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SEDDS: NANOCARRIER SYSTEM FOR A NEW CARDIAC DYSFUNCTION DRUG

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ABSTRACT
The aim of this research was to screen and select components for formulation development of a self-emulsifying drug delivery systems of LASSBio-294 (3,4-methylenedioxybenzoyl-2- thienylhy-drazone) for oral administration. The screened components were long and medium chain oils, surfactants with Hydrophile–Lipophile Balance between 4 to 17 and an alkane diol and glycil esther or glycol ether as cosurfactants. The selection was driven by the ability of selected surfactants to emulsifying the selected oils as well as by the properties of the emulsions such as mean diameter of the droplets, polydispersity index, zeta potential and turbidity. LASSBio-294 solubility was evaluated quantitatively in oils, surfactants and cosurfactants and by compatibility using DSC. Mean diameter, polydispersity index and zeta potential were evaluated by dynamic light scattering method, as well as turbidity. Labrafac PG, Labrasol, and Transcutol HP were selected as the best promising components for SEDDS development of LASSBio294. Although LASSBio-294 is more soluble in the surfactants than in the screened oils, the components were rapidly dispersed into fine droplets with mean diameter 176.83 nm, polydispersity index 0.216, turbidity 91.97 NTU, and zeta potential - 38.43 mV. The screening and selection of components allowed obtaining a nanosized emulsion with low polydispersity and electrostatic stability. This formulation in the SEEDS form is promising with carrier of LASSBio-294.

KEYWORDS: Drug Delivery Systems, Emulsion, Lipids, Nanoparticles, Oral Drug Delivery, Poorly water-soluble drug, Self-emulsifying, Surfactant, Bioavailability, Biomimetics.

1. INTRODUCTION
LASSBio 294 (L294), 3,4-methylenedioxybenzoyl-2-thienylhydrazone, is a new compound that represents an alternative therapy for cardiac dysfunction. It has been investigated as a potent positive cardiac inotropic agent with vasodilator properties. The identification of its actuation allowed the suggestion of a novel mechanism for a positive cardio inotropic effect that is based on a pronounced increased accumulation of calcium into the sarcoplasmic reticulum (Barreiro, 2002). In terms of physicochemical properties, L294 is a weakly acidic drug (pKa = 10.8) that is poorly insoluble in water. Therefore, it remains unionised in the stomach and intestine.

The low solubility of many new drug candidates is a substantial challenge facing pharmaceutical development (Lipinski, 2000). For oral administration, drug solubility is one of the rate-limiting parameters to achieve their desired concentration in systemic circulation for pharmacological response (Chaudhary et al., 2012). In particular, permeability is affected by a number of factors, including lipophilicity, the molecular size of drug, and its affinity to influx or efflux transporter proteins (Chaudhary et al., 2012).

The absorption of orally administered drugs depends on both complex biological processes (i.e. passive membrane penetration, active transport mechanisms and metabolism in the gastrointestinal (GI) tract) and physicochemical properties, such as solubility, dissolution rate and dissociation constants (Wessel et al., 1998). Besides the above-cited factors, bioavailability also depends on first-pass metabolism, pre-systemic metabolism and susceptibility to efflux mechanism. However, among the above-cited factors, solubility is the primary control of drug absorption in the GI tract and further drug bioavailability (Savjani et al., 2012,

Kostewicz et al., 2002). In addition, the amount of drug solubilised within the GI tract is affected by the characteristics of the GI fluids, e.g. volume, pH, level of surfactants and the physicochemical characteristics e.g. lipophilicity and pKa.

Several studies have demonstrated that SEDDS improve the bioavailability of a number of compounds (Barakat, 2010, Kang et al., 2004, Kommuru et al., 2001, Nielsen et al., 2008, Patel and Vavia, 2007, Shafiq et al., 2007). It is believed that this increase in bioavailability is achieved by presenting drugs in a solubilised state (Bates and Sequeira, 1975, Kadu et al., 2011) and by increasing the rate and extent of drug release, as well as the stability of the emulsion (Mahapatra et al., 2014). SEDDS are reported to assist the absorption of poorly soluble drugs by facilitating the formation of an emulsion that is capable of maintaining the drug in solution for the duration of its transit through the GI tract (Fatouros et al., 2007, Pouton, 2000).

SEDDS are mixtures of oil, surfactant, cosurfactant and drug that increase the bioavailability of poorly water-soluble drugs. When orally administered, the conceptual basis of SEDDS action relies on the substance's ability of *in situ* formation of a fine oil-in-water (o/w) micro/nanoemulsion under gentle agitation following dilution by aqueous phases, such as GI fluids (Chaudhary et al., 2012). The digestive motility of the stomach and intestine provides the agitation required for self-emulsification *in vivo* (Nazzal et al., 2002, Patel and Vavia, 2007, Shah et al., 1994). Therefore, SEDDS are good candidates for oral delivery of hydrophobic drugs with adequate solubility in oils or oil/surfactant blends (Kommuru et al., 2001, Tang et al., 2007).

The advantages of these drug delivery systems include not only improved drug solubilisation but also enhanced release and absorption properties due to the large interfacial surface area provided by the micro/nanoemulsion (Nekkanti et al., 2010). Other advantages include increased stability of drug molecules and the possibility of administering the formulation in gelatine capsules (Pouton, 2000).

Lipid (oil phase) is an essential component of SEDDS formulations. Not only can the lipid facilitate self-emulsification but also has the propensity to augment the fraction of drug transported via the intestinal lymphatic system, thereby increasing its absorption from the GI tract (Porter et al., 2007). Modified long- and medium-chain triglyceride oils with varying degrees of saturation or hydrolysis, have been widely used for the design and development of SEDDS formulations (Singh et al., 2009b). These oils offer different types of formulations and physiological advantages because their degradation products should be similar to the natural products of intestinal digestion; furthermore, many cases have shown the ability to reduce or eliminate the influence of food on the absorption of drugs (Chen, 2008, Joshi et al., 2008).

The most important criterion for the screening of components for nano/microemulsion is the drug's solubility in oils, surfactants and cosurfactants. The ability of SEDDS to maintain the drug in solubilised form is influenced by the solubility of the drug in the oily phase (Thankachen et al., 2014). An important criterion for selection of surfactants is the HLB value, and then the issues that govern the selection of a surfactant are its HLB and safety. Surfactants can solubilise relatively high amounts of hydrophobic drug compounds. However, if the surfactant and cosurfactant largely contribute toward solubilisation, then there isn't a risk of drug precipitation (Thankachen et al., 2014). The most widely recommended emulsifiers include non-ionic surfactants because of their relatively high HLB values (Fernandez-Tarrio et al., 2008, Wakerly et al., 1986) and because they are also considered safer than ionic surfactants (Nielsen et al., 2008, Wakerly et al., 1986, Swenson et al., 1994).

Although SEDDS represent a solution for the drug delivery of poorly soluble drugs, the adequate formulation influences the drug absorption and therapeutic effects. Droplet size is a critical factor in self-emulsification performance because it determines the rate and extent of drug release as well as its absorption (Tarr and Yalkowsky, 1989). The smallest particle size of the emulsion droplets may lead to faster absorption and improve bioavailability (Yadav et al., 2014).

This paper approaches the preliminary step to SEDDS formulation of L294 that is the screening and selection of its components. It considered commercially available components, such as long- and medium-chain oils, surfactants with HLB values between 4 and 17 and, alkane diol and glycol ether as cosurfactants. All products are approved for oral administration and belong to the generally regarded as safe category. The criteria were based on the substances' solubility and emulsifying capability as well as on the properties of the emulsions formed, such as droplet size, polydispersity index, zeta potential and turbidity characteristics.

2. MATERIALS AND METHODS

2.1 Chemicals

Labrasol, Transcutol HP, Lauroglycol 90, Labrafac PG, Maisine 35-1, Labrafac Lipophile WL 1349, Capryol 90, Peceol and Labrafil M1944CS were generously donated by Gattefossé (France). Kolliphor HS15, Kolliphor P188, Cremophor EL and Cremophor RH40 were received from BASF (Germany). Castor oil, corn oil, Tween 20, Tween 60 and Tween 80 were obtained as gift samples from Croda (Brazil). Other oils, surfactants and cosurfactants were of pharmaceutical grade. All other chemicals and solvents were of analytical grades.

L294 was 3, 4-methylenodioxybenzoyl-2-thienylhydrazone and was used as the model drug. Its

molecular structure and physicochemical properties are shown in Figure 1 and Table 1, respectively.

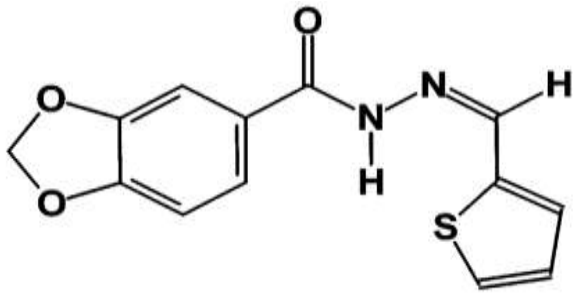


Figure 1: Molecular structure of 3,4-methylenodioxybenzoyl-2-thienylhydrazone (LASSBio 294, L294).

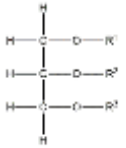
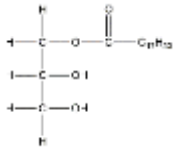
2.2 Apparent Solubility Assays

L294's solubility in oils (Table 2), surfactants (Table 3) and cosurfactants (Table 4) was individually determined in shake flasks. Briefly, an excess amount of L294 (approximately 0.5 g) was added to 50 mL Erlenmeyer flasks containing 25 mL of each tested vehicle. The flasks were homogenised in a vortex mixer (Vortex-2 Genie, Scientific Industries, USA) for 2 min to enhance the drug mixture. Then, the flasks were kept under constant agitation for 48 h at 37°C in an orbital shaker (Dubnoff 304-D, Nova Ética, Brazil). After equilibrium was achieved, the samples were centrifuged at 3,500× g for 15 min (Thermo Scientific Sorval Legend Mach 1.6R) for non-solubilised drug removal. The supernatant was collected, suitably diluted in a proper solvent and spectrophotometrically analysed at λ = 318 nm using an UV-VIS spectrophotometer (PharmaSpec UV-1700, Shimadzu, Japan) to access the amount of solubilised L294. Pure water was used as a control. The experiment was performed in triplicate, and results were represented as a mean value (in $mg.g^{-1}$) ± standard deviation (S.D.).

Table 1: L294 Physicochemical Properties.

Property	Value
Molecular Formula	$C_{13}H_{10}N_2O_3S$
Molecular Weight	274.3 $g.mol^{-1}$
Log *P*	2.52
pKa	10.8
Melting Range	205-210 ºC

Table 2: Oils used in Solubility Tests.

General Class	Cas Number	Compound	Molecular Formula	HBL	Trade Name
Fixed Oils	8001-30-7	Corn oil	$C_{22}H_{25}NO_6$	n.a.	Super Refined Corn Oil
	8001-79-4	Castor Oil	$C_{57}H_{104}O_9$	n.a.	Super Refined Castor Oil
	73398-61-5	Medium-chain triglycerides	Mixing mainly of caprylic acid ($C_8H_{16}O_2$) + capric acid ($C_{10}H_{20}O_2$)	2	Labrafac LipophileWL1349
Propylene glycol esters	68583-51-7	Propylene glycol dicaprylocaprate		2	Labrafac PG
Glycerides	68424-61-3	Glyceryl monolinoleate	Mixing mono, di and triglycerides of linoleic acid	4	Maisine 35-1
	25496-72-4	Glyceryl monooleate (Type 40)		3	Peceol

n.a.: non applied.

Table 3: Surfactants used in Solubility Tests

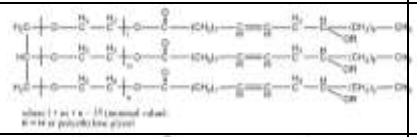
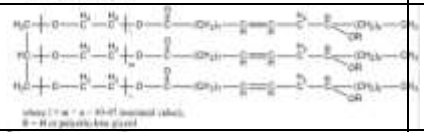
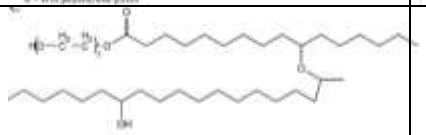
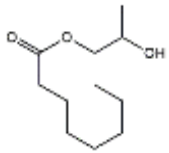
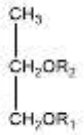
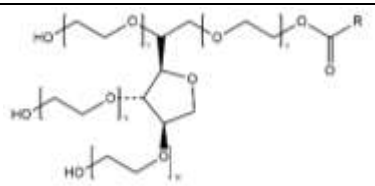
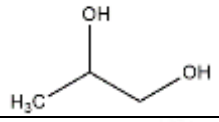
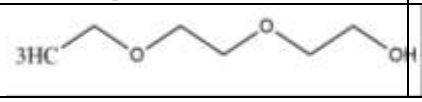
General Class	Cas Number	Compound	Molecular Formula	HBL	Trade Name
Polyoxyethylene castor oil	61791-12-6	Polyoxyl 35 castor oil		12-14	Cremophor EL
	61788-85-0	Polyoxyl 40 Hydrogenerated castor oil		14-16	Cremophor RH40
Polyoxyethylene stearate	70142-34-6	Polyoxyl 15 Hydroxystearate		14-16	Kolliphor HS 15
Polyoxyglycerides	69071-70-1	Oleoyl polyoxyl-6 glycerides	Mixing of triglycerides, PEG-6 and Oleic acid	4	Labrafil M1944CS
	61791-29-5	Caprylocaproylpolyoxyl-8 glycerides	Mixing of triglycerides, PEG-8 and Caprylic/Capric acids	14	Labrasol
Propylene glycol esters	85883-73-4	Propylene glycol monocaprylate (Type II)		6	Capryol 90
Propylene glycol esters	27194-74-7	Propylene glycol monolaurate (type II)		5	Lauroglycol 90
Polysorbates	9005-64-5	Polyoxyethylene 20 sorbitan monolaurate		17	Tween 20
	9005-67-8	Polyoxyethylene 20 sorbitan monostearate		15	Tween 60
	9005-65-6	Polyoxyethylene 20 sorbitan monooleate		15	Tween 80

Table 4: Cosurfactants/co-solvents used in Solubility Tests.

General Class	Cas Number	Compound	Molecular Formula	Trade Name
Alkane diols and triols	57-55-6	1,2-propanediol		Propylene glycol
Glycol ether	111-90-0	Diethylene glycol monoethyl ether		Transcutol HP

2.3 Excipients' compatibility

On the basis of the solubility results, Cremophor EL, Labrafac PG, Capryol 90, Maisine 35-1, Transcutol HP, Labrasol, propylene glycol, castor oil, Tween 20, Tween 80 and Cremophor RH40 were selected for evaluation of the thermal characteristics of their interactions with L294 with a differential scanning calorimeter (DSC). Samples (approximately 2mg.) containing the drug that was solubilised in selected vehicles, pure L294 and unprocessed vehicles were carefully sealed in lidded, heat-resistant aluminium pans; the lid of each pan was crimped onto its surface. Thermal analyses were conducted at a range of −20°C to 300°C under nitrogen gas flow (50mL/min) at a heating rate of 10°C min^{-1} using a DSC822e, Mettler-Toledo (Spain) equipment.

2.4 Emulsification Capability

Surfactants were individually screened on the basis of their ability to emulsify the selected oil phase (Maisine 35-1, castor oil, or Labrafac PG) in the absence of the drug. The emulsification ability was evaluated from 2mL of surfactant (Cremophor EL, Labrasol, Tween 20, Tween 80, Capryol 90, Cremophor RH40) that was added to 2mL of the selected oily phase and thoroughly mixed; then, 0.1 mL of this pre-concentrate mixture was diluted to 100mL with distilled water. To screen the cosurfactants, on the basis of their efficacy to improve the emulsification, 4mL of surfactant was mixed with 2 mL of Transcutol HP or propylene glycol ($Smix_{surfactant:cosurfactant}$ 2:1). The selected oil (6mL) was added to this mixture (oil:Smix 1:1) and then 0.1 mL of the resulting mixture was diluted to 100mL with distilled water and gently agitated.

All emulsions were allowed to stand for 2h and then were analysed for phase separation/precipitation by visual observation. Afterwards, they were characterised by droplet size, polydispersity index, zeta potential and turbidity measurements.

The droplet size, polydispersity index (PDI) and zeta potential were determined by dynamic light scattering at a 90° fixed angle with a Zetasizer Nano ZS (Malvern Instruments, UK).

The droplet size was measured as mean diameter by means of the Stokes-Einstein equation. It was also described in terms of intensity and number distributions. The intensity distribution is proportional to the sixth power diameter ($I\alpha d^6$) and amplifies the signal by showing all diameters in the sample. Number distribution is proportional to the first power diameter ($N\alpha d$) and shows the predominant diameters. The polydispersity (PDI) reflects the dispersion of the particle diameters.

Zeta potential measurements were carried out on the same diluted sample; the equipment operated at 25°C with an electric field strength of 23 $V.cm^{-1}$. The zeta potential values were calculated according to the Smoluchowski equation.

The turbidity of the emulsions was measured using a HACH 2100P Turbidimeter (HACH Co., Germany) and expressed in nephelometric turbidity units (NTU). The measurements were performed on 30 mL of sample stored in a clear screw-capped sample vials. The equipment was previously calibrated according to opalescence standards. All experiments were performed at least in triplicate.

3. RESULTS AND DISCUSSION

3.1 Excipient Selection

Solubility studies were performed to identify a suitable oily phase, surfactant and cosurfactant that possess a good solubilizing capacity for L294 in order to develop SEDDS. Thus, for the present study, vehicles from different categories such as long chain triglycerides, medium chain triglycerides, synthetic monoglyceride oils and synthetic triglycerides of different HLB values were selected so that the highest solubility of L294 could be achieved.

The screening of components for the SEDDS was done by evaluating the solubility and compatibility of L294 in the individual oils, surfactants and cosurfactants. Identification of the solubility of the drug in oils, surfactants and cosurfactants is important to achieve optimum drug loading (Pouton, 2000, Shafiq et al., 2007). The obtained solubility results are shown in Figure 2.

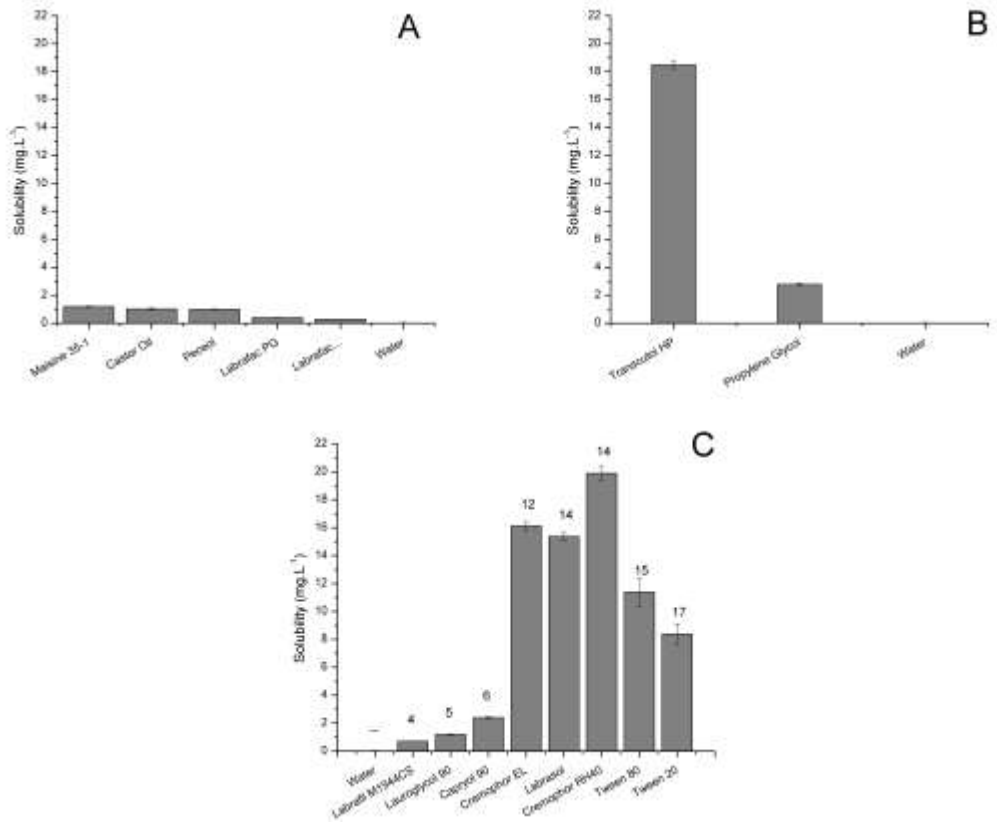


Figure 2: Solubility of L294 in (A) oils (B) cosurfactants and (C) surfactants. The number above each surfactant bar represents the HLB values.

The solubility of L294 in water was 0.006 mg.g^{-1}, thereby confirming that it is a poorly water-soluble drug. In addition, it can be proven by its partition coefficient, $logP_{(octanol/water)}$, which is 2.52. L294 is also a weak acid (pKa 10.8), which causes it to remain 99.9% non-ionised in the stomach's pH. However, its clinical efficacy is reduced by poor water solubility, which limits its effective oral absorption and bioavailability after administration, according to pharmacokinetic studies reported by Braga et al. (2011).

Figure 2A shows that L294 has low miscibility in the assayed oils (≅ 1 mg.g^{-1}), as expected for drugs with intermediate partition coefficients (2 < *logP* < 4) in natural lipids (Porter *et al.*, 2008). L294's solubility ranged from highest to lowest, as per the following list, in Maisine 35-1 (1.182 mg.g^{-1}), castor oil, Peceol, MCT, Labrafac PG and Labrafac Lipophile WL1349, while its solubility was higher in Transcutol (18.8 mg.g-1) than in propylene glycol (Figure 2B). Figure 2C shows the solubility of L294 in the various surfactants. The highest solubility was found in surfactants with HLB values between 12 and 17. Cremophor RH40 (19.888 mg.g^{-1}), Cremophor El (16.114 mg.g^{-1}) and Labrasol (15.357 mg.g^{-1}) demonstrated the highest solubility for L294 among the tested surfactants. Similar results were presented by Shafiq et al. (2007) using other poorly water-soluble drugs. The more lipophilic surfactants may play the role of hydrophilic oils in the formulations. Capryol (propylene glycol monocaprylate–type II HLB 6) is a compound that is classified as a water-insoluble surfactant and it is most often included as an oil phase in SEDDS formulations (Azeem et al., 2009). However, the Capryol was used as a cosurfactant (Salimi et al., 2014) or surfactant in SEDDS, SMEDDS, or microemulsion formulations (Farah et al., 1994). In the surfactants, the solubility of the drug was higher in Cremophor RH40. Labrasol, a medium-length alkyl chain surfactant, was chosen as a surfactant for its good drug solubility (15.36 mg.g^{-1}) and because it was recommended for its enhanced intestinal absorption of drugs (Eccleston, 1994). In their studies with candesartan cilexetil, Nekkanti et al. (2010) found that Labrasol provided the highest drug solubility; they also discovered good solubility in Cremophor EL and Tween.

Finally, Transcutol HP presented higher solubility with L294 (18.48 mg.g^{-1}) than propylene glycol (2.78 mg.g^{-1}). Co-solvents, including ethanol, propylene glycol and PEG, have a serious limitation of evaporating from within sealed gelatine capsules, eventually leading to the precipitation of drug inside the shell. Transcutol is more stable than other cosurfactants and less volatile than ethanol and others volatile cosolvents (Borhade et al., 2009). Transcutol HP was also described to solubilise other poorly water-soluble drugs, such as oridonin (Zhang et al., 2008) and clotrimazole (Borhade et al., 2012).

The stability and efficiency of oral absorption of a drug compound from SEDDS or other formulations depends on many formulation-related parameters, such as surfactant concentration, oil/surfactant ratio, polarity of the emulsion, droplet size and charge, all of which in essence determine self-emulsification ability. Therefore, Labrafac PG, Labrasol and Transcutol HP were selected as the oil phase, surfactant and cosurfactant.

3.2 Excipient Compatibility

On the basis of the results of the miscibility studies, thermal analysis was used to detect any interaction between L294 and the tested vehicles (Labrafac PG, Labrasol and Transcutol HP) that improved L294's solubility.

The metastable-to-stable phase transition is an uncontrolled, independent process. In particular, self-assembled lipid suspensions exhibit phase transitions in which the underlying driving mechanisms and dynamics are not well understood (Jacoby et al., 2015). DSC is a well-established method that is often used as a qualitative technique to characterise physical and chemical changes in either the enthalpy or heat capacity of a crystalline lipid. Azeem et al. (2009) evaluated the presence of thermostable states in Smix with Cremophor EL, Labrasol, Tween 20, Tween 80, Capryol 90, castor oil and propylene glycol and found that all of them were stable.

The DSC method was used to observe the thermal changes in L294, the vehicles (oil, surfactant, or cosurfactant) and a mixture of L294 and the vehicles (Figures 3 through 5).

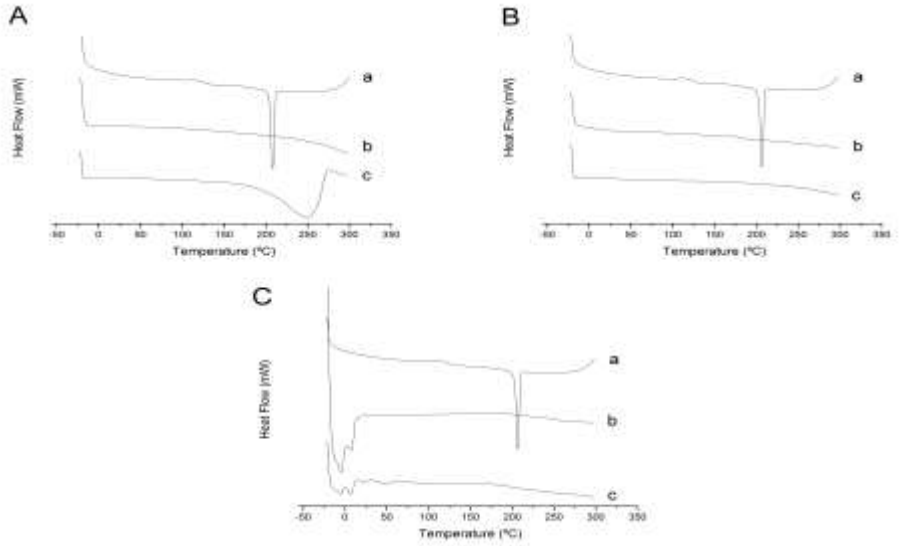


Figure 3: DSC thermogram of L294 in tested oils: (A) Labrafac PG, (B) Castor Oil, and (C) Maisine 35-1: (a) L294, (b) oil + L294, (c) oil.

L294 DSC presented an endothermic melting event between 205°C and 210°C and degradation or decomposition was not observed in the studied temperature range. The DSC curve of pure L294 exhibited a narrow endothermic event at 207.62°C (Figure 3) with onset at 198°C and recovery at 213°C that corresponds to the melting point of the high-purity material and low molecular weight; however, at the evaluated temperature range, it was not possible to observe its degradation or decomposition. The decrease from baseline in the DSC curves (Figures 3 through 6, curve a), starting at 100°C, shows the glass transition temperature of L294. This result show that L294 is not 100% crystalline, the same thermal events is not observed for oil + L294 (b).

Based on the thermal analysis data, no thermal event associated with metastable-to-stable phase transition was found in the L294–oil, L294–surfactant and L294–cosurfactant mixtures. The L294 mixture with Labrafac PG (Figure 3A), castor oil (Figure 3B) and Maisine 35-1 (Figure 3C) showed an absence of any definite melting endothermic peak between 205°C and 210°C due to L294's complete miscibility in the presence of these solvents.

Parmar et al. (2011) found that Cremophor EL, Labrasol, Cremophor RH40, Capryol 90, Lauroglycol 90, Transcutol HP and Labrafil M1944CS excipients showed no definite interaction between the drug and selected vehicles. In this study, the results analyses show that L294 solubilises in the surfactants (Figure 4), except for Capryol 90. This result of the DSC curve (Figure 4B–b) shows a heterogeneous interaction between L294 and Capryol 90 (175°C), and an endothermic event at 250°C corresponds to the surfactant excess. Meanwhile, Figure 5 shows a homogeneous interaction between L294, Transcutol HP (Figure 5A) and propylene glycol (Figure 5B) that peaked at 150°C. The DSC curves of the L294–Capryol (4B curve b), L294–Transcutol (5A curve b) and L294–propylene glycol (5B curve b) mixtures all

highlight the beginning of the glass transition phase at 100°C. These results should also indicate that L294 might be in an amorphous state in all vehicles tested, except for Capryol 90, Transcutol and propylene glycol. Although amorphous solids have a molecular order they do not reach the same structural order of crystals. As a result of this less ordered structure, solubility of most amorphous solids is greater than the corresponding crystalline solid. The high-energy amorphous forms and SEDDS are a crystal engineering technologies can be applied to pharmaceutical substances to improve drug solubility (Derle et al., 2010, Khadka et al., 2014).

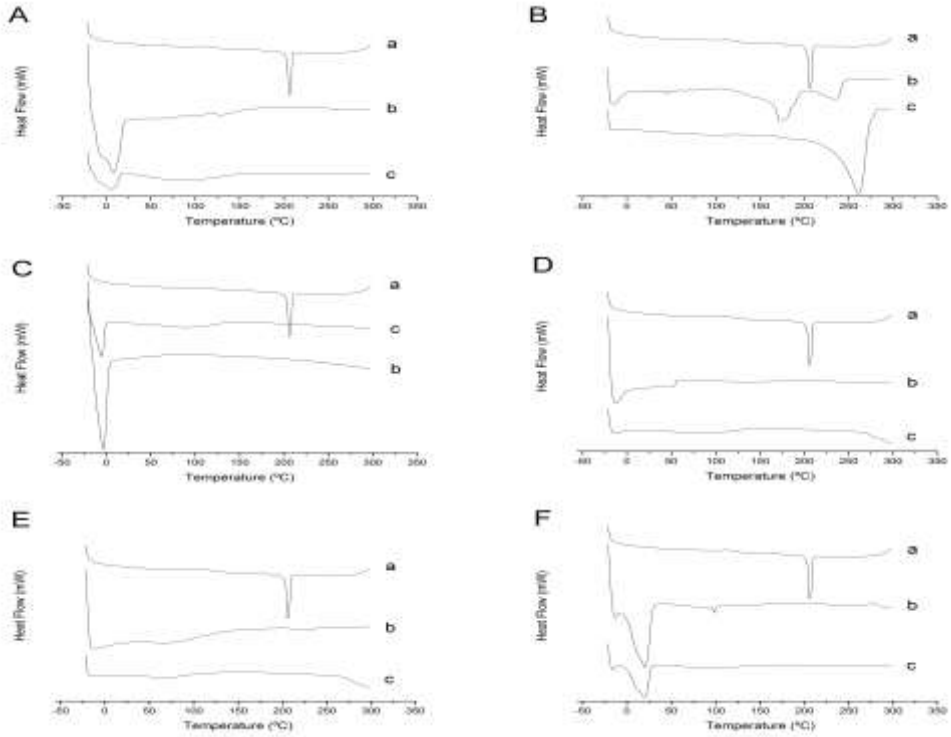


Figure 4: DSC thermogram of L294 in tested surfactants: (A) Cremophor EL, (B) Capryol 90, (C) Labrasol, (D) Tween 20, (E) Tween 80 and (F) Cremophor RH40: (a) L294, (b) surfactant + L294, (c) surfactant.

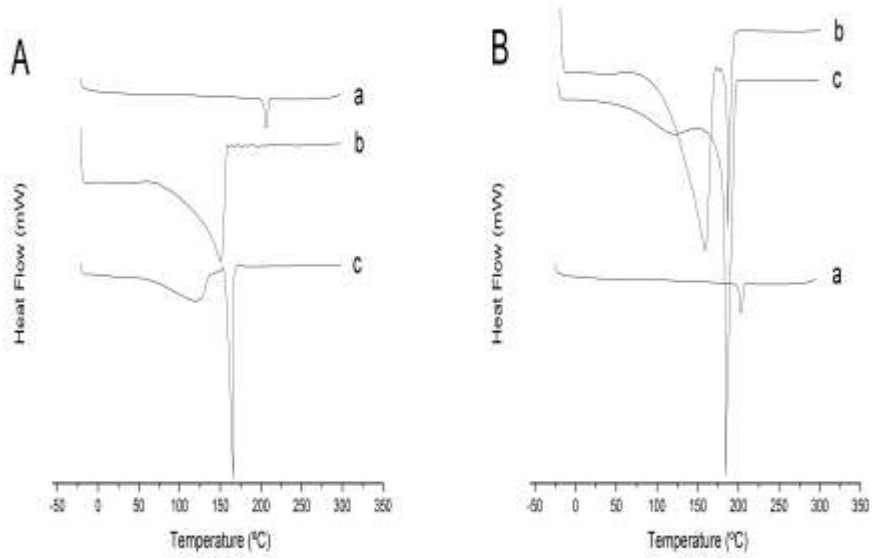


Figure 5: DSC thermogram of L294 in tested cosurfactants: (A) Transcutol HP and (B) Propylene glycol: (a) L294, (b) cosurfactant + L294, (c) cosurfactant

DSC was applied to L294's SEDDS formulation (Labrafac PG, Labrasol and Transcutol HP) with and without L294 present to confirm the alteration of the physical state of the drug. The narrow peak at 207.62°C for pure L294 (Figure 6) infers the presence of the crystalline form of the drug. No representative peaks for a L294–Transcutol interaction can be observed in DSC curves corresponding to samples of SEDDS + L294 (Figure 6 curve b) and SEDDS (Figure 6 curve c). The

analysis of this result shows that both the drug and cosurfactant are soluble in the SEDDS formulation.

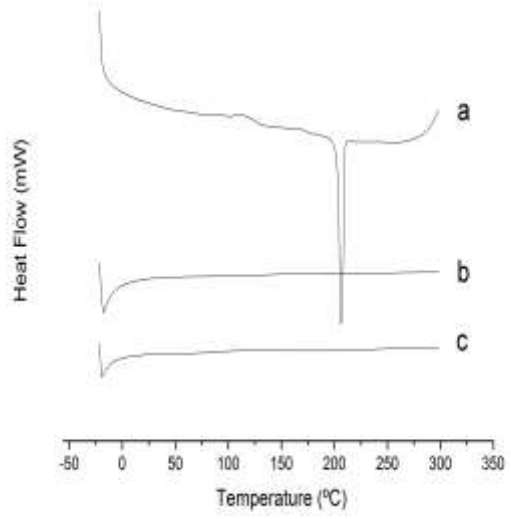


Figure 6: DSC thermogram of L294 in SEDDS formulation (Labrafac PG, Labrasol and Transcutol HP): (a) L294, (b) SEDDS + L294, (c) SEDDS.

3.3 Emulsification Capacity

Self-emulsification ability depends on many formulation-related parameters, such as surfactant concentration, oil/surfactant ratio, polarity of the emulsion, droplet size and charge. Thus, only very specific pharmaceutical excipient combinations will lead to efficient self-emulsifying systems. Surfactant is an essential component of all SEDDS formulations; the selection of surfactants and cosurfactants are primarily governed by their emulsification efficiency in the selected oily phase. The selection of a suitable surfactant for a SEDDS formulation is critical for the development of a stable formulation because it adheres to the oil–water interface, reduces the interfacial energy and forms a mechanical barrier against the coalescence of oily droplets (Kommuru et al., 2001, Nazzal et al., 2002).

Labrafac PG was the only oil used as an oil phase due to preliminary results showing that Maisine 35-1 and castor oil exhibited poor emulsification properties with all surfactants tested (data not shown). Six surfactants (Tween 20, Tween 80, Labrasol, Cremophor RH40, Capryol 90 and Cremophor EL) were tested to evaluate their emulsification ability and were selected based on their solubility and compatibility results. The self-emulsification was visually assessed to measure the apparent spontaneity of nanoemulsion formation (Gershanik and Benita, 1996, Gursoy et al., 2003). SEDDS, when diluted in water, were found to be non-turbid and bluish-transparent in appearance, indicating spontaneous emulsification. All the resulting nanoemulsions were transparent with some opalescence in appearance and did not show any sign of phase separation.

Emulsification results of surfactants in the Labrafac PG oil phase are presented in Table 5. Final selection of surfactants and cosurfactants was based on their emulsification ability, which was determined by physiochemical properties, such as turbidity measurements, droplet size, zeta potential and polydispersity index of the resulting nanoemulsion.

Labrafac PG is a highly miscible solvent with small droplet size and good PDI for all surfactants tested. The droplet size of all the formulations was determined by photon correlation spectroscopy and reported in number-based diameters (N) and intensity (I). The results (Table 5) showed that droplet size was <180 nm (N), intensity was <230 nm (I) and PDI was <0.250. Droplet size distribution is one of the most important characteristics of nanoemulsion for stability evaluation and is a critical step in the pathway of enhancing drug bioavailability (Shah et al., 1994, Tarr and Yalkowsky, 1989). Smaller nanoemulsion particle size leads to larger interfacial surface area, thus promoting rapid absorption and improved bioavailability (Shukla and Patel, 2010).

Table 5: Emulsification Properties of Surfactants with Labrafac PG.

Surfactants	Droplet Size (nm)		PDI	Zeta Potential (mV)	Turbidity (NTU)
	Intensity	Number			
Cremophor EL (HLB= 12-14)	123.10 ± 5.27 (24.36-342.00)	48.99 ± 16.55 (15.69-190.10)	0.144	-9.74	71.1
Labrasol (HLB= 14)	199.13 ± 3.45 (91.28-458.70)	146.77 ± 4.67 (78.82-396.10)	0.204	-14.70	132.3
Tween 80 (HLB= 15)	128.60 ± 2.42 (50.75-295.30)	76.22 ± 1.15 (43.82-255.00)	0.101	-6.03	65.4
Tween 20 (HLB= 17)	165.60 ± 4.56 (58.77-396.10)	96.83 ± 5.8 (50.75-342.00)	0.178	-18.83	129.0
Cremophor RH40 (HLB= 14-16)	136.80 ± 6.25 (50.75-342.00)	80.62 ± 5.38 (43.82-255.00)	0.110	-7.62	89.0

The nanoemulsion for all surfactants tested with Labrafac PG was negatively charged and its zeta potential became less negative, increasing from −6.03 to −18.83 mV. Labrasol and Tween 20 presented more

negative zeta potentials, −14.7 mV and −18.83 mV respectively, than all others surfactants tested. The zeta potential is used to identify the charge of the droplets and the negative charge on the nanoemulsion; it is possibly imparted by the free fatty acids present in the inert oil phase and/or the surfactants used since the latter materials are mostly derivatives of fatty acids (Gershanik and Benita, 1996). Zeta potential can be also related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability. Therefore, nanoemulsions with high zeta potential (negative or positive) are electrically stabilised (Patel et al., 2011).

Turbidity measurements can be carried out to determine the rapid equilibrium reached by the dispersion and the reproducibility of this process (Gursoy et al., 2003). All turbidity values were below 140 NTU; a decrease in turbidity values results in a corresponding decrease in droplet sizes, confirming the emulsification efficiency of the SEDDS.

Results also indicated that Cremophor EL and Tween 80 had very good ability to emulsify Labrafac PG, followed by Labrasol and Tween 20, whereas Capryol 90 did not possess similar emulsification ability (data not shown). These surfactants have an HLB value between 12 and 17, which is very useful in enabling the selection of the best type of emulsifier for any given oil phase. Sagitani (1981) suggested that a proper surfactant HLB value was a key factor for the formation of an emulsion with small droplets. These results are in agreement with the findings reported by Warisnoicharoen et al. (2000), in which the results concluded that emulsification is also influenced by the structure and chain length of the surfactant. Cremophor EL, Labrasol, Tween 20 and Tween 80 rendered very good nanoemulsions that required a short emulsification period.

The HLB value has proven very useful in choosing the best type of emulsifier for any given oil phase. It was observed that surfactants with high HLB values (12–17) showed better emulsification ability. This may be due to the hydrophilicity of the surfactants, which enables rapid and facile dispersion of the oil in the aqueous phase as a very fine oil-in-water emulsion (Constantinides, 1995) and/or rapid spreading of the formulation in the aqueous media (Porter et al., 2008). This would keep the drug at the site of absorption for a relatively prolonged period of time, thus facilitating effective absorption by preventing the precipitation of drug compound within the GI lumen (Shah et al., 1994). Although the droplet size values obtained did not precisely correspond to the order of the HLB values of the surfactants, this indicates that nanoemulsification was also influenced by other factors, which may include the structure and chain length of the surfactant (Warisnoicharoen et al., 2000).

The method employed for screening SEDDS excipients helped us understand the emulsification efficiency of various surfactants for a selected oil phase. It also helped rapidly screen a large pool of cosurfactants available for perioral delivery. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of a single surfactant, usually necessitating the addition of a cosurfactant. The presence of cosurfactants decreases the bending stress of interfaces and grants the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsions over a wide range of compositions (Eccleston, 1994). Thus, after surfactant selection, a cosurfactant was selected based on its ability to increase the self-emulsification spontaneity of the oil/surfactant system. The droplet size, polydispersity index, zeta potential and turbidity results that were obtained by using two cosurfactants, Transcutol HP and propylene glycol, in combination with the selected surfactants and Labrafac PG as the oil phase are shown in Tables 6 and 7, respectively.

Table 6: Emulsification Properties of Transcutol HP with different Surfactants and Labrafac PG.

Surfactants	Droplet Size (nm)		PDI	Zeta Potential (mV)	Turbidity (NTU)
	Intensity	Number			
Cremophor EL (HLB= 12-14)	186.93 ± 6.62 (50.75-615.10)	82.27 ± 11.68 (37.84-396.01)	0.219	-13.33	217.67
Labrasol (HLB= 14)	228.83 ± 6.89 (91.28-531.20)	176.83 ± 12.51 (78.82-458.70)	0.216	-38.43	91.97
Tween 80 (HLB= 15)	(333.77 ± 11.85 (68.06-712.40)	113.83 ± 7.35 (50.75-531.20)	0.193	-19.60	285.33
Tween 20 (HLB= 17)	156.27 ± 2.92 (68.06-396.10)	99.47 ± 3.21 (58.77-295.30)	0.221	-19-90	89.47
Cremophor RH40 (HLB= 14-16)	182.40 ± 4.51 (43.82-531.20)	81.72 ± 3.42 (32.67-342.00)	0.172	-8.88	190.30

From all resultant nanoemulsions, only Labrasol and Tween 20 showed an average droplet size of <180 and 230 nm in both number-based diameter (N) and intensity (I), respectively, PDI <0.25 and turbidity <140 NTU in both cosurfactants tested. Transcutol HP and propylene glycol, along with Cremophor EL and Tween 80, resulted in a

nanoemulsion with unacceptable turbidity (>200 NTU); meanwhile, both cosurfactants decreased the turbidity of Labrasol or Tween 20 when used with Labrafac PG.

Table 7: Emulsification Properties of Propylene Glycol with different Surfactants and Labrafac PG.

Surfactants	Droplet Size (nm)		PDI	Zeta Potential (mV)	Turbidity (NTU)
	Intensity	Number			
Cremophor EL (HLB= 12-14)	194.80 ± 7.28 (58.77-615.10)	95.56 ± 4.81 (43.82-396.10)	0.255	-15.40	290.67
Labrasol (HLB= 14)	218.37 ± 2.58 (105.70-458.70)	168.9 ± 9.22 (91.28-458.70)	0.292	-40.73	85.23
Tween 80 (HLB= 15)	242.17 ± 5.76 (43.82-1281.00)	86.27 ± 4.24 (21.04-531.20)	0.387	-18.40	364.33
Tween 20 (HLB= 17)	170.43 ± 5.03 (50.75-458.70)	84.36 ± 3.94 (37.84-342.00)	0.335	-22.33	101.67
Cremophor RH40 (HLB= 14-16)	234.50 ± 5.95 (32.67-955.40)	53.60 ± 4.72 (28.21-342.00)	0.363	-11.23	388.3

The cosurfactants exerted a more significant impact on the zeta potential of the nanoemulsion (from −8.88 to −40.73 mV) that was formed than when only surfactants were used in the formulation (from −6.03 to −18.83 mV). Moreover, it was found that the absolute zeta potential increased by as much as 20 mV (from −18.83 to −38.43 mV) when Labrasol and Transcutol were used together. Zainol et al. (2012) demonstrate that this phenomenon can be attributed to cosurfactant function in admixture with surfactant. In the study, they used the same surfactant: co-surfactant (S_{mix} 2: 1) rate used in this study. The emulsion stability is directly related to the magnitude of the surface charge and the zeta potential is a stability indicative parameter in colloidal systems means the system will resist aggregation. The reason for this behaviour could be attributed to the strong repulsive Coulomb force between charged particles, which counterbalances the Van der Waals attraction force (Balakumar et al., 2013, Stachurski and Michalek, 1996). Generally, an increase in electrostatic repulsive forces between microemulsion droplets prevents the coalescence of microemulsion droplets. On the contrary, a decrease in electrostatic repulsive forces will cause phase separation (Gupta et al., 2011, Zhang et al., 2008). High absolute (positive and negative) zeta potential values (above +30 or −30 mV) should preferably be achieved in most of the emulsions prepared in order to ensure the creation of a high-energy barrier against coalescence of the dispersed droplets (Yang and Benita, 2000). In general, the zeta potential value of ±30 mV was sufficient for the stability of the system (Müller et al., 2001). Hence, the nanoemulsion that resulted from the combination of Labrafac PG, Labrasol and Transcutol complied with the zeta potential requirements for stability (−38.43 mV) and good separation.

Gupta et al. (2011)'s study, in which nanoemulsification capacity was increased by the addition of Transcutol HP or propylene glycol cosurfactants, obtained similar results. Propylene glycol formulations presented results slightly better than Transcutol HP formulations; however, the latter showed good turbidity and solubility for L294; and along with Labrasol, could easily be used to manufacture SEDDS for oral administration.

The emulsification studies clearly distinguished the ability of various surfactants to emulsify Labrafac PG as well as the ability of cosurfactants to improve the emulsification of selected surfactants. All cosurfactants utilised in this study increased the spontaneity of the nanoemulsion's formation. Synthetic surfactants have an inherent toxicity; therefore, their usage should be considered prior to use in a pharmaceutical formulation. Moreover, large amounts of surfactants may cause gastrointestinal and skin irritation when administered orally and topically, respectively. Non-ionic surfactants are relatively less toxic than their ionic counterparts and typically have lower CMCs (Azeem et al., 2009).

Non-ionic surfactants, such as Labrasol, are often screened for the development of SEDDS due to their relatively low toxicity, are generally considered safer than ionic surfactants and are usually acceptable for oral ingestion (Shafiq et al., 2007). Non-ionic surfactants are also reported to provide higher stability to emulsions over a wider range of pHs and ionic strengths (Swenson et al., 1994).

Among all the vehicles tested with L294, Labrafac PG (oil), Labrasol (surfactant) and Transcutol HP (cosurfactant) proved to be the most promising vehicles for SEDDS formulation. Labrafac PG (propylene glycol dicaprylocaprate) is a mixture of propylene glycol dicaprylate (50%–80%) and propylene glycol dicaprate (20%–40%), Labrasol is a mixture of acylglycerols and PEG esters, and Transcutol HP is an ethylene oxide derivative, mainly purified diethylene glycol monoethyl ether. Labrasol is a self-emulsifying excipient that is used to improve the oral bioavailability of poorly water-soluble drugs. Because it is a mixture of acylglycerols and PEG esters, this compound is a substrate for naturally occurring digestive lipases. Labrasol is a macrogolglyceride that is able to form microemulsions in gastrointestinal fluids. The main fatty acids present are

caprylic and capric acids. Many studies have shown that Labrasol increases the oral bioavailability of various drugs, such as simvastatin (Kang et al., 2004), gentamicin and glycyrrhizin (Hu et al., 2001), low molecular weight heparin (Rama Prasad et al., 2004), ezetimibe (Bandyopadhyay et al., 2012), dexibuprofen (Balakrishnan et al., 2009), ganciclovir (Shen et al., 2011), anti-cancer agent SR13668 (Green et al., 2011), etodolac (Barakat, 2010), coenzyme Q10 (Kommuru et al., 2001) and nimodipine (Yi et al., 2008). Moreover, Labrasol was reported to enhance the intestinal absorption of drugs (Hu et al., 2001, Koga et al., 2006, Prasad et al., 2003).

Transcutol HP, which is a strong solubiliser with low toxicity, has a long history of safe use as a solvent in many products, including pharmaceuticals, cosmetics and food applications (Sullivan Jr et al., 2014). Transcutol HP is a hydrophilic cosurfactant that increases the spontaneity of nanoemulsion formation (Borhade et al., 2012). This surfactant improved drug loading and spontaneous fine emulsion formation (Kang et al., 2004). Transcutol HP was reported to enhance bioavailability of itraconazole (Hong et al., 2006, Woo et al., 2008), exemestane (Singh et al., 2009a) and albendazol (Torrado et al., 1997, Torrado et al., 1996).

4. CONCLUSION

Selection of surfactants as self-emulsifying vehicles should be taken into account on that basis of their compatibility with the drug and emulsification capacity; therefore, pre-formulation studies were performed to evaluate solubility, drug–excipient compatibility and emulsification capability. The most efficient and best self-emulsification processes were observed in systems containing Labrafac PG and Labrasol. The addition of a cosurfactant, Transcutol HP, improved the spontaneity of self-emulsification. The self-emulsification characteristic -s of this formulation were a droplet size of 176.83 nm, polydispersity index equal to 0.216, potential zeta of −38.43 mV and turbidity <92 NTU. The vehicles evaluated with this pre-formulation were compatible with L294. Further work should be done to develop SEDDS formulations with Labrafac PG, Labrasol and Transcutol HP.

REFERENCES

1. AZEEM, A., RIZWAN, M., AHMAD, F. J., IQBAL, Z., KHAR, R. K., AQIL, M. & TALEGAONKAR, S. 2009. Nanoemulsion Components Screening and Selection: a Technical Note. AAPS Pharm Sci Tech, 10: 69-76.
2. BALAKRISHNAN, P., LEE, B.-J., OH, D. H., KIM, J. O., LEE, Y.-I., KIM, D.-D., JEE, J.-P., LEE, Y.-B., WOO, J. S., YONG, C. S. & CHOI, H.-G. 2009. Enhanced oral bioavailability of Coenzyme Q10 by self-emulsifying drug delivery systems. International Journal of Pharmaceutics, 374: 66-72.
3. BALAKUMAR, K., RAGHAVAN, C. V., SELVAN, N. T., PRASAD, R. H. & ABDU, S. 2013. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. Colloids and Surfaces B: Biointerfaces, 112: 337-343.
4. BANDYOPADHYAY, S., KATARE, O. P. & SINGH, B. 2012. Optimized self nano-emulsifying systems of ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids and Surfaces B: Biointerfaces, 100: 50-61.
5. BARAKAT, N. S. 2010. Enhanced oral bioavailability of etodolac by self-emulsifying systems: in-vitro and in-vivo evaluation. Journal of Pharmacy and Pharmacology, 62: 173-180.
6. BARREIRO, E. J. 2002. Estratégia de simplificação molecular no planejamento racional de fármacos: a descoberta de novo agente cardioativo. Química Nova, 25: 1172-1180.
7. BATES, T. R. & SEQUEIRA, J. A. 1975. Bioavailability of micronized griseofulvin from corn oil-in-water emulsion, aqueous suspension and commercial tablet dosage forms in humans. Journal of Pharmaceutical Sciences, 64: 793-797.
8. BORHADE, V., PATHAK, S., SHARMA, S. & PATRAVALE, V. 2012. Clotrimazole nanoemulsion for malaria chemotherapy. Part I: Preformulation studies, formulation design and physicochemical evaluation. International Journal of Pharmaceutics, 431: 138-148.
9. BORHADE, V. B., NAIR, H. A. & HEGDE, D. D. 2009. Development and Characterization of Self-Microemulsifying Drug Delivery System of Tacrolimus for Intravenous Administration. Drug Development and Industrial Pharmacy, 35: 619-630.
10. BRAGA, R. C., TÔRRES, A. C. B., PERSIANO, C. B., ALVES, R. O., FRAGA, C. A. M., BARREIRO, E. J. & DE OLIVEIRA, V. 2011. Determination of the cardioactive prototype LASSBio-294 and its metabolites in dog plasma by LC–MS/MS: Application for a pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis, 55: 1024-1030.
11. CHAUDHARY, A., NAGAICH, U., GULATI, N., SHARMA, V. K. & KHOSA, R. L. 2012. Enhancement of solubilization and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. Journal of Advanced Pharmacy Education & Research, 2: 32-67.
12. CHEN, M.-L. 2008. Lipid excipients and delivery systems for pharmaceutical development: A regulatory perspective. Advanced Drug Delivery Reviews, 60: 768-777.
13. CONSTANTINIDES, P. 1995. Lipid Microemulsions for Improving Drug Dissolution and Oral Absorption: Physical and Biopharmaceutical Aspects. Pharmaceutical Research, 12: 1561-1572.

14. DERLE, D., PATEL, J., YEOLE, D., PATEL, A. & PINGLE, A. 2010. Particle engineering techniques to enhance dissolution of poorly water soluble drugs. International Journal of Current Pharmaceutical Research, 2: 10-15.
15. ECCLESTON, J. 1994. Microemulsions. In: SWARBRICK, J. & BOYLAN, J. C. (eds.) Encyclopedia of Pharmaceutical Technology. New York, USA: Marcel Dekker.
16. FARAH, N., LAFORET, J. P. & DENIS, J. 1994. Self-microemulsifying drug delivery systems for improving dissolution of drugs: in vitro/in vivo evaluation. Pharmaceutical Research, 11: 202.
17. FATOUROS, D. G., KARPF, D. M., NIELSEN, F. S. & MULLERTZ, A. 2007. Clinical studies with oral lipid based formulations of poorly soluble compounds. Therapeutics and Clinical Risk Management, 3: 591-604.
18. FERNANDEZ-TARRIO, M., YAÑEZ, F., IMMESOETE, K., ALVAREZ-LORENZO, C. & CONCHEIRO, A. 2008. Pluronic and Tetronic Copolymers with Polyglycolyzed Oils as Self-Emulsifying Drug Delivery Systems. AAPS PharmSciTech, 9: 471-479.
19. GERSHANIK, T. & BENITA, S. 1996. Positively Charged Self-Emulsifying Oil Formulation for Improving Oral Bioavailability of Progesterone. Pharmaceutical Development and Technology, 1: 147-157.
20. GREEN, C., SWEZEY, R., BAKKE, J., SHINN, W., FURIMSKY, A., BEJUGAM, N., SHANKAR, G., JONG, L. & KAPETANOVIC, I. 2011. Improved oral bioavailability in rats of SR13668, a novel anti-cancer agent. Cancer Chemotherapy and Pharmacology, 67: 995-1006.
21. GUPTA, S., CHAVHAN, S. & SAWANT, K. K. 2011. Self-nanoemulsifying drug delivery system for adefovir dipivoxil: Design, characterization, in vitro and ex vivo evaluation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 392: 145-155.
22. GURSOY, N., GARRIGUE, J.-S., RAZAFINDRATSITA, A., LAMBERT, G. & BENITA, S. 2003. Excipient effects on in vitro cytotoxicity of a novel paclitaxel self-emulsifying drug delivery system. Journal of Pharmaceutical Sciences, 92: 2411-2418.
23. HONG, J.-Y., KIM, J.-K., SONG, Y.-K., PARK, J.-S. & KIM, C.-K. 2006. A new self-emulsifying formulation of itraconazole with improved dissolution and oral absorption. Journal of Controlled Release, 110: 332-338.
24. HU, Z., TAWA, R., KONISHI, T., SHIBATA, N. & TAKADA, K. 2001. A novel emulsifier, Labrasol, enhances gastrointestinal absorption of gentamicin. Life Sciences, 69: 2899-2910.
25. JACOBY, G., COHEN, K., BARKAN, K., TALMON, Y., PEER, D. & BECK, R. 2015. Metastability in lipid based particles exhibits temporally deterministic and controllable behavior. Sci. Rep., 5.
26. JOSHI, M., PATHAK, S., SHARMA, S. & PATRAVALE, V. 2008. Solid microemulsion preconcentrate (NanOsorb) of artemether for effective treatment of malaria. International Journal of Pharmaceutics, 362: 172-178.
27. KADU, P. J., KUSHARE, S. S., THACKER, D. D. & GATTANI, S. G. 2011. Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS). Pharmaceutical Development and Technology, 16: 65-74.
28. KANG, B. K., LEE, J. S., CHON, S. K., JEONG, S. Y., YUK, S. H., KHANG, G., LEE, H. B. & CHO, S. H. 2004. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. International Journal of Pharmaceutics, 274: 65-73.
29. KHADKA, P., RO, J., KIM, H., KIM, I., KIM, J. T., KIM, H., CHO, J. M., YUN, G. & LEE, J. 2014. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian Journal of Pharmaceutical Sciences, 9: 304-316.
30. KOGA, K., KUSAWAKE, Y., ITO, Y., SUGIOKA, N., SHIBATA, N. & TAKADA, K. 2006. Enhancing mechanism of Labrasol on intestinal membrane permeability of the hydrophilic drug gentamicin sulfate. European Journal of Pharmaceutics and Biopharmaceutics, 64: 82-91.
31. KOMMURU, T. R., GURLEY, B., KHAN, M. A. & REDDY, I. K. 2001. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. International Journal of Pharmaceutics, 212: 233-246.
32. KOSTEWICZ, E. S., BRAUNS, U., BECKER, R. & DRESSMAN, J. B. 2002. Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media. Pharmaceutical Research, 19: 345-349.
33. LIPINSKI, C. A. 2000. Drug-like properties and the causes of poor solubility and poor permeability. Journal of Pharmacological and Toxicological Methods, 44: 235-249.
34. MAHAPATRA, A. K., MURTHY, P. N., SWADEEP, B. & SWAIN, R. P. 2014. Self-Emulsifying Drug Delivery Systems (SEDDS): An Update from Formulation Development to Therapeutic Strategies. International Journal of PharmTech Research, 6: 546-568.
35. MÜLLER, R. H., JACOBS, C. & KAYSER, O. 2001. Nanosuspensions as particulate drug formulations in therapy: Rationale for development and what we can expect for the future. Advanced Drug Delivery Reviews, 47: 3-19.

36. NAZZAL, S., SMALYUKH, I. I., LAVRENTOVICH, O. D. & KHAN, M. A. 2002. Preparation and in vitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation. International Journal of Pharmaceutics, 235: 247-265.
37. NEKKANTI, V., KARATGI, P., PRABHU, R. & PILLAI, R. 2010. Solid Self-Microemulsifying Formulation for Candesartan Cilexetil. AAPS Pharm Sci Tech, 11: 9-17.
38. NIELSEN, F. S., PETERSEN, K. B. & MÜLLERTZ, A. 2008. Bioavailability of probucol from lipid and surfactant based formulations in minipigs: Influence of droplet size and dietary state. European Journal of Pharmaceutics and Biopharmaceutics, 69: 553-562.
39. PARMAR, N., SINGLA, N., AMIN, S. & KOHLI, K. 2011. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids and Surfaces B: Biointerfaces, 86: 327-338.
40. PATEL, A. & VAVIA, P. 2007. Preparation and in vivo evaluation of SMEDDS (self-microemulsifying drug delivery system) containing fenofibrate. The AAPS Journal, 9: E344-E352.
41. PATEL, J., PATEL, A., RAVAL, M. & SHETH, N. 2011. Formulation and development of a self-nanoemulsifying drug delivery system of irbesartan. Journal of Advanced Pharmaceutical Technology & Research, 2: 9-16.
42. PORTER, C. J. H., POUTON, C. W., CUINE, J. F. & CHARMAN, W. N. 2008. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Advanced Drug Delivery Reviews, 60: 673-691.
43. PORTER, C. J. H., TREVASKIS, N. L. & CHARMAN, W. N. 2007. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nature Reviews Drug Discovery, 6: 231-248.
44. POUTON, C. W. 2000. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. European Journal of Pharmaceutical Sciences, 11, Supplement 2: S93-S98.
45. PRASAD, Y. V. R., PUTHLI, S. P., EAIMTRAKARN, S., ISHIDA, M., YOSHIKAWA, Y., SHIBATA, N. & TAKADA, K. 2003. Enhanced intestinal absorption of vancomycin with Labrasol and d-α-tocopheryl PEG 1000 succinate in rats. International Journal of Pharmaceutics, 250: 181-190.
46. RAMA PRASAD, Y. V., MINAMIMOTO, T., YOSHIKAWA, Y., SHIBATA, N., MORI, S., MATSUURA, A. & TAKADA, K. 2004. In situ intestinal absorption studies on low molecular weight heparin in rats using Labrasol as absorption enhancer. International Journal of Pharmaceutics, 271: 225-232.
47. SAGITANI, H. 1981. Making homogeneous and fine droplet O/W emulsions using nonionic surfactants. Journal of the American Oil Chemists' Society, 58: 738-743.
48. SALIMI, A., SHARIF MAKHMAL ZADEH, B., HEMATI, A. A. & AKBARI BIRGANI, S. 2014. Design and Evaluation of Self-Emulsifying Drug Delivery System (SEDDS) Of Carvedilol to Improve the Oral Absorption. Jundishapur Journal of Natural Pharmaceutical Products, 9: e16125.
49. SAVJANI, K. T., GAJJAR, A. K. & SAVJANI, J. K. 2012. Drug Solubility: Importance and Enhancement Techniques. ISRN Pharmaceutics, 2012; 195727.
50. SHAFIQ, S., SHAKEEL, F., TALEGAONKAR, S., AHMAD, F. J., KHAR, R. K. & ALI, M. 2007. Development and bioavailability assessment of ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics, 66: 227-243.
51. SHAH, N. H., CARVAJAL, M. T., PATEL, C. I., INFELD, M. H. & MALICK, A. W. 1994. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. International Journal of Pharmaceutics, 106: 15-23.
52. SHEN, Y., LU, Y., JV, M., HU, J., LI, Q. & TU, J. 2011. Enhancing effect of Labrasol on the intestinal absorption of ganciclovir in rats. Drug Development and Industrial Pharmacy, 37: 1415-1421.
53. SHUKLA, J. B. & PATEL, S. J. 2010. Formulation and Evaluation of Self Micro Emulsifying System of Candesartan Cilexetil. International Journal of Pharmacy & Pharmaceutical Sciences, 2: 143-146.
54. SINGH, A., CHAURASIYA, A., AWASTHI, A., MISHRA, G., ASATI, D., KHAR, R. & MUKHERJEE, R. 2009a. Oral Bioavailability Enhancement of Exemestane from Self-Microemulsifying Drug Delivery System (SMEDDS). AAPS Pharm Sci Tech, 10: 906-916.
55. SINGH, B., BANDOPADHYAY, S., KAPIL, R., SINGH, R. & KATARE, O. P. 2009b. Self-Emulsifying Drug Delivery Systems (SEDDS): Formulation Development, Characterization, and Applications. Critical Reviews in Therapeutic Drug Carrier Systems, 26: 427-451.
56. STACHURSKI, J. & MICHALEK, M. 1996. The Effect of the Zeta-Potential on the Stability of a Nonpolar Oil-in-Water Emulsion. Journal of Colloid and Interface Science, 184: 433-436.
57. SULLIVAN JR, D. W., GAD, S. C. & JULIEN, M. 2014. A review of the nonclinical safety of Transcutol®, a highly purified form of diethylene glycol monoethyl ether (DEGEE) used as a pharmaceutical excipient. Food and Chemical Toxicology, 72: 40-50.

58. SWENSON, E. S., MILISEN, W. & CURATOLO, W. 1994. Intestinal Permeability Enhancement: Efficacy, Acute Local Toxicity, and Reversibility. Pharmaceutical Research, 11: 1132-1142.
59. TANG, J.-L., SUN, J. & HE, Z.-G. 2007. Self-Emulsifying Drug Delivery Systems: Strategy for Improving Oral Delivery of Poorly Soluble Drugs. Current Drug Therapy, 2: 85-93.
60. TARR, B. & YALKOWSKY, S. 1989. Enhanced Intestinal Absorption of Cyclosporine in Rats Through the Reduction of Emulsion Droplet Size. Pharmaceutical Research, 6: 40-43.
61. THANKACHEN, D. K., MATHEWS, M. M. & JOSEPH, J. 2014. Self emulsifying drug delivery system: an approach to improve the solubility of poorly water soluble drugs. International Journal of Universal Pharmacy and Bio Sciences, 3: 572-591.
62. TORRADO, S., LÓPEZ, M. L., TORRADO, G., BOLÁS, F., TORRADO, S. & CADÓRNIGA, R. 1997. A novel formulation of albendazole solution: oral bioavailability and efficacy evaluation. International Journal of Pharmaceutics, 156: 181-187.
63. TORRADO, S., TORRADO, S., CADORNIGA, R. & TORRADO, J. J. 1996. Formulation parameters of albendazole solution. International Journal of Pharmaceutics, 140: 45-50.
64. WAKERLY, M. G., POUTON, C. W., MEAKIN, B. J. & MORTON, F. S. 1986. The effect of surfactant HLB on the self-emulsifying efficiency of non-ionic surfactant-vegetable oil mixtures. Journal of Pharmacy and Pharmacology, 38: 2P-2P.
65. WARISNOICHAROEN, W., LANSLEY, A. B. & LAWRENCE, M. J. 2000. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. International Journal of Pharmaceutics, 198: 7-27.
66. WESSEL, M. D., JURS, P. C., TOLAN, J. W. & MUSKAL, S. M. 1998. Prediction of Human Intestinal Absorption of Drug Compounds from Molecular Structure. Journal of Chemical Information and Computer Sciences, 38: 726-735.
67. WOO, J. S., SONG, Y.-K., HONG, J.-Y., LIM, S.-J. & KIM, C.-K. 2008. Reduced food-effect and enhanced bioavailability of a self-microemulsifying formulation of itraconazole in healthy volunteers. European Journal of Pharmaceutical Sciences, 33: 159-165.
68. YADAV, P. S., YADAV, E., VERMA, A. & AMIN, S. 2014. Development, Characterization, and Pharmacodynamic Evaluation of Hydrochlorothiazide Loaded Self-Nanoemulsifying Drug Delivery Systems. The Scientific World Journal, 2014; 274823.
69. YANG, S. C. & BENITA, S. 2000. Enhanced absorption and drug targeting by positively charged submicron emulsions. Drug Development Research, 50: 476-486.
70. YI, T., WAN, J., XU, H. & YANG, X. 2008. A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs. European Journal of Pharmaceutics and Biopharmaceutics, 70: 439-444.
71. ZAINOL, S., BASRI, M., BASRI, H. B., SHAMSUDDIN, A. F., ABDUL-GANI, S. S., KARJIBAN, R. A. & ABDUL-MALEK, E. 2012. Formulation Optimization of a Palm-Based Nanoemulsion System Containing Levodopa. International Journal of Molecular Sciences, 13: 13049-13064.
72. ZHANG, P., LIU, Y., FENG, N. & XU, J. 2008. Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. International Journal of Pharmaceutics, 355: 269-276.