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Original Article

Development and Characterization of Self- Microemulsifying Drug Delivery System for Improvement of Bioavailability of Cefdinir

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Abstract

Oral route is the most common and preferred route for the drug administration due to convenience and ease of administration. Technology Catalysts International reported in 2002 that approximately 35-40% of all new chemical compounds suffer from poor aqueous solubility. Therefore, enhancing drug dissolution became one of the major challenges for pharmaceutical scientists over the past decade. Lipid formulations and in particular SMEDDS/ SNEDDS Self-Micro emulsifying Drug Delivery Systems can induce a considerable increase in dissolution rate Class II-IV drugs are considered the best candidates for intervention by formulation e.g. in self-emulsifying dosage forms. *Aim:* Cefdinir is a poorly water-soluble drug with varying bioavailability. The main purpose of present work was to develop self-micro emulsifying drug delivery system (SMEDDS) for enhancing solubility and bioavailability of Cefdinir is indicated for the treatment of bronchitis as well as for the treatment ofear, nose, throatdisorder. *Materials and Method:* Cefdinir had highest solubility in labrafac with comparison to other lipid vehicles. Emulsification study results were shown that tween 20 has highest solubility capacity of oil was higher (0.8528 ± 0.4075mL) than other surfactant.Sotween 20 was selected as surfactant. From the result were shown that PEG 400 has highest solubility capacity of oil (2.65 ± 1.801 mL). So PEG 400 was selected as co-surfactant. The formulation of Cefdinir SMEDDS was optimized by a simplex lattice design. The optimal formulation of SMEDDS was comprised of 20% oil (Labrafac), 60% surfactant (Tween-80) and 20% co-surfactant (PEG-400). *Results and Discussion:* Pseudo-ternary phase diagrams were constructed to identify the efficient self-emulsification region. Optimal ratio of surfactant to co-surfactant was selected to be 4:1. A suitable SMEDDS formulation should have a minimum self emulsification time, maximum% Transmittance, maximum time require to 20% of drug release.The individual desirability for each response was calculated and batch F2 showed the highest overall desirability therefore this batch considered to be the best batch. In order to obtain both high %Transmittance and high Cumulative %release, the appropriate ratio of components was chosen for optimized formulation, which consisting of oil (20%), surfactant (60%), co-surfactant (20%).The average globule size of SMEDDS containing Cefdinir was about 87.60 nm when diluted in water. No significant variations in globule size and In vitro diffusion studies showed remarkable increase in dissolution of drug. Order of drug release was F-2> F-4> F-1 > F-7> F-3> F-6 > F-5. *Conclusion:* The data suggest use of SMEDDS to provide great potential as an alternative to traditional oral formulations of Cefdinir.

Keywords: Cefdinir; Self-Micro Emulsifying Drug Delivery System (SMEDDS); Simplex lattice Design; Globule Size.

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Introduction

Introduction of Self Micro-Emulsifying Drug Delivery System

It is generally accepted that many of today's new chemical entities are poorly water-soluble and pose a challenge in developing an optimum solid oral dosage form. Oral route has been the major route of drug delivery for the treatment of various chronic diseases like cancer. However, oral delivery of approximately 40% of the drug compounds is limited because of low aqueous solubility, which leads to limited oral bioavailability, high intra and inter subject variability and lack of dose proportionality. To overcome the above discussed drawbacks, various other formulation strategies have been adopted including the use of, nanoparticles, solid dispersions and permeation enhancers. In recent years, much attention has focused on lipid-based formulations to improve the oral bioavailability of poorly water-soluble drug compounds. In fact, the most popular approach is the incorporation of the drug compound into inert lipid vehicles such as oils and surfactant dispersions, self-emulsifying formulations, emulsions and liposomes with particular emphasis on self-microemulsifying drug delivery systems (SMEDDS).

Cefdinir is a BCS class-IV compound it has low solubility and low permeability. Oral bioavailability of cefdinir is 16-25%. In current work cefdinir solubility will be enhanced by using SMEDDS which include various oils, surfactants/co-surfactants, solvents/ co-solvents [1-2].

Nevertheless, oral delivery of over one-half of the drug compounds through gastrointestinal (GI) tract gets diminished owing to their high lipophilicity and consequently poor aqueous solubility. Oral bioavailability of such drugs, being primarily a function of their solubility and dissolution, tends to exhibit inadequate magnitude with high intra- and inter-subject variability. Besides, oral bioavailability also depends upon a multitude of other drug factors such as stability in GI fluids, intestinal permeability, resistance to metabolism by cytochrome P450 family of enzymes present in gut enterocytes and liver hepatocytes, and interaction with efflux transporter systems like P-glycoprotein.Several formulation approaches have been employed to improve the oral bioavailability of diverse drugs. Amongst these, oral lipid-based SMEDDS have proved their immense potential in improving the poor and inconsistent drug absorption of many poorly water-soluble drugs, especially following their administration after meals [3-10].

Composition of SMEDDS

Drugs

Generally, SMEDDS are prepared for drugs possessing poor water-solubility.

Surfactant

Surfactants are having amphiphilic character. They help in solubilisation of lipophilic drug compounds. In GI lumen, this prevents precipitation of drug. So that the drug exists in solution form in lumen for prolonged time. Nonionic surfactants possessing high HLB value are widely employed. The role of surfactant is to enhance absorption of drug, because of induction of permeation changes in biological membrane. It is reported that a cationic emulsion show greater absorption than an anionic emulsion. To form a stable SMEDDS, 30-60% concentration of surfactant is used.

Lipids/Oils

Vegetable oil, mineral oil, lanolin, silicon oil, fatty acids, animal oil etc are utilized in SMEDDS. Mono-/di-/tri-glycerides are widely used in SMEDDS formulation because they enhance the dissolution rate of drug in the intestinal medium. It is also to be assumed that this glyceride form a droplet which carry drug, C *In vitro* dissolution studies are carried out for so that the metabolism of drug is protected. Polyethylene glycol and polyglycolyzed glycerides in along with vegetable oils have been utilized to solubilise lipophilic drugs. Galactolipids show good emulsifying properties, similar to those of phospholipids. The main difference between phospholipids and galactolipids include the former possess charge, while later is non-ionic and regarded as being safe for long-term use [11].

Co-Solvents

Various organic solvents are used as cosolvents such as ethanol, propylene glycol and polyethylene glycol, which may help to dissolve large amounts of drug in liquid base.

Viscosity Enhancers

The viscosity of the emulsions can be altered by the use of additional material such as acetyl alcohol, tragacanth, beeswax and stearic acids etc.

Polymers

Polymer matrix (inert) present in 5 to 40% w/w,

which is not ionizable at physiological pH and able to form matrix. Examples are hydroxyl propyl methyl cellulose, ethyl cellulose, etc [12].

Mechanism of self-emulsification

Self-emulsification occurs when the entropy change that favors dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional emulsion is a direct function of the energy required to create a new surface between the oil and water phases and can be described by the equation:

DG=SNipri2s

Where, DG is the free energy associated with the process (ignoring the free energy of mixing), N is the number of droplets of radius r and s represents the interfacial energy. The two phases of emulsion tend to separate with time to reduce the interfacial area, and subsequently, the emulsion is stabilized by emulsifying agents, which form a monolayer of emulsion droplets, and hence reduces the interfacial energy, as well as providing a barrier to prevent coalescence [13-17].

Emulsification Process

The emulsification process may be associated with the ease with which water penetrates the oil-water interface with the formation of liquid crystalline phases resulting in swelling at the interface thereby resulting in greater ease of emulsification. However, for system containing co- surfactant, significant partitioning of components between the oil and aqueous phases may take place leading to a mechanism described as "diffusion and stranding", where by the oil is solubilized, leading to migration in to the aqueous phase.

Dilution Phases

Upon dilution of a SMEDDS formulation, the spontaneous curvature of the surfactant layer changes via a number of possible liquid crystalline phases. The droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, hexagonal phase, lamellar phase, cubic phase and various other structures until, after appropriate dilution, a spherical droplet will be formed again (Figure 1).

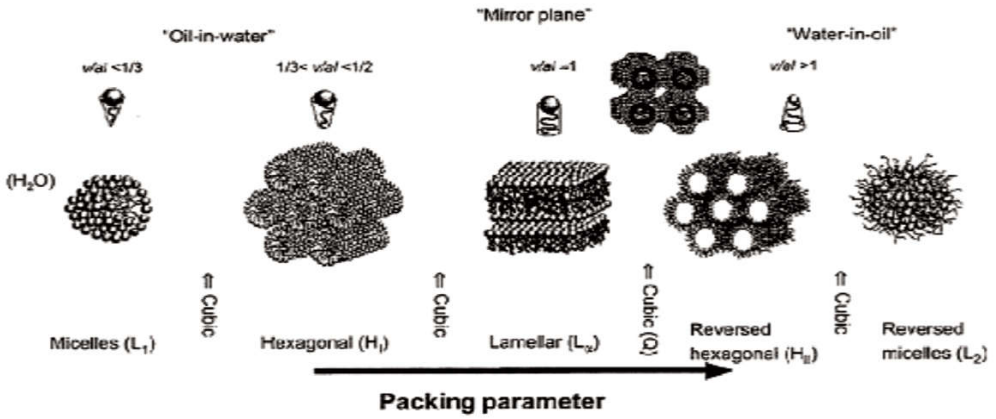


Fig. 1: Representation of the commonly encountered phases upon addition of water to an oil surfactant combination

Many roles have been ascribed to the occurrence of liquid crystalline phases upon aqueous dilution of a lipid formulation. Early work of Groves and Mustafa related the emulsification behavior to the phase behavior of the surfactant-oil mixtures with systems forming liquid crystals showing shorter emulsification times. The authors suggested that the ease of emulsification could be associated with the passage of water into the droplet, more precisely the ease with which the solvent may penetrate into the liquid crystalline phases formed on the surface of the droplet. The structures formed upon dilution have been ascribed an important role in the stability of the diluted micro emulsion and the rate of drug release [18].

Advantages of SMEDDS

Improvement in Oral Bioavailability

Dissolution rate dependant absorption is a major factor that limits the bioavailability of numerous poorly water soluble drugs. The ability of SMEDDS to present the drug to GIT in solubilised and micro emulsified form (globule size between 1-100 nm) and

subsequent increase in specific surface area enable more efficient drug transport through the intestinal aqueous boundary layer and through the absorptive brush border membrane leading to improved bioavailability. E.g. In case of halofantrine approximately 6-8 fold increase in bioavailability of drug was reported in comparison to tablet formulation [19].

Ease of Manufacture and Scale-up

Ease of manufacture and scale- up is one of the most important advantages that make SMEDDS unique when compared to other drug delivery systems like solid dispersions, liposomes, nanoparticles, etc., dealing with improvement of bio-availability. SMEDDS require very simple and economical manufacturing facilities like simple mixer with agitator and volumetric liquid filling equipment for large-scale manufacturing. This explains the interest of industry in the SMEDDS.

Reduction in Inter-Subject and Intra-Subject Variability and Food Effects

There are several drugs which show large inter-subject and intra-subject variation in absorption leading to decreased performance of drug and patient non-compliance. Food is a major factor affecting the therapeutic performance of the drug in the body. SMEDDS are a boon for such drugs. Several research papers specifying that, the performance of SMEDDS is independent of food and, SMEDDS offer reproducibility of plasma profile are available [20].

Ability to Deliver Peptides that are Prone to Enzymatic Hydrolysis in GIT

One unique property that makes SMEDDS superior as compared to the other drug delivery systems is their ability to deliver macromolecules like peptides, hormones, enzyme substrates and inhibitors and their ability to offer protection from enzymatic hydrolysis. The intestinal hydrolysis of prodrug by cholinesterase can be protected if Polysorbate 20 is emulsifier in micro emulsion formulation [21]. These systems are formed spontaneously without aid of energy or heating thus suitable for thermo labile drugs such as peptides.

No Influence of Lipid Digestion Process

Unlike the other lipid-based drug delivery systems, the performance of SMEDDS is not influenced by the lipolysis, emulsification by the bile salts, action of pancreatic lipases and mixed micelle formation. SMEDDS are not necessarily digested before the drug is absorbed as they present the drug in micro-emulsified form which can easily penetrate the mucin and water unstirred layer.

Increased Drug Loading Capacity

SMEDDS also provide the advantage of increased drug loading capacity when compared with conventional lipid solution as the solubility of poorly water soluble drugs with intermediate partition coefficient are typically low in natural lipids and much greater in amphilic surfactants, co surfactants and co-solvents.

Disadvantages of SMEDDS

One of the obstacles for the development of SMEDDS and other lipid-based formulations is the lack of good predicative *in vitro* models for assessment of the formulations.

- Traditional dissolution methods do not work, because these formulations potentially are dependent on digestion prior to release of the drug.
- This in*vitro* model needs further development and validation before its strength can be evaluated.
- The drawbacks of this system include chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30-60%) which irritate GIT.
- Moreover, volatile co solvents in the conventional self-microemulsifying formulations are known to migrate into the shells of soft or hard gelatin capsules, resulting in the precipitation of the lipophilic drugs [22].

Factor of SMEDDS

Nature and dose of the Drug

Drugs which are administered at very high dose are not suitable for SMEDDS unless they exhibit extremely good solubility in at least one of the components of SMEDDS, preferably lipophilic phase. The drugs which exhibit limited solubility in water and lipids (typically with log P values of approximately 2) are most difficult to deliver by SMEDDS. The ability of SMEDDS to maintain the drug in solubilised form is greatly influenced by the solubility of the drug in oil phase. As mentioned above if surfactant or co-surfactant is contributing to

the greater extent in drug solubilization then there could be a risk of precipitation, as dilution of SMEDDS will lead to lowering of solvent capacity of the surfactant or co-surfactant. Equilibrium solubility measurements can be carried out to anticipate potential cases of precipitation in the gut. However, crystallization could be slow in the solubilising and colloidal stabilizing environment of the gut. Pouton's study reveal that such formulations can take up to five days to reach equilibrium and that the drug can remain in a super-saturated state for up to 24 hours after the initial emulsification event. It could thus be argued that such products are not likely to cause precipitation of the drug in the gut before the drug is absorbed, and indeed that super-saturation could actually enhance absorption by increasing the thermodynamic activity of the drug. There is a clear need for practical methods to predict the fate of drugs after the dispersion of lipid systems in the gastro-intestinal tract.

Polarity of the Lipophilic Phase

The polarity of the lipid phase is one of the factors that govern the drug release from the microemulsions. The polarity of the droplet is governed by the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular weight of micronized for their propensity to inhibit crystallization and, thereby, generate and maintain the supersaturated state for prolonged time periods. A supersaturable drug delivery system (S-SMEDDS) of paclitaxel was developed selfmicroemulsifying employing HPMC as a precipitation inhibitor with a conventional SMEDDS formulation. *In-vitro* dilution of the S-SMEDDS formulation resulted in formation of a microemulsion, followed by slow crystallization of paclitaxel on standing. This result indicated that the system was supersaturated with respect to crystalline paclitaxel, and the supersaturated state was prolonged by HPMC in the formulation. In the absence of HPMC, the SMEDDS formulation underwent rapid precipitation, yielding a low paclitaxel solution concentration. A pharmacokinetic study showed that the paclitaxel SMEDDS formulation produced approximately a 10-fold higher maximum concentration (Cmax) and a 5-fold higher oral bioavailability (F~9.5%) compared with that of the orally administered Taxol formulation (F~ 2.0%) and the SMEDDS formulation without HPMC (F~1%). Applying the supersaturable SMEDDS approach, a reduced amount of surfactant can be used with HPMC in order to produce a temporarily supersaturated state with reduced solubilization. Thus a high free drug concentration would be obtained through generating and maintaining a supersaturated state in vivo and to increase the driving force for absorption. It is worth emphasizing that the significantly reduced amount of surfactant used in the S-SMEDDS formulation approach provides a better toxicity/safety profile than the conventional SMEDDS formulations. However, the underlying mechanism of the inhibited crystal growth and stabilized super saturation by means of these polymers is poorly understood even although several studies have been carried out to investigate this [23].

Biological Relevance of Solubility

In the oral route drugs must enter the systemic circulation to exert a therapeutic effect. Figure 1 illustrates the steps that a solid oral formulation passes through in order to get into the blood stream. First, the drug in its solid dosage form disintegrates. Then the solid drug particles dissolve within an aqueous environment (gastrointestinal tract) into drug molecules. The extent and rate at which drug molecules go into solution is determined by the drug solubility and dissolution rate, respectively. This is then followed by permeation of the drug molecules into the bloodstream through the intestinal membrane [24].

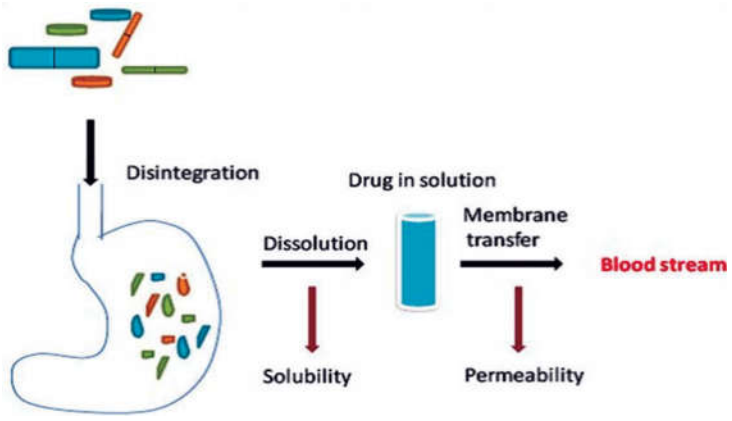


Fig. 2: Pathway of dosage form to reach blood stream

Two critical rate determining steps in the absorption of orally administered drugs are [25]

- Rate of dissolution
- Rate of drug permeation through biomembrane

Therapeutic effectiveness of a drug depends upon the bioavailability which is mostly dependent on the solubility of drug molecules [26]. Solubility behavior of drugs remains one of the most challenging aspect in formulation development. Because of their low aqueous solubility, up to 40% of new chemical entities fail to reach market despite exhibiting potential pharmacodynamic activities. Poorly aqueous soluble drugs are associated with slow drug absorption leading eventually to inadequate and variable bioavailability [27,28]. Oral absorption of a drug can be influenced by variety of factors, such as the physicochemical properties (e.g., pKa, solubility, stability, diffusivity, lipophilicity, polar-nonpolar surface area, presence of hydrogen bond functionalities, particle size and crystal form), physiological conditions (e.g., gastrointestinal pH, blood flow, gastric emptying, small intestinal transit time, colonic transit time and absorption mechanisms) and type of dosage form (e.g., tablet, capsule, solution, suspension and emulsion).

Biopharmaceutical Classification System (BCS)

The BCS system classifies immediate release solid oral dosage forms on the basis of solubility and permeability parameters. Fundamentally, the BCS is a scientific framework for classifying drug substances according to their aqueous solubility and their intestinal permeability. The BCS also takes account of the dissolution of the drug product and hence covers the three main factors which govern the rate and extent of drug absorption from immediate release (IR) solid oral dosage forms (e.g. tablets, capsules):

- Dissolution rate
- Solubility &
- Permeability

Table 1: Biopharmaceutical classification system for drugs

BCS class	Solubility	Permeability
Class I	High	High
Class II	Low	High
Class III	High	Low
Class IV	Low	Low

Table 2: Descriptive solubility profile [31]

Descriptive term	Parts of solvent required
Very soluble	Lessthan1
Freely soluble	From1to10
Soluble	From10to30
Sparingly soluble	From30to100
Slightly soluble	From100to1000
Very slightly soluble	From 1000to10000
Practically in soluble	10000 or more

Class I category drugs are defined as the drugs with the highest solubility and permeability, and therefore are readily absorbed when administered orally. The remaining classes II-IV suffer from poor solubility, permeability, or both and in turn affect the amount of absorption or bioavailability of the drug [29]. Solubility is a predetermined and rate limiting step for absorption, especially for class II drugs. According to Lipinksi, solubility is a much larger issue for drug discovery than permeability [30].

On the molecular level, solubility involves dissolution; the breaking of intermolecular attractions between solute-solute, solvent-solvent, and the formation of new interactions between solute-solvent [31]. It is these interactions, which are identified as ionic, van der Waals, and hydrogen bonding, which govern solubility. The first step is to free a solute molecule from its cavity. Next is to create a cavity in the solvent. Several factors play a role in determining the solubility of a compound. These include compound structure, pH, and temperature, physical state of the compound when placed in solution either solid or liquid, composition and physical conditions of solvent.

Solubility Measurements

Commonly measurements are taken by the traditional shake- flask method. Excess drug is added to solvent at desired temperature and shaken for 24 h, or for 7 days. The excess drug is removed from filtration, and the dissolved amount is detected by high pressure liquid chromatography or ultra-violet

spectroscopy or mass spectrometry detection [33].

Formulation Approaches to enhance solubility

There are different approaches available and reported in literature to enhance the solubility of poorly water soluble drugs [33]. The techniques are chosen on the basis of certain aspects such as properties of drug under consideration, nature of excipients to be selected and nature of intended dosage form.Approaches to enhance solubility are commonly based on chemical or physical modifications. Here different most exploited approaches for enhancement of solubility are illustrated below.

Physical Modification

- Particle size modification
- Micronization
- Nanosuspension

Modification of the Crystal Habit

- Polymorphs
- Pseudopolymorphs

Drug Dispersion in Carriers

- Eutectic mixtures
- Solid dispersions
- Solid solution
- Complexation
- Solubilization by surfactants
- Nanotechnology based approaches
- Chemical modification
- Formation of soluble prodrug
- Formation of salt of the compound
- Preparation of covalent drug conjugates

Dosage Forms from Self-Emulsifying System

Self-Emulsifying Capsule

It is a capsules containing liquid or semisolid form of self-emulsifying system. In the GIT, the capsules get dispersed to SES uniformly in the fluid to micron size, enhancing bioavailability. Second type of self-emulsifying capsule is solid SES filled into capsule.

Self-Emulsifying Tablets

S.nazzal et al developed self nanoemulsified tablet dosage form of Ubiquinone. The main objectives of this study were to study effect of formulation ingredients on the release rate of Ubiquinone and to evaluate an optimized self nanoemulsified tablets formulation. The first prepared self nanoemulsion system containing Ubiquinone was prepared as nanoemulsion, this nanoemulsion was adsorbed by granular materials and then compressed to form tablets. The optimized formulation of coenzyme Q10 self nanoemulsified tablet dissolution profile showed that 80 90% drug release took place in 45 minute [35].

Self-Emulsifying Beads

Self-emulsifying system can be formulated as a solid dosage form by using less excipient. Patil and Paradkar discovered that deposition of SES into microporous polystyrene beads was done by solvent evaporation. Porous polystyrene beads with complexinternal void structures were typically produced by co-polymerising styrene and divinyl benzene. It is inert and stable over a wide range of pH, temperature and humidity. Geometrical features, such as bead size and pore architecture of PPB, were found to govern the loading efficiency and in vitro drug release from SES loaded PPB [36].

Self-Emulsifying Microsphere

You et al. formulated solid SE sustained release microspheres using the quasi emulsion solvent diffusion method for the spherical crystallization technique. Zedoary turmeric oil release behavior could be controlled by the ratio of hydroxypropyl methylcellulose acetate succinate to Aerosil 200 in the formulation. The plasma concentration time profiles were achieved after oral administration of such microspheres into rabbits, with a bioavailability of 135.6% with respect to the conventional liquid SMEDDS [37].

Self-Emulsifying Nano Particle

Nanoparticle technology can be applied to the formulation of self-emulsifying nanoparticle. One of the solvent was injection; in this method the prepared molten lipid Mass contained lipid, surfactant and drug. This lipid molten mass was injected drop wise

into a non-solvent system. This is filtered and dried to get nanoparticles. By these method 100 nm size particles with 70 75% drug loading efficiency was obtained. Second technique is sonication emulsion diffusion evaporation; by this method co load 5 flurouracil and antisense EGFR plasmids into biodegradable PLGA/O CMC nanoparticles. The mixture of PLGA and O CMC had a SE effect; with no additional surfactant required. Trickler et al. developed a novel nanoparticle drug delivery system consisting of chitosan and glycerylmonooleatefor the delivery of paclitaxel. These chitosan/GMO nanoparticles, with bioadhesive properties increased cellular association and were prepared by multiple emulsion (o/w/o) solvent evaporation methods.

Application of Submicron Emulsion

Cosmetics

Submicron emulsion has recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredients in particular skin layers. Due to their lipophilic interior they are more suitable for the transport of lipophilic compounds than liposomes. Similar to liposomes they support the skin penetration of active ingredients and thus increase their concentration in skin. Another advantage is the small sized droplet with its high surface area allowing effective transport of the active to skin.

New Jersey-Based TRI

K Industries and its parent company Kemira have launched a new nano-based gel aimed at enhancing the efficacy of a wide range of skin care products. Kemira Nano Gel is said to be a unique submicron emulsion carrier system that has been designed around easy formulation, combined with the added benefits brought about by its nanotechnology properties. Antimicrobial- Antimicrobial submicron emulsions are oil-in-water droplets that range from 200 to 600nm. They are composed of oil and water and are stabilized by surfactants and alcohol. The submicron emulsion has a broad-spectrum activity against bacteria, enveloped virus, fungi and spores. The submicron particles are thermodynamically driven to fuse with lipid containing organism. The fusion is enhanced by the electrostatic attraction between the cationic charge of emulsion and anionic charge on pathogen. When enough nanoparticles fuse with pathogens, they release part of energy trapped within emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lyses and death.

Bio-Terrorism Attack

Based on their antimicrobial activity, research has begun on use of submicron emulsion as a prophylactic medication, a human protective treatment, to protect people exposed to bio-attack pathogens such as anthrax and ebola.

Mucosal Vaccines

Submicron emulsions are being used to deliver either recombinant proteins or organisms to a mucosal surface to produce an immune response. The first application, an influenza vaccine and an HIV vaccine, can proceed to clinical trials. The submicron emulsion causes proteins applied to the mucosal surface to be adjuvant and it facilitates uptake by antigen-presenting cells.

Non-Toxic Disinfectant Cleaner

A breakthrough nontoxic disinfectant cleaner for use in commercial markets that include healthcare, hospitality, travel, food processing, and military applications has been developed by *invitro* systems, Inc. that kills tuberculosis and a wide spectrum of viruses, bacteria and fungi in 5-10 min without any of the hazards posed by other categories of disinfectants. The product needs no warning labels. It does not irritate eyes and can be absorbed through the skin, inhaled, or swallowed without harmful effects.

Cell Culture Technology

Cell cultures are used for *in vitro* assays or to produce biological compounds, such as antibiotic or recombinant proteins. To optimize cell growth, the culture medium can be supplemented with a number of defined molecules or with blood serum. Up to now, it has been very difficult to supplement the media with oil-soluble substance that are available to the cells, and only small amounts of these lipophilic compounds could be absorbed by the cells. Submicron emulsions are a new method for the delivery of oil-soluble substances to mammalian cell cultures. The delivery system is based on a nanoemulsion which is stabilized by phospholipids. These nanoemulsions are transparent and can be passed through 0.1 mm filters for sterilization. Nanoemulsion droplets are easily taken up by the cells. The encapsulated oil-soluble substances therefore have a high bioavailability to cells in culture. The advantage of using nanaoemulsions in cell culture technology are batter uptake of oil-soluble supplements in cell culture, improve growth and vitality of cultures cells, and allowance of toxicity

studies of oil-soluble drugs in cell cultures.

Cancer Therapy

The effects of the formulation and particle composition of gadolinium (Gd)-containing lipid NE (Gd-nanoLE) on the bio-distribution of Gd after its intravenous (IV) injection in D1-179 melanoma-bearing hamsters were evaluated for its application in cancer neutron-capture therapy. Bio-distribution data revealed that Brij 700 and HCO-60 prolonged the retention of Gd in the blood and enhanced its accumulation in tumors [38].

Material and Method

Table 4.1: Material and reagent used in present work

Materials	Background/Role
Cefdinir	API
Oleic acid	Oil
Castor oil	Oil
Cod liver oil	Oil
Sun flower oil	Oil
Soybean oil	Oil
Palm oil	Oil
Corn oil	Oil
Labrafac	Oil
Cremophore	Surfactant
Labrafil 1944	Surfactant
Span-20	Surfactant
Tween-80	Surfactant
Propylene glycol	Solvent
Poly Ethylene Glycol-400	Solvent
Transcutol	Solvent

Table 4.2: Instruments and apparatus used in current work

Sr. No.	Instruments/Apparatus	Company
1	Digital balance	Shimadzu
2	pH meter	Labtronics
3	Dissolution test apparatus	Electrolab dissolution test
4	UV-Visible Spectrophotometer	Labtronics
5	Brookfield viscometer	Brookfield viscometer
6	Sonicator	Ultrasonic bath

Spectrophotometric Estimation of Cefdinir

In the present study cefdinir will quantitatively analyze by UV-Visible spectrophotometer in dissolution fluid. Standard curve of Cefdinir will be generated in Methanol and 0.1N HCl.

Preparation of 0.1 N HCl

8.5ml of hydrochloric acid diluted with distilled water to produce 1000 ml.

Determination of ëmax of Cefdinir

Weigh accurately require amount of drug will dissolve in 0.1 N HCl. A stock solution will be prepared by withdrawing 10 ml of the above solution and made up to 100 ml. Make a serial dilution up to appropriate microgram. Then ëmax of Cefdinir will be measured by using UV spectroscopy.

Preparation of Standard Curve for Cefdinir in Methanol

Weigh accurately require amount of Cefdinir and transfer in 100 ml of volumetric flask and volume will be made up with methanol to the mark. Then standard curve of Cefdinir will be measured by using UV spectroscopy.

Preparation of Standard Curve for Cefdinir in 0.1 N HCl

Weigh accurately require amount of Cefdinir and transfer in 100 ml of volumetric flask and volume will be made up with 0.1N HCl to the mark. Then standard curve of Cefdinir will be measured by using UV spectroscopy.

Preliminary Study

Solubility Studies

Solubility studies will be conducted by placing an excess amount of cefdinir in a 2mL micro tube containing 1mL of the vehicle, and the mixture will be heated at 60^0C in a water bath to facilitate the solubilization using a vortex mixer. Mixtures will be equilibrated at 25^0C for 48h in a water bath. The

equilibrated samples will be centrifuged at 3000×*g* for 15min to remove the un dissolved cefdininr. The supernatant will be taken and diluted with methanol for quantification of Cefdinir by UV Spectro photometer [67].

Emulsification Study for Surfactant and Co-Surfactant

The surfactant and co-surfactant evaluated on the basis of their potential to emulsify the selected oil phase. In this study 10% solutions of different surfactant and co-surfactants will be prepared with vigorous vortex. If a uniform clear solution will be visually obtained, the addition of oil will be continued until the solution became cloudy and the total amount of oil added will be recorded [68].

Formulation of Self-Microemulsifying Drug Delivery System Containing Cefdinir

Construction of Pseudo-Ternary Phase Diagrams

Pseudo ternary phase diagrams of oil, surfactant/co-surfactant (S/Co-S), and water will be developed using water titration method. The mixtures of oil and S/Co-S at certain weight ratios will dilute with water in a drop wise manner. For each phase diagram at a specific ratio of S/Co-S (i.e. 1:1, 1:3 and 3:1 wt/wt), a transparent and homogenous mixture of oil and S/Co-S will be form by vortexing for 5 minutes. Then each mixture will be titrated with water and visually observed for phase clarity. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred will be derived from weight measurements. These values will be used to determine boundaries of microemulsion domain corresponding to chosen value of oils, as well as S/Co-S mixing ratio. To determine effect of drug addition on micro emulsion boundary, phase diagrams will be also constructed in presence of drug using drug-enriched oil as hydrophobic component. Phase diagrams will be constructed using Tri plot v1-4 software.

Preparation of SMEDDS Formulations

A series of SMEDDS formulations will prepare using S/Co-S combination and selected oil by using Simplex Lattice Design. The actual concentrations of oil, surfactant and co-surfactant will be transformed based on the simplex lattice design so that minimum concentration corresponds to zero and maximum concentration corresponds to one. Briefly, accurately weighed cefdinir will place in a glass vial, and require quantity of oil, surfactant, and co-surfactant will add. Then all components will be mixed by gentle stirring and vortex mixing and will be warmed at 40°C on a magnetic stirrer, until cefdinir will be perfectly dissolved. The mixture will be store at room temperature until further use [69].

Formulation Optimization

Simplex lattice design will be used to optimize the formulation of SMEDDS containing cefdinir. The concentrations of oil (X1), surfactant (X2) and co-surfactant (X3) will be chosen as the independent variables. The emulsification time, % Transmittance and cumulative %release in 20 minute will be taken as responses (Y), respectively. The equation for simplex lattice model is described as follows:

$$Y=\beta_o+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3$$

Where *Y* is the dependent variable and *âi* is the estimated coefficient for the factor *Xi*. The major effects (*X1, X2,* and *X3*) represent average results of changing one factor at a time from its low to high value, the interactions *X1X2, X2X3, X1X3,* and *X1X2X3*, and polynomial terms show how the responses change when two or three factors change simultaneously.

According to simplex lattice design and the selected concentration ranges of oil, surfactant and co-surfactant, seven different formulations of SMEDDS containing cefdinir is constructed.

The responses for seven formulations will be used to fit an equation for simplex lattice model [70-71]. Which then can predict properties of all possible formulations. With the aid of Microsoft Excel, the model equation will develop to represent the relationship between the self-emulsification time, %transmittance and cumulative %release in 20 min the measured characteristics.

Characterization

Refractive Index and Turbid metric Evaluation

The Self-Micro emulsifying system (SMEDDS) will add to 0.1N hydrochloric acid (250 ml) and purified water (250 ml) under continuous stirring (50 rpm) on a magnetic plate at ambient temperature. Then Refractive index of system will be measured by using an Abbe's Refracto meter and turbidity will be measured by measuring % transmittance at 286.4 nm in UV-Visible spectrophotometer [67].

Measurement of Droplet Size and Zeta Potential

Droplet size distribution and zeta potential of

SMEDDS will be determined using Zetatrac. Zetatrac utilizes a high frequency AC electric field to oscillate the charged particles. The Brownian motion power spectrum is analyzed with Modulated Power Spectrum (MPS) technique, a component of power spectrum resulting from oscillating particles. Samples will be diluted to 250 ml with purified water. Diluted samples will be directly placed into cuvette and measure particle size and zeta potential. Zetatrac is controlled by Microtrac FLEX Operating Software.

Drug Content

Cefdinir from pre-weighed SMEDDS will be extracted by dissolving in 25 ml methanol. Then methanolic extract will be separated out and cefdinir content in methanolic extract will analyze spectrophotometrically (UV-Visible spectrophotometer) at 286.4 nm, against standard methanolic solution of cefdinir [72].

Measurement of Viscosity

Viscosity of cefdinir SMEDDS will be measured by using Brookfield viscometer at 25°C temperature. Spindle S-61 will be selected for measurement of viscosity of various SMEDDS formulation. Viscosity of SMEDDS will be measured at 30 rpm before dilution and after dilution with aqueous phase (250 ml) [73].

Measurement of PH

pH of cefdinir SMEDDS will be measured by using pH meter at room temperature. pH of SMEDDS will measure before dilution and after dilution with aqueous phase (250 ml) [73].

Self-Emulsification and Precipitation Assessment

Evaluation of the self-emulsifying properties of SMEDDS formulations will be performed by visual assessment as previously reported. In brief, different compositions will be categorized on speed of emulsification, clarity and apparent stability of resultant emulsion. Visual assessment will be performed by drop wise addition of pre-concentrate (SMEDDS) into 250 ml of distilled water. This will be done in a glass beaker at room temperature, and contents will gently stirr magnetically at 50-100 rpm. Precipitation will be evaluated by visual inspection of resultant emulsion after 24 hours. The formulations will be categorized as clear (transparent or transparent with bluish tinge), nonclear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours) [36].

In Vitrodiffusion Studies

In vitro diffusion studies were carried out for all formulations using dialysis technique. One end of pre-treated dialysis membrane tubing was with thread and then diluted SMEDDS was placed in it. The other end of tubing was also secured with thread and was allow to rotate freely in dissolution vessel of USP 24 type II dissolution test apparatus (Electrolab TDT-06P, India) that contained 250 ml dialyzing medium maintained at 37 ± 0.5 °C and stirred at 50 rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through whatman filter paper (No. 41), were analyzed spectrophotometrically at 286.4 nm for Cefdinir content. The data was analyzedusing the software.

Accelerated Stability Tests: Centrifugation and Freeze–Thaw Cycle

Cefdinir SMEDDS were diluted with 250 ml and 900 ml aqueous phase (distilled water and 0.1 N HCL) and centrifuged at 5000 rpm for 30 min. In addition, it was subjected to freeze–thaw cycle by storing it at -20°C for 24 hour and then for another 24 hourat 40°C. Micro emulsions were observed visually for phase separation and drug precipitation.

Result and Discussion

Spectrophotometric Estimation of Cefdinir

In the present study Cefdinir has been quantitatively analysed by UV-Visible spectrophotometer in dissolution fluid. Standard curve Cefdinir has been generated in methanol and 0.1N HClandphosphate buffer (pH 6.8).

Determination of ëmax of Cefdinir

100 µg/ml solution of Cefdinir in methanol was scanned in UV range of 200 to 400nm. Cefdinir showed maximum absorbance at 286.4 nm. Thus 286.4nm was taken as ëmax. Similar procedure was adopted with 0.1N HClandëmax in 0.1N HCl was also found out to be 286.4 nm. Figure 5.1, 5.2 and 5.3 shows absorbance spectra of Cefdinir in methanol, 0.1 N HCl and phosphate buffer (pH 6.8) respectively.

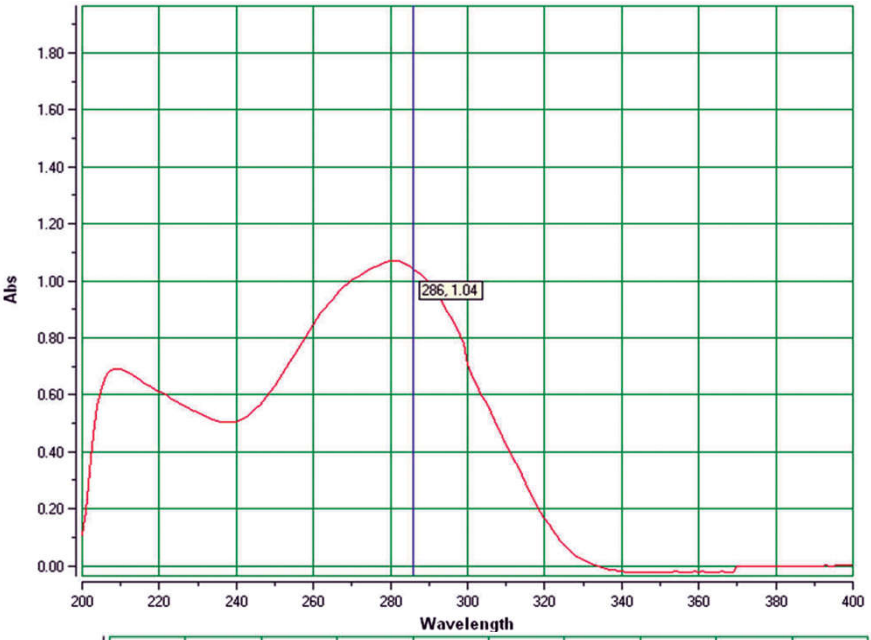


Fig. 5.1: UV spectrum of cefdinir in 0.1 N HCl

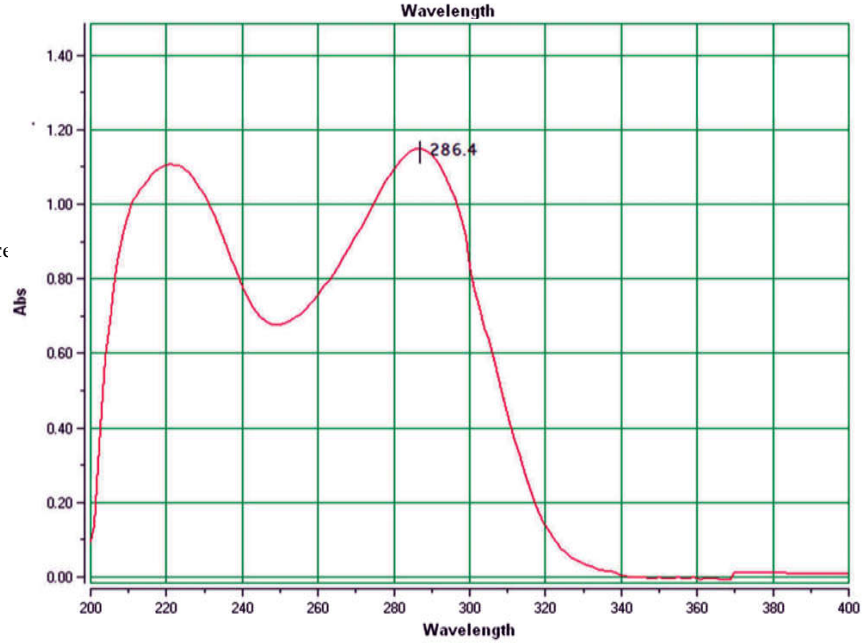


Fig. 5.2: UV spectrum of ce in methanol)

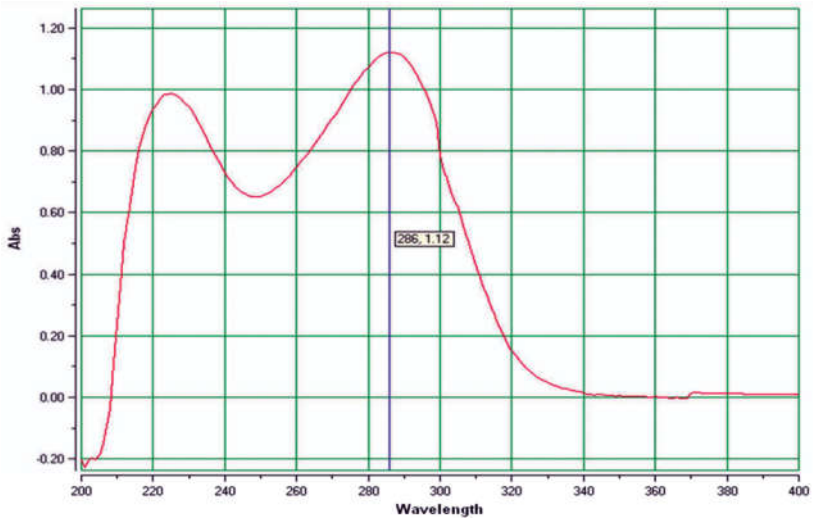


Fig. 5.3: UV spectrum of cefdinir in phosphate buffer (pH 6.8)

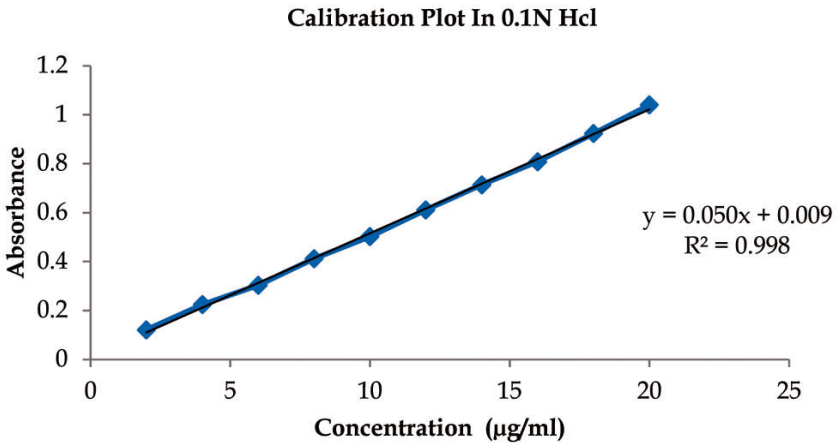


Fig. 5.4: Standard curve of cefdinir in 0.1 N HCl)

Table 5.1: Standard curve data of cefdinir in 0.1 N HCl)

Sr. no	Concentration (µg/ml)	Absorbance I	II	III	Average absorbance	Standard deviation
1	0	0	0	0	0	0
2	2	0.134	0.121	0.119	0.12466667	0.008144528
3	4	0.263	0.224	0.22	0.23566667	0.023755701
4	6	0.336	0.303	0.313	0.31733333	0.016921387
5	8	0.403	0.412	0.424	0.413	0.010535654
6	10	0.54	0.502	0.522	0.52133333	0.01900877
7	12	0.624	0.611	0.591	0.60866667	0.016623277
8	14	0.751	0.714	0.699	0.72133333	0.026764404
9	16	0.857	0.808	0.855	0.84	0.027730849
10	18	0.949	0.923	0.949	0.94033333	0.015011107
11	20	1.057	1.04	1.053	1.05	0.008888194

Preparation of standard curve for cefdinir in 0.1 N HCl

Weigh accurately 10 mg ofCefdinir and transfer in 100 ml of volumetric flask and volume was made up to the mark with 0.1 N HCl. Aliquots were taken from prepared stock solution and were appropriately diluted to prepare 2, 4, 6, 20 µg/ml and then absorbance were taken at 286.4 nm, keeping 0.1N HCl as blank solution. Data and Figure of standard curve were shown in Table 5.1 and Figure 5.4 respectively.

Results of Weighted Linear Regression Analysis

R square 0.998

R square Y=0.050x + 0.009

Linearity was observed between 0-10 µg/ml concentrations of Cefdinir, therefore the drug obeys beer's law.

Preparation of Standard Curve for Cefdinir in Methanol

Weigh accurately 10 mg of Cefdinir and transfer in 100 ml of volumetric flask and volume was made up to the mark with methanol. Aliquots were taken from prepared stock solution and were appropriately diluted to prepare 2, 4, 6, 20 µg/ml and thenabsorbance were taken at 286.4 nm, keeping methanol as blank solution. Data and Figure of standard curve were shown in Table 5.2 and Figure 5.5 respectively.

Results of Weighted Linear Regression Analysis

R square 0.997

R square Y=0.05x + 0.105

Linearity was observed between 0 -10 µg/ml concentrations of Cefdinir, therefore the drug obeys beer's law.

Preparation of Standard Curve for Cefdinir in Phosphate buffer (pH 6.8)

Weigh accurately 10 mg ofCefdinir and transfer in 100 ml of volumetric flask and volume was made up to the mark with phosphate buffer(pH 6.8). Aliquots were taken from prepared stock solution and were appropriately diluted to prepare 2, 4, 6, 20 µg/ml and then, absorbance were taken at 286.4 nm, keeping phosphate buffer as blank solution. Data

Table 5.2: Standard curve data of cefdinir in methanol

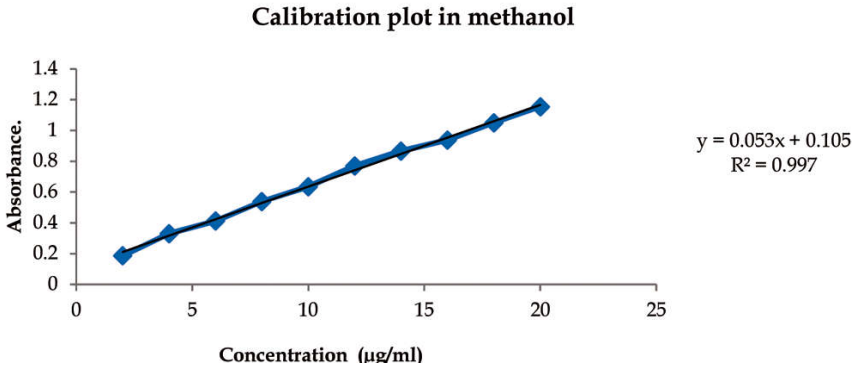


Fig. 5.5: Standard curve of cefdinir in methanol

Table 5.2: Standard curve data of cefdinir in methanol

Sr. no	Concentration (µg/ml)	Absorbance 1	2	3	Average absorbance	Standard deviation
1	0	0	0	0	0	0
2	2	0.187	0.155	0.17	0.17066667	0.016010413
3	4	0.33	0.3	0.302	0.31066667	0.016772994
4	6	0.412	0.44	0.414	0.422	0.015620499
5	8	0.539	0.512	0.53	0.527	0.013747727
6	10	0.635	0.633	0.63	0.63266667	0.002516611
7	12	0.77	0.733	0.755	0.75266667	0.018610033
8	14	0.867	0.855	0.868	0.86333333	0.007234178
9	16	0.938	0.955	0.944	0.94566667	0.008621678
10	18	1.05	1.02	1.03	1.03333333	0.015275252
11	20	1.154	1.125	1.13	1.13633333	0.015502688

Table 5.3: Standard curve data of cefdinir in phosphate buffer

Sr. no	Concentration (µg/ml)	Absorbance I	II	III	Average absorbance	Standard deviation
1	0	0	0	0	0	0
2	2	0.166	0.153	0.123	0.14733333	0.022052967
3	4	0.274	0.25	0.221	0.24833333	0.026539279
4	6	0.369	0.36	0.311	0.34666667	0.031214313
5	8	0.413	0.452	0.415	0.42666667	0.021962089
6	10	0.532	0.552	0.501	0.52833333	0.025696952
7	12	0.634	0.649	0.613	0.632	0.018083141
8	14	0.701	0.761	0.72	0.72733333	0.030664855
9	16	0.839	0.856	0.813	0.836	0.021656408
10	18	0.955	0.923	0.922	0.93333333	0.018770544
11	20	1.128	1.01	1.05	1.06266667	0.06001111

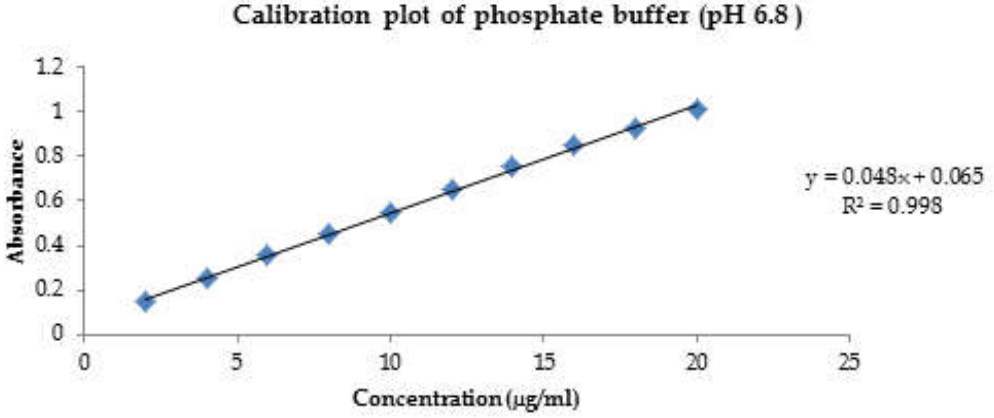


Fig. 5.6: Standard curve of cefdinir in phosphate buffer pH 6.8)

and Figure of standard curve were shown in Table 5.3 and Figure 5.6 respectively.

Results of Weighted Linear Regression Analysis

R square 0.998

R square Y = 0.048x + 0.065

Linearity was observed between 0 -10 µg/ml concentrations of Cefdinir, therefore the drug obeys beer's law.

Preliminary Study

Selection Cefdinir SMEDDS Components

Solubility Study

Screening of SMEDDS formulation involves formulation composition should be simple, safe, non-toxic and compatible. It should possess good solubility and large efficient self-micro emulsification region which should be found in pseudo-ternary

Table 5.4: Solubility of cefdinir in various oils, surfactants and co-surfactants

All Excipiants solubility data			
Sr. no.	**Name of Excipiants**	**Type of Excipiants**	**Solubility (mg/ml)***
1	soya been oil	Oil	0.568 ± 0.5685
2	palm oil	Oil	0.0835 ± 0.0473
3	castor oil	Oil	0.431 ± 0.3146
4	cod liver oil	Oil	0.236 ± 0.3949
5	oleic oil	Oil	0.542 ± 0.5388
6	sunflower oil	Oil	0.166 ± 0.0449
7	Olive oil	Oil	0.0331 ± 0.0057
8	Labrafac	Oil	1.087 ± 0.159
9	Tween-20	Surfactant	0.8528 ± 0.4075
10	Tween-80	Surfactant	0.68 ± 0.5600
11	Cremophore RH 40	Surfactant	0.71 ± 0.014
12	Labrafil M 1944	Surfactant	0.721 ± 0.007
13	Span-80	Surfactant	0.8065 ± 0.811
14	PEG	Co-Surfactant	1.4495 ± 0.5551
15	PEG-400	Co-Surfactant	2.65 ± 1.801
16	Transcutol	Co-Surfactant	0.547 ± 0.057

Note= *Data presented as a mean value ± standard error, n = 2
Abbreviation = PEG (Poly ethylene glycol)

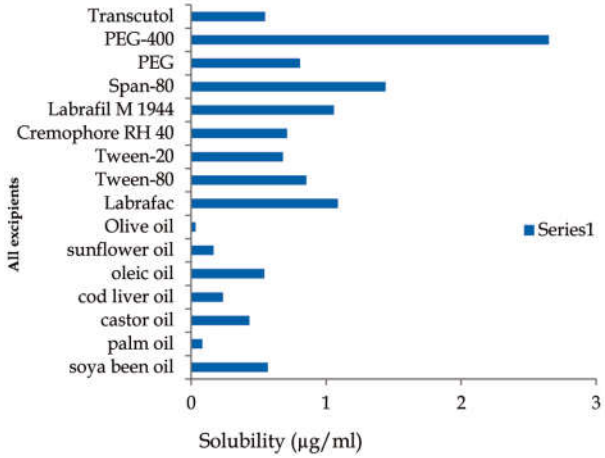


Fig. 5.7: Solubility studies of cefdinir in various oils, surfactants and co-surfactants data expressed as mean ± SD (*n*= 2)

phase diagram, and have efficient droplet size after forming micro emulsion.

Vehicles should have good solubilizing capacity of drug substance, which is essential for composing SMEDDS. The results of solubility of Cefdinir in various vehicles were shown in Table 5.3 and Figure 5.4. Cefdinir had highest solubility in labrafac, tween-80 and polyethylene glycol-400 as liquid vehicle, surfactant and co-surfactant respectively with comparison to others. So, labrafac as oil, tween-80 as surfactant and polyethylene glycol-400 as co-surfactant was selected for optimal SMEDDS formulation resulting in improved drug loading capabilities. Furthermore, with respect to its safety, labrafac, tween-80 and PEG-400 are included in the FDA Inactive Ingredients Guide.

Emulsification Study

The surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of SMEDDS that can provide the correct curvature at interfacial region for SMEDDS.

Emulsification study results were shown that tween 80 has highest solubility capacity of oil was higher than other surfactant. So, tween 80 was selected as surfactant then selection of co-surfactant this study performed again. From the result were shown that PEG 400 has highest solubility capacity of oil. There tween 80 and PEG 400 were selected as surfactant and co-surfactant respectively.

Table 5.5: Emulsification study

Surfactants*	mL of oil**	Co-Surfactants*	mL of oil**
Tween 80	0.68 ± 0.5600	PEG	1.4495 ± 0.5551
Tween 20	0.8528 ± .4075	PEG-400	2.65 ± 1.801

*10 %v/v surfactant aqueous solution, **mL of labrafac oil

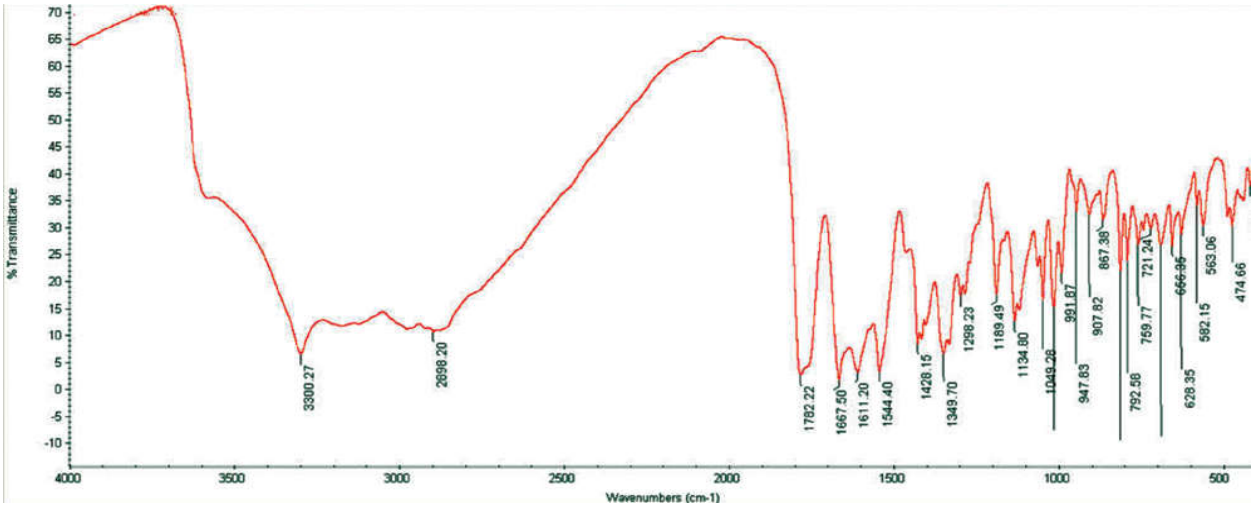


Fig. 5.8: FTIR spectra of cefdinir

FT-IR spectra of Cefdinir figure 5.8 exhibited principal peaks at 3300.27 cm-1 (= CH- H-), 2898.20 cm-1 (C-H stretching), and 1782.22 cm-1 presence of ester group.

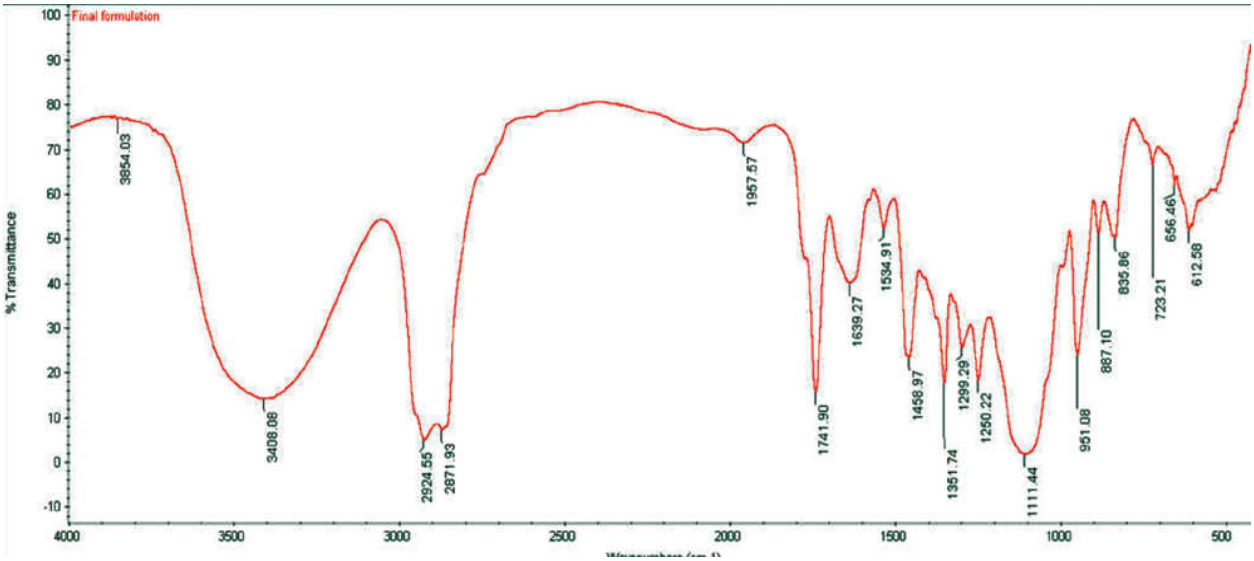


Fig. 5.9: FTIR spectra of SMEDDS formulation

FTIR spectra of SMEDDS formulation (figure 5.7) exhibited principal peaks at 3408.08 cm-1 (-OH{broad peak}), 2924.55 cm-1 (C-H streaching), and 1741.90 cm-1 presence of ester group

Fourier Transforms Infrared Spectroscopy (FT-IR) Studies

FT-IR study was carried out to determine possible drug interaction with excipients whichutilised in the formation of Cefdinir SMEDDS.

All these peaks clearly indicate that they are very much closely similar to the peaks of pure drug.

Formulation of Self-Micro emulsifying Drug Delivery System (SMEDDS)

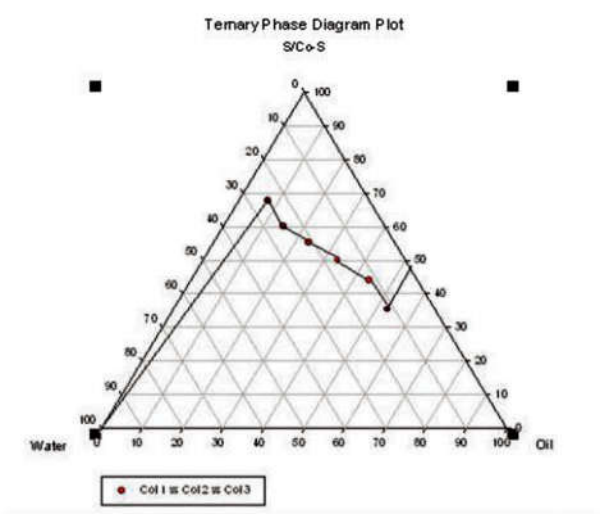
Containing Cefdinir

Pseudo-Ternary Phase Diagrams

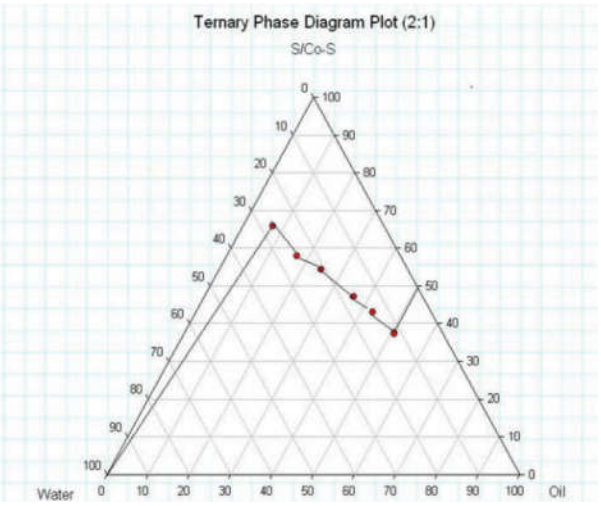
Phase diagrams were constructed to obtain optimum concentrations of oil, water, surfactant, and co-surfactant. SMEDDS form fine oil–water emulsions with only gentle agitation, upon its introduction into aqueous media.

Phase behavior investigations of this system demonstrated suitable approach to determining water phase, oil phase, surfactant concentration, and co-surfactant concentration with which transparent, one phase low-viscous micro emulsion system was formed.

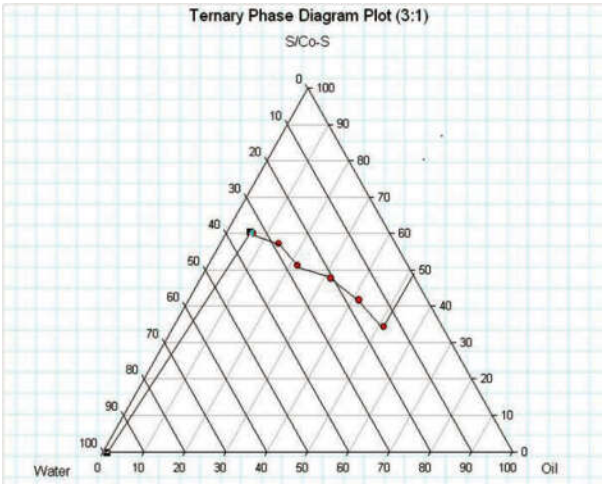
Since free energy required to form an emulsion is very low, formation is thermodynamically spontaneous. Surfactants form a layer around emulsion droplets and reduce interfacial energy as well as providing a mechanical barrier to coalescence. The visual test is measured apparent spontaneity of emulsion formation.



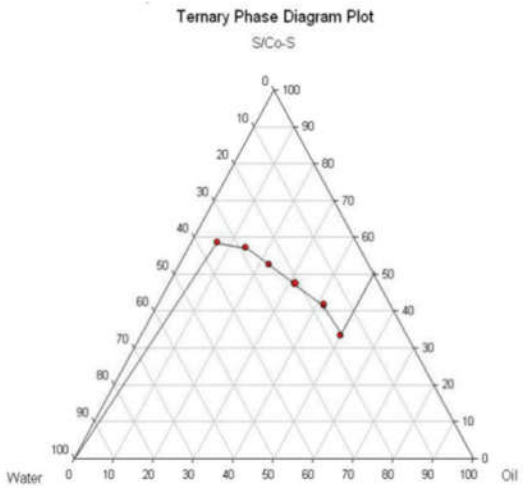
(a) S/Cos ratio 3:1



(b) S/Cos ratio 2:1



(c) S/Cos ratio 3:1



(d) S/Cos ratio 4:1

Fig. 5.10: Pseudo-Ternary Phase Diagrams (a) S/Cos ratio 1:1 (b) S/Cosratio 1:3 (c) S/Cos ratio 3:1 (d) S/Cos ratio 4:1)

The series of SMEDDS were prepared and their self-micro emulsifying properties were observed visually. Pseudo-ternary phase diagrams were constructed to identify the self-micro emulsifying regions and to optimize concentration of oil (Figure 5.10).

It was observed that increasing concentration of surfactant such as tween-80 in SMEDDS formulation increased spontaneity of self- emulsification region. Therefore, much higher concentration of surfactant, much higher self-emulsifying region in phase diagrams. The ratio of surfactant to co-surfactant was very effective to a stable and efficient SMEDDS formation. The phase diagrams were constructed at ratio of surfactant/co-surfactant 1:1, 2:1, 3:1, 4:1 (w/w). However, stability of self-emulsifying droplets from ratio of S/Co-S = 1:1, 2:1, 3:1 (w/w) was decreased because of precipitation after a few hours. So, ratio of S/Co-S = 4:1 was chosen in formulation. Figure shows phase diagrams which identify area of stable micro emulsion in presence of Cefdinir when diluted with aqueous media.

However, excessive amount of co-surfactant will cause system to become less stability for its intrinsic high aqueous solubility and lead to droplet size increasing as a result of expanding interfacial film. Hence, optimal ratio of surfactant to co-surfactant was selected to be 4:1.

Preparation of SMEDDS Formulations

A series of SMEDDS formulations were prepared using Tween 20 and PEG 400 as S/Co-S combination and labrafac as oil by using Simplex Lattice Design. The actual concentrations of oil, surfactant and co-surfactant were transformed based on the simplex lattice design so that minimum concentration corresponds to zero and maximum concentration corresponds to one (Shown in Table 5.6). Briefly, accurately

weighedCefdinir was placed in a glass vial, and require quantity of oil, surfactant, and co-surfactant was added. Then all components were mixed by gentle stirring and vortexmixing and were warmed at 40°C on a magnetic stirrer, until Cefdinir was perfectlydissolved. The mixture was store at room temperature until further use.

Table 5.6: Developed formulations with their actual and transformed value as per simplex lattice design

Formulation code	Dose of Cefdinir	Components (in ml)		
		Labrafac	Tween-80	PEG 400
F-1	10 mg	0.1	0.65	0.25
F-2	10 mg	0.2	0.60	0.20
F-3	10 mg	0.3	0.55	0.15
F-4	10 mg	0.4	0.50	0.10
F-5	10 mg	0.5	0.45	0.05
F-6	10 mg	0.6	0.30	0.10
F-7	10 mg	0.7	0.20	0.10

Formulation Optimization

Simplex lattice design was used to optimize the formulation of SMEDDS containing Cefdinir. The concentrations of oil (X1), surfactant (X2) and co-surfactant (X3) were chosen as the independent variables. The emulsification time and Cumulative %release in 20 minute was taken as responses (Y), respectively. The equation for simplex lattice model is described as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{23}X_1X_3 + b_{123}X_1X_2X_3 \quad (1)$$

Where *Y* is the dependent variable and βi is the estimated coefficient for the factor *Xi*. The major effects (*X1, X2,* and *X3*) represent average results of changing one factor at a time from its low to high value, the interactions *X1X2, X2X3, X1X3,* and *X1X2X3*, a polynomial term.

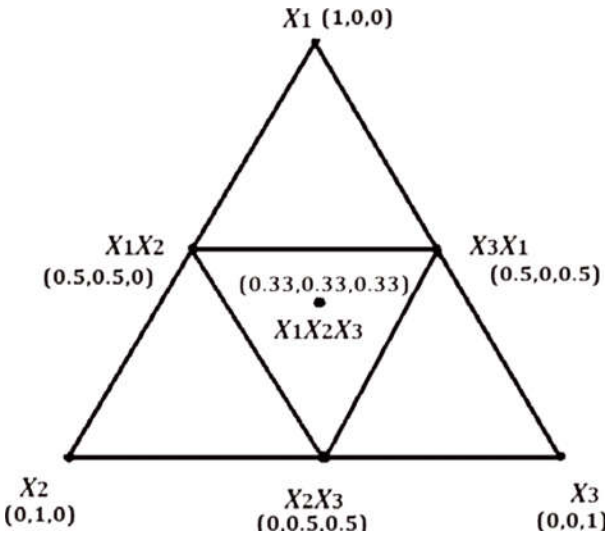


Fig. 5.11: Equilateral triangle representing simplex lattice design for three components

Table 5.8: Code representation of formulation by simplex lattice design

Formulation code	Code Representation	Concentration (Transformed value)		
		Oil	Surfactant	Co- Surfactant
F-1	X1	1	0	0
F-2	X2	0	1	0
F-3	X3	0	0	1
F-4	X1X2	0.5	0.5	0
F-5	X2X3	0	0.5	0.5
F-6	X1X3	0.5	0	0.5
F-7	X1X2X3	0.33	0.33	0.33

Table 5.9: Emulsification time (*Y1*) and cumulative percent release in 20 minute (*Y2*) of seven different formulations as per simplex lattice design

Formulation code	formulation component			Emulsification time sec (Y1)	Cumulative Percent release in 20(Y2)
	Oil	Surfactant	Co- Surfactant		
F-1	1	0	0	39	24.12
F-2	0	1	0	25	30.24
F-3	0	0	1	47	10.15
F-4	0.5	0.5	0	50	28.15
F-5	0	0.5	0.5	32	9.16
F-6	0.5	0	0.5	36	10.15
F-7	0.33	0.33	0.33	49	19.21

According to simplex lattice design and the selected concentration ranges of oil, surfactant and co-surfactant, seven different formulations of SMEDDS containing Cefdinir was constructed. The results of their emulsification time, % Transmittance and cumulative % release in 20 minute were given in Table 5.9.

With the help of Microsoft Excel, the fitted results are shown in Equations (2), (3):

$$Y_1 = 39X_1 + 25X_2 + 47X_3 + 72X_1X_2 + 8X_2X_3 - 28X_1X_3 + 168\ X_1X_2X_3 \quad (2)$$

$$Y_2 = 24.12X_1 + 30.24X_2 + 10.15X_3 + 3.92X_1X_2 - 44.76X_2X_3 - 27.94X_1X_3 + 142.56X_1X_2X_3 \quad (3)$$

Equations (2) and (3) can be used to calculate the predicted values for other formulations in the design space. Equation 2 showed β2 < β1 that means X1 has highest effect on emulsification time. Additionally β12 and β23 had positive value which showed synergistic effect of emulsification time. Whereas, β13 had positive value which showed synergistic effect of emulsification time. Equation-3 showed β1<β2 that means X2 has highest effect on amount of Cefdinir release. Additionally β12 had positive value which showed synergistic effect of Cefdinir release in 20 minutes. β23and β13had antagonistic effect on Cefdinir release because â23 and â13had negative value. The chosen concentrations of surfactant, co-surfactant and oil were introduced into above Equations (2) and (3).

A ternary contour plot can be used to examine relations between four dimensions where three of those dimensions represent components of a mixture (i.e., the relations between them is constrained such that values of three variables add up to the same constant). One typical application of this graph is when the measured responses from an experiment depends on relative proportions of three components (Oil, Surfactant and Co-surfactant) that are varied in order to determine an optimal combination of those components.

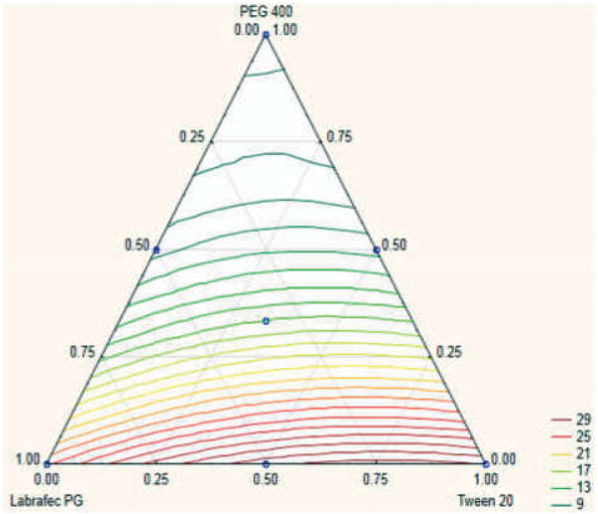
Graphics of Emulsification time and Cumulative % release in 20 minute were constructed in form of Ternary contour plots (Stastetica 12.o version), and optimized formulation was chosen by superimposing ternary contour plots of three responses, which were shown in Figure 5.7. combination of responses in one desirability requires the calculation of individual functions. A suitable SMEDDS formulation should have a minimum self emulsificationtime,maximum time required to 20% of drug release. The individual desirability for each response was calculated and batch F2 showed the highest overall desirability therefore this batch considered to be the best batch.

As shown in Table 5.8 and Figure 5.10, as the emulsification time of SMEDDS formulation was decreases,Cumulative %release increase. In order to obtain low emulsification time and high Cumulative %release, the appropriate ratio of components was chosen for optimized formulation, which consisting of oil, surfactant and co-surfactant.

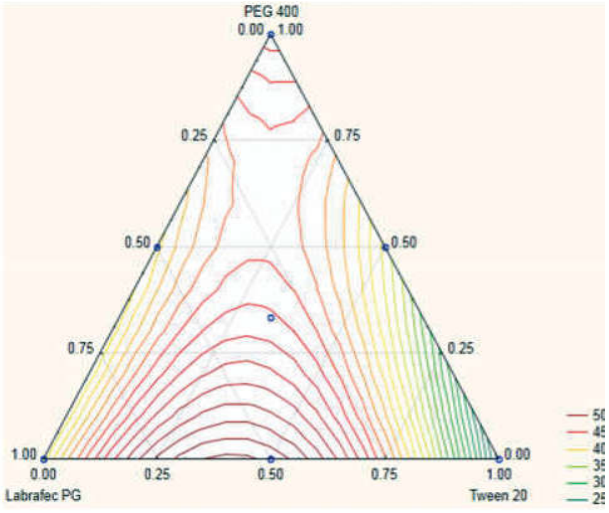
Characterization

Refractive Index and Turbidimetric Evaluation

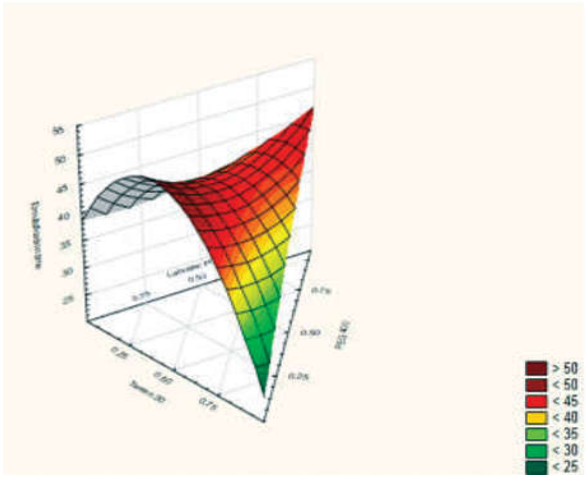
The refractive index and % transmittance of various formulations were shown in Table 5.10.



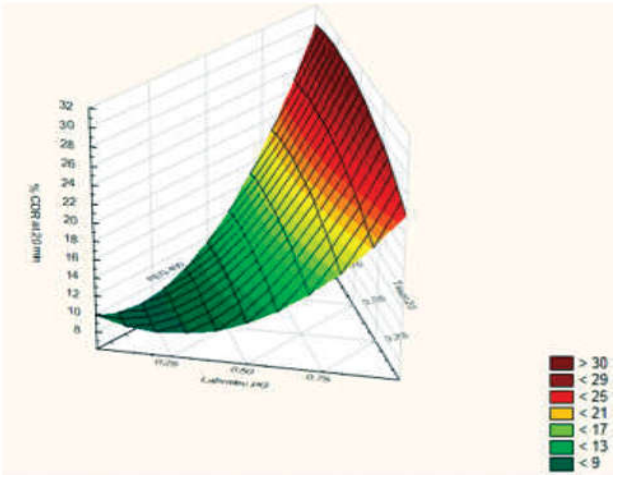
(a) Ternary Contour plot for % CD



(B) Contour Plot for Emulsification time



(c) Surface plot for emulsification time



(d) Surface plot for % CDR

(e) Overlay between % CDR and Emulsification Time

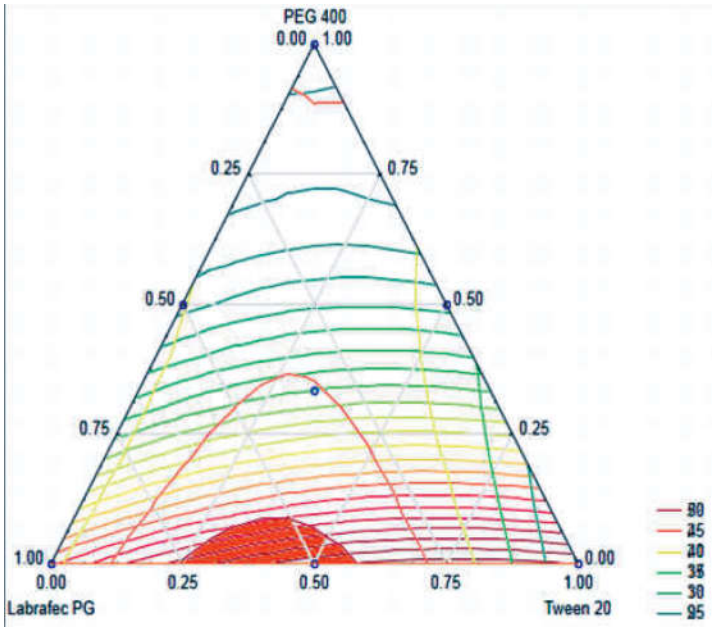


Fig. 5.12: Ternary contour plots (a) Ternary Contour plot for % CDR (B) Contour Plot for Emulsification time (c) Surface plot for emulsification time (d) Surface plot for % CDR

Optimized formulation (F-2) had refractive index similar to refractive index of water (1.333) and percent transmittance >97%. The refractive index and percent transmittance data prove that transparency of system.

Measurement of Droplet Size and Zeta Potential

Droplet size distribution following self-micro emulsification is a critical factor toevaluate self-micro emulsion system. Droplet size is thought to have an

Table 5.10: Refractive index and %transmittance of various SMEDDS formulations

Formulation code	Refractive Index		% Transmittance	
	0.1 N HCL (250 ml)	Water (250 ml)	0.1 N HCL (250 ml)	Water (250 ml)
F-1	1.492	1.516	80.23	85.23
F-2	1.246	1.335	97.35	99.26
F-3	1.43	1.405	80.22	87.56
F-4	1.116	1.126	70.86	76.75
F-5	1.22	1.293	60.95	68.95
F-6	1.12	1.22	58.75	59.73
F-7	1.1	1.14	50.34	55.56

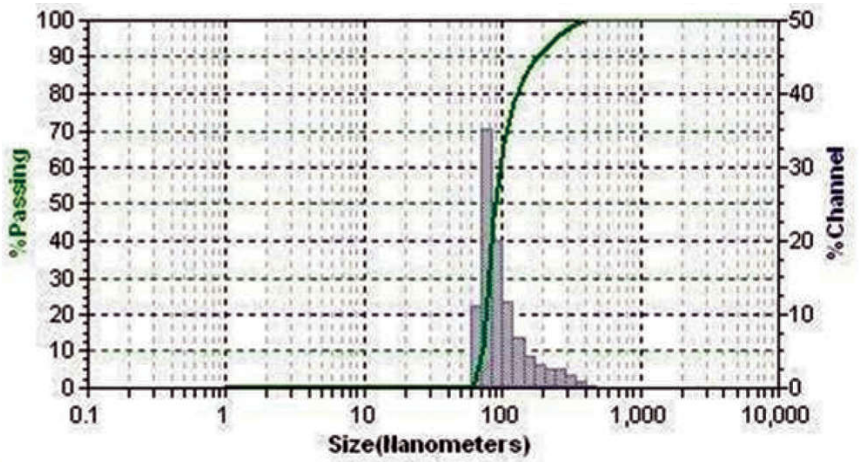


Fig. 5.13: Droplet size analysis of SMEDDS formulation F-2

effect on drug absorption as has been illustrated in several papers. The smaller droplets have largerinterfacial surface area will be provided for drug absorption.

The optimized formulation (F-2) have droplet size 87.60 nm (% passing is 50percent).

Droplet size analysis graph were shown in Figure-5.13.

Cefdinir SMEDDS (F-2) was diluted with distil water, and resulted zeta potential was 24.12mV. Several studies have reported that the zeta potential played an important role The charge of oil droplets of SMEDDS is another property that should be assessed for increased absorption. The charge of oil droplets in SMEDDS was negative due to presence of free fatty acid, the zeta potential of optimized formulation was 24.12 mV. In general the zeta potential value of ±30mV is sufficient for the stability of microemulsion.In our formulation, it is -30.92 which means complies with requirement of zetapotential for stability [73].

Drug Content

Drug content of SMEDDS formulation can be found by methanolicextractof SMEDDS wasanalysed spectrophotometrically (UV-Visible Spectrophotometer, Shimadzu) at 286.4 nm, against standard methanolic solution of Cefdinir. Drug content of various for mulations shown in Table 5.11.

Measurement of Viscosity and pH of Cefdinir SMEDDS

Viscosity of Cefdinir SMEDDS was measured by using Brookfield viscometer at 25°C temperature. Spindle S-61 was selected for measurement of viscosity of various SMEDDS formulation. Viscosity measurement was done at 30 rpm before and after dilution with water. PH of Cefdinir SMEDDS formulation was measured by using pH meter at room temperature. pH of SMEDDS formulations were also measured before and after dilution with 0.1 N HCl. Viscosity and pH data of SMEDDS formulation was shown in Table 5.12.

Table 5.11: Drug content in various SMEDDS formulations

Formulation code	Drug Content		Average
	I	II	
F-1	84.86	82.81	83.835
F-2	90.92	91.33	91.125
F-3	80.12	81.33	80.725
F-4	78.22	79.76	78.99
F-5	82.12	82.89	82.505
F-6	83.66	82.98	83.32
F-7	76.76	75.7	76.23

Table 5.12: Viscosity and pH of various SMEDDS formulations

	Dilution	Formulation code						
		F-1	F-2	F-3	F-4	F-5	F-6	F-7
Viscosity	Before	85.6	152	97.3	89	87	82.2	73.2
	After	1.35	1.88	1.78	1.35	1.25	1.22	1.12
pH	Before	7.831	7.5	7.2	7.01	7.21	7.05	7.03
	After	1.2	1.32	1.22	1.2	1.28	1.25	1.25

Table 5.13: Self-emulsification and precipitation of various SMEDDS formulations

Formulation code	Dispersion Time(Second)	Clarity	Precipitation
F-1	34	clear	Stable
F-2	28	clear	Stable
F-3	30	clear	Stable
F-4	33	Non clear	Stable
F-5	35	Non clear	Stable
F-6	40	Non clear	Stable
F-7	42	Non clear	Stable

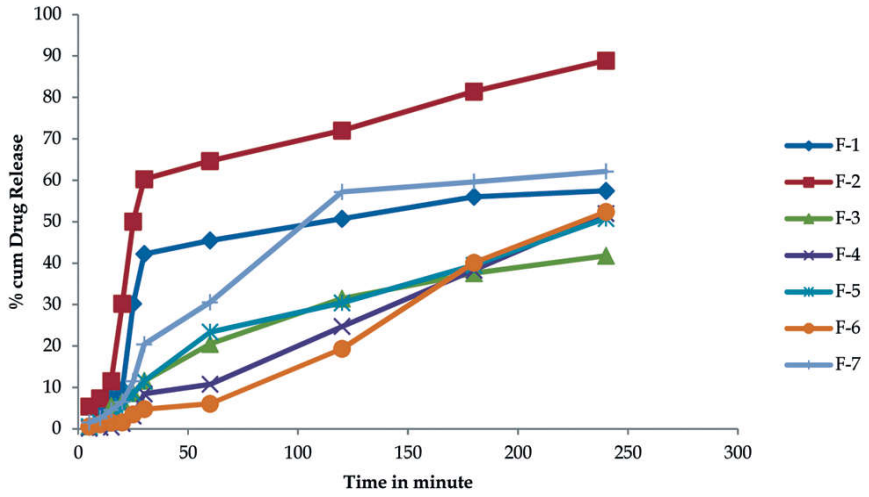


Fig. 5.14: Comparison of dissolution profile of various SMEDDS formulations

Self-Emulsification and Precipitation Assessment

The results of self-micro emulsification and precipitation studies were shown in Table 5.13.

Formulation F-2 and F-3 showed less dispersion time, clear and stable micro emulsion.

Other formulations were showed greater dispersion time as compare to optimizedformulation (F-2) and they were stable micro emulsion but not clear [74].

In VitroDissolution Studies

Alternatively, for evaluating the *in vitro* performance of SMEDDS, drug diffusion studies using the dialysis technique are well documented in literature [75-77]. The drug release profile was shown in Figure 5.14.

In case of SMEDDS (F-2), more than 30% of Cefdinir was released in 20 minute. The release percentage of Cefdinir from SMEDDS form was significantly higher than that of other Cefdinir Drug formulation.

It could suggest that Cefdinir dissolved perfectly in SMEDDS form could be released due to small droplet size, which permits a faster rate of drug release into aqueous phase and it could affect bioavailability. The release rate of Cefdinir from SMEDDS(F-2) was faster than SMEDDS than other formulation.

Accelerated Stability Studies of Cefdinir SMEDDS

The effect of centrifugation and freeze–thaw cycling on phase separation of Micro emulsion and precipitation of drug is shown in Table 5.14. Both accelerated tests are carried out to ascertain stability of Micro emulsion under stress conditions. Optimized formulation of Micro emulsion (F-2) did not exhibit any drug precipitation, phaseseparation after centrifugation confirming its stable nature. Similarly, Optimized formulation of Micro emulsion (F-2) survivedfreeze–thaw cycling as it was found to be reconstituted without anyphase separation, drug precipitation after exposure to freeze–thawcycling.

Table 5.14: Accelerated stability data of various SMEDDS formulations

Accelerated Study	Parameter	Formulation code						
		F-1	F-2	F-3	F-4	F-5	F-6	F-7
Centrifugation	phase separation	No	No	No	Slight	No	Slight	Slight
	Drug precipitation	No	No	No	Yes	No	Yes	Yes
Freeze-thaw cycle	phase separation	No	No	No	No	No	No	No
	Drug precipitation	No	No	No	No	No	No	No

Table 5.15: Stability data of SMEDDS formulation (F-2)

Time (Hours)	Storage condition	Observation
24	25 ± 3°C	No phase separation
	40 ± 2°C/75 ± 5%	No phase separation
48	25 ± 3°C	No phase separation
	40 ± 2°C/75 ± 5%	No phase separation
120	25 ± 3°C	No phase separation
	40 ± 2°C/75 ± 5%	No phase separation
240	25 ± 3°C	No phase separation
	40 ± 2°C/75 ± 5%	No phase separation
720	25 ± 3°C	No phase separation
	40 ± 2°C/75 ± 5%	No phase separation

Summery and Conclusion

Cefdiniris a BCS Class II drug and is water insoluble, with varying bioavailability. Cefdinir exhibits very low dissolution profile in the gastro intestinal fluid which might be attributed to its hydrophobic characteristic. The potential of SMEDDS was explored successfully for oral delivery of poorly water-soluble Cefdinir. SMEDDS are isotropic mixtures made up of oil, surfactant and sometimes co-surfactant or co-solvent.

Solubility study of Cefdinir was carried out in presence of various oil, surfactant and co-surfactant. Cefdinir had highest solubility in Labrafac PG, tween-80 and polyethylene glycol-400 as liquid vehicle, surfactant and co-surfactant respectively with comparison to others. So, Labrafac PG as oil, tween-80 as surfactant and polyethylene glycol-400 as co-surfactant was selected for optimal SMEDDS formulation resulting in improved drug loading capabilities.

Phase diagrams were constructed to obtain optimum concentrations of oil, water, surfactant, and co-surfactant. It was observed that increasing concentration of surfactant such as tween-80 in SMEDDS formulation increased spontaneity of self-emulsification region. Therefore, much higher concentration of surfactant, much higher self-emulsifying region in phase diagrams. The phase diagrams were constructed at ratio of surfactant/co-surfactant 1:1, 2:1, 3:1, 4:1 (w/w).

Simplex lattice design was used to optimize the formulation of SMEDDS containing Cefdinir. The concentrations of oil (X1), surfactant (X2) and co-surfactant (X3) were chosen as the independent variables. The emulsification time and Cumulative %release in 20 minute were taken as responses (Y), respectively. The optimal formulation of SMEDDS was comprised of 20% oil (Labrafac PG), 60% surfactant (Tween-20) and 20% co-surfactant (PEG-400).

Droplet size distribution following self-micro emulsification is a critical factor to evaluate self-micro

emulsion system. The smaller droplets have larger interfacial surface area will be provided for drug absorption. The optimized formulation (F-2) have droplet size 87.60 nm (% passing is 50).

In vitro release study was carried out to understand characteristics of drug release from SMEDDS. In case of SMEDDS (F-2), more than 99% of Cefdinir was dissolved. Order of drug dissolution was F-2> F-4> F-1 > F-7> F-3> F-6 > F-5.

Conclusion

The potential of SMEDDS was explored successfully for oral delivery of poorly water-solubleCefdinir. SMEDDS are isotropic mixtures made up of oil, surfactant and sometimes co-surfactant or co-solvent. In an aqueous environment a homogeneous, transparent, isotropic and thermodynamically stable dispersion will result, the formation of which is improved by gentle agitation, *in vivo* provided by gastrointestinal motility. The formulation of Cefdinir SMEDDS was optimized by a simplex lattice design. Solubility study was showed that highest solubility of Cefdinir in oleic acid as compare to other materials. The optimized formulation of SMEDDS was comprised of 20% oil (Labrafac PG), 40 % surfactant (Tween-20) and 20% co-surfactant (PEG-400). Pseudo-ternary phase diagrams were constructed to identify the efficient self-emulsification region. The average globule size of SMEDDS containing Cefdinirwas about 87.60 nm when diluted in water. SMEDDS had also shown that after dilution of formulation there was no precipitation and phase separation found. In vitro dissolution studies revealed that release of Cefdinirfrom SMEDDS was faster. Our studies illustrated potential use of SMEDDS for delivery of hydrophobic compounds, such as Cefdinir.

Experience with self-micro emulsifying drug delivery system (SMEDDS) reveals that this is a fruitful approach to improve the solubility and bioavailability ofCefdinir. Now, further study required to convert SMEDDS formulation in to powdered form which will either fill in capsule or compress the tablet. In vivo pharmacokinetic and pharmacodynamic study also required to be carried out to evaluate its efficiency in improving oral bioavailability of poorly water soluble drug.

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