterile manufacturing and trained personnel assistance, so reducing the cost of the health treatment [24]. Pharmaco-economic analyses were performed in clinical trials to evaluate the economic effectiveness of various oral drugs and to make a contrast with the cost of infusion administration [25].

In 2006, Cassidy, J. *et al.* [26] compared the costs for oral administration of capecitabine and intravenous administration of 5-fluorouracil/leucovorin (5-FU/LV), that are two chemotherapeutic drugs. The total costs of the two therapies were calculated by evaluating the following direct medical cost:

Cost of chemotherapy drugs;

Cost of visits for drug administration;

Cost of hospital use;

Cost of physician consultations for adverse events and for treating them;

Cost of ambulance trips.

Data analysis showed that when the 'societal costs' were added, the total costs were approximately £3500 for the oral capecitabine versus £8500 of 5-FU/LV. For this reason, based on the economic effectiveness, they termed capecitabine as a 'dominant' treatment strategy.

Besides, higher drug dosage may lead to side effects and wastage of the drugs, which is not economically tolerable, especially for some kinds of expensive drugs [27].

1.3. Challenges in the oral drug delivery

Regardless of many advantages, the development of oral delivery route still represents a great challenge owing to peculiar physicochemical properties of lipophilic drug candidates, and physiological barriers such as gastrointestinal instability, pre-systemic metabolism and efflux pump [28]. Upon oral administration, lipophilic drug in a dosage form is easily ingested by patients, travels in the GIT passing through an extremely various environment. When drug transits from a strong acidic pH in stomach to basic environment of the intestine, it encounters harsh pH changes, but also different digestive enzymes and the resident microflora [6, 29]. After the digestive journey, only a fraction of dose is available to systemic circulation for execution of therapeutic response [30]. In view of this, the principal challenges to the oral delivery are classified into physicochemical properties of drugs and physiological barriers posed by human body (Figure 1.2).

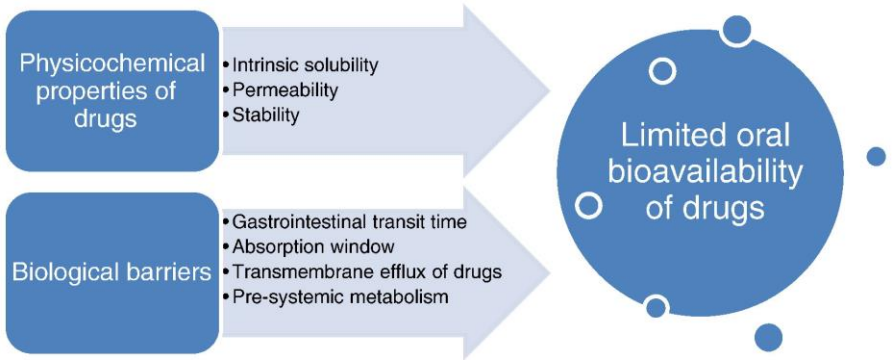

Figure 1.2 Schematic representation of the various challenges to the oral delivery of drugs.[24]

1.3.1. Solubility of drug substances

A plenty of organic materials are poorly soluble in water. The poorly water-soluble drugs are typical examples. Poor solubility of a drug is in most cases associated with poor bioavailability. As reported by CA Lipinski [31], 31.2% of 2246 compounds synthesized in academic laboratories between 1987 and 1994 had solubility equal to or less than 20 μg/ml. Furthermore, in drug discovery, about 40% of new drug candidates display poor solubility in water, which leads to low bioavailability, erratic absorption, high intra-subject and inter-subject variability and lack of dose proportionality [32]. From a physicochemical point of view, poor aqueous solubility and low dissolution rate are the major factors that affect oral delivery of many existing lipophilic drugs [33]. Improving the drug solubility might only solve one aspect of the problem but it is a starting point to design efficient pharmaceutical formulations [25].

1.3.2. Gastrointestinal transit

Human digestive system is complicatedly designed to safely, selectively, and effectively absorb as many nutrients as possible from our diet. In the case of drug

delivery, after the oral administration, drug candidates have to reach final absorption site – intestine. However, the gastrointestinal tract (GIT) presents various chemical and enzymatic barriers that affect delivery of drugs [34]. During the drug transit, the pH of the GI tract lumen rises from the strongly acidic (pH 1.0–2.0) in the stomach, to 5.0–6.0 in the duodenum, to basic (pH 7.0-9.0) in the jejunum [35]. On the other hand, variety of enzymes that include lipases and proteases also function to initiate foodstuff digestion and destroy unwanted pathogens and toxins [36]. Furthermore, the gastrointestinal transit time is another factor that significantly affects oral bioavailability and efficacy of many drugs. Many efforts have been done to enhance the duration for absorption, like the dosage form mucoadhesive. The use of mucoadhesives can increase local drug concentrations for absorption enhancement, improve the efficiency for prolonging drug resistance time, and in some cases restrict absorption to a specific site in the intestine [37, 38]. So far, various types of approaches have been successfully developed to extend the gastrointestinal transit time, further to improve the intestinal permeability and to enhance the oral bioavailability [39].

1.3.3. Drug metabolism and efflux pump

The metabolism of drug candidates and their efflux in the intestine during the absorption process represent another problem arising when the drugs are orally administered [40]. Drug metabolism is the biochemical modification of pharmaceutical substances or xenobiotics respectively by living organisms, usually through specialized enzymatic systems before reaching the systemic circulation. The rate of metabolism determines the duration and intensity of a drug's pharmacological action [41]. After oral administration, the drug is absorbed by the digestive system and enters into the liver via the portal vein, where a fraction of absorbed dose is metabolized [42]. In the issue, the systemic availability of the drug

is greatly reduced, in turn, affecting the amount of the drug reaching the final absorption sites. Transmembrane efflux of drugs is a mechanism responsible for moving foreign compounds, like drug substance, toxic substances, and antibiotics, out of the cell via a clinically significant systematic transportation system such as P-glycoprotein (P-gp), flurochrome efflux, methotrexate efflux (folates), etc. [24, 43, 44]. P-glycoprotein (P-gp) is extensively distributed and expressed in the intestinal epithelium where it pumps drugs back into the intestinal lumen. P-gp inhibitors are explored for overcoming multidrug resistance and poor bioavailability problems of various drug substrates [45]. Therefore, the drug metabolism is considered as major contributor for low oral bioavailability of many drugs.

1.4. Approaches for enhancement of oral bioavailability

The common approaches to improve the systemic bioavailability of drugs are to deliver them by alternative administration routes such as oral, transdermal, nasal, vaginal or rectal. Among these routes, oral administration is the most convenient way to achieve the desired therapeutic effects.

Numerous pharmaceutical scientists have logically focused on oral administration route to effectively enhance the bioavailability of the drug substances. The key approaches to maximize oral drug absorption are described as follows:

(1) By using efflux pump inhibitors to improve the efficiency of drug transport;

(2) By using permeation enhancers to inhibit drug degradation and improve permeability;

(3) Modifying the physicochemical properties of drugs for improving drug solubility, stability and dissolution rate;

(4) Designing the specialized formulation such as nanoparticles, micro-particles and liposomes that improve the drug solubility and protect drugs from harsh environment of the gastrointestinal tract;

(5) Developing stimuli-responsive systems for controlled drug delivery [46, 47]. Figure 1.3 summarizes the various strategies that have been investigated and proposed to improve the oral bioavailability of drug substances.

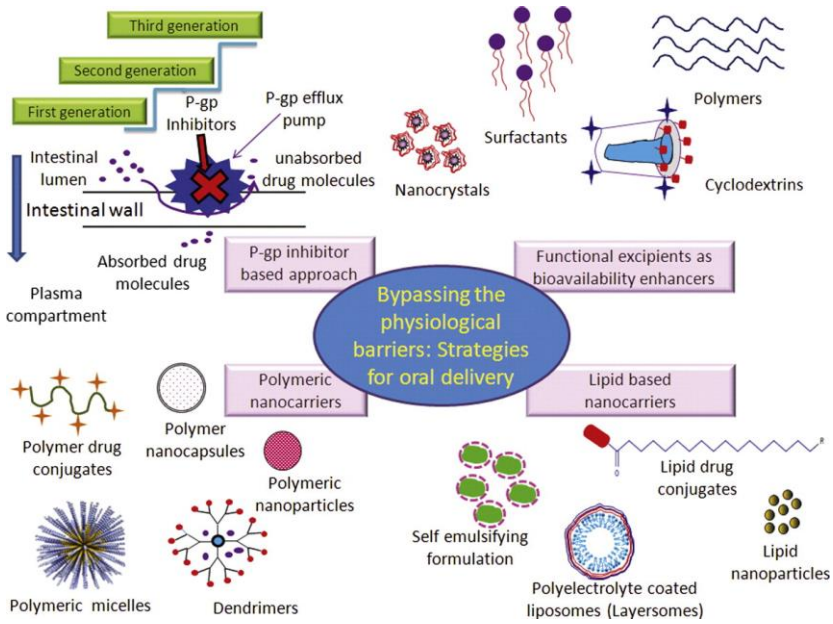

Figure 1.3 Strategies to improve the oral bioavailability of drug substances [24].

1.4.1. The use of efflux pump inhibitors

In recent years, the impact of efflux pumps on the therapeutic activity of drugs has been well established. The efflux transporters such as P-gp, the breast cancer resistance protein (BCRP) and the multidrug resistance related protein (MRP), which have been identified to be over-expressed in tumor cells, are also widely distributed throughout normal tissues in humans [48]. In the view of this, approaches to identify efflux pump substrates and inhibitors as well as strategies to overcome the barrier caused by efflux pumps have been investigated (Figure 1.4). Several studies have demonstrated the possibility of using P-glycoprotein inhibitors as an attempt to improve the efficiency of drug transport across the epithelia, thus resulting in

enhanced oral bioavailability. As reported by Kwak *et al* [49], HM30181, a newly developed P-gp inhibitor, showed promising results for increasing oral absorption of some drugs. Nevertheless, the efflux pump inhibitor approach is scarcely used clinically owing to associated clinical complications such as suppression of immune system thus causing long term medical complications[24]. Therefore, safer alternatives with similar properties can be sought to enable the safe use for chronic therapy.

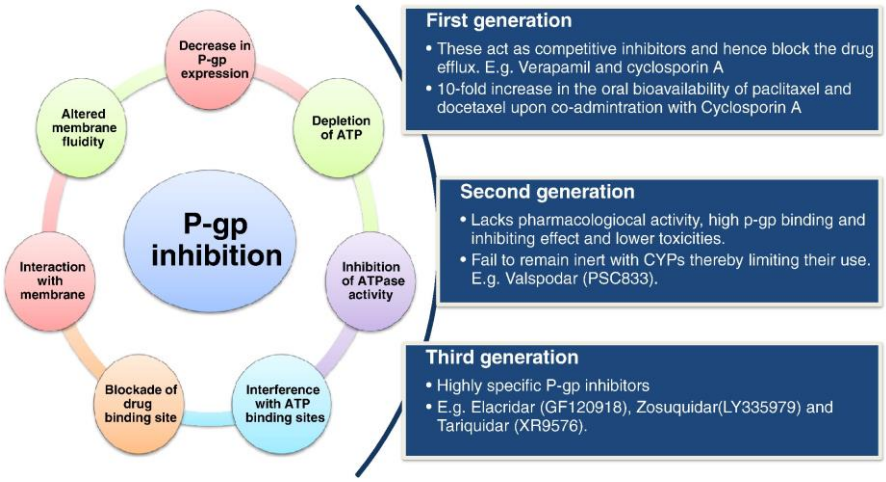

Figure 1.4 Various P-gp based approaches for improving the oral bioavailability of drugs.[24]

1.4.2. The use of permeation enhancers

The membrane permeation, which is governed by the drug lipophilicity limits the therapeutic efficacy of many drugs. Permeation enhancers can improve the permeation of drug substances through intestinal barriers. In general, permeation enhancers improve drug absorption by the following mechanisms:

(1) Disruption and opening of tight junctions to increase paracellular permeability;

(2) Decrease of in the mucus viscosity;

(3) Increase of membrane fluidity specific to their category [2, 50].

A large variety of permeation enhancers have been studied to improve the intestinal permeability of drugs. These include lipids, surfactants, fatty acids, medium chain glycerides, chitosan and other derivatives. Mechanistically, they are found to modulate the activity of the P-gp efflux pump, increase drug solubility, facilitate wetting, and then increase permeability across the gastrointestinal tract. Some of these enhancers have been developed to the stage of initial clinical trials. Several enhancers seem to have potential to improve oral bioavailability without causing significant gastrointestinal tract damages [51].

1.4.3. Modification of the physicochemical properties of drugs

The physicochemical properties of drug substances dramatically influence their performance. Modification of the physicochemical properties of the drug molecules has already been confirmed to be an important approach for the development of effective oral delivery systems. In order to exert maximum therapeutic action, the drugs must be absorbed into the systemic circulation via passive diffusion to achieve high plasma concentrations. For the poor water soluble drugs, dissolution rate of the drugs is regarded as the limiting step for the absorption process, so their solubility should corresponds to the dissolution rate in gastrointestinal tract to achieve effective absorption. The drug molecular modification for solubility increase can be achieved by various approaches, including salt and prodrug formation, complexation, polymorphism or preparation of analogues. The molecular size of drugs is another factor that affects their bioavailability and absorption. Recently, various nanonization approaches have been sought to increase the dissolution rates of numerous drugs. Nanonization can lead the improvement on drug solubility and pharmacokinetics, further it may also decrease systemic side-effects [52].

1.4.4. The use of specialized formulation vehicles

Numerous specialized strategies have been attempted to enhance the bioavailability of drugs by using of lipid based formulation (liposomes, lipid–drug conjugates, layersomes, nano/micro-emulsion, self-emulsifying drug delivery systems), polymer based formulation (polymeric micelles, polymeric nanoparticles), nanocarrier based approaches (nanosuspension, carbon nanotubes, nanocrystals) to successfully deliver lipophilic drugs via the oral route. These approaches improve the oral bioavailability of lipophilic drugs by different mechanisms including improved drug solubilisation, absorption and protection against enzymatic and physicochemical degradation. Furthermore, smaller droplet/particle size of these systems increases the interface between the lipophilic droplet and the aqueous gut medium to facilitating a homogeneous and wide distribution of the drug along the GIT.

1.4.5. Stimuli-responsive drug delivery systems

The therapeutic efficacy of the drug delivery systems depends on the capacity to release the drug to the specific region at the right time with a desired dosage to achieve the therapeutic response. Various stimuli-responsive materials which are sensitive to physical stimuli (temperature, electric charge, electrochemical, light, magnetic, and ultrasonic), chemical stimuli (pH, ionic, and redox), or biological stimuli have been sought for controlled drug delivery systems. For instance, pH-sensitive systems have been widely used for drug delivery in colon targeted release and cancer therapy according to the pH change in different tissues such as tumour and normal tissues, extracellular and cellular, gastric fluids and intestinal tract.

2. Nanotechnology in oral drug delivery

Nanotechnology has been defined as “ the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel

applications” [53]. Recently, nanotechnologies have gained attention to enhance the oral bioavailability of drugs in their dosage forms, especially lipophilic drugs. The most acclaimed and prospective nanotechnology strategies used in oral drug delivery include lipid based nanoparticles (nanoemulsions, self-nanoemulsifying drug delivery system (SNEDDS), solid lipid nanoparticles, lipid nanocapsules nanosuspension, liposomes, layersomes, liquid crystalline nanoparticles, lipid-drug conjugates); Polymer based nanocarriers (polymeric nanoparticles, polymeric micelles, polymer-drug conjugates); Drug nanocrystals; Dendrimers; Carbon nanotubes; Silica and silicon nanoparticles; Nanogels and so on. Moreover, nanotechnology-based therapeutic products had been validated through the improvement not only for the previously approved drug substances, but also for many new drug candidates [54]. The use of nanotechnology in oral drug delivery may radically change the way we exploit drugs and the way we take drugs, thus providing an ideal approach for chemotherapy [55]. Numerous types of nanocarriers and formulations available for oral delivery have been used as delivery vehicles to develop effective therapeutic modalities, as shown in Figure 1.5. The variety and advantages associated with them have been discussed in the subsequent sections. The potential mechanisms responsible for enhanced oral delivery observed with nanocarriers are shown in Figure 1.6.

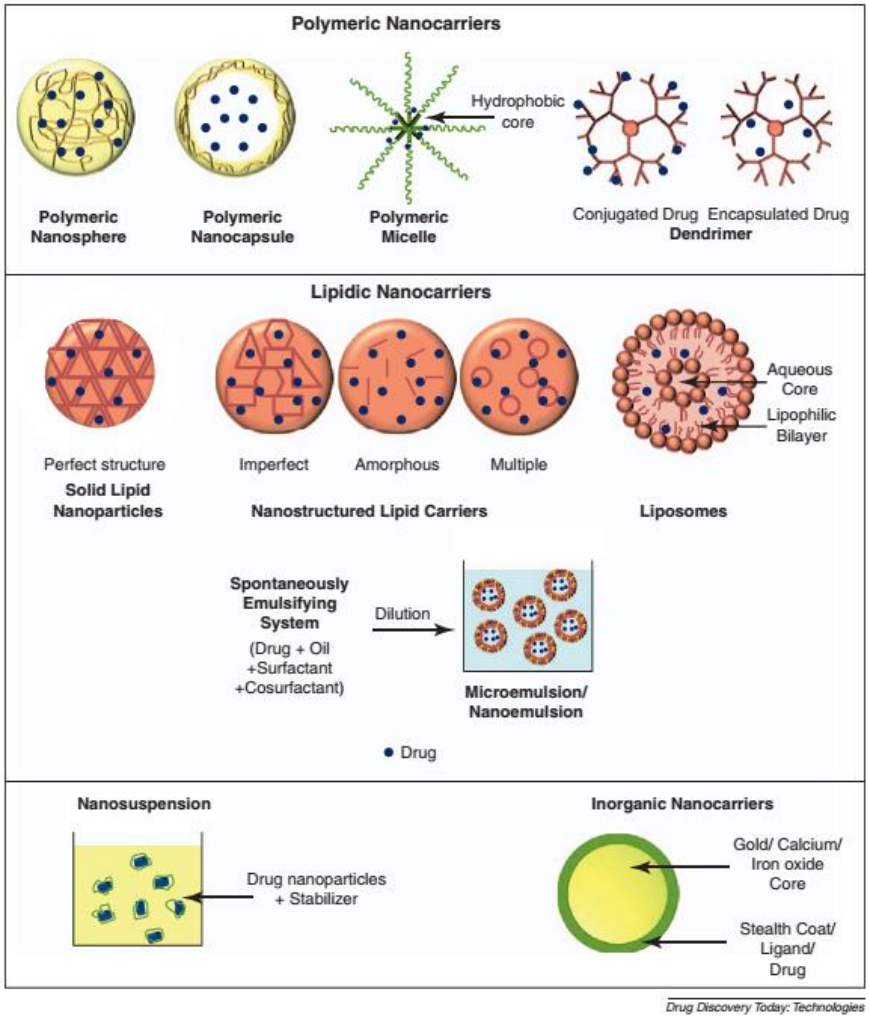

Figure 1.5 Examples of various nano-architectures available for oral drug delivery.[1]

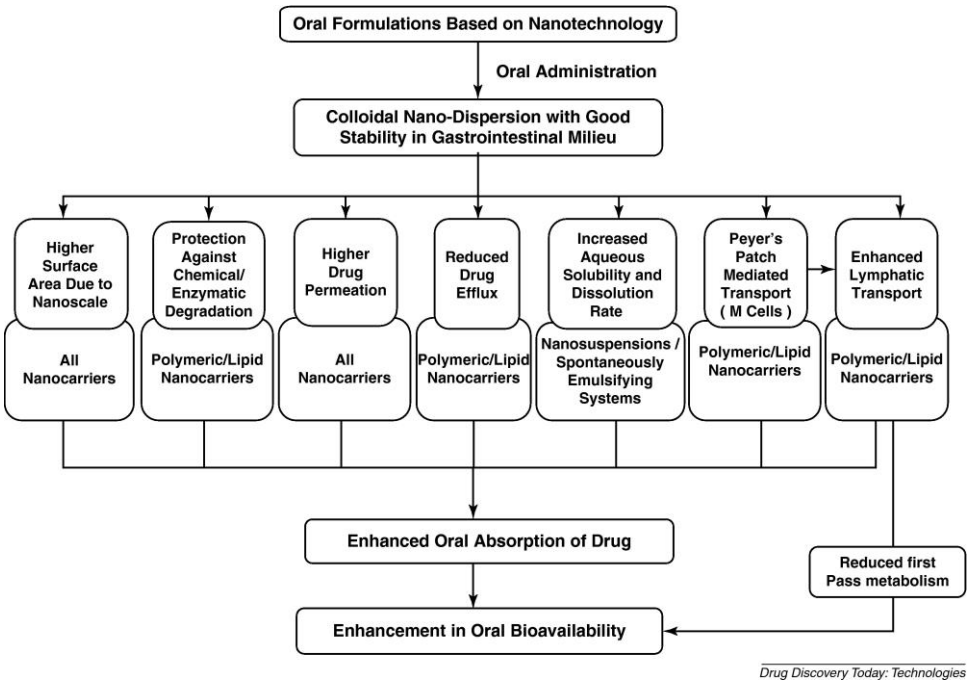

Figure 1.6 Overview of nanocarriers-mediated mechanisms leading to enhanced oral drug delivery.[1]

2.1. Lipid based nanoparticles

2.1.1. Nanoemulsions

Nanoemulsions are non-equilibrium, heterogeneous systems composed of oil droplets dispersed in an aqueous medium and stabilized by surfactant molecules. In a nanoemulsion, the oil droplets serve as the reservoir for hydrophobic drugs [52]. Moreover, nanoemulsions are regarded as kinetically stable, isotropic and transparent without any apparent coalescence during the long time storage. The nanoemulsions are usually stabilized by large amount of surfactants, which can improve drug solubilisation, protect active compound against physicochemical and enzymatic degradation and modify the permeability of the GIT membrane. Non-ionic surfactants are commonly preferred due to their less toxicity, less affected by pH and ions than ionic and amphiphilic surfactants, and better compatibility with

biological systems [56]. Combinations of different surfactants have also been employed to decrease the droplet size and improve the stability of nanoemulsions. Methods used for the production of nanoemulsions include high-pressure homogenization, microfluidization, ultrasonication, spontaneous emulsification and so on [57]. The advantages of nanoemulsions are increased drug loading, tissue targeting and enhanced permeability.

2.1.2. Lipid-drug conjugates

To overcome the limitation of limited loading capacity for highly potent hydrophilic drugs and drug expulsion during storage, lipid-drug conjugates have been made. Lipid-drug conjugates nanoparticle are prepared either by formation of a salt with a fatty acid or alternatively by covalent linkage (e.g. to ester or ethers) [58]. Further process is perform an aqueous surfactant solution to a nanoparticle formulation using high pressure homogenisation [59]. The lipids that can be used for formulation of lipid–drug conjugates include phospholipids, fatty acids such as stearic acid, oleic acid, docosahexaenoic acid, etc. and lipoamino acids [24].

2.1.3. Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are composed of melt-emulsified solid lipids like highly purified triglycerides, monoglycerides, hard fats, complex glyceride mixtures as matrix materials. As they are derived from biodegradable and compatible lipids, SLN represents a comparatively stable system with protective effects against serious drug toxicity and harsh external environment in comparison to the conventional nanoparticles. In addition, they also offer the advantages of avoidance of organic solvents in their preparation, controlled release of drugs and excellent tolerability [60]. Of the available methods for preparation, cold high-pressure

homogenisation process, hot homogenization of melted lipids at elevated temperatures and microemulsion technology are considered as the most feasible methods for large scale production of SLNs [61]. Although solid lipid nanoparticles (SLNs) have attracted increasing attention due to its advantages, SLNs have several limitations, for example, low loading efficiency for some drugs which owing to the densely packed lipid crystal network. Furthermore, SLNs also show considerable expulsion of the drug during storage [3]. The schematic structure of SLNs is shown in Figure 1.7.

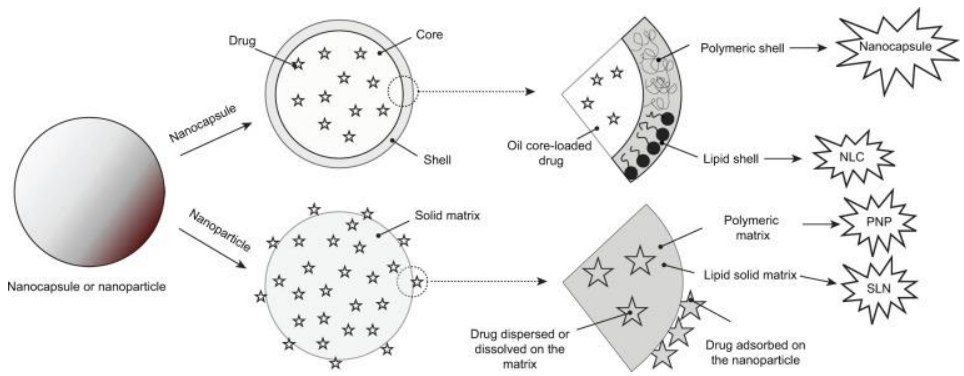

Figure 1.7 Schematic differences between nanocapsule, polymeric nanoparticle (PNP), and solid lipid nanoparticle (SLN) drug delivery systems.[62]

2.1.4. Lipid nanocapsules (LNCs)

Lipid nanocapsules (LNCs) provide a new nanotechnology which contributes to oral drug delivery development. LNCs are another kind of lipid nanoparticles, composed of an internal liquid or semi-liquid oil core and an external lipid layer solid as a core-shell structure [63]. LNCs with the unique properties such as controlled release profiles and high bioavailability, represent a promising biocompatible drug delivery platform in nanometer range with narrow size distribution [64]. The phase inversion temperature (PIT) method proposed by Shinoda and Saito [65] led to lipid

nanocapsules preparation with good mono-dispersion. LNCs prepared by PIT method is based on three main components: an oil phase, an aqueous phase and a non-ionic surfactant. Furthermore, the temperature cycling process crossing the phase-inversion zone (PIZ) plays another role on LNCs formulation. Increasing the number of cycles promotes LNC formation and improves the quality of LNC dispersion [66]. Recently, many lipophilic drugs have been developed in LNCs form for instance, ibuprofen loaded LNCs for pain treatment; indinavir, an inhibitor of HIV1 protease; various hydrophobic anticancer agents. Consequently, LNCs provide an attractive drug delivery approach for highly lipophilicity drug substances that are usually unsuitable for oral use.

2.1.5. Nanosuspensions

Nanosuspensions are nanoscale colloidal dispersion of solid drug particles which are stabilized by surfactants, polymers or a combination of both. The key difference from conventional suspensions is that the particle size distribution of the solid particles in nanosuspensions is usually < 1 μm [67]. Nanosuspensions engineering processes presently used are media milling, high pressure homogenization, microprecipitation-high pressure homogenization, emulsion diffusion method and melt emulsification method. Owing to the enhanced drug solubility, increased surface-volume ratio of the nanocrystals, and improved dissolution rate, oral nanosuspensions have been specifically used. Furthermore, nanosuspensions are available in various dosage formats such as tablets, pellets, and capsules following different manufacturing techniques [18]. Nevertheless, the major challenges in nanosuspensions preparation are maintaining colloidal stability and particle size of the nanosuspensions during storage. The appropriate selection of the surfactants and/or steric stabilizers and the method of fabrication have been sought to prevent

the nanocrystal aggregation to achieve the nanosuspensions with long-term storage and physiological stability.

2.1.6. Liposomes

Liposomes are a form of self-assembled lipid bilayer vesicles which composed of one or more aqueous compartments are completely enclosed by hydrophilic and/or hydrophobic molecules. Due to the core (aqueous)-shell (lipidic) structure, liposomes are available for encapsulating hydrophilic drugs in the aqueous core, hydrophobic agents in the lipidic shell, meanwhile, amphiphilic molecules distributed through the hydrophobic-hydrophilic layers. In addition, using biologically and natural lipids makes liposomes highly biocompatible and suitable for in vivo use [68]. Recently, research on liposomes technology has been extensively investigated for the delivery of various therapeutic and bioactive agents, decreasing toxicity and increasing their accumulation at target sites. Nitesh Kumar *et al* [69] developed lecithin-based silymarin liposomes. The results showed that incorporating phytosomal form of silymarin in liposomes had better *in vitro* and in vivo hepatoprotection and better anti-inflammatory effects in histopathological changes. Therefore, liposomes can be used in the oral delivery of lipophilic drugs to increase its oral bioavailability.

2.1.7. Liquid crystalline nanoparticles (LCNPs)

Liquid crystalline nanoparticles (LCNPs), which combine the properties of both liquid and solid states, are self-assembled from polar amphiphilic lipids in the presence of excess water. LCNPs are generally prepared by dispersing the liquid crystalline matrix formed into water phase using high-energy fragmentation, such as ultrasonication, microfluidization, or homogenization [70]. Normally, LCNPs enhance the oral bioavailability of lipophilic drug by improvement of bioadhesiveness,

membrane fusing properties, superior encapsulation, solubilization, etc. [24] . Ni Zeng *et al* [70] developed self-assembled LCNPs consisting of soy phosphatidylcholine and glycerol dioleate for oral delivery of paclitaxel. The results of this study suggest that LCNPs could be a promising approach for enhancing the oral bioavailability of lipophilic drugs and agents.

2.1.8. Self-nanoemulsifying drug delivery system (SNEDDS)

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that rapidly form fine oil-in-water (o/w) nanoemulsions when introduced into aqueous medium under mild agitation [59]. In the human body, the agitation required for formation of nanoemulsions is provided by digestive motility of the gastrointestinal tract [34]. In comparison with the ready to use nanoemulsions or nanosuspensions, SNEDDS have shown many advantages such as: physical or chemical stability profile improvement in long term storage; possibility of filling into soft/hard gelatin capsules, which results in attractive commercial viability and patient acceptability; no palatability-related issues.

In recent years, SNEDDS have attracted more and more attention as the mean to enhance the oral bioavailability of poorly soluble and highly metabolized drugs. Nevertheless, conventional SNEDDS also require a relatively large amount of surfactants, which may induce GI irritation and side-effects. In order to achieve a safe and efficient delivery system for the poor oral bioavailability drugs, we have designed a novel self-nanoemulsifying drug delivery system with high proportion lemon essential oil as carrier for lipophilic drugs.

2.2. Polymer based nanocarriers

2.2.1. Polymeric nanoparticles

Polymeric nanoparticles are submicronic solid particles where drug is encapsulated or adsorbed onto particles. With the increasing study on polymers, polymeric nanoparticles have emerged as a promising approach in oral drug delivery field due to their unique properties such as improved drug stability, the duration of the therapeutic effect and to minimize drug degradation and metabolism etc.[3]. A variety of biodegradable and biocompatible polymers have been used in the research of polymeric nanoparticle preparation include starch, chitosan, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), etc. [55]. These polymers can be used either separately or combined with each other. The advantages of polymeric nanoparticles can be their high stability in the gastrointestinal tract, protection and controlled release of the incorporated drugs, flexibly modulating, and offering targeting with improved cellular uptake. However, the potential challenge for polymeric nanoparticles is associated with the polymer toxicity and the residues of organic solvents during the preparation. In addition, some of the synthetic polymers are highly hydrophobic and not friendly to hydrophilic drugs. These limitations of polymeric nanoparticles should be addressed in the future studies.

2.2.2. Polymeric micelles

Polymeric micelles are nanosized supramolecular constructs (Figure 1.8) formed by amphiphilic molecules consisting of an inner hydrophobic core and an outer hydrophilic entity [71]. As a core-shell structure, the hydrophobic core acts a reservoir for lipophilic drugs whereas the hydrophilic shell protects the drugs to avoid the inactivation and increase the bioavailability and retention.

Two main methods have been commonly used to produce drug-loaded polymeric micelles. Direct dissolution involves dissolving both polymer and drug in an aqueous solvent. Alternatively, organic solvents are employed when both polymer and drugs

are highly hydrophobic [71]. As reported by literatures, polymeric micelles are stable in terms of both thermodynamic and kinetics, imparting overall structural stability. Moreover, polymeric micelles allow a multifunctional design to achieve integrated diagnostic and therapeutic functions and molecular targeting capabilities [52]. Nevertheless, more efforts are still required in order to overcome the challenges, for examples, low drug loading, low permeability in transport through intestinal membrane.

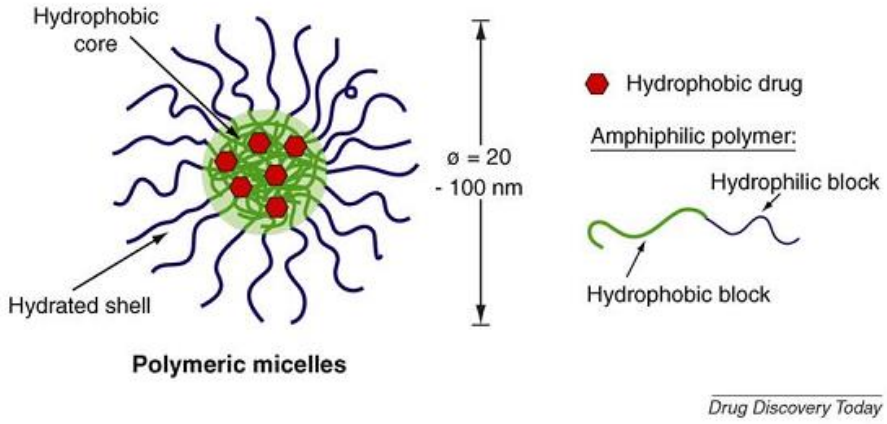

Figure 1.8 Schematic of polymeric micelles.[52]

2.2.3. Polymer-drug conjugates

By definition, polymer-drug conjugates are formed by the conjugation of a biocompatible polymeric carrier and low-molecular weight biologically active molecule(s) through a biodegradable linker. One of the major differences between polymer–drug conjugates and other nanocarriers that contain physically entrapped drugs is that the drug molecules are covalently bound to the polymers [72]. Mostly, the presence of polymers increases the solubility of hydrophobic drugs, modifies drug dispersion profile, extends plasma circulation half-life, and improves its pharmacokinetic profile, in turn, enhancing the oral bioavailability of the drugs. On the other hand, the biodegradable linker can also become active by triggering drug

release under certain conditions, such as a change in pH or in the presence of enzymes, such as esterases, lipases or proteases [73]. A pH-sensitive amphiphilic dendritic polyrotaxane drug-polymer conjugate by covalently linked doxorubicin (DOX) and dendritic polyrotaxane has been designed and successfully fabricated by Yang Kang *et al.*[74]. This pH-sensitive drug-polymer conjugate showed a significantly faster drug release at mildly acidic condition while without burst release in aqueous at a physiological pH of 7.4. The results proved that this conjugate has tremendous potentials for targeted cancer therapy.

2.3. Drug nanocrystals

Besides liposomes, nanocrystals are the most successful nanocarriers when considering the first marketed products as well as the total number of commercial products and in clinical phases [59]. Nanocrystals are nanosized crystals of pure drug particles with the surfactants or polymeric steric stabiliser absorbed onto the surface of drugs. Thus, drug nanocrystals possess a 100% drug loading in contrast to polymer or lipid-based nanoparticles. As we known, decrease in particle size provides a greater surface area in the diffusion layer and leads increase of the drug dissolution rate, furthermore, enhancing the absorption (Figure 1.9). Industrially, the drug nanocrystals are produced with four main technologies, including top-down (e.g. pearl milling, high pressure homogenisation), bottom-up (e.g. precipitation) and combination (sonication–precipitation) and chemical approaches.

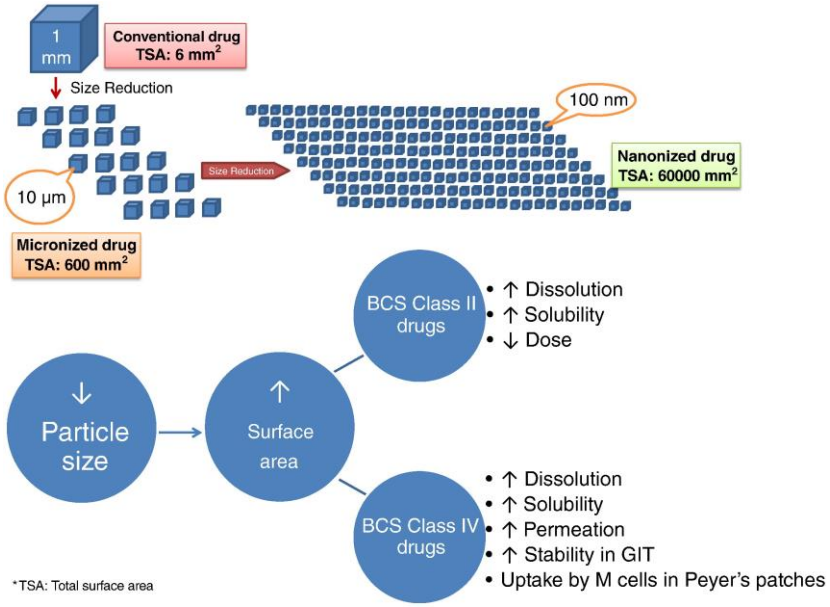

Figure 1.9 Mechanistic representation of absorption via nanocrystals.[24]

2.4. Dendrimers

Dendrimers are the new artificial well-defined polymeric nanostructures exhibiting tree-like architecture that consist of a hydrophobic central core, branching units and terminal functional groups. Dendrimers possess definite molecular weight, shape, size and specific physicochemical properties including host–guest entrapment properties [24]. Unlike many traditional polymeric nanocarriers, dendrimers can be manufactured in almost any size whereas the diameters are commonly 10-20nm. In addition, dendrimers also have a narrow polydispersity and well defined spherical shape with a variety of terminal functional groups. These unique structural nanosized macromolecules offer multiple ways for incorporation of plenty of drugs which pose oral delivery challenges. First, drug molecules can be physically encapsulated in the core of the dendrimers. Second, drug molecules can be chemically conjugated to the functional end groups on the dendrimer surface during or after synthesis. Third, dendrimer drug networks can be formed. As an

approach for the oral bioavailability enhancement, dendrimers provide many potential mechanisms. First, the dendrimers entrap the drugs to prevent the drug degradation from harsh gastrointestinal tract. Next, dendrimers may act as permeability enhancers and alter the barrier of the intestinal epithelium, thereby improve the drug absorption. Last, the dendrimer-drug conjugate may be transported across the intestinal epithelium by itself [55]. The properties of dendrimers such as size, surface charge and conformation significantly affect the drug delivery and absorption in the GIT. Moreover, larger dendrimers have been found be more toxic, in comparison with the smaller ones. Conclusively, dendrimers are promising delivery system, but more efforts should be required to overcome challenging biological barriers.

2.5. Others

Except the above mentioned strategies, many other nanotechnologies are also employed in the oral drug delivery, for example, carbon nanotubes, silica and silicon nanoparticles, nanogels and so on. Carbon nanotubes possess unique hollow cylindrical structures, high surface area, conductivity, optical and potential higher absorption capabilities, allow the incorporation of drug molecules for controlled and site-specific delivery [75]. Silica/silicon nanoparticles offer a high absorption capacity, mesoporous channel to change the crystalline state of the drugs and the possibility to tailor the physicochemical properties [76]. The biocompatibility, chemical properties and mesoporous structure make silica/silicon nanoparticles an excellent alternative for drug delivery application. Nanogels are commonly used for oral controlled drug delivery with the advantages such as thermodynamic compatibility with water, enviro-intelligent, stimuli-sensitive and sustained release.

3. Self-emulsifying drug delivery systems (SEDDS)

3.1. Overview of SEDDS

3.1.1. Basic concepts

Self-emulsifying drug delivery systems (SEDDS) are emulsion pre-concentrates or anhydrous forms of emulsion. These systems (SEDDS) are ideally isotropic mixtures of drugs, oils and surfactants, sometimes containing co-surfactant or co-solvents. Upon mild agitation followed by dilution with aqueous media, SEDDS can form fine oil-in-water emulsions spontaneously [77]. In gastrointestinal tract of human body, the agitation required for formation of emulsions is provided by gastric mobility, the aqueous media are gastrointestinal fluids. In comparison with ready-to-use emulsions, which are metastable dispersed forms, SEDDS possess improved physical and/or chemical stability profile upon long-term storage, and also easy manufacture property. Thus, for the lipophilic drugs that exhibit poor water solubility and rate−limited dissolution, SEDDS may offer an improvement in the rate and extent of absorption and result in more reproducible blood−time profiles [33].

3.1.2. Types of SEDDS

SEDDS include both self-microemulsifying drug delivery systems (SMEDDS) and self-nanoemulsifying drug delivery systems (SNEDDS). SMEDDS indicate the formulations producing transparent microemulsions with droplets size range between 100 and 250 nm while SNEDDS form emulsions with the globule size range lower than 100 nm [77]. The term ‘droplet’ is used to describe micelles, mixed micelles which exist in the emulsions. In details, the microemulsion is a thermodynamically stable colloidal dispersion consisting of small spheroid particles (comprised of oil, surfactant, and possibly co-surfactant) dispersed within an aqueous medium and thus in equilibrium. In contrast, the nanoemulsion is non-equilibrium colloidal dispersion system that over time spontaneously will exhibit

coalescence of the dispersed droplets [78, 79]. However, nanoemulsions can have a relatively high kinetic stability, and in this case it will be difficult to distinguish on the previous basis micro and nano-emulsions [79]. Actually, the structure of the droplet in both nanoemulsion and microemulsion are very similar: the non-polar tails of surfactant molecules protrude into the lipophilic core formed by the oil, while the polar head groups protrude into the surrounding aqueous phase (Figure 1.10).

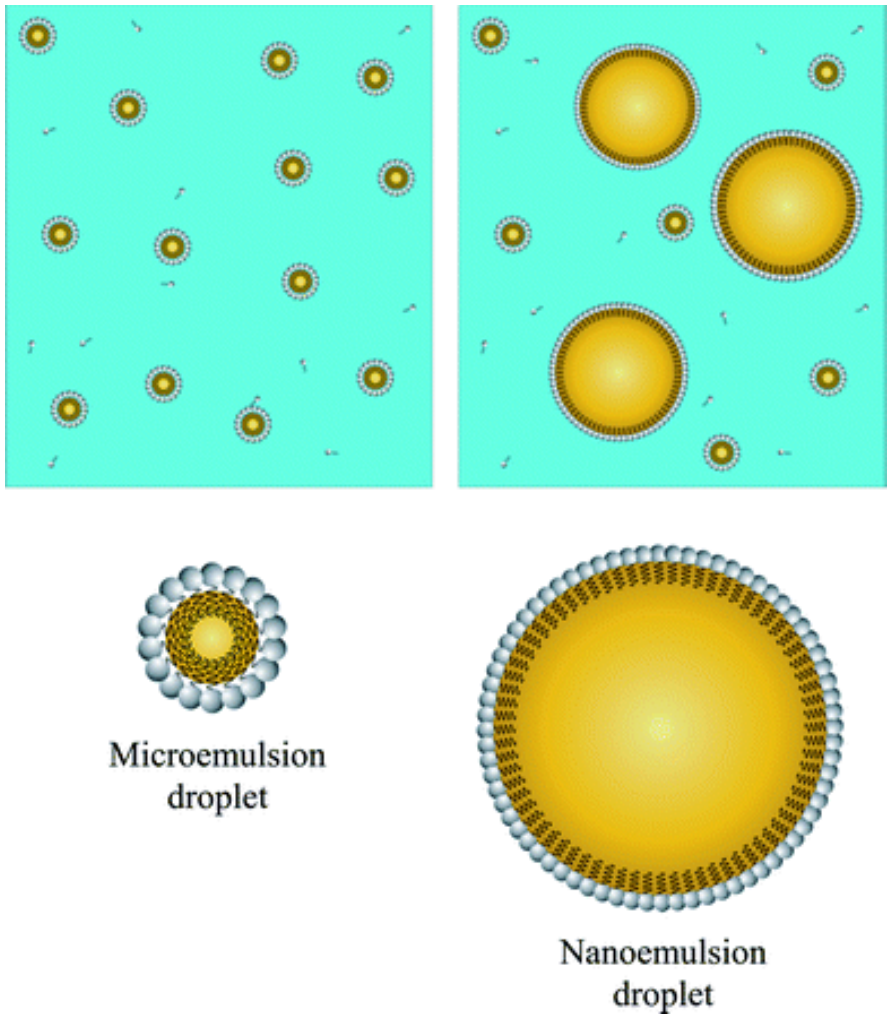

Figure 1.10 Schematic of microemulsions droplet and nanoemulsions droplet formed from oil, water and surfactant [78].

3.1.3. Advantages of SEDDS

Self-emulsifying drug delivery systems are new approach for enhancing the oral bioavailability of lipophilic drugs. They offer a number of advantages over the conventional micro/nanoemulsions systems owing to their interesting properties. Potential advantages of SEDDS include:

Long-term stability

As anhydrous formulations, SEDDS possess the improved physicochemical stability profile upon long-term storage in contrast with nanoemulsions that contain water;

Patient compliance

The SEDDS formulations can be filled into unit dosage forms, such as soft/hard gelatin capsules, which improves patient acceptability and commercial viability [80];

Palatability

No palatability-related issues in comparison with other formulations/tablets, as SEDDS formulations can be filled into capsules [80];

Ease of manufacture & scale-up

Ease of manufacture and scale-up are key factors that govern success in its industrial applicability. The methods employed for the fabrication of SEDDS formulations, such as simple mixed with an agitator and volumetric liquid filling equipment, offer easy manufacture at large-scale and economic benefits as well;

Quick onset of action

Quick onset of action is required in many conditions, such as inflammation, hypertension and angina [34, 80]. SEDDS have capacity to enhance the oral absorption of the drugs, which would result in quick onset of actions. Study from Taha *et al*.[81] showed that the t_{max} (t_{max} is the term used to describe the time at which the maximum plasma concentration of a drug after administration is observed.) is reduced considerably when comparing the pharmacokinetic analysis of SEDDS

and conventional formulation without any additives. Many other literatures can be found which reflect the potential of SNEDDS to increase the bioavailability of drug;

Reduction in the drug dose

SEDDS offer improved drug-loading capacity and oral bioavailability or therapeutic effect for numerous hydrophobic drugs owing to the drug solubility in excipients. The enhancement in drug-loading and bioavailability can be translated into reduction in the drug dose and dose-related side effects of many hydrophobic drugs.

3.1.4. Limitations of SEDDS

Each strategy has specific advantages and limitations. Limitations of SEDDS are:

- High content of surfactant in the SEDDS formulation may irritate gastrointestinal tract and result in toxicity. This problem can be solved by designing and optimizing SEDDS with decreased amount of surfactants.
- There is no effective *in vitro* model for the assessment of the SEDDS formulation [82].
- Presence of high amount of surfactant or co-solvent may cause the degradation and instability of the drugs [83].
- Soft gel or hard gel capsule must be sealed effectively, due to the possibility of component loss and leak.
- In addition, SEDDS are not very suitable for controlled drug release.

3.2. Formulation of SEDDS

3.2.1. Excipients screening for SEDDS

With plenty of liquid excipients available, ranging from oils through biological lipids and hydrophobic and hydrophilic surfactants to water-soluble co-surfactant/ co-solvents, there are various combinations that could form colloidal emulsions [77, 84]. Pharmaceutical acceptability and toxicity issues of the excipients used make the

screening of excipients really critical. Hence, it is essential to optimize the quantities of the SEDDS components at the initial selection. Self-emulsification has been shown to be specific to the nature and amount of the components; the ratio of oil/surfactant; and the temperature at which self-emulsification occurs [85, 86]. Supporting these facts, only a few of specific pharmaceutical excipient combinations could form fine self-emulsifying systems. The following points should be considered in the SEDDS excipients selection:

(I) Drug solubility in different oil, surfactants and co-surfactant/ co-solvents;

(II) The final selection of oil, surfactant and co-surfactant/co-solvent based on solubility studies and the preparation of the ternary phase diagrams [77].

These excipients are discussed below.

Oil phase

Oil is the most important excipient which can solubilize the lipophilic drug in a specific amount. Unmodified edible oils provide the most natural basis as lipid vehicles, but their poor lipophilic drug dissolution and their relative difficulty in efficient self-emulsification markedly reduce their use [87]. Long chain triglyceride and medium chain triglyceride oils with different degrees of saturation have been used in the design of SEDDS. Hydrolysed or modified vegetable oils have contributed widely to the success of SEDDS because of their biocompatibility and physiological advantages.

Surfactant

Surfactants with amphiphilic character help the solubilisation of lipophilic drugs so preventing their precipitation in the gastrointestinal lumen. Non-ionic surfactants are frequently selected for fabrication of SEDDS due to their less toxicity and

because typically possess low critical micelle concentration, in comparison with their ionic surfactants [87]. Non-ionic surfactants possessing high HLB value are widely employed for the immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous phase, providing a good self-emulsifying performance [88].

Co-surfactant

Stable interfacial tension is rarely achieved by the use of single surfactants, usually necessitating the addition of a co-surfactant. The presence of a co-surfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsions over a wide range of composition [89].

Aqueous phase

The droplet size, stability and performance of emulsion formed from SEDDS formulations is influenced by the nature of the aqueous environment where formulations would be introduced. Therefore, pH and ionic content of the aqueous phase should be accurately considered in the SEDDS designing [34]. The physiological environment has pH ranges varying from pH 1.2 (pH in stomach) to 7.4 and greater (pH of blood and intestine). In addition, the presence of various ions in the GIT significantly affects the properties of nanoemulsions generated from SEDDS [34].

3.2.2. Mechanism of SEDDS

The mechanism by which self-emulsification takes place is not yet well understood. Nevetheless, it has been suggested by Reiss *et al* [90] that self-emulsification occurs when the entropy change favoring dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of emulsion

formulation is a direct function of the energy required to create a new surface between the oil and water phases and can be described by

$$\Delta G = \sum_i (N_i 4\pi r_i^2 \sigma) \quad [91]$$

Where

ΔG --- the free energy associated with the process (ignoring the free energy of mixing);

N --- the number of droplets of radius r and s is the interfacial energy;

r --- the radius of globules;

σ --- the interfacial energy.

The two formed phases of the emulsion will tend to separate with time to reduce the interfacial energy and thus reduce the free energy of the system. The conventional emulsifying agents stabilize emulsions, reduce the interfacial energy by forming a monolayer around the emulsion droplets, and in turn, provide a barrier to coalescence. In this case, the separation of the two phases is merely being delayed as these emulsions are still thermodynamically unstable. Emulsification requiring very little input energy involves destabilization through contraction of local interfacial regions. It is necessary for the interfacial structure to show no resistance against surface shearing in order for emulsification to take place [86]. The potential mechanisms responsible for improvement in oral bioavailability by self-nanoemulsifying drug delivery system are elucidated in Figure 1.11.

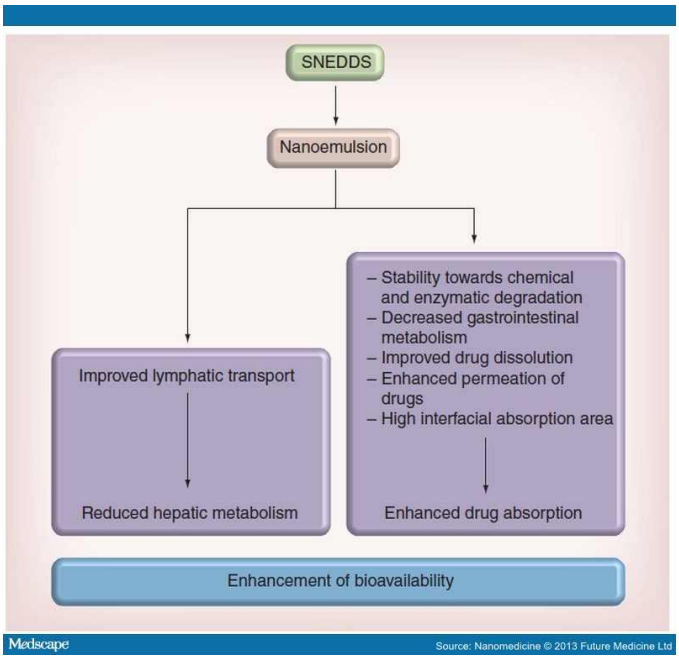

Figure 1.11 Mechanisms of self-nanoemulsifying drug delivery systems to improve bioavailability. [92]

3.2.3. The factors influencing the phenomenon of SEDDS

A thorough understanding of the spontaneous nanoemulsification process and physicochemical and biopharmaceutical properties of components used for the fabrication of SEDDS would be of great help for developing successful formulations of SEDDS.

The factors influencing the formation of SEDDS can be summarized as following:

- The physicochemical nature and concentration of oily phase, surfactant and cosurfactant;
- The ratio of the excipients, especially the ratio of oil and surfactant;
- The temperature, pH and ionic content of the aqueous phase where nanoemulsification would occur;

- Physicochemical properties of the drug, such as hydrophilicity/lipophilicity, pKa. and polarity [34].

3.3. Characterization of SEDDS

3.3.1. Ternary phase diagrams

Construction of ternary or pseudo-ternary phase diagrams is usually employed in the development of SEDDS. Ternary phase diagrams enable comparison of different surfactants and their synergistic effect with co-surfactant. They can also help to determine the optimum concentration ranges of different excipients and to identify the self-emulsification regions. The boundaries of different phase regions can easily be assessed visually.

3.3.2. Emulsification time

With the purpose of quantifying the efficiency of emulsification of SEDDS. Pouton [93] employed the rotating paddle to promote emulsification in a crude nephelometer. This enabled an estimation of the time taken for emulsification. On completion of emulsification, the SEDDS samples were taken for particle sizing by photon correlation spectroscopy, and further by other characterizations.

3.3.3. Turbidity measurement

The turbidity measurements can be carried out to identify the efficient self-emulsification by establishing whether the dispersion reaches equilibrium rapidly and in a reproducible time [94]. These measurements are commonly carried out on turbidity meters, and also can be processed in terms of spectroscopic characterization of optical clarity (i.e. absorbance of suitably diluted aqueous dispersion at 400 nm) [95].

3.3.4. Droplet size

Droplet size is a decisive factor in self-emulsification performance because it will determine the rate and extent of drug release, as well as the stability of the emulsion [80]. Dynamic light scattering (DLS) techniques, photon correlation spectroscopy and microscopic techniques are mainly used for the determination of the emulsion droplet size. DLS is ideal for measuring particles or droplets in the diameter of 3 nm to 3 mm. Droplet size distributions can be further verified by cryogenic transmission electron microscopy (cryo-TEM), which offers the possibility to observe the droplet's size and shape.

3.3.5. Zeta potential

Zeta potential is used to identify the charge of the oil droplets of SEDDS. The charge of the oil droplets in conventional SEDDS is negative due to the presence of free fatty acids [96]. For the droplets in SEDDS emulsions, a high zeta potential will confer stability and long shelf life. When the potential is low, attractive forces may exceed this repulsion and the emulsion may break and aggregate. Some investigators consider zeta potential as secondary characterization parameter for SEDDS, because SEDDS are preconcentrate mixture of drug in oil and surfactant and emulsified in vivo only. The zeta potential of SEDDS emulsion is commonly investigated using Malvern ZetaSizer [86].

3.3.6. Morphology

The morphology of the nanoemulsion droplets can be evaluated by Cryo-Transmission Electron Microscopy (Cryo-TEM), small-angle neutron scattering and

small-angle X-ray scattering. Cryo-TEM and small-angle neutron scattering offer the advantage of vizualising the particle sizes and shapes. Furthermore, droplets size distributions can be further verified by cryo-TEM. Small-angle X-ray scattering is used to determine the microscale or nanoscale structure of particle systems in terms of such parameters as averaged particle sizes, shapes, distribution and surface-to-volume ratio [77].

3.3.7. Viscosity.

The rheological properties of the SEDDS formulations are useful to assess their ability to be filled in the soft or hard gelatin capsules. The viscosity of formulations should not be high to create problem in pourability. Conversely, low viscosity may lead to leakage from the capsules.

3.4. Dosage Forms from SEDDS

SEDDS are usually limited by liquid dosage forms, because many excipients used in SEDDS are not solid at room temperature. In view of the advantages and limitations of SEDDS, various dosage forms of SEDDS have been extensively exploited in recent years, as they frequently represent more effective alternatives to conventional liquid SEDDS.

3.4.1. Dry emulsions

Dry emulsion formulations are typically prepared from oil in water (O/W) emulsions containing a solid carrier in the aqueous phase by freeze-drying, spray drying or rotary evaporation. The dry emulsions spontaneously disperse in vivo or when exposed to an aqueous solution. Dry emulsions can be used for further preparation of capsules and tablets. [32]. A exciting finding in this field is the newly

developed enteric-coated dry emulsion formulation, which is potentially applicable for the oral delivery of peptide and protein drugs [97].

3.4.2. Self-emulsifying sustained/controlled-release tablets

In order to greatly reduce the amount of solidifying excipients required for transformation of SEDDS into solid dosage forms, Patil *et al* [98] developed a gelled SEDDS. The patent diclosed by Schwarz *et al* [99] showed that SE tablets are of great utility in obviating adverse effect. Inclusion of indomethacin into self-emulsifying tablets could increase the penetration efficiency through the gastrointestinal mucosal membranes, potentially reducing gastrointestinal bleeding. The newest improvement in the field of self-emulsifying tablet is the self-emulsifying osmotic pump tablet, where the elementary osmotic pump system was the carrier of the self-emulsifying tablet [32].

3.4.3. Self-emulsifying suppositories

Some investigators proved that Solid-SEDDS could increase not only gastrointestinal adsorption but also rectal/vaginal adsorption [100] . Glycyrrhizin, which barely achieves therapeutic plasma concentrations by the oral route, can obtain fine therapeutic levels for chronic hepatic diseases by either vaginal or rectal self-emulsifying suppositories [32].

3.4.4. Self-emulsifying implants

Researches on self-emulsifying implants have signifacantly improved the utility and application of solid-SEDDS. Carmustine (BCNU) is a chemotherapeutic agent used to treat malignant brain tumours. However, its short half life hinders its therapeutic efficacy. In order to enhance its stability and intestinal permeablity, a self-emulsifying system of carmustine was designed and fabricated into wafers with

flat and smooth surface by compression molding. The results demonstrated that the self-emulsifying system increased the *in vitro* half-life of BCNU up to 130 min compared with 45 min of intact BCNU. The *in vitro* release of BCNU from self-emulsifying PLGA wafers was prolonged to 7 days [101].

4. Oral controlled drug delivery systems

4.1. Overview of oral controlled drug delivery systems

4.1.1. Historical perspective

Controlled drug delivery technology has progressed for more than 60 years. This progression began in 1952 with the introduction of the first sustained release formulation. In the years 1950-80, a first generation (1G) of sustained drug delivery system was developed for oral and transdermal sustained release, while during 1980–2010 a second generation (2G) comprised the development of zero-order release systems, self-regulated drug delivery systems, long-term depot formulations, and nanotechnology-based delivery systems [102]. A third generation (3G) of drug delivery systems is excepted to develop drug delivery formulations primarily based on today's necessities, and focus on understanding the biological barriers. Figure 1.12 describes the three generations of drug delivery systems.

Controlled drug delivery systems were developed to increase patient compliance and acceptability. Years ago it was common to take a drug 4 and more times a day by oral administration. Making the drug administration once/twice-a-day resulted in dramatic improvement in patient convenience and compliance. Benefit for the patient, drug effectiveness and better compliance are the main advantages of controlled drug delivery systems. Moreover, the introduction of novel administration or release methods rendered old drugs again effective and useful [103].

Delayed release, sustained release, and repeat action formulations are the three most common controlled release formulations [104, 105]. A common example of

delayed release is the enteric formulation of tablets or capsules [106], in which drug will not be released in gastric fluid (harsh acidic environment), and will remain protected until it reaches the intestine (neutral environment). In sustained release formulations, a portion of drug is released immediately, and the remaining drug is released slowly over an extended period of time. In fixed dosage combination (FDC), combination of immediate release (IR) and sustained release (SR) for single drug or double drugs are used[107, 108].

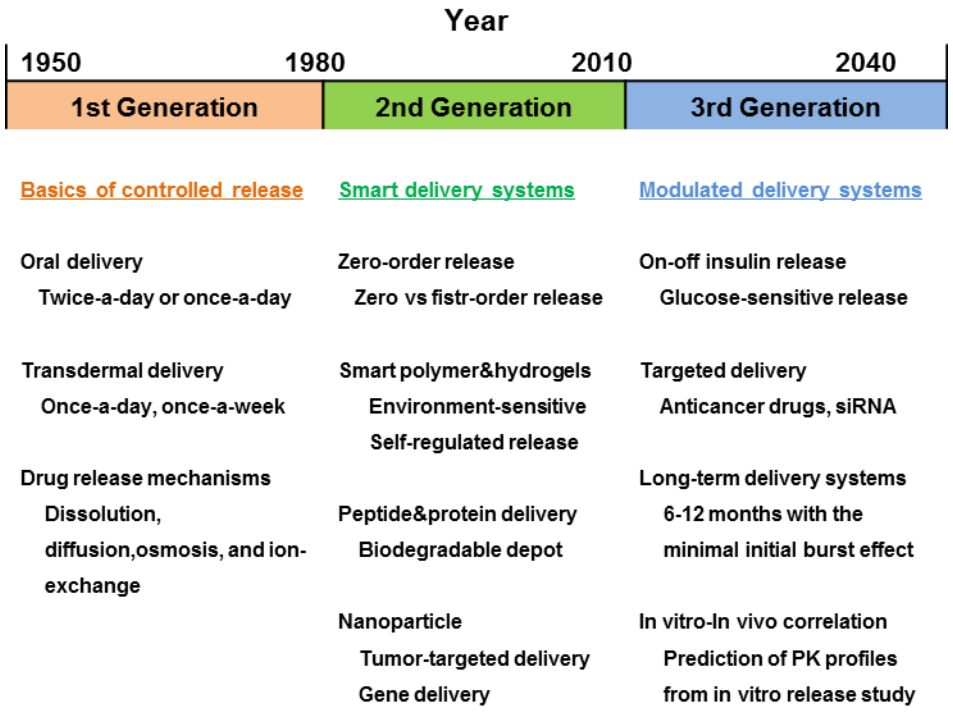

Figure 1.12 Evolution of controlled drug delivery systems since 1950. [102]

4.1.2. Limiting factors for oral controlled drug delivery formulations

There are a few unique properties of the gastrointestinal tract (GIT) that make development of oral controlled drug delivery rather difficult. These limiting factors and disadvantages can be classified into:

(1) Relatively short gastric emptying and intestinal transit time [109];

(2) Nonuniform absorption abilities of different segments of GIT [110];

(3) Pre-systemic clearance [111] ;

(4) Poor absorption of peptide and protein drugs [112];

(5) Difficult *in vitro–in vivo* correlations;

(6) Higher cost of some controlled drug delivery formulations [113].

4.2. Classification and mechanisms of oral controlled drug delivery systems

In general, for most of the pharmaceutical industry, drug delivery has induced simple, fast-acting responses (conventional forms) via oral or injection delivery routes. Problems include reduced potencies because of partial degradation (first pass metabolism), toxic levels of administration (in cases of excess dose), increased costs associated with excess dosing, and compliance issues due to administration pain [113]. A useful classification of current controlled release drug delivery systems based on mechanistic considerations will be outlined below [114]. This classification provides a systematic account of principles behind the design of various oral controlled release products.

4.2.1. Membrane Systems

Membrane systems, by virtue of its rate controlling membrane, are generally non-disintegrating. Products are usually developed like drug core surrounded by a rate controlling membrane (e.g., microcapsules, coated pellets, beads, or coated tablets). Drug release from membrane systems is generally controlled by osmotic pumping or solution–diffusion mechanism. The osmotic-controlled drug release (OROS™) concept for controlling delivery is based on dissolved drug being transported in a controlled manner from the dosage form to the external media under the influence of osmotic pressure [115]. Dissolution-controlled release can be obtained by slowing

the dissolution rate of a drug in the gastrointestinal medium, by incorporating the drug with insoluble polymer, and coating drug particles/ granules with polymeric materials of varying thicknesses [116]. The rate-limiting step for the dissolution of drug is the diffusion across the aqueous boundary layer [19].

4.2.2. Matrix Systems

Matrix systems are actually introduced by drug dissolved or dispersed in a carrier matrix (e.g., beads, pellets, or tablets). Drug release from matrix type products are mostly regulated by mechanisms such as (1) diffusion, (2) swelling/erosion, (3) geometry/area changes, and (4) nonuniform drug distribution. A good example of marketed pulsatile product is Drixoral Cold & Allergy Sustained-Action Tablets (pseudoephedrine sulfate and dexbrompheniramine maleate; Schering-Plough) that produce two pulses of drug release separated by several hours [19, 117].

4.2.3. Hybrid Systems

Hybrid systems comnmonly are the combinations of membrane and matrix systems (e.g., coated pellets or beads embedded in a tablet matrix,core press coated tablets, or tablets in a capsule). Hybrid systems can be disintegrating or nondisintegrating. Hybrid chronotherapeutic dosage forms have been designed based on osmotically controlled-release, membrane-coated beads (delayed release), press-coated tablets, or the combination of erodible polymer coating and a drug matrix (e.g., beads, pellets, and tablets) [118]. Typical chronotherapeutic product examples include Covera-HS (verapamil HCl; Pfizer), Verelan PM (verapamil HCl; Schwarz), and InnoPran XL (propranolol HCl; Reliant) [19]. All of these systems can be in either single-unit or multiple-unit dosage forms.

4.3. Preformation consideration for controlled release formulations

4.3.1. Particulate and mechanical properties' consideration for drug substances

The physicochemical properties of drug substances have a large impact on the selection of controlled release formulations and manufacturing process. The drug's physicochemical properties to be considered include molecular size, lipophilicity, solubility, protein binding, polar surface area, and charge or rotatable bonds. These properties will ultimately influence the permeability of a compound across the lung epithelial barrier [113].

4.3.2. Stability and compatibility

A drug substance is usually more stable by itself than in a formulation with excipients, and as the drug concentration decreases, the stability deteriorates in a corresponding manner. A forced degradation study encompasses a comprehensive assessment of degradation under various stress conditions including acid, base, heat, light, and oxidative conditions which is necessary to demonstrate the stability of the drug substances. Furthermore, pH–stability profile and stabilization are especially useful for controlled release dosage forms since the drug is retained in a matrix or within coating membranes together with a pH modifier. *In vitro* pH-stability studies may help predict performance in first-time in human studies.

Besides aboved mentions, compatibility of excipient and drug is very important parameter for the formation of controlled drug delivery systems. Despite the importance of drug–excipient interaction, no standard method is generally accepted among pharmaceutical scientists and most methods reported in the literature have poor predictive values [119].

4.3.3. Solubility consideration

Solubility of the drug substance is a fundamental property that should be evaluated early in the development of controlled release dosage forms. In reality, it is difficult to predict the aqueous solubility due to the complicated solubilization procedure and solid-phase chemistry of the drug candidates [120]. A variety of approaches are employed at different phases of drug discovery and development such as in silico, kinetic and equilibrium solubility. A lack of solubility affects the ability of drug to achieve efficacious and toxicologically relevant exposures in animals [120]. For drugs with high solubility, dissolution rate can be slowed down by embedding the drug in a matrix or enclosing the drug within a film, whereas for drugs with low solubility, it is more difficult to shift the controlling mechanism from solubility to formulation [19].

4.4. Lipids in oral controlled release drug delivery

4.4.1. Lipids in oral drug delivery

Lipids not only vary in structures and physiochemical properties, but also in their digestibility and absorption pathway, therefore, selection of lipid excipients and dosage form has a pronounced effect on the biopharmaceutical aspects of drug absorption and distribution both *in vitro* and *in vivo* [121]. In particular, the following properties and behaviors of lipids can play key roles:

- Lipids may have amphiphilic structures that determine their capability to self-assemble in aqueous environments. Such behavior can have a critical effect on drug disposition kinetics in the gastrointestinal tract [115].
- Lipids can act as solvents, leading to drug being present in the gastrointestinal fluids thereby overcoming the drug dissolution step [122].

- Lipids may be digestible. Digestion of dietary and formulation lipids can lead to generation of colloidal structures in the GIT, providing transient solubilization of drug, and reducing the propensity for precipitation prior to absorption.

4.4.2. Mechanisms of controlled release using lipids

The mechanisms of controlled release can be summarized as follows, matrix controlled release; gastroretention; stimulation of lymphatic transport.

Matrix plays as a barrier to slow the appearance of dissolved drug in gastrointestinal fluids, by inhibiting diffusion , or by requiring erosion of the matrix before exposure of undissolved drug particles [115]. Gastroretention strategies aim to retain the dosage form in the stomach, preventing transit before complete drug release. Prolonged retention coupled with slow drug release can prolong drug absorption and therapeutic effect. Drug transport via lymph is increased with increasing amounts of co-administered long-chain lipids swelling the chylomicrons and providing a greater pool for drugs to directly entrter systemic circulation. Such lymphatic transport avoids hepatic first-pass metabolism experienced by drug molecules transported via the portal blood system [115].

4.4.3. Technologies for controlled release using lipids

Lipid-based formulations range from simple lipid solutions to complex systems incorporating triglycerides, partially digested triglycerides, semisynthetic ester glycerides, lipophilic and hydrophilic surfactants and cosolvents [123, 124]. The formulation can influence digestibility, dispersion and solubilization of the lipid vehicle *in vivo*, in turn, influencing drug absorption. Solid lipid particles are composed of melt-emulsified lipids, which are solid nature. They offer the advantages of avoidance of organic solvents, resulting in a comparatively stable system with protective effects against serious drug toxicity [125]. Slow erosion of the

lipid controls drug release, prolonging plasma residence and inhibit peak plasma concentrations. *In vitro* release is slowed in comparison to other formulations [126, 127].

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Chapter II Objectives and outline

Self-emulsifying drug delivery systems (SEDDS) represent a vital tool in enhancing oral bioavailability of lipophilic drugs. Lipophilic drugs can be dissolved in SEDDS formulations, enabling them to be administered as a unit dosage form for oral administration.

The overall objective of the present thesis was to improve the solubility, dissolution rate, potentially the intestinal permeability and bioabavailability of l of lipophilic drugs by using self-nanoemulsifying drug delivery systems (SNEDDS) for oral administration.

The main objective implies the following specific objectives:

1. *Design and optimization of novel self-nanoemulsifying drug delivery systems (SNEDDS) with a high proportion of essential oil as carrier.*

   Surfactants are indispensable component for self-emulsifying formulations. However, usage of large amount of surfactants would induce irritation to gastrointestinal tract (GIT) and moreover toxicity. A compromise must be reached between the surfactant toxicity and self-emulsifying capacity of the formulation.

   In our work, essential oil was using to replace part of the surfactant for reducing the potential toxicity of the formulation. As will be shown, our high essential oil containing SNEDDS formulations possess excellent self-emulsfiying property, stability and suitable *in vitro* drug release profile, while the drug loading capacity didn't decrease.

2. *Development of a pH-sensitive self-emulsifying formulation (pH-SNEDDS) to avoid the release of the drug in the stomach and protect it from its harsh acidic environment, that is paticularly important for acid-labile lipophilic drugs.*

Orally administered bioactive compounds have to resist the harsh acidic fluids or enzyme digestion in stomach, in order to reach their absorbed destination in small intestine. The use of pH-sensitive self-nanoemulsifying drug delivery systems (pH-SNEDDS) could overcome the drug degradation in the stomach while enhancing drug solubility and dissolution rate. Our *in vitro* characterization studies showed that pH-SNEDDS would protect the acid-labile drug from harsh acidic gastric-like fluids while providing excellent self-emulsification in the intestinal tract.

Chapter III Design and optimization of self-nanoemulsifying formulations for lipophilic drugs

The purpose of the current study was to develop and optimize novel self-nanoemulsifying drug delivery systems (SNEDDS) with a high proportion of essential oil as carriers for lipophilic drugs. Solubility and droplet size as a function of the composition were investigated, and a ternary phase diagram was constructed in order to identify the self-emulsification regions. The optimized SNEDDS formulation consisted of lemon essential oil (oil), Cremophor RH40 (surfactant) and Transcutol HP (co-surfactant) in the ratio 50:30:20 (v/v). Ibuprofen was chosen as the model drug. The droplet size, ζ-potential and stability of the drug-loaded optimized formulations were determined. The stability of SNEDDS was proved after triple freezing/ thawing cycles and storage at 4 °C and 25 °C for 3 months. *In vitro* drug release studies of optimized SNEDDS revealed a significant increase of the drug release and release rate in comparison to the Ibuprofen suspension (80% versus approximately 40% in 2 h). The results indicated that these SNEDDS formulations could be used to improve the bioavailability of lipophilic drugs.

1. Introduction

Various approaches have been proposed to increase dissolution and bioavailability of lipophilic drugs [1-3]; among those, the use of self-emulsifying drug delivery systems has been suggested [4, 5]. Self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of oil, a surfactant, a co-surfactant and a drug which can rapidly form fine oil-in-water emulsions upon mild agitation in an aqueous media [6]. Depending on the droplet size, SEDDS can be categorized as self-microemulsifying drug delivery systems (SMEDDS, droplet size range between

100 and 250 nm) and self-nanoemulsifying drug delivery systems (SNEDDS, droplets smaller than 100 nm) [7]. The larger interfacial area of SNEDDS improves the efficiency of drug release and absorption, resulting in the decrease of drug dosage and administration frequency [8]. Moreover, SNEDDS would protect the drug from the enzymes of the GI and reduce the first-pass effect [6].

Considering the advantages of SNEDDS and the shortages of previous research, in the present chapter, a novel high essential-oil-contained self-nanoemulsifying drug delivery system was carefully investigated with the aim of enhancing the solubility and dissolution of lipophilic drugs. Figure 3.1 demonstrates the human digestive tract with self-nanoemulsifying drug delivery system.

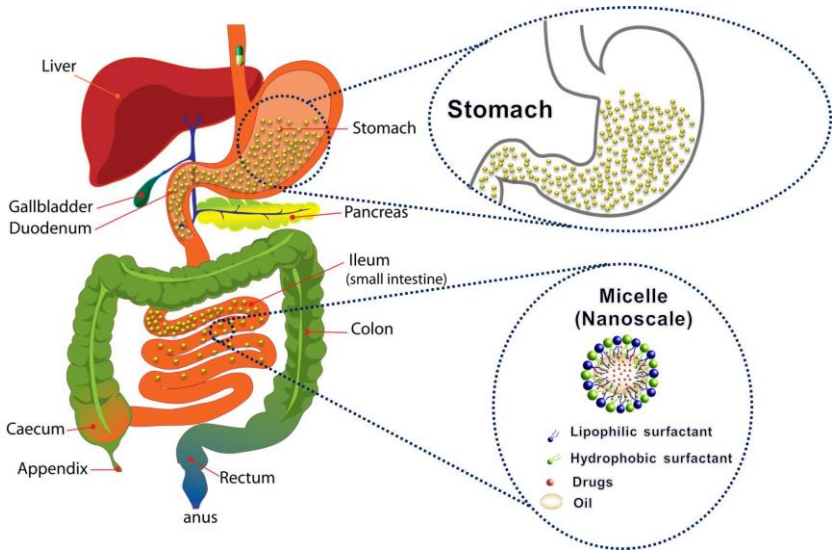

Figure 3.1 Schematic outline of the human digestive tract with self-nanoemulsifying drug delivery system.

Large amount of surfactants would induce irritation to the gastrointestinal system, thus requiring a balance between the surfactant toxicity and self-emulsifying capacity of the formulation [9]. The optimization of SNEDDS was performed in terms of solubility, droplet size, drug loading and *in vitro* drug release.

Ibuprofen, an anti-inflammatory drug belonging to class II of the biopharmaceutical classification [4, 10, 11] that is poorly soluble in acid solutions, such as gastric fluid (21 mg l^{-1} at pH 1.2) [4, 10, 11], was chosen as the model drug (Figure 3.2).

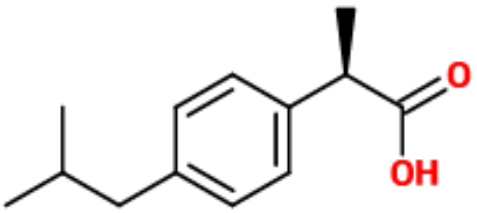

Figure 3.2 Molecular structure of ibuprofen.

2. Materials and methods

2.1. Materials

Ibuprofen, lemon essential oil, anise essential oil, castor essential oil, soybean oil, Span80 and Cremophor RH40 were purchased from Sigma-Aldrich (Milan, Italy). The Labrasol, Labrafil M 1944CS, Labrafil M 2125CS, Capryol 90 and Transcutol HP were received as free samples from Gattefosse (Saint-Priest, France). The Capmul MCM C8 EP was obtained from ABITEC Corporation (Janesville, USA). The acetonitrile and methanol (analytical reagent grade) were purchased from Sigma-Aldrich (Milan, Italy). Deionized water was used through the whole study.

2.2. Drug solubility

The solubility of Ibuprofen in various oils, surfactants and cosurfactants was analyzed by high performance liquid chromatography (HPLC). The excess amount of Ibuprofen (approximately 500 mg) was added to a 2 ml sealed vial containing 1 ml of each selected oil, surfactant or co-surfactant. The mixture was vortex-mixed, then stirred in a shaking water bath at 38.0 ± 0.5 ℃ for 48 h to facilitate the dissolution

and was finally centrifuged at 3000 g for 15 min with a SIGMA 2-16 Centrifuge (SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The aliquots of the supernatant were filtered using a 0.2 μm PTFE filter membrane to remove the undissolved Ibuprofen. The filtrates were diluted with a mobile phase (acetonitrile: methanol (6:4 v v−1) with 4 g L−1 of chloroacetic acid, adjusted to a pH of 3.0 with ammonium hydroxide) and analyzed by HPLC (column Kinetex C18 100A, working temperature of 30 ℃, flow rate of 1 ml min−1 with a 20 μl injection volume, Jasco intelligent UV-1570, Jasco Corporation, Japan). The assays were repeated in triplicate; the mean and standard deviation (σ) were calculated.

2.3. Surfactant and oil miscibility

The oil and surfactant in the ratio of 1:1 were shaken at 40 °C in 3 ml transparent glass vials. The miscibility was monitored optically and considered to be good when the mixture was transparent.

2.4. Construction of ternary phase diagrams

Ternary phase diagrams of the selected oils, surfactants and co-surfactants at various proportions were constructed to identify the self-emulsification regions. A total of 54 formulations were investigated with various proportions of oil, surfactant and co-surfactant for each system. The self-emulsification was observed using the modified visual examination method reported by Villar et al [12]. Briefly, 200 μl formulations were added drop by drop to 500 ml deionized water or simulated gastric fluid (0.01 M HCl solution) at 38.0 ± 0.5 ℃; the mixtures were gently stirred with a magnetic bar to simulate the gastrointestinal wriggle and were observed to classify the emulsifying property. The mixtures were considered well dispersed when the formulation spread quickly in water and was clear or milk-white color with no phase separation or coalescence after the stirring stopped (Figure 3.3). Four

formulations are showed in Figure 3.4, panels A and B correspond with self-nanoemulsifying systems showing “Good” emulsification capacity, in contrast with panels C and D show systems with “Bad” emulsification capacity [12]. All the measurements were repeated three times. The ternary phase diagrams were constructed using Origin software (OriginLab Corporation, USA).

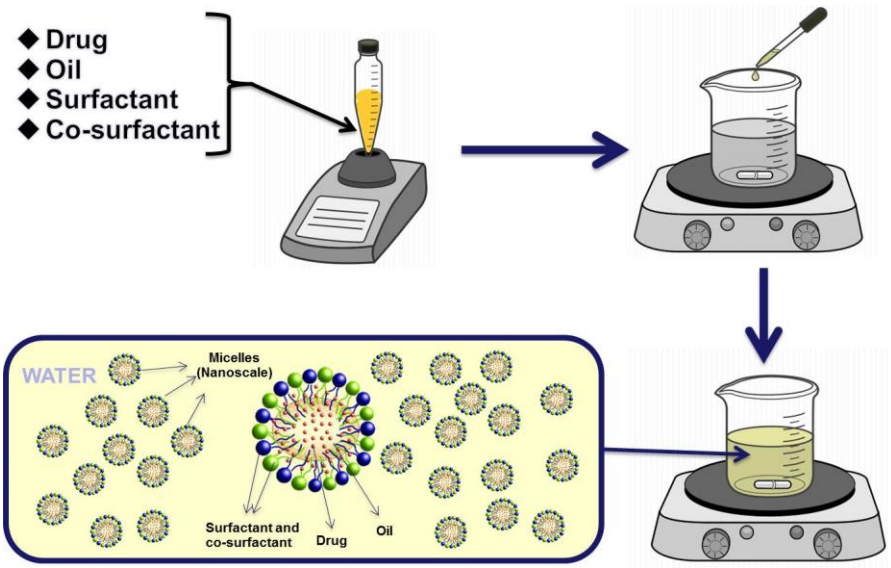

Figure 3.3 Set-up for preparation of nanoemulsions by the self-emulsification method.

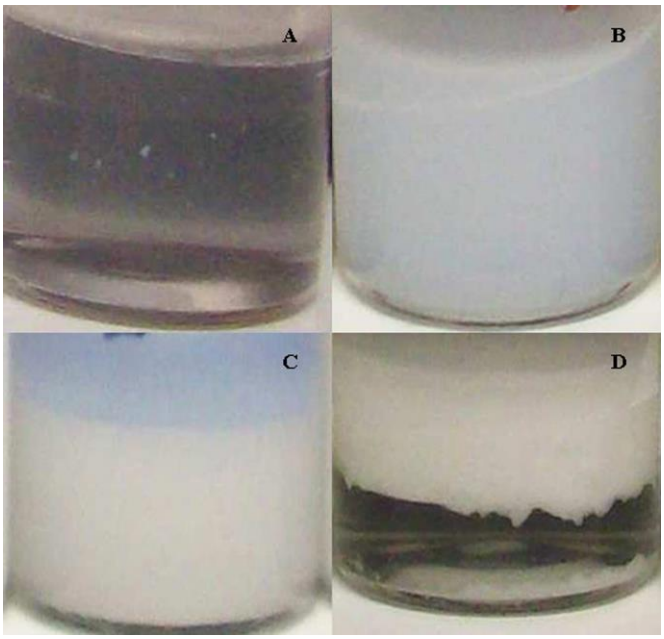

Figure 3.4 Formulations classified as “Good” for emulsifying ability (A and B) and formulations classified as “Bad” for emulsifying ability (C and D) [12].

2.5. Droplet size

The droplet size was determined through dynamic light scattering (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK) at a scattering angle of 90° at 25 ℃. All the SNEDDS emulsions were diluted five times with deionized water in a disposable cuvette, and the content was gently mixed. The average droplet size and polydispersity index (PDI) were calculated. Three consecutive measurements for each sample were made, and the results were presented as the mean and standard deviation.

2.6. Optimization and characterization of Ibuprofen-loaded SNEDDS

2.6.1. Solubility in optimized formulations

The solubility of Ibuprofen in optimized formulations was evaluated as in section 2.2. The concentrations were detected at the wavelength of 264 nm where there

was no UV light absorption of the other components. The assays were repeated in triplicate, and the results were represented as the mean and standard deviation.

2.6.2. Evaluation of viscosity

The viscosity of each ibuprofen-loaded formulations were measured by Physica MCR 301(Anton Paar, Graz, Austrian) at 25±0.5 ℃ in triplicate. Samples were formulated 12 h before the measurements for purpose of stabilization. 1-2 ml sample was put on the plate and equilibrated for analysis. Measurements were performed at shear rates from 0 to 100 s^{-1}.

2.6.3. Self-emulsification time and appearance

Followed the process of emulsification, visual observation was used to determine the self-emulsification time for each SNEDDS emulsion. Begin timing after the formulation was added completely, and stop until the homogenous emulsion was formulated. The appearance of emulsions was monitored and categorize as: clear, translucence and cloudy.

2.6.4. Droplet size and ζ-potential measurements

The droplet size and ζ-potential measurements were performed at 25 ℃ with a Zetasizer Nano-ZS dynamic light scattering apparatus (Malvern Instruments, Worcestershire, UK), as in paragraph 2.5.

2.6.5. Formulation stability

Selected Ibuprofen-loaded formulations underwent three consecutive freezing-thawing cycles to assess their stability. Each cycle consisted of freezing the formulation at 4 ℃ for 24 hours in the refrigerator, followed by heating at 65 ℃ for 48 hours in an incubator. The droplet size, PDI and ζ-potential of the emulsions were

determined after each cycle, and moreover every month on formulations stored at 4 ℃ and 25 ℃ for up to three months.

2.6.6. Morphological characterization

The morphology of the optimal Ibuprofen-loaded SNEDDS was assessed by TEM (Philips CM12 microscope operating at 120 kV). The SNEDDS emulsion was diluted 100 times with 0.01 M HCL solution (simulated gastric fluid) and mixed by gently shaking. One drop of diluted emulsion was placed on the TEM copper grids; we removed the excess liquid with a filter paper and placed them in a hood until complete drying. Subsequently, the grid was stained with a 2% phosphotungstic acid solution for 30 seconds.

2.6.7. *In vitro* drug release study

For the *in vitro* release studies, a dialysis membrane tubing (MWCO: 3500 Da, Spectrum®) was soaked in deionized water for 24 h before use. 2 ml of Ibuprofen-loaded SNEDDS emulsion (containing 30 mg Ibuprofen) and 2 ml Ibuprofen suspension (30 mg Ibuprofen in phosphate-buffered saline as the control) were sealed in dialysis tubings suspended in glass beakers containing 500 mL of simulated gastric fluid (0.01 M HCl solution) or simulated intestinal fluid (phosphate buffer saline, pH 6.8, SIGMA) as the release medium, magnetically stirred at 100 rpm at 38 ± 0.5 ℃, as shown in Figure 3.5. 2 ml of aliquot were periodically taken, replaced with an equal amount of fresh release medium and filtered through a 0.2 μm PTFE membrane filter. The content of the drug was analyzed by HPLC.

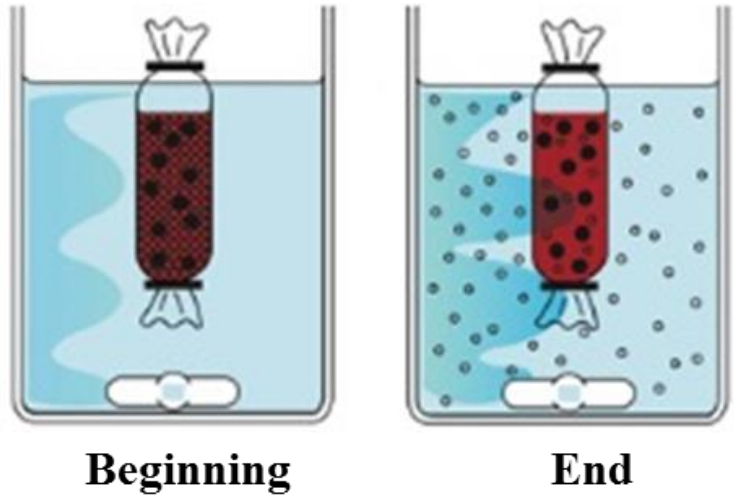

Figure 3.5 Schematic models illustrating the *in vitro* drug release study.

2.7. Statistics

The statistical analysis was performed using GraphPad Prism software (GraphPad Software Inc., California, USA). An one-way analysis of variance (ANOVA) was carried out on the results.

3. Results and discussion

3.1. Solubility of Ibuprofen in various vehicles

The drug-loading capacity of the SNEDDS formulations depends on the solubility of Ibuprofen in the various vehicles of the system, which was determined by solubility studies. The results are presented in Figure 3.6. Among the four oils that have been tested, Ibuprofen has similar solubility in anise essential oil and lemon essential oil (about 120 mg ml^{-1}), which is better than castor essential oil and soybean oil (about 90 mg ml^{-1}). Surfactant has a pivotal role in stabilizing nanoemulsions, its nature and amount determining droplet size and stability [13]. Nonionic surfactants are commonly preferred because of their lower toxicity and higher stability to pH and ions than ionic and amphiphilic surfactants [14]. The

hydrophilic-lipophilic balance (HLB) is a measure of the degree to which a substance is hydrophilic or lipophilic [15]. A HLB value of 20 defines a fully hydrophilic molecule, while a value of 0 defines a lipophilic one [16]. The stability of emulsions depends also on the ratio between the high HLB and low HLB surfactant amounts [12, 17]. As shown in Figure 3.6, among all the investigated surfactants, Ibuprofen exhibited quite higher solubility in Transcutol HP (HLB 4.2), 694 ± 30 mg ml^{-1}; Labrasol (HLB 14), 598 ± 12 mg ml^{-1}; Cremophor RH40 (HLB 13), 339 ± 21 mg ml^{-1}; and Capryol 90 (HLB 6), 306 ± 10 mg ml^{-1} that have been selected for further investigations.

Moreover, the optimal formulation is not only determined by the drug solubility but also by the emulsification efficiency and surfactant synergistic effect.

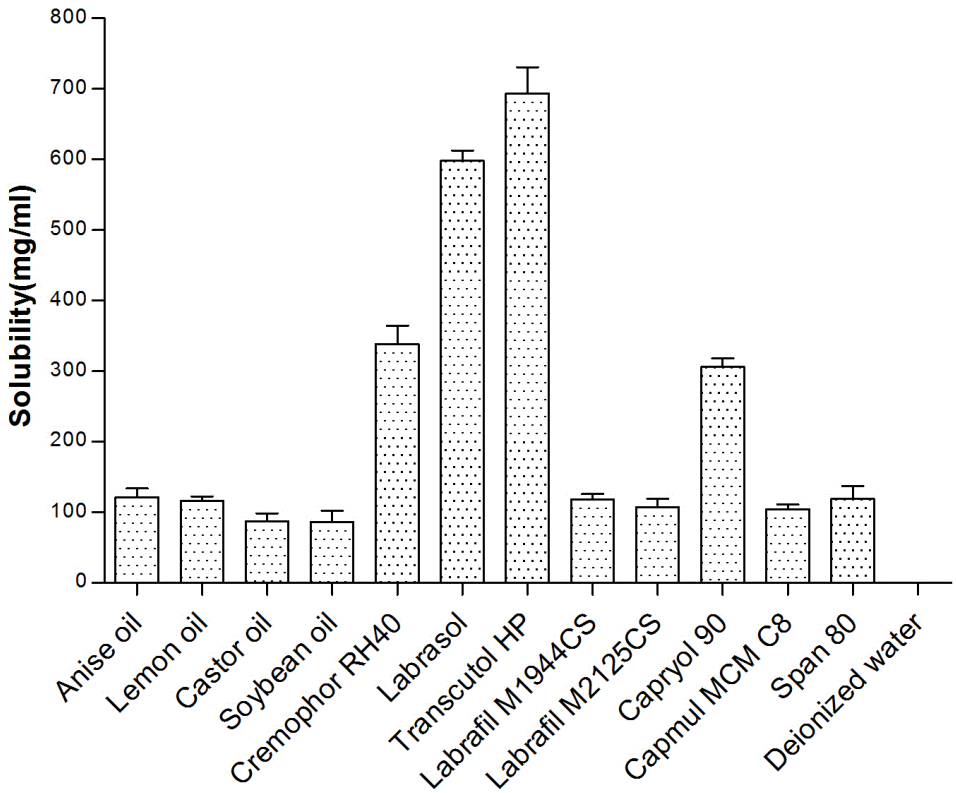

Figure 3.6 Solubility of Ibuprofen in various vehicles; each value is expressed as mean ±σ (n = 3).

3.2. Construction of ternary phase diagrams

The ternary phase diagrams of SNEDDS were constructed to screen the optimized SNEDDS. Before the construction of ternary phase diagrams, the miscibility between high HLB surfactants and oils was investigated to select the best components. While Labrasol (HLB 14) was poorly mixed with castor essential oil and soybean oil, other mixtures resulted in clear or milky homogenous solutions.

Due to the fact that all surfactants are potentially irritant or are poorly tolerated [18], therefore large amounts of surfactants may cause gastrointestinal tract irritation [19]; systems which contain a higher proportion of essential oil should be preferred. The ternary phase diagrams of SNEDDS selected according to the previous criteria are shown in Figures 3.7 (A)–(C). The shadow areas enclosed in the triangle represented the self-emulsification regions. SNEDDS made of lemon essential oil, Labrasol and Transcutol HP (Figure 3.7 (B)) showed the largest self-emulsification region, with an improved self-emulsification capacity at decreasing the oil component amounts, thanks to the reduction in interfacial tension caused by higher content of the surfactant [20]. At a Cremophor RH40 (HLB 13) concentration higher than 60% (Figure 3.7 (A)) and a Labrafil M1944CS (HLB 4) concentration higher than 50% (figure 2(C)), self-emulsification didn't occur, confirming that emulsification is determined not only by the surfactant or co-surfactant but also by the synergistic effect of the two.

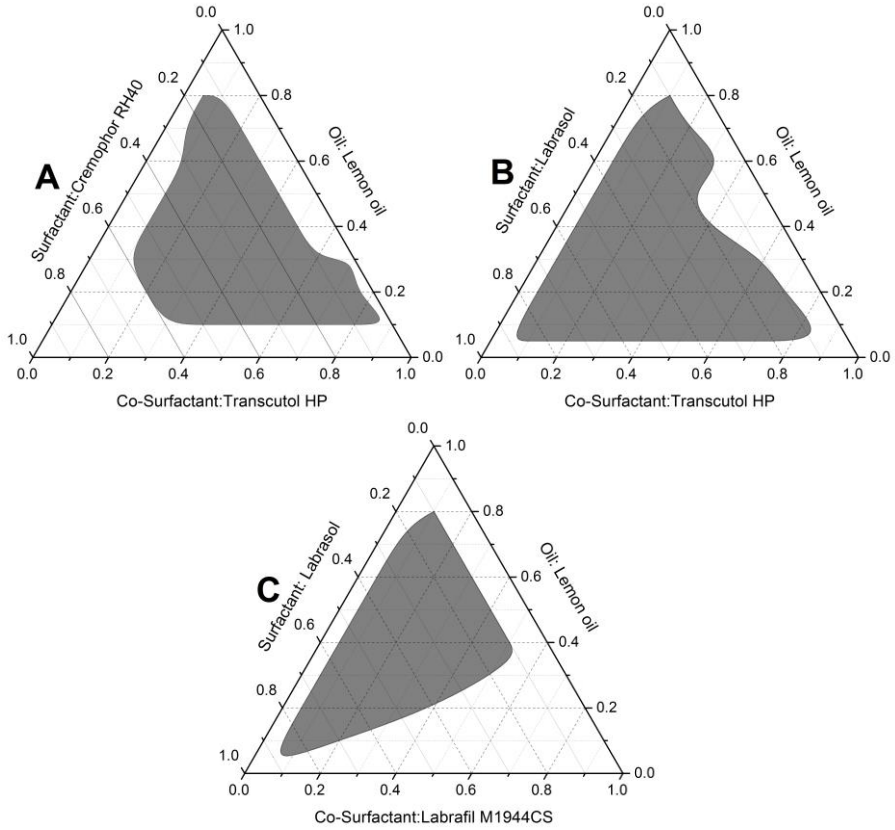

Figure 3.7 Ternary phase diagrams for SNEDDS: (A) Lemon oil/Cremophor RH40/Transcutol HP; (B) Lemon oil/Labrasol/Transcutol HP; (C) Lemon oil/Labrasol/Labrafil M1944CS. The shadow areas represent the self-emulsification regions.

3.3. Droplet size analysis

As reported in [18, 21], smaller droplet sizes induce a higher intestinal absorption rate. The ternary contour of SEDDS as a function of lemon essential oil, Cremophor RH40 and Transcutol HP amounts (Figure 3.8) indicated that self-emulsification occurred with a droplet size smaller than 750 nm, both with phosphate buffer saline (PBS, pH 6.8) and simulated gastric acid (SGA, pH 2.0).

With the increasing surfactant amount, the droplet size in emulsion decreased for both cases. Moreover, droplets were smaller in a SGA medium.

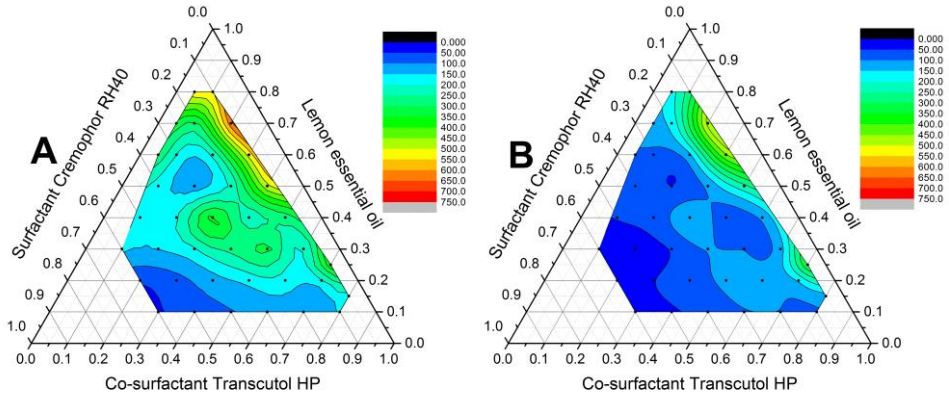

Figure 3.8 Ternary contour for droplet size of the lemon oil/Crempohor RH40/Transcutol HP system: (A) Emulsified with PBS (Phosphate buffered saline, pH 6.8); (B) Emulsified with SGA (Simulate gastric acid, pH 2.0). The colors represent different droplet sizes (from 0 to 750 nm).

3.4. Optimization and characterization of Ibuprofen-loaded SNEDDS

3.4.1. Solubility studies in optimized formulations

Eight formulations (F1–F8, Table 3.1) with the smaller droplet size have been selected for the further optimization studies. Ibuprofen solubility increased in a higher amount surfactant and a co-surfactant containing formulations. However, a high level of surfactant and co-surfactant is reported to induce irritation and other negative gastrointestinal issues.

Table 3.1 Composition of optimized SNEDDS formulations and Ibuprofen solubility.

Formulation number	Lemon oil/Crempohor RH40 (Surf)/Transcutol HP (Co-surf) [v/v/v, %]	Solubility *(±σ)* [mg/ml]
F1	70/20/10	219±4
F2	60/30/10	241±3
F3	60/20/20	277±5
F4	50/30/20	299±1
F5	50/20/30	334±5
F6	40/50/10	285±3
F7	40/40/20	321±2
F8	40/20/40	392±3

3.4.2. Evaluation of viscosity, emulsification time and emulsion appearance for ibuprofen-loaded SNEDDS formulations

The viscosity of the SNEDDS formulations is relevant for the manufacturing of formulation filled in soft or hard gelatin capsules [22, 23]. Too low viscosity of the formulations would hinder the capsule sealing effectively and enhance the probability of leakage, whereas too high viscosity may create the problems of pourability and emulsification capacity [22-25]. Ibuprofen content was chosen as 200 mg/ml, because higher drug contents reduced the self-emulsification capacity. The results showed an increase of the formulation viscosity with increasing surfactant proportion in the formulations with values for the optimal formulations ranging from 7.0 ± 0.1 to 42.0 ± 0.2 centipoise, depending on the formulation composition (Table 3.2). The measured values are in agreement with the values required for the above described filling process. [26].

Apart from the viscosity, the emulsification time and emulsion appearance were also observed. The self-emulsification time of all formulations was less than 20 seconds, and decreased with the decrease of viscosity. It means that SNEDDS

formulations could disperse quickly and completely under gentle agitation. Except emulsion F1 (7.0 ± 0.1 centipoise) that was cloudy after dilution, other emulsions appeared translucent, this being perhaps related to the larger droplet size of formulation 1.Viscosity was a crucial in affecting the emulsifying efficiency but played a negligible role on the droplet size. This results well agreed with some previous studies [2, 27-29].

Table 3.2 Viscosity, emulsification time and emulsion appearance of the optimized ibuprofen-loaded SNEDDS formulations, ibuprofen contents was 200mg/ml.

Formulation number	Viscosity *(±σ)* [mPa•s]	Self-emulsification time (±σ) [s]	Emulsion appearance
F1	7.0 ± 0.1	8 ± 2	Cloudy
F2	10.6 ± 0.1	10 ± 2	Translucence
F3	8.0 ± 0.1	9 ± 2	Translucence
F4	14.2 ± 0.2	10 ± 2	Translucence
F5	9.5 ± 0.1	9 ± 2	Translucence
F6	42.0 ± 0.2	18 ± 2	Translucence
F7	33.1 ± 0.3	15 ± 2	Translucence
F8	25.9 ± 0.2	13 ± 2	Translucence

3.4.3. Droplet size and ζ-potential

Droplet size, PDI and ζ-potential of the optimized SNEDDS in SGA with (200 mg ml^{-1}) and without Ibuprofen are listed in Table 3.3. In agreement with [24], a slight increase in droplet size is observed for the Ibuprofen-loaded SNEDDS. This can be attributed to the preferential dissolution of the drug in the interfacial film (formed by the surfactant and co-surfactant) that increases the interfacial tension. Moreover, the

addition of the drug could induce surfactant aggregation, thus reducing its efficiency. The PDI values are below 0.40, which indicates that the droplets are uniform in size.

The ζ-potential is correlated to the electrostatic repulsion and aggregation of the droplets. High positive or negative ζ-potential values (higher electrostatic repulsive forces) prevent coalescence, thus conferring stability of the emulsions [11, 22]. As shown in Table 3.3, all the SNEDDS emulsions had high negative ζ-potential values. The negative charges are due to the presence of free fatty acids in the surfactant and cosurfactant [14, 30]. The ζ-potential of the Ibuprofen-loaded SNEDDS was found to range between −35 ± 1 and −46 ± 1 mV, which indicated that the emulsions were stable. The ζ-potentials of Ibuprofen-loaded emulsion showed higher negative charges because of the negatively charged carboxyl groups in the Ibuprofen molecule.

The droplet size of F4 with ibuprofen was found to be 31 ± 3 nm (Figure 3.9 A) with PDI of 0.20 ± 0.02. The zeta potential of the emulsion developed by F4 was found to be 38 ± 1 mV (Figure 3.9 B). The conductivity of the emulsion was 0.109 ± 0.009 mS/cm, which means the emulsion was fine oil in water (conductivity > 10 µS/cm) [19].

Table 3.3 Droplet size, DPI and ζ-potential of 200µl optimized SNEDDS in 500 ml SGA (pH 2.0) at room temperature, with and without drug.

Formulation number	Without drug			With drug (200mg/ml)		
	Droplet size (±σ) [nm]	DPI (±σ)	ζ-potential (±σ) [mV]	Droplet size (±σ) [nm]	DPI (±σ)	ζ-potential (±σ) [mV]
F1	98 ± 1	0.21±0.01	-33±1	138±7	0.28±0.08	-38±1
F2	71 ± 5	0.19±0.01	-31±1	98±8	0.19±0.02	-35±1
F3	87 ± 6	0.32±0.04	-31±1	93±7	0.27±0.05	-37±1
F4	13 ± 3	0.18±0.02	-33±1	31±3	0.20±0.02	-38±1
F5	67 ± 8	0.31±0.04	-34±1	87±5	0.24±0.04	-40±1
F6	41 ± 5	0.22±0.05	-42±1	53±8	0.31±0.02	-46±1
F7	30 ± 4	0.34±0.08	-38±1	70±5	0.32±0.01	-42±2
F8	47 ± 7	0.25 ±0.05	-39±1	57±7	0.29±0.01	-44±1

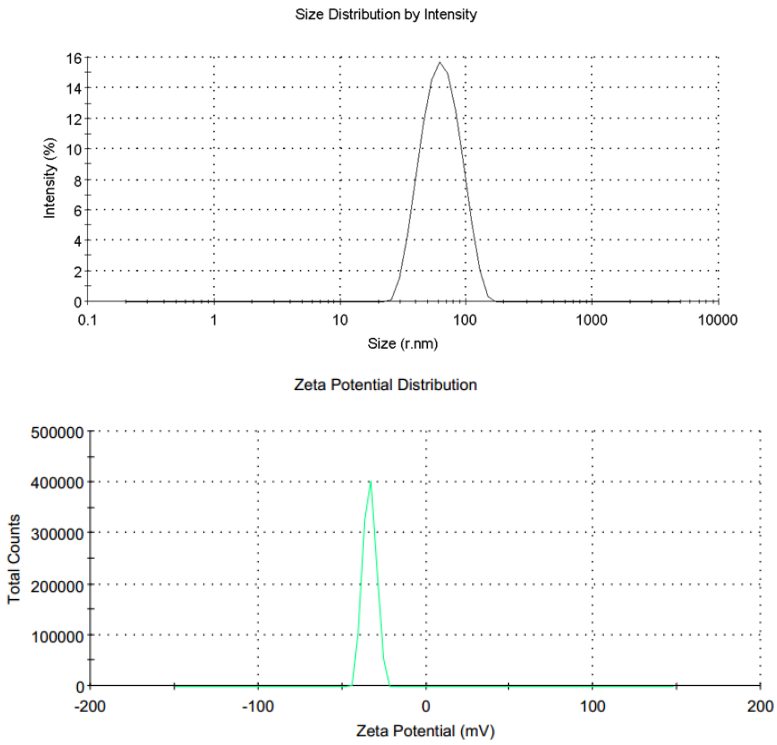

Figure 3.9 Droplet size distribution (A) and zeta potential (B) of ibuprofen-loaded F4 emulsion.

3.4.4. Formulation stability

The stability of F4, i.e. the formulation producing the smallest droplets after three freezing/thawing cycles, is summarized in Table 3.4. The droplet size increased with no significant changes of the ζ-potential after three freezing/thawing cycles. Moreover, the formulation didn't exhibit any drug precipitation or phase separation during the whole process. No marked difference of droplet size was observed for formulations stored at 4 ℃ or 25 ℃ (Table 3.5). The above findings indicated that this Ibuprofen-loaded formulation is thermodynamically stable.

Table 3.4 Effects of freezing/thawing cycles on the dynamic characteristics of nanoemulsions obtained from F4 (Lemon oil/Crempohor RH40/ Transcutol HP with ratio 50/30/20, v/v/v) containing 200mg/ml Ibuprofen in SGA (pH 2.0, 500ml).

Freezing/thawing cycle	Droplet size (±σ) [nm]	DPI (±σ)	ζ-potential (±σ) [mV]
-	31±3	0.20±0.02	-38±1
First	36±8	0.22±0.02	-39±2
Second	43±5	0.15±0.02	-38±2
Third	44±4	0.19±0.03	-39±2

Table 3.5 Effects of storage conditions on the dynamic characteristics of nanoemulsion obtained from F4 (Lemon oil/Crempohor RH40/ Transcutol HP with ratio 50/30/20, v/v/v) containing 200mg/ml Ibuprofen in SGA (pH2.0, 500ml).

Storing Time [months]	Temp=4℃		Temp=25℃	
	Droplet size (±σ) [nm]	DPI (±σ)	Droplet size (±σ) [nm]	DPI (±σ)
1	36±3	0.21±0.02	36±5	0.23±0.03
2	34±3	0.35±0.01	33±4	0.29±0.02
3	33±5	0.25±0.03	34±3	0.35±0.02

3.4.5. Morphological characterization

The morphology of F4 Ibuprofen-loaded emulsion droplets was observed by TEM. As shown in Figure 3.10, droplets are spherical with a diameter range of 20–40 nm, according to the light scattering data (Table 3.3).

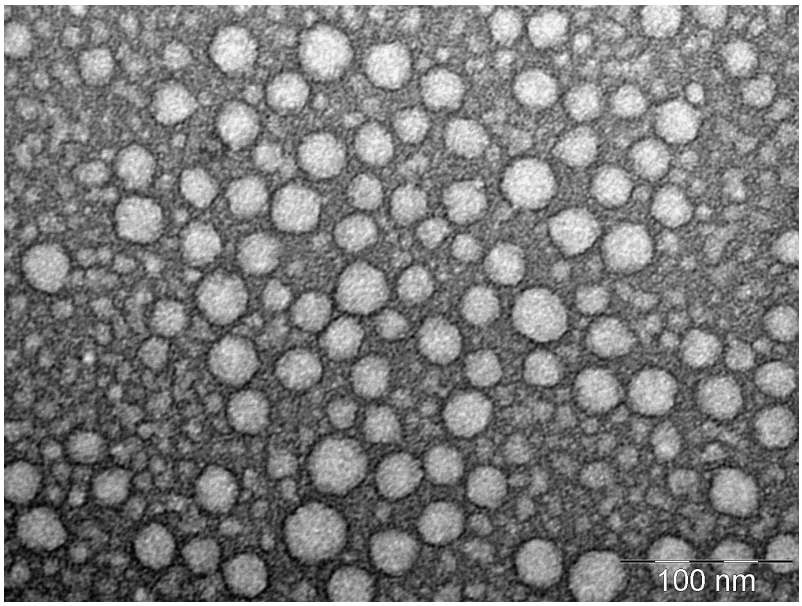

Figure 3.10 TEM image of F4 Ibuprofen-SNEDDS (lemon oil/ Crempohor RH40/Transcutol HP with ratio 50/30/20, v/v/v) nanoemulsion.

3.4.6. Drug *in vitro* release study

The Ibuprofen *in vitro* release in SGA for the eight selected optimal formulations emulsified in PBS and Ibuprofen suspended in PBS was evaluated for 4 h at 38 ℃ (Figure 3.11), following the previously described method. The drug release from SNEDDS was significantly greater than that of the Ibuprofen suspension. In 2 h, all the SNEDDS released approximately 80% of drug, with respect to 40% of the Ibuprofen suspension. All the SNEDDS released almost all drug in 4 h, with just a small difference among the different SNEDDS that are consistent with the droplet sizes (Table 3.3). In addition, the release from SNEDDS was faster, further supporting the hypothesis that nano-scale emulsions can improve the release of lipophilic drugs.

On the other hand, the droplet size was related with the pH of dilution medium, which maybe affect the drug release efficiency. Figure 3.12 represents the release profile of three batches emulsions which were formulated with PBS and SGA, then released in the both media. B1 was diluted with PBS (pH=6.8), released in SGA (pH=2.0); B2 was diluted with SGA, released in SGA; B3 was diluted with SGA, released in PBS. Comparing B1 with B2, the pH change of dilution media did not bring marked difference, this possibly because the droplet size of both batches was small enough and had weak effect on the drug release. Comparing the release of B2 and B3, the release in PBS is more effective than that in SGA. A possible explanation was that ibuprofen exhibited acidity, which renders it less soluble at low pH. Similar results were reported earlier [31].

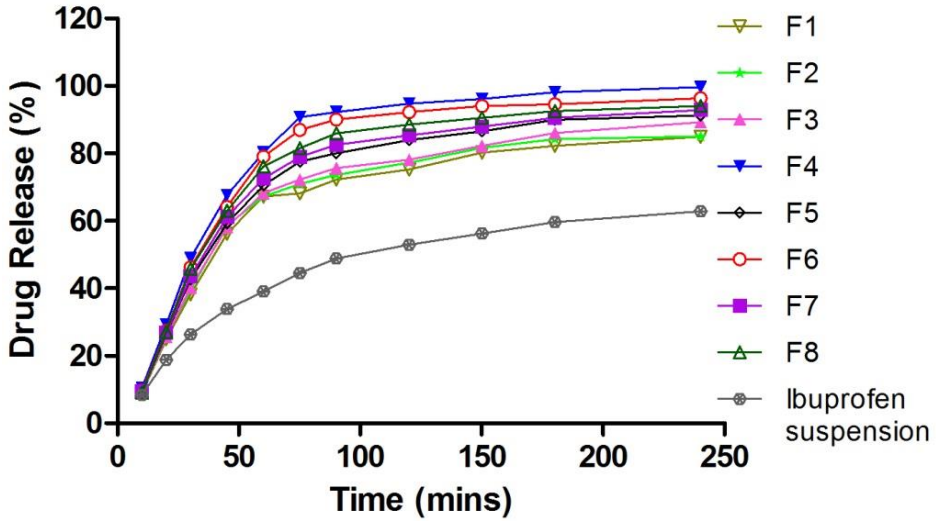

Figure 3.11 *In vitro* release profile of Ibuprofen suspension and Ibuprofen-SNEDDS (Emulsified with PBS, pH = 6.8, 10 ml) in SGA (pH = 2.0, 500 ml).

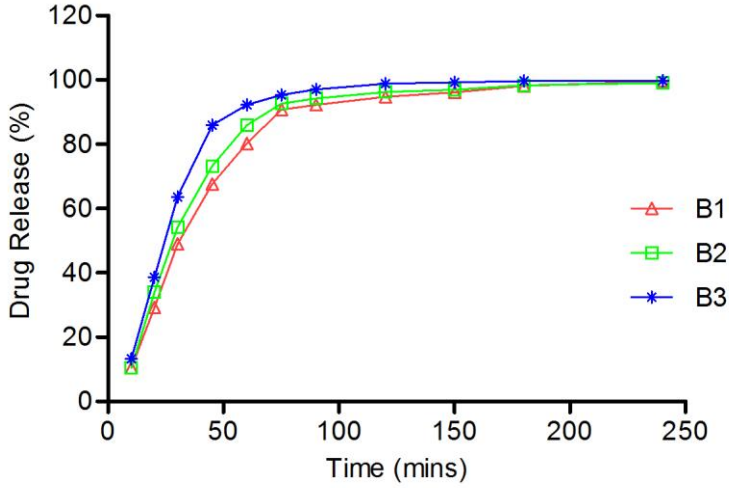

Figure 3.12 *In vitro* release profile of F4 in both SGA and PBS. B1-Dilution with PBS, release in SGA; B2-Dilution with SGA, release in SGA; B3-Dilution with SGA, release in PBS.

4. Conclusion

In the present chapter, a novel SNEDDS was successfully designed as a stable, high essential oil ratio (50%) and high drug-loaded (approximate 20%) formulation for the solubility and dissolution rate enhancement of Ibuprofen, chosen as a model for the lipophilic drug. The formulation composition and pH of the emulsifying medium significantly impacted the droplet size. The stability study confirmed that the SNEDDS formulations could withstand various storage conditions with excellent stability. The *in vitro* drug release study demonstrated that the release from SNEDDS was more efficient when compared with the drug suspension. Under these circumstances, the present SNEDDS would be a promising novel system to improve the lipophilic drug's dissolution rate and potentially the bioavailability.

Acknowledgments

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Chapter IV A novel pH-sensitive self-nanoemulsifying drug delivery system for acid-labile lipophilic drugs

Oral administration is the most convenient way of all the drug delivery routes. Orally administered bioactive compounds must resist the harsh acidic fluids or enzyme digestion in stomach, to reach their absorbed destination in small intestine. This is the case for silibinin, a drug used to protect liver cells against toxins that has also been demonstrated *in vitro* to possess anti-cancer effects. However, as many other drugs, silibinin can degrade in the stomach due to the action of the gastric fluid. The use of pH-sensitive self-nanoemulsifying drug delivery systems (pH-SNEDDS) could overcome the drawback due to degradation of the drug in the stomach while enhancing its solubility and dissolution rate.

In this paper we have investigated pH-sensitive self-nanoemulsifying formulations containing silibinin as model drug. Pseudo-ternary phase diagrams have been constructed in order to identify the self-emulsification regions under different pH. Solubility of silibinin in selected formulations has been assessed and stability of the pure drug and of the silibinin loaded pH-SNEDDS formulations in simulated gastric fluid had been compared. Droplet size of the optimized pH-SNEDDS has been correlated to pH, volume of dilution medium and silibinin loading amount. TEM (Transmission electron microscopy) studies have shown that emulsion droplets had spherical shape and narrow size distribution. *In vitro* drug release studies of the optimal pH-SNEDDS indicated substantial increase of the drug release and release rate in comparison to pure silibinin and to the commercial silibinin tablet. The results indicated that pH-SNEDDS have potential to improve the biopharmaceutics properties of acid-labile lipophilic drugs.

1. Introduction

Oral drug delivery is the most favorable route for drug administration. However, nearly half of the currently drugs exhibit low solubility in water, which leads to limited oral bioavailability, developments and clinical applications [1, 2]. Various approaches such as the use of lipid nanoparticles [3], liposomes [4] and self-emulsifying formulations [5], have been developed to improve the bioavailability and dissolution rate of poor water-soluble drugs. Among them, self-nanoemulsifying drug delivery systems (SNEDDS), spontaneously forming nano-droplets emulsion in water have acquired growing interest. SNEDDS are isotropic mixtures of drug, surfactant and co-surfactant that can rapidly form fine oil-in-water emulsions upon mild agitation in an aqueous media with a droplet size in the range 50-200 nm [6, 7]. The dissolution of lipophilic drug in these nano-droplets combined with the small size and the larger surface area results in higher loading and improved bioavailability of the drug [8, 9].

Generally drug absorption occurs at the small intestine where absorption is more effective due to the presence of villi and microvilli [10]. To reach the intestine (pH 7.0-9.0) [11, 12], drugs must however resist the extremely low pH (pH 1.0-2.0) and enzymes in the stomach. Furthermore, some drugs could irritate the stomach, and, in addition, some lipophilic drugs have poor enteral absorption.

Silibinin (also known as silybin), is a potent and principal component of silymarin extracted from the silybum marianum (Milk thistle) [13]. Silibinin has been used as a natural remedy for hepatitis, cirrhosis and recently has been reported to possess anticancer activity [14]. Unfortunately, silibinin is poorly bioavailable, due to its degradation in the gastric fluid, low water solubility and poor enteral absorption [15-17].

In order to prevent degradation of acid-labile lipophilic drugs in the stomach, several approaches have been attempted. Among those, pH sensitive drug carriers

have been proposed also to exploit the physiological pH gradient between gastric juice and the intestinal tract [18, 19].

So far, there are no publications on self-nanoemulsifying systems displaying pH sensitive properties. The aim of the present study was to develop a pH-sensitive self-nanoemulsifying drug delivery system (pH-SNEDDS) to increase solubility and dissolution of silibinin, thereby enhancing its oral bioavailability potentially. This formulation could moreover protect the drug from the acidic degradation in the stomach while facilitating the release in small intestine thanks to self-nanoemulsification (Figure 4.1). Drug solubility and loading in the formulations, nano-emulsions droplet size and stability, and *in vitro* drug release have been evaluated.

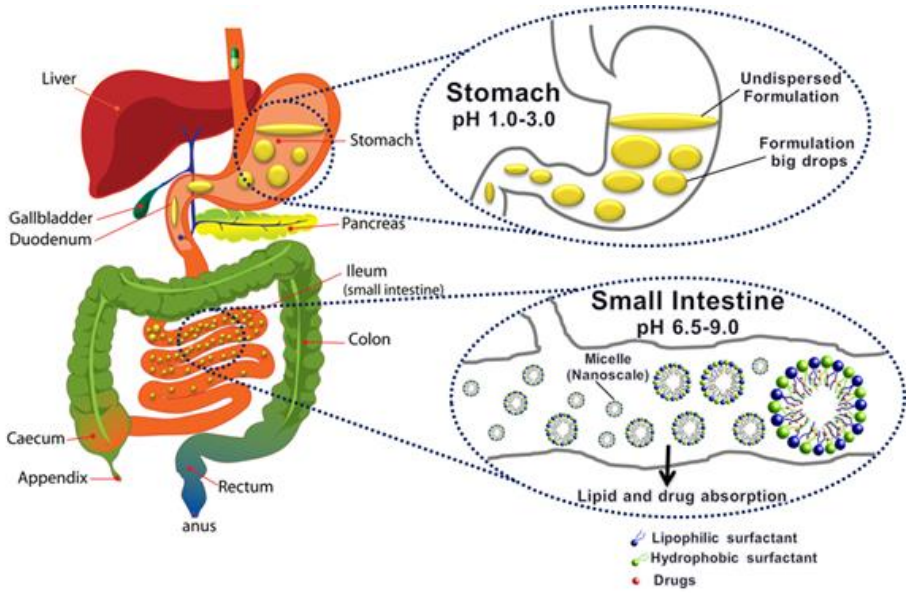

Figure 4.1 Schematic outline of the human digestive tract with pH-sensitive self-nanoemulsifying drug delivery system.

2. Materials and methods

2.1. Materials

Silibinin and Oleic acid were purchased from Sigma Aldrich S.r.l (Milan, Italy). Mono/diglycerides of caprylic acid (Capmul MCM C8 EP) was received as gift sample from ABITEC Corporation (Janesville, United states). Tablets (Cardo mariano) containing 11 mg silibinin in 500 mg excipients were purchased from ALCH Co. (Giarre, Italy). All other chemicals used were analytical reagent grade. Deionized water was used through the whole study.

2.2. Construction of pseudo-ternary phase diagrams

Mixtures of low and high HLB (hydrophilic-lipophilic balance) surfactants are necessary for developing stable emulsions [8, 20]. Different ratios of oleic acid (as precursor of the hydrophilic surfactant) and Capmul MCM C8 (as hydrophobic surfactant), in the range 1:9 to 9:1, were used to identify the self-emulsification regions at 37.0±0.5 ℃ through the construction of pseudo-ternary phase diagrams.

Oleic acid is a fatty acid, which included in the normal human diet as a part of animal fats and vegetable oils. Meanwhile, Capmul MCM C8 EP is a proven pharmaceutical excipient which meets the requirements of the European Pharmacopoeia Monograph for “Glycerol Monocaprylate” Type I [21]. The usage of Capmul MCM C8 EP for oral bioavailability enhancement was firstly reported by Panayiotis et al [22] for enhancing intestinal absorption of an RGD peptide in 1995, as well as the site-specific drug delivery[23].

Additionally, the modified visual examination method reported by Villar et al [7] was used to determine the self-emulsification regions. Briefly, the above formulations were magnetically stirred for 1 day, and 250 μl of each formulation was added drop by drop into 50 ml sodium phosphate buffer solution (PBS, pH range was between 6.8 and 8.0) under gentle magnetic stirring at 37.0±0.5 ℃ (Figure 4.2). The generated mixtures with clear or milk-white color were considered as self-emulsifying emulsion. All the assays were repeated three times. The pseudo-ternary

phase diagrams were constructed using Origin software (OriginLab Corporation, USA).

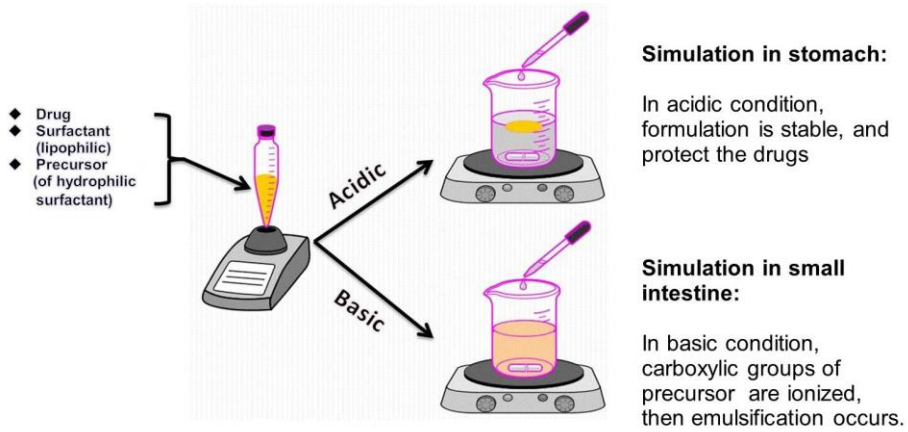

Figure 4.2 Set-up for preparation of nanoemulsions by the pH-sensitive self-emulsification method.

2.3. pH stability of silibinin

Silibinin stock solution (300 mg silibinin in 10 ml ethanol) was added into various buffer solutions (pH 1.0, 3.0, 6.8, 7.0, 7.2, 7.4 and 8.0, in order to simulate all physiological pHs), at a concentration of about 100 ppm silibinin. Solutions were mixed under gentle magnetic stirring at 37.0±0.5 ℃ for 4 hours and aliquots of 200 μl were periodically sampled and the amount of silibinin was determined by UV spectrophotometry (Nanodrop, Thermo Scientific, Wilmington, USA) at 288 nm wavelength. A calibration curve was constructed to correlate the height of the UV peaks to the weight concentration of silibinin in solution, using the method reported by Sooväli L et al [24]. Degradation was taken as the ratio between the silibinin content after the stability assay and the initial silibinin loading. Three consecutive measurements were made for each sample, and the results were presented as the mean and standard deviation.

2.4. Optimization and characterization of silibinin loaded pH-SNEDDS

2.4.1. Drug solubility in formulations

The solubility of silibinin in various formulations was measured by Nanodrop spectrophotometry. An excess of silibinin (approximately 200 mg) was placed in 1 ml different ratios oleic acid/Capmul MCM C8 formulations in sealed vials and the mixture was vortex-mixed at 37.0±0.5 ℃ for 48 hours in a water-bath to facilitate the dissolution. Finally, drug saturated formulations were centrifuged at 10000 x *g* for 30 min with a SIGMA 2-16 Centrifuge (SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The supernatant was filtered through a 0.2 μm PTFE filter membrane to remove the undissolved silibinin, and filtrates were diluted and analyzed by Nanodrop. The pure formulations without drug were used as reference. This assay was repeated in triplicate for each formulation.

2.4.2. Silibinin stability in the SNEDDS formulation in simulated gastric fluid

Silibinin stability was studied in simulated gastric fluid (SGF, 0. 1 M HCl solution with 0.9% NaCl) at pH 1.0. In brief, 10mg silibinin was introduced to 5ml of each formulation and vortex-mixed for 24 hours at room temperature. After attaining equilibrium, the formulations were added into SGF at 37.0±0.5 ℃ under gentle magnetic stirring for 4 hours, and aliquots of 20 μl were taken out periodically and filtered through 0.2 μm PTFE filter membrane for the analysis by Nanodrop. Each sample was studied in triplicate.

2.4.3. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy was carried out to detect the possible chemical property change of silibinin in excipients and formulations. The FT-IR spectra in the range of 650 - 4000 cm^{-1} for pure silibinin, physical mixtures of silibinin with Capmul MCM C8 EP and oleic acid, and silibinin loaded pH-SNEDDS formulations were observed at a resolution of 2 cm^{-1} using Spectrum One

spectrometer with ATR correction (Perking Elmer, Waltham, MA, USA) with Zinc Selenide crystal.

2.4.4. Differential scanning calorimetry (DSC)

The thermal characteristics of silibinin powder, physical mixture of drug with excipients, and optimal SNEDDS formulation were investigated using a differential scanning calorimetry (Mettler DSC 30, Mettler-Toledo, OH, USA). The samples were placed in aluminum pans, while an empty pan was used as reference. The DSC scans were recorded at a heating rate of 10 ℃/min from 25 ℃ to 250 ℃ under a nitrogen flow (100ml/min).

2.4.5. Determination of droplet size

Droplet size of emulsions was measured by dynamic light scattering (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK) at a scattering angle of 90°. The liquid pH-SNEDDS emulsions were filled in a disposable cuvette after diluting five times with deionized water, and shaken gently to mix thoroughly. All measurements taken at room temperature were repeated three times, and the values of average diameters and standard deviation (σ) were determined.

2.4.6. Formulation stability study

The stability of optimal drug-loaded formulations was evaluated by exposing the formulations to three freeze-thaw cycles, which consisted of freezing at 4 ℃ for 24 h followed by thawing at 65 ℃ for 24 h in an incubator. The droplet size, PDI and ζ-potential of the emulsions were investigated after each cycle. Moreover, accelerated stability of formulations were evaluated for droplet size and PDI at 4 ℃ and 25 ℃ for up to 6 months, respectively.

2.4.7. Droplets morphology characterization

Transmission electron microscope (TEM, Philips CM12 microscope, Netherland) was employed to study the morphology of silibinin loaded pH-SNEDDS emulsions. One drop of emulsion was placed on a carbon coated copper grid and the water removed by drying in the hood. Subsequently, samples were stained with 2% (v/v) phosphotungstic acid solution and dried again before the analysis. The operating voltage of TEM was 120 kV.

2.4.8. *In vitro* drug release

The *in vitro* drug release of silibinin loaded pH-SNEDDS at 37.0±0.5 ℃ was evaluated as follows. 30 ml of drug loaded pH-SNEDDS emulsions (containing 10 mg silibinin), 10 mg silibinin in PBS at pH 7.4 as the control and milled commercial silibinin tablet suspension (equivalent to 10 mg silibinin in PBS of pH 7.4) were introduced into sealed dialysis membrane tubings (MWCO: 12-14000 Da, Spectrum ®). Tubings were suspended in glass beakers containing 500 mL simulated intestinal fluid (sodium phosphate buffer solution, pH 7.4) as release medium, magnetically stirred at 100 rpm. An aliquot (200 µl) of the medium was periodically collected, replaced with an equal amount of fresh medium, and analyzed for the content of silibinin by Nanodrop spectrophotometry. All measurements were performed in triplicate.

2.5. Statistics

All the results were represented as mean and standard deviation (σ). Statistical analysis was performed with GraphPad Prism software (GraphPad Software Inc., California, U.S.A) using one way analysis of variance (ANOVA).

3. Results and discussion

3.1. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams of pH-SNEDDS have been constructed to identify the self-emulsifying regions for the optimized formulations. Before the construction of the pseudo-ternary phase diagram, a series of formulations have been screened to assess their pH responsiveness.

The *pseudo-ternary phase diagrams* of the selected pH-SNEDDS at various pH are shown in Figure 4.3. The whole gray area represent the self-emulsification region in the pH range 6.8-8.0 while the light gray area focusing on the self-emulsification region at pH 6.8 -7.0. For pH>7.0, the emulsification region is wider due to the fact that above that pH more free carboxylic groups of oleic acid are ionized [25, 26]. Consequently, the synergistic effects between hydrophilic and lipophilic surfactant are more effective [27].

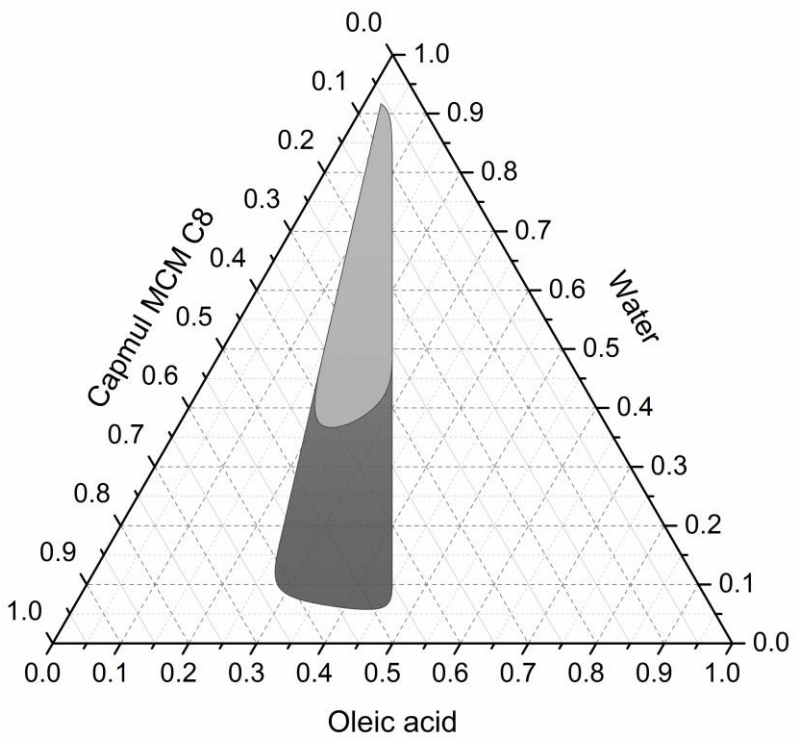

Figure 4.3 Pseudo-ternary phase diagram for pH-SNEDDS: The whole gray area represents the self-emulsification region between pH 6.8 and 8.0, while the area in light gray is the self-emulsification region at pH 6.8 -7.0.

3.2. Optimization and solubility study of pH-SNEDDS

Three pH-SNEDDS formulations have been selected from the pseudo-ternary phase diagram for further optimization. Their composition and silibinin solubility data are reported in Table 4.1. The higher silibinin solubility of F1 is consistent with the higher amount of the hydrophobic component Capmul MCM C8 EP.

Appearance of F2 pH-SNEDDS in various buffer solutions from pH 1.0 to 8.0 is illustrated in Figure 4.4. pH-SNEDDS formulations are stable in acidic medium (pH 1.0 and 3.0), being able to resist harsh gastric fluids and protect the drug. Emulsification is only partial at pH 6.8, while improves at higher pH consistently with the pH range (7.0 to 9.0) of the small intestine [11, 12].

Table 4.1 Composition of optimized pH-SNEDDS formulations and silibinin solubility. Data expressed as $\mu \pm \sigma$ (n = 3).

Formulation number	Oleic acid/ Capmul MCM C8 EP [v/v,%]	Silibinin solubility (±σ) [mg/ml]
F1	30/70	89.1±8.7
F2	40/60	71.9±9.5
F3	50/50	29.9±7.7

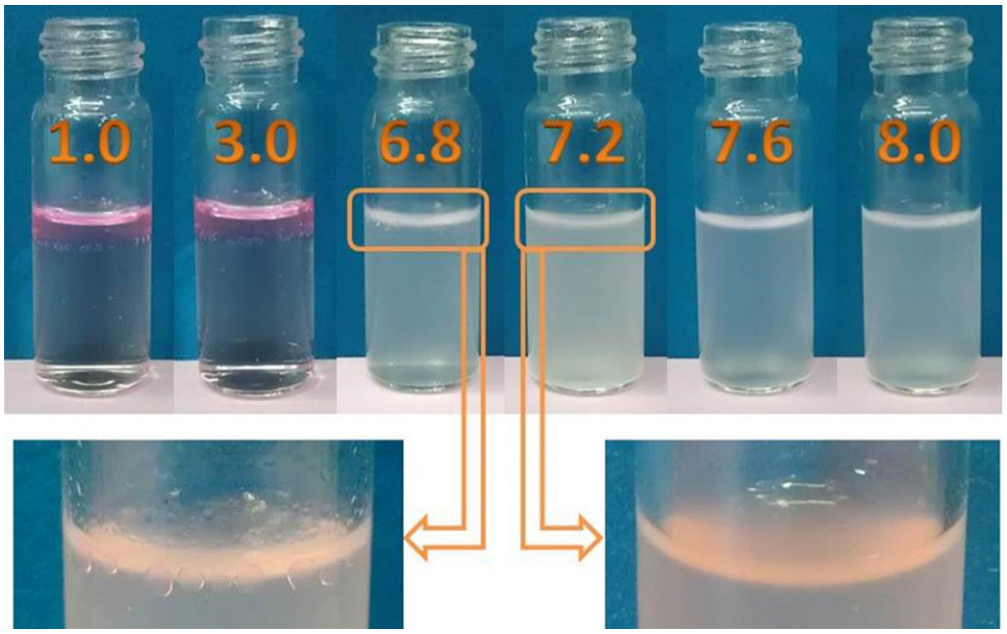

Figure 4.4 Photographs of pH-SNEDDS (F2) at various pH (up) and enlargements of the interface layers (down) at pH6.8 (left) and 7.2 (right). DiI, a lipophilic dye, was added to distinguish the formulations.

3.3. pH stability of silibinin

Besides the poor water solubility, utilization of silibinin is also limited by its degradation in gastric fluid [15, 28]. Figure 4.5 A indicates that silibinin concentration in the solution decreased by 80% in the first 5 minutes at pH 1.0 and 3.0, with degradation of only about 10% in 4 hours under basic pH between 7.2 and 8.0. The above finding is in agreement with the results reported by Patel A. *et al* [16].

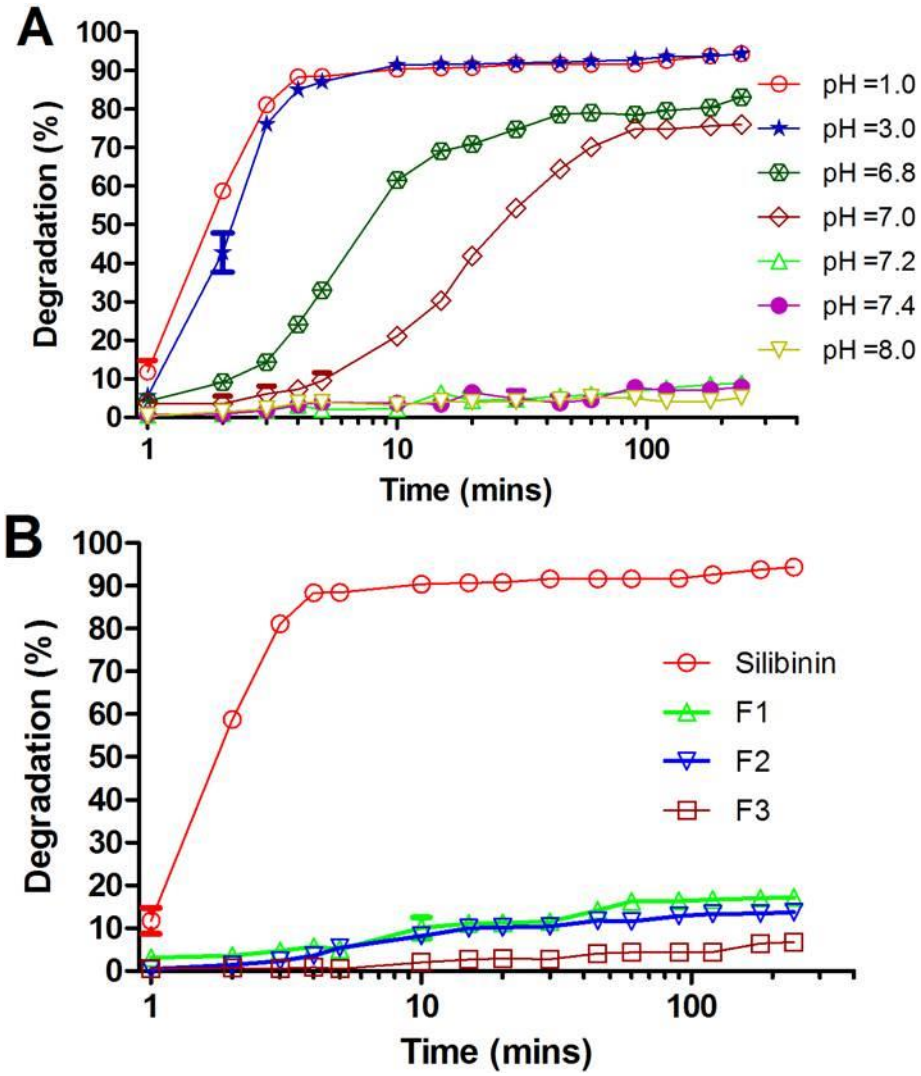

Figure 4.5 Degradation of pure silibinin at various physiological pH (A) and silibinin in selected formulations in simulated gastric fluid (pH=1.0, B). The results are presented as μ ± σ (n=3).

3.4. Silibinin stability in formulation

Studies of silibinin stability in formulations during gastric incubation (pH=1.0) at 37±0.5 ℃ for 4 hours (Figure 4.5 B) , showed that silibinin degrades less than 20% from the optimized formulations in comparison with the pure silibinin (more than

90%), thus indicating that pH-sensitive self-emulsifying formulations protect silibinin from simulated acidic fluids.

3.5. Fourier transformed infrared spectroscopy (FTIR)

FTIR spectra of pure silibinin, physical mixture of silibinin with Capmul MCM C8 EP and oleic acid, silibinin loaded optimal pH-SNEDDS formulations are demonstrated in Figure 4. The characteristic peaks at 3452 cm−1 can be attributed to the presence of hydroxyl group (–OH), while the peak at 1631 cm−1 is associated with the C=O stretching of the carboxylic acid group (–COOH). Similar FTIR observation are reported by the works of Tan *et al.* [29] and Pooja D *et al* [30]. The spectra of both physical mixtures and silibinin loaded optimal formulations (F1 and F2) don't show any changes in characteristic peak position from silibinin spectrum, indicating the absence of chemical nature change of silibinin in the formulations.

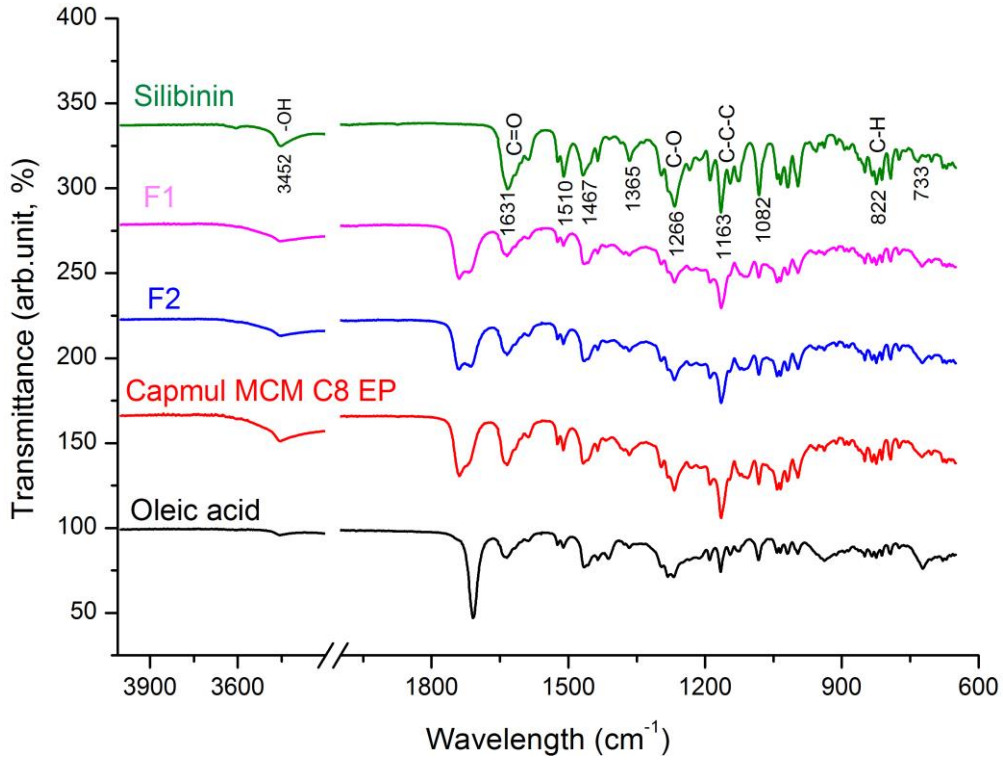

Figure 4.6 FTIR spectra of pure silibinin, physical mixtures of silibinin with Capmul MCM C8 EP and oleic acid, and silibinin loaded optimal pH-SNEDDS formulations (F1 and F2).

3.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to investigate the thermal behavior of the pure silibinin and the excipients with silibinin (Figure 4.7). Silibinin showed an endothermic peak at 166.31 ℃ with onset at 147.79 ℃ and endset at 174.76 ℃ that corresponds to the melting point of silibinin in crystalline form. No endothermic peaks were found in the physical mixtures of optimal formulations, Capmul MCM C8 EP, and oleic acid, indicating that silibinin must be molecularly dissolved in an amorphous state in the formulations.

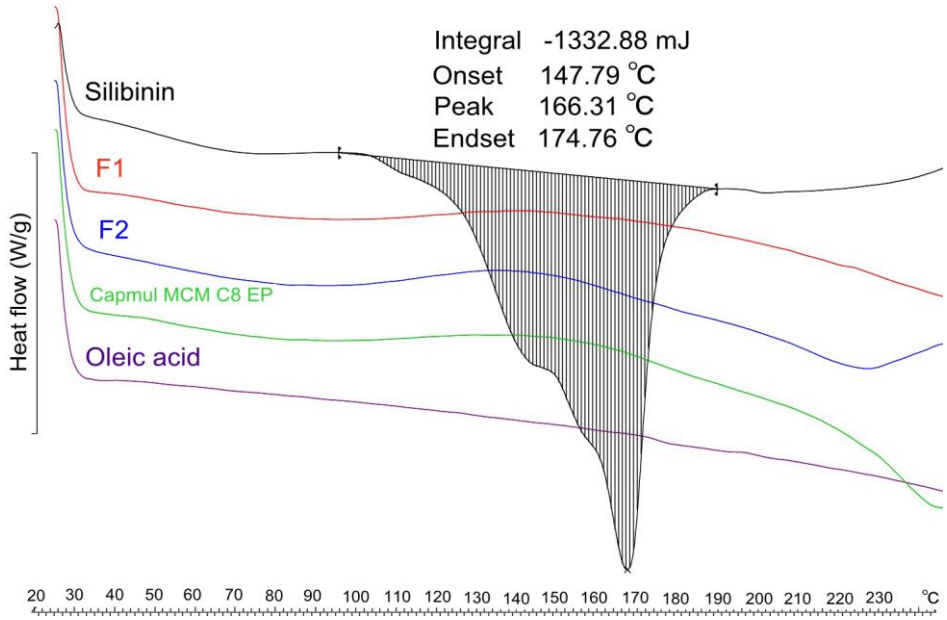

Figure 4.7 Differential scanning calorimetry thermogram of pure silibinin, physical mixtures of silibinin with Capmul MCM C8 EP and oleic acid, silibinin loaded optimal pH-SNEDDS formulations (F1 and F2).

3.7. Determination of droplet size

The droplet size distribution of emulsions is one of the most important factors for the self-emulsification performance. The smaller the droplet size, the higher the drug dissolution and intestinal absorption rate is. Due to the low solubility of silibinin in F3, only F1 and F2 have been compared in the following assays.

3.7.1. Droplet size at different pH

The effect of the emulsifying medium pH on the droplet size of silibinin pH-SNEDDS emulsions is reported in Figure 4.8. A slight increase in droplet size is observed for the silibinin loaded emulsions in comparison to that without drug for both F1 and F2. This can be attributed to the drug dissolution in the oil-water interfacial films, which increases the interfacial tension, and leads the droplet size enlargement. In addition, the presence of drug could induce surfactant aggregation, thus reducing the efficiency of surfactants [31]. An increase of pH from 7.0 to 8.0 resulted in the decrease of droplet size. F2 has smaller droplets than F1 under the same pH conditions.

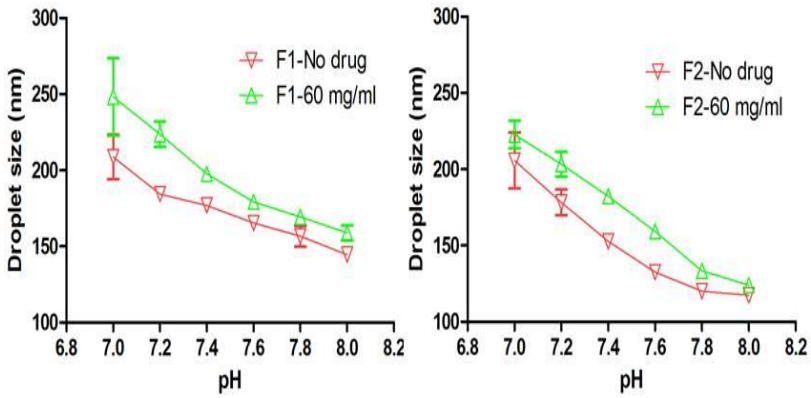

Figure 4.8 Effect of emulsifying medium pH on the droplet size of silibinin loaded pH-SNEDDS emulsions. Formulations were diluted a 200 fold. Each value is represented as μ ± σ (n=3).

3.7.2. Droplet size by dilution medium volume

The effect of dilution medium volume on droplet size is showed in Figure 4.9 A for formulations with and without drug. The dilution does not appreciably affect the droplet size, which proves the formulation stability under the variable dilution conditions that could result after the oral administration.

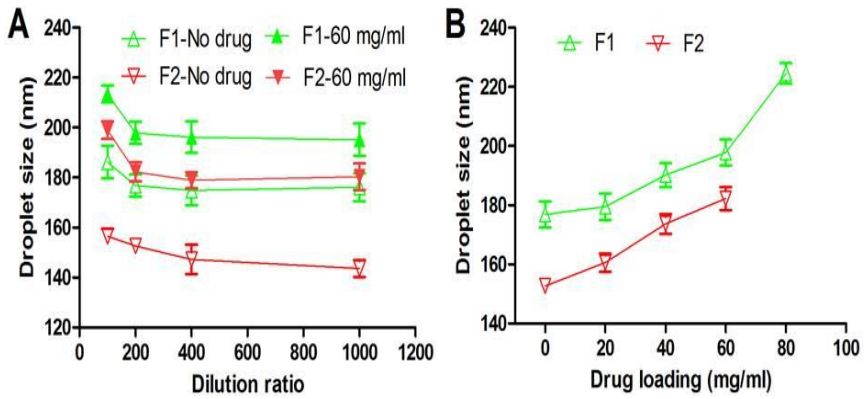

Figure 4.9 Effect of dilution medium volume (A) and silibinin loaded amount (B) on droplet size of pH-SNEDDS emulsions. Dilution medium was sodium phosphate buffer solution with pH 7.4. Each value is represented as μ ± σ (n=3).

3.7.3. Droplet size by different drug loading

The droplet size profiles of pH-SNEDDS emulsion from each formulation have been estimated by gradually increasing drug loading (Figure 4.9B). In this investigation, a noticeable increase of the droplets diameter has been observed at increasing drug loading for both formulations, however still mantaining the size in the nano-range. For F2, maximum test drug loading is 60 mg/ml, because of the relatively limited dissolution of silibinin in this formulation (71.9 ± 9.5 mg/ml).

3.8. Formulation stability

The results of characterization of F1 with silibinin after three freeze/thaw cycles were summarized in Table 4.2. The droplet size slightly increased with no significant changes of the ζ-potential. The accelerated stability of F1 was also investigated under different storage conditions (Table 4.3). The results suggested that no significant changes occurred on droplet size and PDI of the formulated emulsions. Thus, it could be concluded that this formulation was thermodynamically stable at harsh storage conditions as well as accelerated conditions.

Table 4.2 Parameters of nanoemulsions obtained from F1 (Oleic acid/ Capmul MCM C8 EP with ratio 30/70, v/v) containing 60 mg/ml silibinin during freeze thaw cycles. Dilution medium was sodium phosphate buffer solution with pH 7.4. Data reported are μ ± σ (n = 3).

Freeze thaw cycle	Droplet size (±σ) [nm]	PDI (±σ)	ζ-potential (±σ) [mV]
Initial	189 ± 11	0.15 ± 0.03	38 ± 3
First	190 ± 9	0.19 ± 0.04	36 ± 4
Second	192 ± 13	0.21 ± 0.02	37 ± 3
Third	193 ± 11	0.22 ± 0.04	38 ± 2

Table 4.3 Accelerated stability data of nanoemulsions obtained from F1 (Oleic acid/ Capmul MCM C8 EP with ratio 30/70, v/v) containing 60 mg/ml silibinin. Dilution medium was sodium phosphate buffer solution with pH 7.4. Data reported are mean ± σ (n = 3).

Time (months)	Temp=4 ℃		Temp=25 ℃	
	Droplet size (±σ) [nm]	PDI (±σ)	Droplet size (±σ) [nm]	PDI (±σ)
1	189 ± 9	0.22 ± 0.02	187 ± 14	0.29 ± 0.04
3	192 ± 11	0.25 ± 0.03	195 ± 9	0.28 ± 0.01
6	197 ± 12	0.28 ± 0.01	191 ± 12	0.30 ± 0.03

3.9. Morphology characterization

The TEM morphology of F1 silibinin loaded pH-SNEDDS emulsion droplets is shown in Figure 4.10. Nano-droplets are spherical in shape, uniform in size from 150 nm to 200 nm, in accordance with dynamic light scattering data (Figure 4.9).

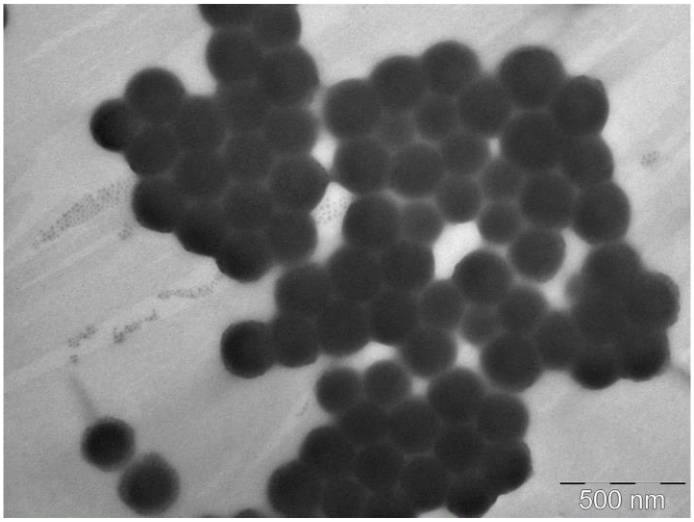

Figure 4.10 TEM image of F1 silibinin pH-SNEDDS (60mg/ml) nanoemulsions. Formulation was diluted a 200 fold in the emulsifying medium (PBS, pH 7.4).

3.10. *In vitro* drug release study

The *in vitro* drug release was carried out for silibinin loaded pH-SNEDDS, silibinin suspension and milled commercial silibinin tablet suspension (Product from ALCH®). As shown in Figure 4.11, silibinin released from suspension was less than 10% in 9 hours, with much lower drug release from the tablets. In contrast, within 9 hours approximately 70% and 80% of silibinin are released from pH-SNEDDS F1 and F2, respectively. The significant release enhancement by pH-SNEDDS can be attributed to its amorphous nature, smaller droplet size and increased surface area [30]. In addition, F2 has shown higher release than F1, because of the relatively smaller droplet size of F2 emulsions, that is consistent with the droplet sizes study (Figure 4.9).

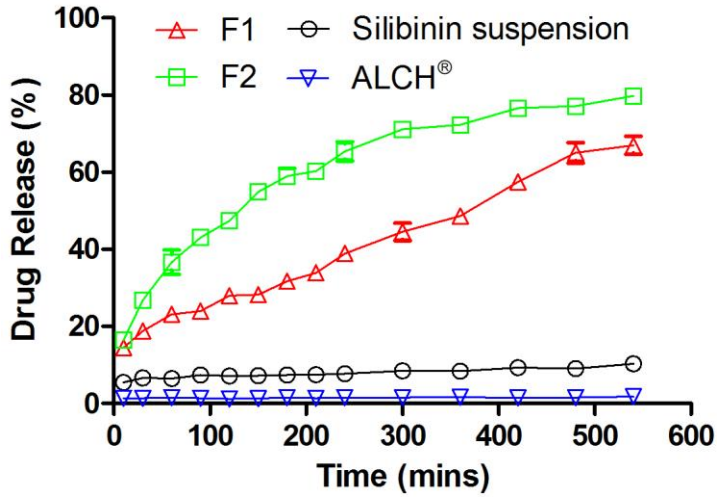

Figure 4.11 Comparison of *in vitro* release of optimized silibinin loaded pH-SNEDDS (Emulsified with PBS, pH=7.4), silibinin suspension and milled commercial silibinin tablet suspension (Product from ALCH®). Data expressed as μ ± σ (n = 3).

4. Conclusions

The present chapter describes an innovative approach for protecting acid-labile bioactive compounds and improving the solubility and dissolution rate of lipophilic drugs by using pH-sensitive self-emulsifying formulations. In particular, pH-SNEDDS protected silibinin from the harsh acidic gastric-like fluids while providing excellent self-emulsification in intestinal tract. Further, the formulation stability study demonstrated that the formulations were stable under various storage conditions. Increasing the emulsifying medium pH leads droplet size decrease, while size significantly increasing together with drug loading. *In vitro* release profile from pH-SNEDDS was much higher than from powder and commercial tablets product, thus resulting more effective as drug carrier.

Accordingly, we concluded that the pH-SNEDDS could enhance the bioavailability of lipophilic drugs, and represent a new route for the oral administration of acid-labile drug delivery systems.

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Chapter V Summary and Future perspectives

1. Summary

The overall objective of the present thesis was to design and optimize self-nanoemulsifying drug delivery systems (SNEDDS) for poor water soluble drugs oral delivery.

Chapter III focused on the development and optimization of a surfactant reduced amount SNEDDS with high proportion of essential oil as carrier for lipophilic drugs. In fact, surfactants are generally toxic, moreover the large amount of surfactants used in SNEDDS could provoke irritation to GI tract.

A second study, described in chapter VI, aimed at exploiting self-nanoemulsifying drug delivery systems for controlled release. In spite of the many efforts that have been done on the design and production of the novel self-emulsifying formulations as alternatives to conventional SEDDS, there is no approved controlled release self-emulsifying product available.

Some drugs are prone to degradation, undesired inactivation or irritation in the GI tract. The developed pH-sensitive self-nanoemulsifying formulations in our work have been shown to be able to protect acid-labile drug, control drug release, increase drug solubility and potentially enhance the oral bioavailability. Combination of SNEDDS and pH sensitive technique represents a new route for the oral administration of acid-labile drug delivery systems.

2. Future perspectives

Since nearly 40% of recent new drug substances are lipophilic, it appears that more drug products will be formulated as SEDDS for the pharmaceutical market in

the very near future. The challenges associated with the formulation of self-emulsifying systems include the selection of right excipients with consideration of their solvent capacity, miscibility, chemical stability, dispersibility, regulatory issues, and so on. Although the potential utility of SEDDS has been known for decades, it is only in recent years that a mechanistic understanding of their impact on drug disposition has emerged [1]. To this end, more predictive *in vitro* models are needed for predicting the changes involving the drug in SEDDS in the gut, so that the fate of the drug *in vivo* can be more reliably monitored [2]. The applications of SNEDDS in other routes of delivery apart from the oral route can be explored. Besides, other techniques may be combined with self-emulsification to develop multifunctional drug delivery systems. With future developments in this novel technology, SEDDS will remove deficiencies associated with delivery of poorly soluble drugs. Thus, this field requires further exploration and research to bring out a wide range of commercially available self-emulsifying formulations [1].

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Scientific Production

Manuscripts in International journals

T.J. Zhao, D. Maniglio, J. Chen, B. Chen, A. Motta and C. Migliaresi, Design and optimization of self-nanoemulsifying formulations for lipophilic drugs, *Nanotechnology 26 (2015) 125102*.

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B. Chen, W. Bonani, T.J. Zhao, A. Motta, J. Chen and C. Migliaresi, Injectable In Situ Forming Fibroin Hydrogel and Drug Delivery (In preparation)

Participation to Congresses, Schools

8-11th July 2015

11th International Symposium on Frontiers in Biomedical Polymers. Riva del Garda, Italy.

Oral presentation. Tianjing Zhao, Devid Maniglio, Jie Chen, Bin Chen, Claudio Migliaresi: "Design and Optimization of pH Sensitive Self-Nanoemulsifying Drug Delivery System for Acid Labile Lipophilic Drugs"

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Summer school on Tissue Engineering and Regenerative medicine. Riva del Garda, Italy.

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Tissue Engineering & Regenerative Medicine International Society, European Chapter Meeting, Genova, Italy.

Poster. Tianjing Zhao, Devid Maniglio, Claudio Migliaresi: "Enhanced oral bioavailability of Ibuprofen by Self-nanoemulsifying Drug Delivery System (SNEDDS)". Published abstract PP235: on-line Journal of Tissue Engineering and Regenerative Medicine, Volume 8, Issue Supplement s1, pages 344-345.

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