International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.2, pp 832-841, 2014-2015

ICONN 2015 [4th -6th Feb 2015]
International Conference on Nanoscience and Nanotechnology-2015
SRM University, Chennai, India

Nanoemulsion for lymphatic absorption: Investigation of Fenofibrate Nanoemulsion system for lymphatic uptake

Raman Sureshkumar[1], K.Gowthamarajan[1]* and Paruchuri Bhavani[1]

**JSS College of Pharmacy (A Constituent college of JSS University Mysore)
Ootacamund.643001, TamilNadu, India**

Abstract: Self nanoemulsifying drug delivery systems are the alternative for the bioavailability enhancement of poorly soluble BCS classII drugs. Self Nanoemulsifying drug delivery system (SNEDDS) for BCS classII drug Fenofibrate was designed using Capryol90 and Cremophore:Ethanol mixture. The oil phase was selected based on intestinal lipid digestion model and the formulation was subjected to animal studies i.e intestinal lymphatic absorption and pharmacokinetic parameter study. The enhancement of bioavailability of SNEDDS was well predicted from AUC, C_{max} and $T_{max}$of the formulations. SNEDDS formulation showed 0.47 folds increase in bioavailability when compared to that of marketed formulation and 0.166 fold increase in bioavailability when compared to pure drug suspension while the marketed formulation exhibited only 0.266 folds increase in bioavailability compared to pure drug suspension for Fenofibrate. *In vivo* lymphatic absorption studies were found to be similar to the *in vivo* plasma studies, with maximum lymphatic concentrations of 22.41 ± 0.51 ng/mL for SNEDDS, 2.74 ± 0.42 ng/mL for marketed formulation and 0.35 ±0.71 ng/mL for pure drug suspension of Fenofibrate.
Key Words: SNEDDS, Fenofibrate, Lipid digestion and Lympahtic absorption.

Introduction:

In the present scenario, oral drug delivery is continuously looking into newer avenues due to the realization of the factors like poor drug solubility and/ or absorption, bioavailability and rapid metabolism[1]. Methods for improving the bioavailability, dissolution rate includes particle size reduction, co-solvency, solid dispersions, complexation, solubilising excipients, pH adjustment, lipid based drug delivery system[2,3]. The lipid based drug delivery system (LBDDS) approach has attracted wide attention in order to enhance drug solubilization in the gastrointestinal tract (GIT) and to improve the oral bioavailability of BCS class II and IV drugs[4,5,6]. LBDDS which includes oils, surfactants and co-surfactants are typically self-dispersing systems often referred to as self-emulsifying drug delivery systems (SEDDS) or self-micro emulsifying drug delivery systems (SMEDDS). SMEDDS upon mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can form fine oil in water (O/W) emulsions i.e. Nanoemulsions (NE)[7]. Usually the average droplet size is between 100 and 500 nm. The particles can exist as oil-in-water and water- in-oil forms[8].The mechanisms behind the augmented bioavailability include enhanced dissolution and solubilization of the co-administered lipophilic drug by stimulation of biliary and pancreatic secretions, prolongation of gastrointestinal tract (GIT) residence time, stimulation of lymphatic transport, increased intestinal wall permeability and reduced

metabolism and efflux activity[9,10].The majority of orally administered drugs gain access to the systemic circulation by direct absorption into the portal blood. However, highly lipophilic compounds may reach the systemic blood circulation via the intestinal lymphatic system[11]. This enhanced absorption pathway from the gastrointestinal tract (GIT) has been shown to be significant contributor for the overall bioavailability of a number of highly lipophilic drugs, including fat soluble vitamins**,** halofantrine, probucol, mepitiostane and others. In addition to increased overall bioavailability of lipophilic molecules, lymphatic transport of a drug provides further advantages, including avoidance of hepatic first pass metabolism, a potential to target the drug into the lymphatic system for the case of specific disease states known to spread via the lymphatics, and improved plasma profile of the drug[12,13]. Hence the present work was focused on the attempt to enhance the bioavailability of BCS ClassII drug Fenofibrate through SNEDDS which undergoes excessive 1st pass effect and Pgp efflux.

Experimental:

2.1 Materials: Fenofibrate was a gift sample from Zim Laboratories. CapmulMCM C8, Capryol90, Maisine, Isopropyl myristate(IPM), Labrafac lipophilic WL 1349(LL), Labrafac(LF), linseed oil, castor oil ,Cremophore El, Ethanol,Trizma maleate, Bile salts, Lecithin, Calcium chloride, Pancreatic lipase and Sodium hydroxide from Sigma inc.

Selection of oil, surfactant and co-surfactant[14,15]:

The solubility of fenofibrate was checked in different oils by shake flask method .The surfactant and co surfactants were screened based on their ability to emulsify the respective oil phase. The ability to emulsify was determined by mixing oil, surfactant and water to produce a uniform emulsion.

Formulation of Nanoemulsion (NE) for Lipolysis:

Based on the solubility study, capryol90 for fenofibrate was selected for SNEDDS formulation. Simple spontaneous emulsification technique was used. The SNEDDS formulations was prepared by dissolving sufficient amount of drug in the respective oil and mixed with surfactant and co surfactant mixture (SCoS 1:1) 2 mL. The resulted emulsion was used for lipolysis experiment.

Lipolysis/Lipid Digestion procedure:

Based on the solubility studies, oil was selected and the drug was solubilized with reference to the dose[16,17]. Christensen et.al method was followed for the study. The lipase solution was prepared according to the method described by Zangenbergto give an activity of 800 US units/mL. A biorelevant media consisting of ingredient as described by Christensen was prepared without any modification. The lipolysis activity was carried out and the influence of pancreatic lipase during the lipolysis process was observed. The Biorelevant media whose pH adjusted to6.5 using 1MNaOH was used. SNEDDS formulation was added, the pH was again adjusted to 6.5 with the use of auto titrator followed by pancreatic lipase solution.5mL samples were withdrawn at 5, 10, 15 and 30 min intervals and centrifuged at 40,000 rpm for 130 min. Inhibition of the pancreatic lipase activity was achieved by adding 4-bromoboronic acid after withdrawing the sample to stop the lipolysis process. Among the three layers formed 1mL was withdrawn from each layer and the volume was made upto 10mLwith acetonitrile and analysed. The same procedure was followed for media without lipase. Media was also prepared without lipase for control study purpose

Formulation development and optimization[18]

Construction of pseudo ternary phase diagrams:

Pseudo ternary phase diagrams were constructed to examine the formation of oil in water SNEDDS with 4 components oil, surfactant, co-surfactant, and aqueous phase. The four component system consists of (i) lipid A (Selected from solubility studies) (ii) Surfactant (S) (iii) Co-surfactant (Cos) and (iv) distilled water (aqueous phase). Surfactant and co-surfactant in each group were mixed in different weight ratios (1:1 to 1:9**)**. For each phase diagram, oil and specific surfactant and co surfactant mixture (SCoS) was mixed in different weight ratios from 1:1, 1:2, and 1:3 in different glass vials. Seventeen combinations of oil and SCoS, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1 , 2:1 were made so that maximum ratios were covered for the study to depict the boundaries of phases precisely formed in the phase diagrams. Only the

SNEDDS regions were plotted in the pseudoternary phase diagram. From the pseudoternary phase diagrams the combination of oil and SCoS were selected and subjected to following evaluation tests for stability.

Evaluation of NE:

Thermodynamic stability studies[19]:

The formulations were subjected to the following stability studies.a) Heating and Cooling Cycle: Temperature ranging between 4°C and 45°C for 48 h. The formulations stable at these temperatures were subjected to centrifugation test. b) Centrifugation was doneat 3500 rpm for 30 min. Formulations that did not show any phase separation were taken for the freeze thaw stress. c) Freeze Thaw Cycle were carried out between a temperature - 21°C and +25°C where the formulation was stored for not less than 48h at each temperature. The formulations that passed the thermodynamic stability tests were taken for further studies.

Characterisation of NE:

Mean droplet size and PDI of the SNEDDS was determined by using Malvern Zetasizer Nano, Series ZEN1002 (Malvern, UK). Abbe-type refractometer (Macro Scientific Works, Delhi) was used for the refractive index.BrookfieldDVEviscometer was used for the determination of viscosity of the formulations[20.]Electro-conductivity of the resultant system was measured by an electro-conductometer (Conductivity meter 305, Systronic). The measurements were made in triplicate at 25±1^{o}C[21]. The surface morphology was done using SEM and TEM analysis[21,22]**.** Qualitative measurements of sizes and size distribution of TEM micrographs were performed using a digital image processing programme.

Drug loading :

From the optimized studies the drug is dissolved in respective oil and mixed with SCoS to form self emulsifying concentrate. Since the solubility of the drug is well above the dose the total amount of drug was presumed to be present in the oil phase.

Release behavior study:

***In vitro* release by USP apparatus I[23]:**

The quantitative *in vitro* release test was performed in 250 mL , pH 6.5 simulated intestinal fluid using USP dissolution apparatus Type I at 50 rpm at 37±0.5^{0}c. The optimized SNEDDS formulation containing single dose of Fenofibrate was filled in size 1cs of hard gelatin capsule**[23]**(CONISNAP). Samples were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, h) and an aliquot amount of dissolution media was replaced. The release of drug from SNEDDS formulation was compared with the marketed tablet formulation and pure drug suspension.

***In vivo* release behavior study:**

The experiments were carried out after getting the approval of the CPCSEA and IAEC, JKK Natraja College of Pharmacy, Komarapalayam. Reg.No.:887/ac/05/CPCSEA and their guidelines were followed throughout the experiment.

Estimation of drug in rat plasma[24]:

Healthy overnight fasted male Sprague-Dawley rats weighing about 350-370gm were used for *in-vivo* experiments. The animals were given water *ad.libitum* during fasting and throughout the experiment. The animals were then divided into 4 groups each comprising three animals. Based on the surface area ratio of rat and man,dose was administered with an oral cannula. Group 1,2 and 3 received marketed formulation, SNEDDS and pure drug suspension respectively and group 4 was used as control which was used for plasma spiking. The animals were anaesthetized and the carotid artery was cannulated. Blood samples (0.5mL) were withdrawn from the carotid artery at0,0.25,0.5,0.75,1,2,4,6,8,12h with a sterile syringe and collected in a RIA vial containing anti-coagulant (0.4 mL of 2.5% sodium citrate), centrifuged at 2500rpm for 4 min and the plasma samples were separated and stored at -20^{0}C. The plasma samples were deprotonated and extraction of drug was done by solid phase extraction (SPE) using ACN-water mixture and analyzed by HPLC. The results of *in vivo* was also subjected to pharmacokinetic treatment.

***In vivo* lymphatic absorption studies[24,25]:**

For lymphatic study Glen A. Edwards *et al.*, 2001 procedure was used without any modifications. All the animals were suitably anesthetized, and cannulation of mesenteric lymph duct for collection of intestinal lymph and thoracic lymph duct for the administration of rehydration solution was done as per the procedure[26]**.** To study the lymphatic absorption, the lymph that drains from the intestinal lymphatic duct was collected at predetermined time intervals for 12h, analyzed for drug concentration and multiplied with the volume of the lymph collected.

Results and Discussion:

Screening of oil, surfactant and cosurfactant:

Solubility is an important criterion in formulation of SNEDDS , as the drug remains in liquid solubilized form in the oil phase. Hence, the oil phase in which the drug shows maximum solubility was selected for this purpose. From table.2 it was evident that Capryol90 shows maximum solubility of fenofibrate i.e.180.24±0.64mg/mL. The increased solubility of the drug could be due to the more affinity towards the respective oils.

Table 1.Solubility of drug in oil

Oil	Fenofibrate(mg/mL)
Capryol 90	**180.24±0.64**
Capmul MCM	110.67±0.98
LF	96.39±0.22
IPM	72.19±0.28
LG	84.59±0.96
LL	126.51±0.37
Oleic acid	115.95±1.64

From the screening studies, the SCoS mixture of Cremophore RH and ethanol for capryol90 was found to produce clear and uniform o/w emulsion.

Effect of lipolysis:

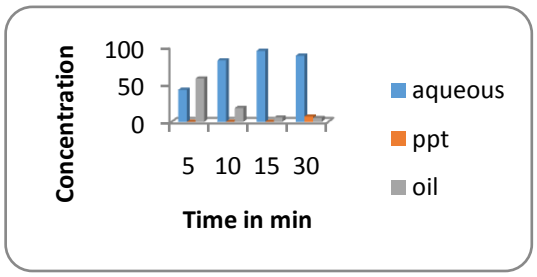


Figure 1percentage of Fenofibrate in different layers.

Lipid Digestion experiments are used (lipolysis) to investigate the fate of drugs in GI fluid. Autotitrator at pH 6.5.was used for lipolysis. pH 6.5was used since the transit time of lipid based formulations in gastric environment was very short hence, once the formulation converted into nanodroplets moves to the small intestine immediately where the maximum action was expected. Lipolysis also used to predict drug partitioning during hydrolysis of TAG (triacylglycerol) and to predict which type of lipid will increase concentration of the drug in the intestinal fluids**.**The physiochemical properties of the drugs influences the extent of drug solubilization in mixed bile salt micelles. Initially 42.53% of fenofibrate was available in the aqueous layer since the drug was having more affinity towards the media and the availability of drug was found to be increased as the lipolysis process was continued until 15 min. The drug starts getting precipitated (7%) from the 30th min. The main reason behind the precipitation of the drug was found to be either supersaturation of the drug or the excipients undergone enzymatic degradation. The precipitated drug needs to get re-dissolved in the

aqueous media to get absorbed. From the lipolysis experiment, the maximum amount of fenofibrate i.e. 88% was found to be present in the aqueous phase at the end of 30^{th} min, while more than 50% of the drug was present in the oil layer initially at 5 min. as the time proceeds the concentration of drug in the oil phase decreases, while it increased in the aqueous phase, which reveals that the hydrolysis of the oil occurred, as the result of digestion process. This shows that a negligible amount of drug present in the precipitated layer, makes the oil phase a suitable candidate for formulation of fenofibrate as SNEDDS.

Nanoemulsion formulation and optimization

Pseudo ternary phase diagram study:

Phase behavioral studies were performed by constructing phase diagrams that depict the boundaries of different phases, as a function of composition, to investigate the structural organization of the emulsions formed. The concentration of surfactant and co-surfactant is responsible for the barrier formation at the interface required to prevent the coalescence of the formed NE. SCoS gets adsorbed at the interface, reducing the energy required for SNEDDS formation thus improving the thermodynamic stability of the SNEDDS formulation. In SCoS ratio 1:1 when surfactant and cosurfactants in equal ratio was used only a small area of SNEDDS was formed with oil solubilized upto 70% with 30% of SCoS. But when co-surfactants was doubled than surfactant (SCoS 1:2) there was a slight increase in SNEDDS region but the oil solubilization increased upto 74% with 28% of SCoS. This may be attributed to the fact that the addition of co-surfactant may lead to greater penetration of the oil phase in the hydrophobic region of the surfactant monomer thereby further decreasing the interfacial tension, which lead to increase in the fluidity of the interface thus increasing the entropy of the system**.** When the concentration of co-surfactant was doubled (SCoS 1:2) SNEDDS area increased considerably with 74% oil solubilized using 28% SCoS. There was no difference in SNEDDS region and the oil solubilization remained same for SCoS 1:3.

Formulation selection:

From each phase diagram different concentrations of oil which could solubilize single dose of drug was selected at 5% intervals (10%, 15%, 20%, 25% and 30%).So that, largest number of formulations could be selected covering the SNEDDS area of the phase diagram. For each percentage of oil selected, only those formulations were taken from the phase diagram which used minimum concentration of SCoS for the formation of SNEDDS and subjected to stability studies.

Evaluation of NE:

Thermodynamic stability studies and dispersibility tests:

The selected formulations from the pseudo ternary phase diagrams were subjected to different thermodynamic stability studies and the formulations which survived thermodynamic stability studies were taken for dispersibility test. Thermodynamic stability studies differentiate those SNEDDS formulations from those of kinetically stable and undergo phase separation. This implies that the formulations contain adequate amounts of SCoS concentration required for SNEDDS formulation, which decreases the energy required for SNEDDS formation. This decreased energy contributes to the stability of NE. The SNEDDS formulation on entering the GI tract undergoes infinite dilution leading to phase separation of the formulation due to poor dispersibility. Formulations which passed the dispersibility studies were certain to remain as SNEDDS upon dispersion in the aqueous environment of the GIT. For oral SNEDDS the process of dilution by the GI fluids will result in the gradual desorption of the surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration.

Table2.Thermodynamic stability studies and dispersibility test.

Formulation	SCoS	Oil %	SCoS %	Aq %	Centri	H&C Cycle	Freeze	Dispersibility
7	**1:1**	**25**	**43.75**	**31.25**	**P**	**P**	**P**	*******

***** Formulation which passed the dispersibility test; P- pass; F- fail**.

Formulations which passed thermodynamic stability tests and dispersibility test were subjected to globule size analysis, refractive index determination, viscosity determination and *in vitro*release studies. It is confirmed by the visual observation by clarity and transparency. The formulations which are clear and free from turbidity are considered here as stable where as others are considered to be unstable.

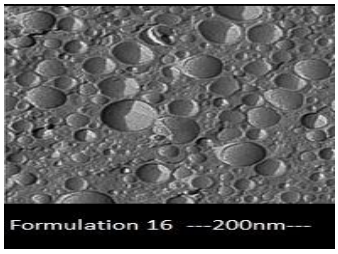
Characterisation of SNEDDS:

The optimized formulation was selected based on least mean particle size and PDI and the same has been supported by SEM and TEM studies.At the same time, the formulations containing capryol90 and SCoScremophore RH and ethanol, resulted in the least mean particle size of 24.31nm with PDI of 0.228 and zeta potential -0.729mV for formulation 7. Hence this was selected as the optimized formulation for fenofibrate. Our formulations reveal that zeta potential did not play a major role in confining stability to the formulation. The other parameters like viscosity,% transmission and conductivity were found to be satisfactory for all the formulations. The results were displayed in the table4. From the above analysis the formulations 7was selected for drug incorporation and *in vitro* and *in vivo* studies.

Table 3.Characterisation of SNEDDS

Formulation	Mean Droplet Size (nm)	PDI	Zeta Potential (mV)	Viscosity (cPs)	Refractive index	% Transmission	Conductivity (µS/cm)
7	**24.31**	**0.228**	**0.729**	**19.84**	**1.364**	**99.48**	**391.37**

The scanning electron microscopic study reveals the external morphology of the nanodroplets. A good analysis of surface morphology of disperse phase in the formulation was obtained through SEM. It was evident that most of the nanodroplets were nearly spherical in shape. In TEM, higher resolution images of the disperse phase were obtained. TEM studies confirm the droplet size obtained by the laser scattering spectroscopy. In the TEM image the SNEDDS appeared dark and the surroundings were bright. The micrograph exhibits, the droplets size of the sample were in the range of NE.A clear picture of the nanodroplets were clearly depicted by SEM and TEM micrographs in fig 1& 2.



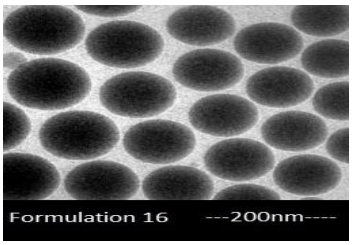


Figure 1 SEM of SNEDDS **Figure 2 TEM of SNEDDS**

Drug loading :

Formulation7 was used for Fenofibrate since the solubility of the drug in capryol90 was well above the dose of the drug, volume of oil phase used was equivalent to the dose of the drug. i.e., fenofibrate 67mg.

***In vitro* release study:**

In vitro dissolution studies were performed at pH6.5 biorelevant media.The solubulization of lipophilic molecule occurs in most cases at upper GI tract in which pancreatic fluids and biliary lipids are secreted and enhance solubulization process and the residence time in upper GI is limited and the transit time in small intestine is 3.5 to 4.5h hence the pH 6.5 was used as a dissolution media. 94.36% with SNEDDS fenofibrate which is maximum formulation comparatively. The reason for this could be the smaller droplet size and PDI, which lead to the increased surface area permitting a faster release rate with a maximum release within 15 min. At the end of 120 min almost all the drug was found in solution did not show any precipitation or aggregation of the particles which was evidenced from the analysis. The release profile suggested that the SNEDDS preserved enhanced *in vitro* dissolution and this would eventually enhance dissolution of drug *in vivo.*

Table4 Comparative *In vitro* dissolution data in pH 6.5 biorelevant media for fenofibrate.

Time (min)	% Cumulative release of fenofibrate		
	SNEDDS	**Marketed**	**Pure drug**
15	94.36±0.59	29.91±0.91	11.54±0.18
30	95.39±0.99	45.18±0.16	12.56±0.28
45	95.41±0.20	52.42±0.99	13.79±0.46
60	96.37±0.78	59.36±0.29	14.54±0.72
90	98.23±0.90	62.10±0.96	16.84±0.64
120	99.64±0.58	64.20±0.78	19.33±0.61

Oneway ANOVA followed by TUKEY-KRAMERS multiple comparison tests.

***In vivo* bioavailability studies and pharmacokinetic data analysis:**

In vivo bioavailability studies were performed to quantify SNEDDS formulation after oral administration and to compare the bioavailability of SNEDDS with that of marketed formulation and pure drug suspension. Pharmacokinetic parameters were calculated from the *in vivo* release of fenofibrate in Sprague-Dawley rats. Since the rate limiting step in the absorption of fenofibrate is dissolution from the formulation and the results from the study reveals that the dispersion of the drug (since the drug is completely dissolved in the oil phase) into the aqueous gastrointestinal environment is the rate limiting step in case of SNEDDS and plays a major role for absorption. It can be explained that, following oral administration, SNEDDS disperse spontaneously to form a SNEDDS in the GI fluid where the active components are present in a solubilized form and the small droplet size provides a large surface area for drug absorption. Such an ultra-fine dispersion of the oil will afford rapid and extensive absorption. In addition high concentration of surfactant in SNEDDS may increase permeability of the oil across the cell membrane, and lymphatic transport through the transcellular pathway. The release rates were quite significant ($p < 0.001$) compared to both pure drug suspension as well as marketed formulation. This was well evidenced from the table6.

Table 5 Comparative *in vivo* bioavailability studies of fenofibrate

Time (h)	Fenofibrate (ng/ml)		
	SNEDDS	**Marketed**	**Pure drug suspension**
0.25	12.44 ± 1.75$^{***\#\#}$	10.44 ± 1.25**	8.42 ± 0.56
0.5	15.79 ± 1.41$^{***\#\#\#}$	11.79 ± 1.71**	9.29 ± 0.64
0.75	19.81 ± 1.10$^{***\#\#\#}$	13.81 ± 1.90***	10.12 ± 0.93
1	20.83 ±1.52$^{***\#\#\#}$	14.83 ±1.92***	10.84±0.065
2	**28.9 ± 1.93$^{***\#\#\#}$**	**18.75 ± 1.13*** **	11.56 ±0.48
4	23.75 ± 1.64$^{***\#\#\#}$	15.17 ± 1.74***	**14.52 ± 0.84**
6	19.66 ±1.59$^{***\#\#\#}$	15.66 ±1.79*	12.15 ± 0.73
8	16.12 ±1.41$^{***\#\#}$	14.12 ±1.51***	11.56 ±1.09
12	12.44 ±1.74$^{**\#}$	13.44 ±1.64**	11.29 ±0.13

Values are expressed as mean ±S.D; n=2

*P<0.05; **P< 0.01;***P<0.001 when compared with pure drug suspension.

$^{\#}$P<0.05; $^{\#\#}$P< 0.01; $^{\#\#\#}$P<0.001 when compared with marketed formulation.

One way ANOVA followed by TUKEY-KRAMERS multiple comparison tests.

Table 6 Pharmacokinetic parameters for fenofibrate

Pk parameters	Fenofibrate		
	SNEDDS	**Marketed**	**Pure drug suspension**
C_{max}(ng/mL)	28.9 ± 1.93	18.75 ± 1.13	14.52±0.84
T max(h)	2	2	4
K_e(h)	0.0981±0.0014	0.165±0.0024	0.3167±0.0019
AUC_{0-t}(ng.h/mL)	228.4388±14.82	177.8438±2.06	96.6925±0.682
$AUC_{0-\infty}$(ng.h/mL)	355.248±23.6	247.177±4.89	99.8186±1.54
F_r	8.0277	1.476	----------
F_r	0.437	----------	----------

SNEDDS containing fenofibrate was well absorbed with a C_{max} of 28.9ng/mL in 2h (T_{max}),when compared with other two.It is also supported by the elimination rate constant which is 0.0981h.SNEDDS formulation showed 0.47 folds increased bioavailability when compared to that of marketed formulation and 0.166 fold increased bioavailability when compared to pure drug suspension while the marketed formulation exhibited only 0.266 folds increase of bioavailability compared to pure drug suspension. These results suggests that better availability of drug was achieved with lipid based SNEDDS . This is because of the small globule size, and eventually higher surface area which permits faster rate of drug release. Being droplets and the drug in solution form, instant absorption should have been taken place. Since the carrier is lipid, it undergoes lipolysis in the presence of bile salts and pancreatic lipases etc. Therefore the surfactant layer around the droplets gets released and is converted to micelles, reverse micelles etc. Sometimes this may lead to precipitation of drug since the drug gets detached from the droplets. This is the case where the surfactants are directly involved in the solubility of the drug.

***In vivo* lymphatic absorption studies:**

In vivo lymphatic absorption studies in Sprague-Dawley rats were performed and the intestinal lymphatic fluid was collected for a period of 12h. The amount of drug that has been absorbed through lymphatic system for SNEDDS formulation was found to reach a maximum of 22.41 ± 0.51ng/mL by 2h after administration of fenofibrate . From then the rate at which drug absorbed via lymphatic route showed a decline.

***In vivo* Lymphatic absorption for fenofibrate**

Time (h)	**Fenofibrate (ng/ml)**		
	SNEDDS	**Marketed**	**Pure drug suspension**
0.25	12.93 ± 0.34	1.08 ± 0.34	0.027 ± 0.04
0.5	16.13 ± 0.28	1.45 ± 0.28	0.16 ± 0.08
0.75	18.07 ± 0.91	1.98± 0.91	0.19 ± 0.01
1	19.79 ±0.46	2.37±0.46	0.26 ±0.06
2	**22.41 ± 0.51**	**2.74 ± 0.42**	0.30 ±0.04
4	19.98 ± 0.42	2.66±0.71	**0.35 ±0.01**
6	17.22 ±0.71	2.54 ± 0.51	0.32 ± 0.02
8	15.34 ±0.34	2.16 ±0.34	0.31 ± 0.01
12	13.76 ±0.51	2.04 ±0.51	0.27 ±0.05

The marketed formulations reached a maximum lymphatic absorption 2h was only 2.74 ± 0.42ng/mL while for pure drug suspension it was only 0.35 ±0.01fenofibrate 4h . Whereas very small quantity of drug available in the lymphatic system for marketed and pure drug suspension. This could be the reason for the low bioavailability of conventional formulation, since most of the drug might have transported through portal blood, which eventually metabolized and lead to decreased concentration of drug in the systemic circulation. The enhanced rate of drug absorption via lymphatics for SNEDDS formulation is due to the lipid nature of the droplets, micelles and reverse micelles formed during lipolysis. It is also evident that the result of lipolysis did not account for precipitation. Hence maximum amount of drug was available for absorption. Moreover the lipid carriers and the surfactants might have involved in overcoming the P-gp efflux, thereby increasing the drug available for absorption which is evident from the lymphatic drug absorption study. The enhanced lymphatic absorption is also attributed to the lipid nature of the SNEDDS which might have triggered the chylomicron synthesis. The GI tract is highly supplied with both lymphatic and blood vessels and hence the materials absorbed across the small intestine (enterocytes) can enter the lymphatic or blood capillaries. However most of the SNEDDS materials are absorbed and transported into the intestinal lymph which is evident from the results.

Conclusion:

From the study it is concluded that SNEDDS can be used as a delivery system to enhance the solubility and bioavailability of BCS ClassII drugs which is well evidenced from the pharmacokinetic and lymphatic uptake study. The study also suggests that SNEDDS could be used to overcome the 1st pass effect and Pgp efflux**.**

References:

1. Vieth M, Siegel MG, Higgs RE, Watson IA, Robertson DH,Characteristic physical properties and structural fragments of marketed oral drugs. J Med Chem 47,2004: 224-232.
2. Fores B, EhrhardtC.Human respiratory epithelial cell culture for drug delivery applications. Eur. J. Pharm. Biopharm. 60(2), 2005: 193-205.
3. Krishna G, Chen K.J, Lin C.C, Nomeir A.A, Permeability of lipophilic compounds in drug discovery using in vitro human absorption model Caco-2. Int. J. Pharm. PharmSci, 222, 2001: 77–89.
4. Amidon, G. L., Robinson, J.R. and Williams, R. L., (Eds.). Scientific Foundations for Regulating Drug Product Quality.American Association of Pharmaceutical Sciences. Alexandria, Virginia. AAPS Press. 1997.
5. Gao D, Delmare D, Narang AS. Stable drug encapsulation in micelles and microemulsions.Int.J. Pharm.345, 2007: 9-25.
6. Ljusberg-Wahren. H., Seier Nielsen. F., Brogard. M., Troedsson. E., Mullertz. A., Enzymatic characterization of lipid-based drug delivery systems, Int. J. Pharm. 298 (2), 2005: 328–332.
7. Shaji J, Joshi V, Self-microemulsifying drug delivery system (SMEDDS) for improving bioavailability of hydrophobic drugs and its potential to give sustained release dosage forms. Indian J. Pharm. Educ. 39(3), 2005:130-135.
8. Shah. P., Bhalodia. D. and Shelat. P. Nanoemulsion: A Pharmaceutical Review. Sys. Rev in Pharmacy, 1(1), 2010: 24-32.
9. Dahan. A., Hoffman. A., Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs. Eur. J. Pharm. Sci. 24, 2005: 381–388.
10. Gershanik. T., Benita. S., Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur. J. Pharm. Biopharm. 50 (1), 2000: 179–188.
11. Dahan. A., Hoffman. A, in: Touitou. E., Barry. B.W. (Eds.), Enhancement in Drug Delivery, CRC Press. 2006: 111–127.
12. Thomson. A.B., C. Schoeller. C., M. Keelan. M., Smith. L., Clandinin. M.T., Lipid absorption:passing through the unstirred layers, brush-border membrane, and beyond, Can.J. Physiol. Pharmacol.71, 1993: 531–555.
13. O'Driscoll. C.M., Griffin. B.T., Biopharmaceutical challenges associated with drugs with low aqueous solubility — the potential impact of lipid based formulations, Adv. Drug Deliv. Rev. 60,2006: 617–624.
14. Bhavesh S. Barot, Punit B. Parejiya, hetal K. Patel, MukeshC.Gohel, Pragna K. Shelat, Microemulsion Based Gel of Terbinafine for the treatment of Onchomycosis: Optimization of Formulation using D-Optimal Design, AAPS Pharm Sci Tech. 13(1),2012: 184-192.
15. Elnaggar Y.S.R, El-Massik M.A, Abdallah O.A. Self-nanoemulsifyingdrug delivery systems of tamoxifen citrate: Design and optimization. International Journal of Pharmaceutics. 2009: 380:133–41.
16. Zangenberg, N.H., Müllertz, A., Kristensen, H.G., Hovgaard, L, A dynamic in vitro lipolysis model II: evaluation of the model. Eur. J. Pharm. Sci. 14, 2001: 237–244.
17. Shafiq S., Shakeel F., Talegaonkar S., Ali J., Baboota S., Ahuja A., Formulation development and optimization using nanoemulsion technique: A technical note. AAPS Pharm.Sci.Tech.8(2),2007:Article 28(E1-E6).
18. Sushil Kumar, SushmaTalegaonkar, Lalit M. Negi and Zeenat I. Khan, Design and Development of Ciclopirox Topical Nanoemulsion of Sublingual Onchomycoses, Ind J. Pharm Edu Res. 46(4), 2012: 303-311.
19. Ghosh, P.K., Majithiya, R.J., Umrethia, M.L., Murthy, R.S.R., AAPS. Pharm. Sci. Tech.7(3),2006 :Article 77.
20. Barea. M. J., Jekins. M. J., Gaber. M. H.. Evaluation of Liposomes Coated with a pH Responsive Polymer. Int. J. harm. 402(1), 2010: 89-94.
21. Samah. N. A, Williams. N. and Heard. C. M., Nanogel Particulates Located within Diffusion Cell Receptor Phases Following Topical Application Demonstrates Up-take into and Migration Across Skin. Int. J. Pharm. 401(1-2), 2010: 72-78.
22. Nagarsenker MS, Date AA. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoximeproxetil. Int. J. Pharm329,2007:166–72.
23. Sunesen. V.H., Pedersen. B.L., Kristensen. H.G., Mullertz. A., In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. Eur. J. Pharm. Sci. 24 (4), 2005: 305–313.

24. Michael Boyd, VericaRisovic, Philip Jull, Eugene Choo, Kishor M. Wasan. A stepwise surgical procedure to investigate the lymphatic transport of lipid-based oral drug formulations: Cannulation of the mesenteric and thoracic lymph ducts within the rat; Journal of Pharmacological and Toxicological Methods. 49, 2004:115– 120.
25. Edwards. G.A., Porter. C.J.H., Caliph. S.M. ,Khoo. S.M., Charman. W.N., Animal models for the study of intestinal lymphatic drug transport. Adv. Drug Deliv. Rev. 50, 2001: 45–60.
26. Anne Christiansen, Thomas Backensfeld, Werner Weitschies. Effects of non-ionic surfactants on in vitro triglyceride digestion and their susceptibility to digestion by pancreatic enzymes.European Journal of Pharmaceutical Sciences. 41, 2010: 376–382.

*****