Whites Science
A Scientific Erudition

DESIGN AND DEVELOPMENT OF SELF-EMULSIFYING DRUG DELIVERY SYSTEMS NOVEL CARDIOACTIVE N-ACYLHYDRAZONE COMPOUND

Beatriz Zanchetta[1,2*], Marco Vinícius Chaud[3*], Maria Helena Andrade Santana[1]

[1]**Department of Engineering of Materials and Bioprocesses, School of Chemical Engineering, University of Campinas, 13083-852 Campinas, SP, Brazil.**

[2]**Department of Research and Innovation, Cristalia Chemical and Pharmaceutical Industry, 13970-970 Itapira, SP, Brazil.**

[3]**Laboratory of Biomaterials and Nanotechnology of the University of Sorocaba, 18023-000 Sorocaba, SP, Brazil.**

ABSTRACT

In this study, the emphasis is placed on a strategy for enhancing the drug/carrier interaction for improved drug solubility, drug-loading capacity, self-emulsification and stability.Preliminary solubility of L294 was determined in various oils, surfactants and cosurfactants. A ternary phase diagram was constructed to identify the self-emulsifying region for the selected systems, using series concentrations of Labrafac PG, Labrasol and Transcutol HP. Self-emulsifying properties, particle size, polydispersibility, and zeta potential were studied after dilution of formulations in water.The results demonstrated the development of a self-emulsifying formulation of L294 in liquid form, which upon contact with aqueous media spontaneously forms a clear nanoemulsion having a small droplet size (around 100 nm). The zeta potential of the selected SEDDS formulation was between −11.09 and −20.50 with a viscosity around 40-60 cP. The optimum formulation consisted of a mixture of Labrafac PG, Labrasol and Transcutol HP.The L294 showed extremely low water solubility (0,006 mg.mL^{-1}), and when formulated in SEDDS, its solubility increased over than 33,000 fold.This study demonstrate that SEDDS can be considered as a very good candidate to optimize the peroral administration of L294.

Keywords: SEDDS, Emulsion Drug Delivery, L294, Oral Dosage Forms, Cardioactive.

INTRODUCTION

LASSBio 294 is a novel cardioactive N-acylhydrazone compound, 3,4-methylenedioxybenzoyl-2-thienylhydrazone (L294).[1; 2] This compound has been described as a potent cardiac inotropic agent with vasodilator properties.[3; 4] L294 was found to improve intracellular Ca^{2+} regulation [2; 5; 6] and prevent myocardial infarction induced by cardiac dysfunction, which could potentially prevent heart failure.[7] In addition, L294 also promoted vasodilation in aortic rings, mediated by the guanylatecyclase/cyclic guanylate monophosphate pathway.[6; 8]

The L294 drug is rapidly absorbed and eliminated after oral administration.[9]Following a single 10 mg oral dose administration, mean peak plasma levels of 550 ng/ml are attained in about 1 h. Based on *in silico* data, L294 should be classified in the class II category under the biopharmaceutical classification system (BCS), i.e., it has good permeability through biological membranes, but exhibits low aqueous solubility. The rate of absorption of any drug is mainly controlled by its dissolution rate in gastrointestinal fluids, especially insoluble hydrophobic drugs. L294 alone have a very low bioavailability, due to its poor solubility in water combined with an insufficient dissolution rate.[9] L294 is a weak acid (pKa predicted properties is 10.8) – it is practically insoluble in water 0.006 mg.g^{-1} (personal data). These parameters make L294 a perfect molecule for the development of SEDDS, aiming the improvement of its bioavailability.

Poorly water-soluble drug candidates are often presented to formulators with considerable technical challenges. A significant number of drugs suffer from the problem of low oral absorption,[10-12]and thus poor oral efficiency. Different

*Corresponding author:
Email:biapharma@gmail.com

http://dx.doi.org/10.20530/IJNN_4_1-12
ISSN 2397-5547© 2016

strategies have been investigated to enhance the bioavailability of poorly absorbed drugs in order to increase their clinical efficacy when administered orally.[13] Innovative lipid-based formulations, such as self-emulsifying drug delivery systems (SEDDS), have been extensively described as effective delivery systems due to their proven ability to enhance bioavailability of lipophilic drugs.[12; 14-17]

SEEDS are drug delivery systems based on a mixture of oils, surfactants, co-solvents and drugs, which results in entropy-favoured spontaneous emulsification on *in situ* exposure, and can sometimes emulsify under conditions of gentle agitation, similar to those that would be encountered in the gastrointestinal tract.[13; 18-21]

The ability of SEDDS to improve oral bioavailability has been shown for curcumin,[22] mitotane,[23]mefenamic acid,[24] Valsartan,[25] to improve the oral absorption of carvediol,[11] inhibition of human efflux transporter ABCC2,[26] and transformation of the crystalline form of the drug to the amorphous form in the SEDDS.[16] SEDDS are among the methods used to improve the oral bioavailability of poorly soluble drugs by presenting and maintaining the drug in a dissolved state, in small droplets of oil, and all over its transit through the gastrointestinal tract.[16; 27; 28] SEDDS can improve oral bioavailability by minimising the effect of pH on drug absorption, increasing drug solubilisation, in which the water-insoluble drug is usually dissolved in the oil phase, therefore enhancing permeation across the intestinal membrane through a wide distribution in the gastrointestinal tract, due to its small droplet size.[29-31] The potential advantages of the SEDDS include 100% drug entrapment capacity, physically stable formulation,[13] with no dissolution step required, and also providing protection against gastric degradation.[13; 32]

Soft gelatin capsules containing SEDDS readily disperse in the stomach to form a fine emulsion;[33] in this case, gastrointestinal motility can provide the agitating effect necessary for emulsification.[33]While the primary mechanism by which SEDDS formulations are thought to improve drug absorption is through elimination of the need for pre-absorptive drug solubilisation in the gastrointestinal tract, other mechanisms may include protection from chemical and enzymatic degradation localised in the aqueous environment of the gastrointestinal tract and promotion of lymphatic drug transport, bypassing the hepatic first pass effect.[13; 15; 19]

In the process of developing SEDDS formulations, different compositions of oils, surfactants and cosurfactants have to be evaluated for identifying the best self-emulsifying region of the system.[34] Self-emulsified formulations are a clear dispersion, which should remain stable on dilution in order to make the hydrophobic drugs remain in solubilised form until absorption.[34; 35]

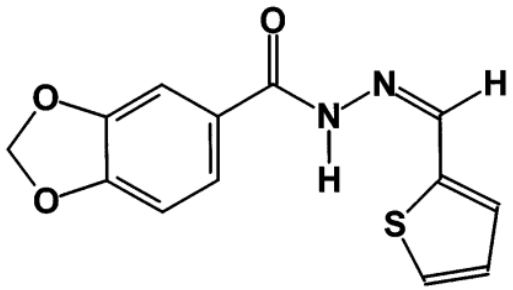

Figure 1: Molecular structure of 3,4-methylenodioxybenzoyl-2-thienylhydrazone (LASSBio 294). Adapted from Costa et al.[6]

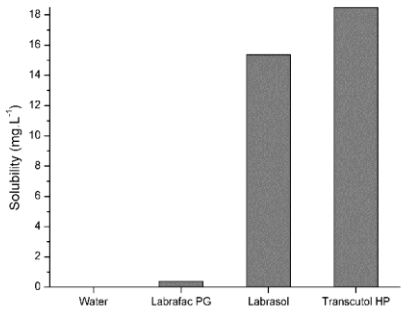

Figure 2: Solubility studies of L294 in selected vehicles

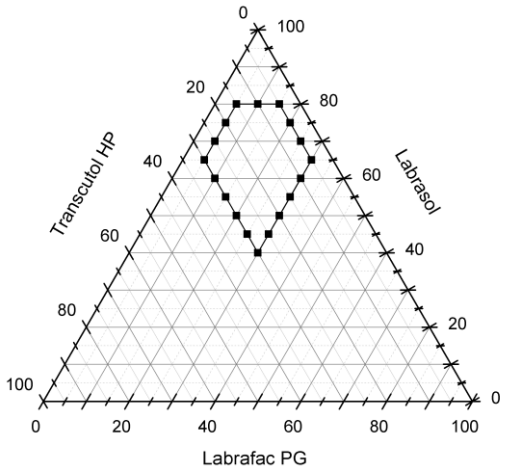

Figure 3: Phase diagram of Labrafac PG, Labrasol and Transcutol HP in water at 25 °C. The area inside the line represents the self-nanoemulsion region.

The present work aims on the design, development and characterisation of a SEDDS formulation loaded with L294. Moreover, this work describes the ability to form nanoemulsion characterisation of formulations on the nanoemulsifying area in the phase diagram.

MATERIAL AND METHODS

Chemicals

Labrasol, Transcutol HP, Lauroglycol 90, Labrafac PG, Maisine 35-1, LabrafacLipophile WL 1349, Capryol 90, Peceol, and Labrafil M1944CS were generously donated by Gattefossé (France). Cremophor EL and Cremophor RH40 were received from BASF (Germany). Castor oil, Tween 20 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 Fig. 1 and Table 1, respectively.

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

Excipient screening

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 (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, then, the flasks were kept under mild constant agitation for 48 h at 37°C in an orbital shaker (Dubnoff 304-D, Nova Etica, Brazil). After equilibrium was achieved, the samples were centrifuged at 3,500g for 15 min (Thermo Scientific Sorval Legend Mach 1.6R) for non-solubilised drug removal. The supernatant was collected, diluted in a proper solvent and analysed at 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 the results were represented as a mean value ($mg.g^{-1}$) ± standard deviation (S.D.).

Table 2: Oils used in Solubility Tests

General Class	Compound	HBL	Trade Name
Fixed Oils	Castor Oil	n.a.	Super Refined Castor Oil
	Medium-chain triglycerides	2	Labrafac LipophileWL1349
Propylene glycol esters	Propylene glycol dicaprylocaprate	2	Labrafac PG
Glycerides	Glycerylmonolinoleate	4	Maisine 35-1
	Glycerylmonooleate (Type 40)	3	Peceol

n.a.: non applied

Construction of phase diagrams

The selected oil (Labrafac PG), surfactant (Labrasol), and cosurfactant (Transcutol HP), based on preliminary screening studies, were used to develop phase diagrams. The ternary phase diagram was constructed in the presence of the drug to identify the self-emulsifying region and the ideal proportions of eachexcipient. To construct the phase diagram, different liquid SEDDS formulations (pre-concentrate) were prepared by solubilisation of L294 in the mixture of excipients, using mild magnetic stirring at room temperature for a maximum period of three hours to obtain a clear solution. The formulations were left standing for 24 h to achieve equilibrium and then stored at room temperature. The ternary phase diagram was plotted, with the composition of oil, surfactant and cosurfactantfixed as each vertices of the triangle. Different oil/surfactant/cosurfactant mixtures were prepared according to the proportion of each point of the triangle.

The level of L294 was fixed at 0.2% w/w of the vehicle. Six different series of the SEDDS (Table 5) were prepared with varying concentrations of oil (5–30%), surfactant (40–80%) and cosurfactant (5–30%). The volume ratio of diluent to pre-concentrate SEDDS formulations was fixed in 100:0.1, considering the volume (≅ 100 mL) of the gastric fluid. [36]

Table 3: Surfactants used in Solubility Tests

General Class	Compound	HBL	Trade Name
Polyoxyethylene castor oil	Polyoxyl 35 castor oil	12-14	Cremophor EL
	Polyoxyl 40 Hydrogenerated castor oil	14-16	Cremophor RH40
Polyoxyglycerides	Oleoyl polyoxyl-6 glycerides	4	Labrafil M1944CS
	Caprylocaproylpolyoxyl-8 glycerides	14	Labrasol
Propylene glycol esters	Propylene glycol monocaprylate (Type II)	6	Capryol 90
Propylene glycol esters	Propylene glycol monolaurate (type II)	5	Lauroglycol 90
Polysorbates	Polyoxyethylene 20 sorbitanmonolaurate	17	Tween 20
	Polyoxyethylene 20 sorbitanmonooleate	15	Tween 80

Table 5: Cosurfactants/co-solvents used in Solubility Tests

General Class	Compound	Trade Name
Alkane diols and triols	1,2-propanediol	Propylene glycol
Glycol ether	Diethylene glycol monoethyl ether	Transcutol HP

Table 6: Different Composition of Formulation Series.

Group	Oil	Surfactant	Cosurfactant
A series	5%	65 – 80%	15 – 30%
B series	10%	60 – 80%	10 – 30%
C series	15%	55 – 80%	5 – 30%
D series	20%	50 – 75%	5 – 30%
E series	25%	45 – 70%	5 – 30%
F series	30%	40 – 65%	5 – 30%

Table 4: Physical Properties of Different L294 SEDDS Formulations

Series	Viscosity (cP)	Droplet Size (nm)		PI	ZP (mV)
		Intensity	Number		
A1	48.40 ± 0.10	149.77 ± 5.97	103.87 ± 3.84	0.095 ± 0.030	-11.09 ± 1.21
A2	40.73 ± 0.06	153.60 ± 3.74	117.27 ± 1.15	0.056 ± 0. 020	-15.20 ± 0.84
B1	58.27 ± 0.06	171.23 ± 5.31	102.10 ± 6.29	0.154 ± 0.008	-19.80 ± 0.52
B2	42.67 ± 0.06	168.83 ± 5.13	118.67 ± 3.89	0.151 ± 0.028	-14.90 ± 1.08
C1	62.57 ± 0.25	177.00 ± 4.76	110.60 ± 5.11	0.249 ± 0.040	-20.50 ± 0.85
C2	49.23 ± 0.06	164.47 ± 2.63	111.70 ± 8.76	0.229 ± 0.007	-15.30 ± 1.15
D1	55.60 ± 0.10	168.83 ± 11.01	111.20 ± 9.48	0.219 ± 0.006	-14.80 ± 0.38
E1	45.83 ± 0.06	173.57 ± 2.07	87.72 ± 3.70	0.272 ± 0.034	-19.80 ± 0.40
F1	42.53 ± 0.06	185.47 ± 2.07	91.19 ± 7.44	0.282 ± 0.034	-20.10 ± 0.40

PI = polydispersity index. ZP = ζ-potentia

emulsify under gentle agitation and dilution were identified in the phase diagram. All formulations were characterised for droplet size, ζ-potential and visual observation after dilution in water. Viscosity measurements and visual observations were taken for the pre-concentrates.

Viscosity

Viscosity can be used as a physical characterisation parameter. Thus, viscosity was measured using the Vibration method, placing 10 mL of undiluted formulation SEDDS in the viscometer SV-10 (A&D Co., Japan). The measures were determined at room temperature by room temperature. The contents were gently stirred, immediately kept standing against light, and were observed for assessment for self-emulsification efficiency, transparency, phase separation and precipitation of L294. Precipitation was evaluated by visual inspection of the resultant nanoemulsion after 24 h. These formulations were categorised as clear (transparent or transparent with bluish tinge), not clear (turbid), stable (with precipitation) or unstable (without precipitation). SEDDS with clear or slightly bluish appearances were classified as nanoemulsions.

Droplet size analysis and ζ-potential

The droplet size and polydispersity index (PDI) of the SEDDS formulation were determined using the dynamic light scattering (DLS) technique – DLS at 90° fixed angle using a Zetasizer Nano ZS (Malvern Instruments, UK). Each formulation was diluted with purified water before analysis. This equipment was also used for ζ-potential measurement and operated at a 25°C, 23 Vcm^{-1} electric field intensity. All studies were repeated three times and the values of the diameters and intensity were used.

The droplet size was measured as the mean diameter using 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 (Nαd) and shows the predominant diameters. The polydispersity (PDI) reflects the dispersion of the particle diameters. The zeta potential values were calculated according to the Smoluchowski equation.

RESULTS

Excipient selection

The selection of oil, surfactant or cosurfactant was governed by previous studies of excipient screening (personal data). The comparative solubility studies of L294 in the oil, surfactant and cosurfactant selected are reported Fig. 2. The aqueous solubility of L294 was about 0.006 $mg.g^{-1}$, indicating that it was poorly water-soluble. L294 has low miscibility in the assayed oils (approximately 1 $mg.g^{-1}$). Solubility of L294 in the surfactants Cremophor EL (HLB 12–14), Labrasol (HLB 14), Cremophor RH40 (HLB 14–16), Tween 80 (HLB 15), and Tween 20 (HLB 17) was between 8 and 20 $mg.g^{-1}$. However, the solubility in Labrafil M1944CS (HLB 4), Capryol 90 (HLB 6), and Lauroglycol 90 (HLB 5) was less than 2.5 $mg.g^{-1}$. Solubility of L294 in the cosurfactantTranscutol HP showed good solubilising capacity (18.5 $mg.g^{-1}$), and poor solubility in propylene glycol (less than 3.5 $mg.g^{-1}$). Amongst the various solvents investigated for equilibrium solubility studies, Labrafac PG, Labrasol and Transcutol HP were selected for construction of ternary phase diagrams.

The lipid is a more important ingredient of the SEDDS formulation[15],among the used oils, Maisine 35-1 and Labrafac PG showed maximum and minimum solubility for L294. Maisine (HLB = 4) had more strength to solve L294 than did Labrafac(HLB = 2). However, Labrafac PG exhibited good emulsification properties with all surfactants tested (personal data). Therefore, lipids can not only solubilise large amount of lipophilic drugs or facilitate self-emulsification but also enhance the fraction of lipophilic drugs transported via the intestinal lymphatic system, thereby increasing its absorption from the GI tract.[15; 37; 38]

A surfactant is to provide the essential emulsifying characteristics to SEDDS,[15] making it possible for large amounts of drug compounds to get dissolved into the system.[18] Surfactants, being amphiphilic compounds, invariably dissolve larger amounts of the hydrophobic drug. The two issues that govern the selection of a surfactant involve the hydrophilic–lipophilic balance (HLB) and safety.[15] The hydrophobically assembled micelles usually consist of amphiphilic compounds that have distinct hydrophobic and hydrophilic domains.[39] Upon exposure to an aqueous medium, the amphiphilic molecules spontaneously self-assemble into supramolecular core/shell structures, and water-insoluble drugs can be loaded into the hydrophobic cores.[40]

The HLB of a surfactant provides important information on its potential and utility in the formulation of SEDDS.[15] For attaining high emulsifying performance, the emulsifier involved in the formulation of SEDDS should have high HLB and high hydrophilicity for immediate formation of o/w droplets and rapid spreading of formulation in aqueous media.[15] Non-ionic surfactants are generally considered for pharmaceutical applications and nanoemulsion formulation since these are less toxic[41] than ionic surfactants, and they are accepted for oral ingestion.[20; 42; 43]As required for SEDDS development, surfactants of high HLB value have a high self-emulsifying capability in the aqueous phase.[44] HLB values in the range of 12–16 favour the nanoemulsion formation.[45] Lipophilic surfactants with an HLB of less than 10 are capable of promoting some emulsification of the oil, but the resulting emulsions are normally too crude (in terms of size) to be useful.[45] Hydrophilic surfactants with an HLB of more than 10 are far superior at these, providing fines and uniform emulsion droplets that are more likely to empty rapidly from the stomach.[46; 47] Thus, for the present study, Labrasol was used as surfactant having, an HLB value equal to 14. Labrasol, a surfactant of medium length alkyl chain, showed higher drug solubility, and is a macrogol glyceride that is able to form microemulsions in GI fluids.[48] Moreover, Labrasol was reported to enhance the intestinal absorption of drugs,[49; 50] and several studies

describesLabrasolas an enhancer of oral bioavailability of various drugs, such as insulin,[51]vancomycin,[49]ezetimibe,[14]ganciclovir,[52]buparvaquone,[53]flurbiprofen,[54]nimodipine,[55] and fizetin.[56] Surfactants form a layer around the emulsion droplets and hence reduce the interfacial energy, as well as provide a mechanical barrier to coalescence.[47]

The addition of a cosurfactant in the formulation containing surfactant was reported to improve dispersibility and drug absorption from the formulation, in addition to reducing interfacial tension and the formation of mechanical barriers to coalescence.[20; 44]Cosurfactants increase surfactant emulsification by penetrating the interfacial surfactant monolayer and their structure and chain length affect their performance.[57; 58]Therefore, it seems that increasing surfactant having a high HLB concentration, the drug's solubility increased. Transcutol has been used as an effective solubilizing agent and a permeability enhancer in emulsifying systems. The blending of Transcutol with the Labrasol mainly helps in improving the emulsification ability of Labrasol, and Kim et al[59] has already used this approach. Furthermore, Labrafac, Labrasol and Transcutol are known bioavailability enhancers.[54; 60; 61]

Ternary phase diagrams

The phase diagram plays an important role in studying the phase behaviour of prepared nanoemulsions.[44]Phase diagrams are normally constructed with the oil phase, surfactant or mixture of surfactant and cosurfactant, and the aqueous phase, which will reveal the regions of liquid crystal, nano/microemulsion and coarse emulsion.[33; 42] In SEDDS, the primary means of assessment of self-emulsification is visual evaluation, and the self-emulsification efficiency can be estimated by determining the droplet size (number diameter and intensity). The visual test is a measure of an apparent spontaneity of emulsion formation. In the dilution study, visually there were no significant differences found in the self-emulsifying performance among series diluted in the same dissolution medium (water).

Oil, surfactant and cosurfactant were selected based on their drug solubility capacity, hydrophilic-lipophilic balance (HLB) values and ability of emulsion formation and biopharmaceutical properties. These ternary phase diagrams were constructed in the presence of L294 to identify the self-emulsifying regions and optimise the concentration of oil, surfactant and cosurfactant in the SEDDS formulation. The effect of the aqueous phase was overlooked for simplicity's sake, and only oil, surfactant and cosurfactant components concentration were used to identify the self-emulsifying region, as described in similar studies.[36; 47; 62]Fig. 3 describes the phase diagrams of the self-emulsifying systems loaded with L294 andcomposed byLabrafac PG oil, Labrasol surfactant and Transcutol HP cosurfactant. The shaded area, corresponding to oil (between 5–30%), surfactant (between 40–80%) and cosurfactant (between 5 and 30%), represents the efficient spontaneous emulsion region after dilution. It was observed that emulsification efficiency was good when Labrafac concentrations were higher than 5% and less than 30% of the SEDDS formulation. However, increasing Labrasolconcentration up to approximately 80%,increased the self-emulsification process spontaneity. Furthermore, increasing the cosurfactantconcentration decreases the self-emulsification process spontaneity. As cosurfactants have very little effect on reducing the interfacial tension, they help the surfactants to reduce the interfacial tension.[47; 63]

Dispersions resulting from water dilution showed to be clear without precipitation and/or phase separation, nor showed signs of cloudiness and were bluish-transparent. All of the liquid SEDDS formulations form clear and slight bluish nanoemulsions in less than 1 min when diluted with the distilled water medium. A desired ratio of components was further determined by investigating the droplet size distributions.

Physicochemical properties of liquid SEDDS

The liquid SEDDS series (A–F, Table 5) showedviscosity values 16 and 63 cP, while the droplet size of the resultant formulations after dilution was found to be between 82 and 493 nm (number). PI was less than 0.35, indicating that the studied self-emulsifying systems had a narrow size distribution.[64] The results of ZP ranged from -5.00 to -21.70 mV. The resultant charge was negative probably due to both surfactants used in the formulation. Based on these results, formulations some (Table 6) have been selected as the most promising nanoemulsion.

The physicochemical properties of the selected formulations were examined as a pre-concentrate, showed viscosity results less than 65 cP, which indicated formulations with good operability.[63] The viscosity of the formulations increases when the concentration of surfactant is above 65%; however, there is no statistical correlation between viscosity and droplet size. It was expected that the increasing cosurfactant concentrations

resulted in smaller droplets formation, since the cosurfactant acts on the reduction of the curvature angle of droplet formation,[65] but in this case, low concentrations of cosurfactant (5–15%) result in a droplet size of around 100 nm.

The PI increases with increases in the oil concentration. Most formulations (76%) have a DS in the range of 100–200 nm, while a smaller proportion of formulations (18%) had a DS of less than 100 nm, and only 6% of formulations exhibited a DS above 200 nm. When one increases the surfactant concentration, the result is a decrease on the mean droplet size. This could be explained by the oil droplet stabilisation since the surfactant molecules would be present at the oil–water interface.[25; 34; 45]

SEDDS selected series, examined as a pre-concentrate, showed viscosity results less than 65 cP, which indicated formulations with good operability.[63] The viscosity of the formulations increases when the concentration of surfactant is above 65%; however, there is no statistical correlation between viscosity and droplet size. It was expected that increasing concentrations of cosurfactant would also result in the formation of smaller droplets because it reduces the angle of the curvature during droplet formation,[65] but in this case, low concentrations of cosurfactant (5–15%) result in a droplet size of around 100 nm.

Viscosity assessmentis necessary on SEDDS formulation for its physical characterisation and also to understand the control of its stability. It is also crucial in determining its ability to be filled in hard or soft gelatin capsules.[30] If the system has very low viscosity, there is an enhanced probability of leakage from the capsule and the system with very high viscosity may have problems with operability.[30; 66]

The droplet size of SEDDS is a critical factor in self-emulsification performance because it determines the rate and extent of drug release, as well as absorption,[67] providing enhancing drug bioavailability.[35] Droplet size under 100 nm is considered desirable.[45; 64]Microemulsions with droplets size under 200 nm were considered acceptable according to Jain et al[68]. A smaller droplet size would bring forth a larger interfacial surface area for drug absorption;[69] therefore, a faster release rate could be achieved.[25; 34; 37; 70]

PI value is a measure of the width of size distribution and ranges from 0 to 1.[64] The values near zero indicate a monodispersed particle population, whereas values >0.5 signify a very broad size distribution.[64] PI <0.1 indicates a homogenous population, while a PI >0.3 indicates a higher heterogeneous dispersion.[71]The PI for all the stable SEDDS formulations was within the acceptable limits.[10]The polydispersity value is inversely proportional to the uniformity of droplet size in the formulation.[25; 72]

The charge of the SEDDS droplets should be assessed,[73; 74]along with polarity of droplets is also a quite important factor at the characterisation of emulsification efficiency.[44; 75] Zeta-potential indicates the degree of repulsion between adjacent particles with the same surface charge in dispersion, and its value can relate to the stability of nanoemulsion in SEDDS. In this particular case, the SEDDS emulsion stability would be granted by a higher zeta potential, either positive or negative. If the potential is low enough, the attraction between droplets would exceed repulsion and the emulsion would break and/or flocculate.[44]Therefore, nanoemulsions from SEEDS with high zeta potential are electrically stabilised.[37; 44]

CONCLUSION

In this study, the emphasis was placed on a strategy for enhancing the drug/carrier interaction for improved L294 solubility, L294-loading capacity, self-emulsification and stability of formulation. SEDDS components were selected based on biopharmaceutical properties and L294 solubility. A L294 SEDDS formulation composed by Labrafac PG (oil) and Labrasol (surfactant) with Transcutol HP (cosurfactant) was effectively developed. A small optimisation was performed based on droplets size, polydispersity, viscosity, and solubility. After extensive optimisation and evaluation, the developed L294-SEDDS were characterised for various qualitative and quantitative attributes. L294-SEDDS was shown to be monodispersed droplets with a size of 100 ± 10 nm, exhibiting negative ζ potential. The optimised L294 SEDDS needed a surfactant content of more than 65% and yielded nanoemulsion of a mean globule size of around 100 nm. This work reported the L294 self-emulsifying systems development in liquid form, in which in contact with water or aqueous media forms a nanoemsulsion without the need for external energy. The L294 showed extremely low water solubility (0.006 $mg.mL^{-1}$), and when formulated in SEDDS, its solubility increased over than 33,000 fold. Further studies should be performed on the evaluation of L294 loaded in SEDDS *in vivo* bioavailability.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Laboratory of Evaluation and Synthesis of Bioactive Substances (LASSBio) of the Federal University of Rio de Janeiro (UFRJ), and especially to Prof. Dr.Eliezer J. Barreiro, for the idealisation of the model. The authors also would like to thank Basf, Gattefossé, and Croda for supplying the excipients. The authors would like to acknowledge FAPESP, CNPq, and CAPES for their financial support.

REFERENCES

1. Sudo RT, Zapata-Sudo G, Barreiro EJ. The new compound, LASSBio 294, increases the contractility of intact and saponin-skinned cardiac muscle from Wistar rats. British Journal of Pharmacology. 2001 Oct;134(3):603–13. Available from: http://dx.doi.org/10.1038/sj.bjp.0704291.

2. Leal CM, Pereira SL, Kümmerle AE, Leal DM, Tesch R, de Sant'Anna CMR, et al. Antihypertensive profile of 2-thienyl-3,4-methylenedioxybenzoylhydrazone is mediated by activation of the A2A adenosine receptor. European Journal of Medicinal Chemistry. 2012 Sep;55:49–57. Available from: http://dx.doi.org/10.1016/j.ejmech.2012.06.056.

3. Silva CLM, Noël F, Barreiro EJ. Cyclic GMP-dependent vasodilatory properties of LASSBio 294 in rat aorta.British Journal of Pharmacology. 2002 Jan;135(1):293–8. Available from: http://dx.doi.org/10.1038/sj.bjp.0704473.

4. Pereira SL, Kummerle AE, Fraga CAM, Barreiro EJ, Sudo RT, Zapata-Sudo G.Vasodilator and antihypertensive effects of a novel N - acylhydrazone derivative mediated by the inhibition of L-type Ca 2+ channels . Fundamental & Clinical Pharmacology. 2012 Sep 7;28(1):29–41. Available from: http://dx.doi.org/10.1111/j.1472-8206.2012.01076.x.

5. Zapata-Sudo G, Sudo RT, Maronas PA, Silva GLM, Moreira OR, Aguiar MIS, et al. Thienylhydrazone derivative increases sarcoplasmic reticulum Ca2+ release in mammalian skeletal muscle. European Journal of Pharmacology . 2003 May;470(1-2):79–85. Available from: http://dx.doi.org/10.1016/s0014-2999(03)01757-6.

6. Costa DG, da Silva JS, Kümmerle AE, Sudo RT, Landgraf SS, Caruso-Neves C, et al. LASSBio-294, A Compound With Inotropic and Lusitropic Activity, Decreases Cardiac Remodeling and Improves Ca2+ Influx Into Sarcoplasmic Reticulum After Myocardial Infarction. Am J Hypertens. 2010 Jul 29;23(11):1220–7. Available from: http://dx.doi.org/10.1038/ajh.2010.157.

7. Braga RC, Alves VM, Fraga CAM, Barreiro EJ, de Oliveira V, Andrade CH. Combination of docking, molecular dynamics and quantum mechanical calculations for metabolism prediction of 3,4-methylenedioxybenzoyl-2-thienylhydrazone. J Mol Model. 2011 Sep 8;18(5):2065–78. Available from: http://dx.doi.org/10.1007/s00894-011-1219-9.

8. Silva AG, Zapata-Sudo G, Kummerle AE, Fraga CAM, Barreiro EJ, Sudo RT. Synthesis and vasodilatory activity of new N-acylhydrazone derivatives, designed as LASSBio-294 analogues. Bioorganic & Medicinal Chemistry. 2005 May;13(10):3431–7. Available from: http://dx.doi.org/10.1016/j.bmc.2005.03.003.

9. Braga RC, Tôrres ACB, Persiano CB, Alves RO, Fraga CAM, Barreiro EJ, et al. 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. 2011 Jul;55(5):1024–30. Available from: http://dx.doi.org/10.1016/j.jpba.2011.02.031.

10. Bansal T, Akhtar N, Jaggi M, Khar RK, Talegaonkar S. Novel formulation approaches for optimising delivery of anticancer drugs based on P-glycoprotein modulation. Drug Discovery Today. 2009 Nov;14(21-22):1067–74. Available from: http://dx.doi.org/10.1016/j.drudis.2009.07.010.

11. Salimi A, Sharif MakhmalZadeh B, Hemati A asghar, AkbariBirgani S. Design and Evaluation of Self-Emulsifying Drug Delivery System (SEDDS) Of Carvedilol to Improve the Oral Absorption. Jundishapur Journal of Natural Pharmaceutical Products. 2014 Jun 21;9(3). Available from: http://dx.doi.org/10.17795/jjnpp-16125.

12. Negi LM, Tariq M, Talegaonkar S. Nano scale self-emulsifying oil based carrier system for improved oral bioavailability of camptothecin derivative by P-Glycoprotein modulation. Colloids and Surfaces B: Biointerfaces.. 2013 Nov;111:346–53. Available from: http://dx.doi.org/10.1016/j.colsurfb.2013.06.001.

13. Gupta S, Kesarla R, Omri A. Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems. ISRN Pharmaceutics. 2013;2013:1–16. Available from: http://dx.doi.org/10.1155/2013/848043.

14. Bandyopadhyay S, Katare OP, Singh B. Optimized self nano-emulsifying systems of ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids and Surfaces B: Biointerfaces. 2012 Dec;100:50–61. Available from: http://dx.doi.org/10.1016/j.colsurfb.2012.05.019.

15. Kohli K, Chopra S, Dhar D, Arora S, Khar RK. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. Drug Discovery Today. 2010 Nov;15(21-22):958–65. Available from: http://dx.doi.org/10.1016/j.drudis.2010.08.007.

16. Bakhle SS, Avari JG. Development and Characterization of Solid Self-emulsifying Drug

Delivery System of Cilnidipine.Chem Pharm Bull. 2015;63(6):408–17. Available from: http://dx.doi.org/10.1248/cpb.c14-00326.

17. Barakat NS. Self-emulsifying system for improving drug dissolution and bioavailability: in vitro/in vivo evaluation. Drug Dev Res 2010; 71(2): 149-158.Available from:http://dx.doi.org/10.1002/ddr.20356.

18. Pouton CW. Formulation of self-emulsifying drug delivery systems. Advanced Drug Delivery Reviews. 1997 Apr;25(1):47–58. Available from: http://dx.doi.org/10.1016/s0169-409x(96)00490-5.

19. Hauss DJ. Oral lipid-based formulations.Advanced Drug Delivery Reviews. 2007 Jul;59(7):667–76. Available from: http://dx.doi.org/10.1016/j.addr.2007.05.006.

20. Porter CJH, Pouton CW, Cuine JF, Charman WN. Enhancing intestinal drug solubilisation using lipid-based delivery systems.Advanced Drug Delivery Reviews. 2008 Mar;60(6):673–91. Available from: http://dx.doi.org/10.1016/j.addr.2007.10.014.

21. Elnaggar YSR, El-Massik MA, Abdallah OY. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. International Journal of Pharmaceutics. 2009 Oct 1;380(1-2):133–41. Available from: http://dx.doi.org/10.1016/j.ijpharm.2009.07.015.

22. Wu X, Xu J, Huang X, Wen C. Self-microemulsifying drug delivery system improves curcumin dissolution and bioavailability. Drug Development and Industrial Pharmacy. 2010 Aug 25;37(1):15–23. Available from: http://dx.doi.org/10.3109/03639045.2010.489560.

23. Attivi D, Ajana I, Astier A, Demoré B, Gibaud S. Development of microemulsion of mitotane for improvement of oral bioavailability. Drug Development and Industrial Pharmacy. 2010 Mar 6;36(4):421–7. Available from: http://dx.doi.org/10.3109/03639040903225083.

24. Sriamornsak P, Limmatvapirat S, Piriyaprasarth S, Mansukmanee P, Huang Z. A new self-emulsifying formulation of mefenamic acid with enhanced drug dissolution.Asian Journal of Pharmaceutical Sciences. 2015 Apr;10(2):121–7. Available from: http://dx.doi.org/10.1016/j.ajps.2014.10.003.

25. Dixit AR, Rajput SJ, Patel SG. Preparation and Bioavailability Assessment of SMEDDS Containing Valsartan.AAPS PharmSciTech. 2010 Feb 25;11(1):314–21. Available from: http://dx.doi.org/10.1208/s12249-010-9385-0.

26. Li L, Yi T, Lam CW. Inhibition of Human Efflux Transporter ABCC2 (MRP2) by Self-emulsifying Drug Delivery System: Influences of Concentration and Combination of Excipients. JPPS. 2014 Oct 4;17(4):447. Available from: http://dx.doi.org/10.18433/j3vp5h.

27. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and “self-microemulsifying” drug delivery systems. European Journal of Pharmaceutical Sciences. 2000 Oct;11:S93–S98. Available from: http://dx.doi.org/10.1016/s0928-0987(00)00167-6.

28. Abdalla A, Klein S, Mäder K. A new self-emulsifying drug delivery system (SEDDS) for poorly soluble drugs: Characterization, dissolution, in vitro digestion and incorporation into solid pellets. European Journal of Pharmaceutical Sciences. 2008 Dec;35(5):457–64. Available from: http://dx.doi.org/10.1016/j.ejps.2008.09.006.

29. Wang DK et al. Development of self-microemulsifying drug delivery systems for oral bioavailability enhancement of α-asarone in beagle dogs. PDA J Pharm Sci Technol. 2006; 60(6): 343-349.

30. Gupta S, Chavhan S, Sawant KK. Self-nanoemulsifying drug delivery system for adefovirdipivoxil: Design, characterization, in vitro and ex vivo evaluation. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2011 Dec;392(1):145–55. Available from: http://dx.doi.org/10.1016/j.colsurfa.2011.09.048.

31. Villar AMS, Naveros BC, Campmany ACC, Trenchs MA, Rocabert CB, Bellowa LH. Design and optimization of self-nanoemulsifying drug delivery systems (SNEDDS) for enhanced dissolution of gemfibrozil. International Journal of Pharmaceutics. 2012 Jul;431(1-2):161–75. Available from: http://dx.doi.org/10.1016/j.ijpharm.2012.04.001.

32. Seo YG, Kim DH, Ramasamy T, Kim JH, Marasini N, Oh Y-K, et al. Development of docetaxel-loaded solid self-nanoemulsifying drug delivery system (SNEDDS) for enhanced chemotherapeutic effect. International Journal of Pharmaceutics. 2013 Aug;452(1-2):412–20. Available from: http://dx.doi.org/10.1016/j.ijpharm.2013.05.034.

33. Kim JY, Ku YS.Enhanced absorption of indomethacin after oral or rectal administration of a self-emulsifying system containing indomethacin to rats.International Journal of Pharmaceutics. 2000 Jan;194(1):81–9. Available from: http://dx.doi.org/10.1016/s0378-5173(99)00367-1.

34. Balakumar K, Raghavan CV, selvan NT, prasad RH, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. Colloids and Surfaces B: Biointerfaces. 2013 Dec;112:337–43. Available from: http://dx.doi.org/10.1016/j.colsurfb.2013.08.025.

35. Swenson ES, Milisen WB, Curatolo W. Pharmaceutical Research. 1994;11(8):1132–42. Available from: http://dx.doi.org/10.1023/a:1018984731584.

36. Hu X, Lin C, Chen D, Zhang J, Liu Z, Wu W, et al. Sirolimus solid self-microemulsifying pellets: Formulation development, characterization and bioavailability evaluation. International Journal of Pharmaceutics. 2012 Nov;438(1-2):123–33. Available from: http://dx.doi.org/10.1016/j.ijpharm.2012.07.055.

37. Gershanik T. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. European Journal of Pharmaceutics and Biopharmaceutics. 2000 Jul 3;50(1):179–88. Available from: http://dx.doi.org/10.1016/s0939-6411(00)00089-8.

38. Devani M, Ashford M, Craig DQM. The emulsification and solubilisation properties of polyglycolysed oils in self-emulsifying formulations.Journal of Pharmacy and Pharmacology. 2004 Mar;56(3):307–16. Available from: http://dx.doi.org/10.1211/0022357022872.

39. Sutton D, Nasongkla N, Blanco E, Gao J. Functionalized Micellar Systems for Cancer Targeted Drug Delivery. Pharmaceutical Research. 2007 Mar 24;24(6):1029–46. Available from: http://dx.doi.org/10.1007/s11095-006-9223-y.

40. Zhang Y, Huang Y, Li S. Polymeric Micelles: Nanocarriers for Cancer-Targeted Drug Delivery. AAPS PharmSciTech. 2014 Apr 4;15(4):862–71. Available from: http://dx.doi.org/10.1208/s12249-014-0113-z.

41. Shah SR. Studies in dissolution enhancement of anti diabitic drug of sulfonylurea class by novel drug delivery strategy. PhD thesis.India: Saurashtra University, 2012.

42. Constantinides PP. Pharmaceutical Research. 1995;12(11):1561–72. Available from: http://dx.doi.org/10.1023/a:1016268311867.

43. Prajapati ST, Joshi HA, Patel CN.Preparation and Characterization of Self-Microemulsifying Drug Delivery System of OlmesartanMedoxomil for Bioavailability Improvement.Journal of Pharmaceutics. 2013;2013:1–9. Available from: http://dx.doi.org/10.1155/2013/728425.

44. Parmar N, Singla N, Amin S, Kohli K. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids and Surfaces B: Biointerfaces. 2011 Sep;86(2):327–38. Available from: http://dx.doi.org/10.1016/j.colsurfb.2011.04.016.

45. Beg S, Jena SS, Patra CN, Rizwan M, Swain S, Sruti J, et al. Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids and Surfaces B: Biointerfaces. 2013 Jan;101:414–23. Available from: http://dx.doi.org/10.1016/j.colsurfb.2012.06.031.

46. Lacy JE, Embleton JK. Delivery systems for hydrophobic drugs.United State;US5645856, 1997.

47. Kommuru T., Gurley B, Khan M., Reddy I. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. International Journal of Pharmaceutics. 2001 Jan;212(2):233–46. Available from: http://dx.doi.org/10.1016/s0378-5173(00)00614-1.

48. Fernandez S, Jannin V, Chevrier S, Chavant Y, Demarne F, Carrière F. In Vitro Digestion of the Self-Emulsifying Lipid Excipient Labrasol® by Gastrointestinal Lipases and Influence of its Colloidal Structure on Lipolysis Rate.Pharmaceutical Research. 2013 May 2;30(12):3077–87. Available from: http://dx.doi.org/10.1007/s11095-013-1053-0.

49. Prasad YVR, Puthli S., Eaimtrakarn S, Ishida M, Yoshikawa Y, Shibata N, et al. Enhanced intestinal absorption of vancomycin with Labrasol and d-α-tocopheryl PEG 1000 succinate in rats. International Journal of Pharmaceutics. 2003 Jan;250(1):181–90. Available from: http://dx.doi.org/10.1016/s0378-5173(02)00544-6.

50. Kang JH, Oh DH, Oh Y-K, Yong CS, Choi H-G.Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS).European Journal of Pharmaceutics and Biopharmaceutics. 2012 Feb;80(2):289–97. Available from: http://dx.doi.org/10.1016/j.ejpb.2011.11.005.

51. Eaimtrakarn S, Rama Prasad YV, Ohno T, Konishi T, Yoshikawa Y, Shibata N, et al. Absorption Enhancing Effect of Labrasol on the Intestinal Absorption of Insulin in Rats. Journal of Drug Targeting. 2002 Jan;10(3):255–60. Available from: http://dx.doi.org/10.1080/10611860290022688.

52. Shen Y, Lu Y, Jv M, Hu J, Li Q, Tu J. Enhancing effect of Labrasol on the intestinal absorption of ganciclovir in rats. Drug Development and Industrial Pharmacy. 2011 May 31;37(12):1415–21. Available from: http://dx.doi.org/10.3109/03639045.2011.582874.

53. Venkatesh G, Majid MIA, Mansor SM, Nair NK, Croft SL, Navaratnam V. In vitro and in vivo evaluation of self-microemulsifying drug delivery system of buparvaquone.Drug Development and Industrial Pharmacy. 2010 Feb 6;36(6):735–45. Available from: http://dx.doi.org/10.3109/03639040903460446.

54. Kim DW, Kang JH, Oh DH, Yong CS, Choi H-G. Development of novel flurbiprofen-loaded solid self-microemulsifying drug delivery system using gelatin as solid carrier.Journal of Microencapsulation. 2012 Jan 10;29(4):323–30. Available from: http://dx.doi.org/10.3109/02652048.2011.651497.

55. Yi T, Wan J, Xu H, Yang X. 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. 2008 Oct;70(2):439–44. Available from: http://dx.doi.org/10.1016/j.ejpb.2008.05.001.

56. Ragelle H, Crauste-Manciet S, Seguin J, Brossard D, Scherman D, Arnaud P, et al. Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice. International Journal of Pharmaceutics. 2012 May;427(2):452–9. Available from: http://dx.doi.org/10.1016/j.ijpharm.2012.02.025.

57. Malcolmson C, Satra C, Kantaria S, Sidhu A, Jayne Lawrence M. Effect of Oil on the Level of Solubilization of Testosterone Propionate into Nonionic Oil-in-Water Microemulsions. Journal of Pharmaceutical Sciences. 1998 Jan;87(1):109–16. Available from: http://dx.doi.org/10.1021/js9700863.

58. Warisnoicharoen W, Lansley AB, Lawrence MJ. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. International Journal of Pharmaceutics. 2000 Mar;198(1):7–27. Available from: http://dx.doi.org/10.1016/s0378-5173(99)00406-8.

59. Kim H-J, Yoon KA, Hahn M, Park E-S, Chi S-C.Preparation and In Vitro Evaluation of Self-Microemulsifying Drug Delivery Systems Containing Idebenone.Drug Development and Industrial Pharmacy. 2000 Jan;26(5):523–9. Available from: http://dx.doi.org/10.1081/ddc-100101263.

60. Woo JS, Song Y-K, Hong J-Y, Lim S-J, Kim C-K. Reduced food-effect and enhanced bioavailability of a self-microemulsifying formulation of itraconazole in healthy volunteers. European Journal of Pharmaceutical Sciences. 2008 Feb;33(2):159–65. Available from: http://dx.doi.org/10.1016/j.ejps.2007.11.001.

61. Zhang P, Liu Y, Feng N, Xu J. Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. International Journal of Pharmaceutics. 2008 May 1;355(1-2):269–76. Available from: http://dx.doi.org/10.1016/j.ijpharm.2007.12.026.

62. Zhao Y, Wang C, Chow AHL, Ren K, Gong T, Zhang Z, et al. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavailability studies. International Journal of Pharmaceutics. 2010 Jan;383(1-2):170–7. Available from: http://dx.doi.org/10.1016/j.ijpharm.2009.08.035.

63. Beg S, Swain S, Singh HP, Patra CN, Rao MB. Development, Optimization, and Characterization of Solid Self-Nanoemulsifying Drug Delivery Systems of Valsartan Using Porous Carriers.AAPS PharmSciTech. 2012 Oct 16;13(4):1416–27. Available from: http://dx.doi.org/10.1208/s12249-012-9865-5.

64. Borhade V, Pathak S, Sharma S, Patravale V. Clotrimazolenanoemulsion for malaria chemotherapy. Part I: Preformulation studies, formulation design and physicochemical evaluation. International Journal of Pharmaceutics. 2012 Jul;431(1-2):138–48. Available from: http://dx.doi.org/10.1016/j.ijpharm.2011.12.040.

65. Patil P, Joshi P, Paradkar A. Effect of formulation variables on preparation and evaluation of gelled self-emulsifying drug delivery system (SEDDS) of ketoprofen. AAPS PharmSciTech. 2004 Sep;5(3):43–50. Available from: http://dx.doi.org/10.1208/pt050342.

66. Patel D, Sawant KK. Oral Bioavailability Enhancement of Acyclovir by Self-Microemulsifying Drug Delivery Systems (SMEDDS).Drug Development and Industrial Pharmacy. 2007 Jan;33(12):1318–26. Available from: http://dx.doi.org/10.1080/03639040701385527.

67. Nekkanti V, Karatgi P, Prabhu R, Pillai R. Solid Self-Microemulsifying Formulation for Candesartan Cilexetil. AAPS PharmSciTech. 2009 Dec 15;11(1):9–17. Available from: http://dx.doi.org/10.1208/s12249-009-9347-6.

68. Jain S, Jain AK, Pohekar M, Thanki K. Novel self-emulsifying formulation of quercetin for improved in vivo antioxidant potential: Implications for drug-induced cardiotoxicity and nephrotoxicity. Free Radical Biology and Medicine. 2013 Dec;65:117–30. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2013.05.041.

69. Li W, Yi S, Wang Z, Chen S, Xin S, Xie J, et al. Self-nanoemulsifying drug delivery system of persimmon leaf extract: Optimization and bioavailability studies. International Journal of Pharmaceutics. 2011 Nov;420(1):161–71. Available from: http://dx.doi.org/10.1016/j.ijpharm.2011.08.024.

70. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, et al. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. International Journal of Pharmaceutics. 2004 Apr;274(1-2):65–73. Available from: http://dx.doi.org/10.1016/j.ijpharm.2003.12.028.

71. Kallakunta VR, Bandari S, Jukanti R, Veerareddy PR. Oral self emulsifying powder of lercanidipine hydrochloride: Formulation and evaluation. Powder Technology. 2012 May;221:375–82. Available from: http://dx.doi.org/10.1016/j.powtec.2012.01.032.

72. Baboota S et al. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Actapharmaceutica (Zagreb, Croatia). 2007; 57(3): 315-32.

73. Gershanik T, Benita S. Positively Charged Self-Emulsifying Oil Formulation for Improving Oral

Bioavailability of Progesterone. Pharmaceutical Development and Technology. 1996 Jan;1(2):147–57. Available from: http://dx.doi.org/10.3109/10837459609029889.

74. Gershanik T et al. Interaction of a Self-Emulsifying Lipid Drug Delivery System with the Everted Rat Intestinal Mucosa as a Function of Droplet Size and Surface Charge. Pharm Res. 1998; 15(6): 863-869.

75. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs.International Journal of Pharmaceutics. 1994 May;106(1):15–23. Available from: http://dx.doi.org/10.1016/0378-5173(94)90271-2.