Madhu Babu A. *et al.* / *International Journal of Pharmacy & Therapeutics*, 3(3), 2012, 287-294.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

DEVELOPMENT AND CHARACTERIZATION OF NOVEL SELF-NANOEMULSION DRUG DELIVERY SYSTEM OF LOW SOLUBILITY DRUG "FENOFIBRATE" FOR IMPROVED ORAL BIOAVAILABILITY

A. Madhu Babu[1]*, B. Prakash Rao[2], K.R.S. Sambasivarao[3], P. Sudhakar[3]

[1]Department of Pharmaceutics, Deevena College of Pharmacy, Suryapeta, A.P, India.
[2]Department of Pharmaceutics, Karnataka College of Pharmacy, Bangalore, Karnataka, India.
[3]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

ABSTRACT

Fenofibrate is insoluble in aqueous solution and the bioavailability after oral administration is low. Self-nanoemulsifying drug delivery systems (SNEDDS) containing fenofibrate have been successfully prepared to improve its bioavailability. SNEDDS is a mixture of lipid, surfactant, and cosurfactant, which are emulsified in aqueous medium under gentle digestive motility in the gastrointestinal tract. Pseudo-ternary phase diagrams composed of various excipients were plotted. Droplet sizes, zeta-potential and long-term physical stability of the formulations were investigated. The invitro drug release profile of self nanoemulsion was carried in phosphate buffer Ph 7.4 for 1 hr by using USP dissolution apparatus type-II device. From the invitro dissolution data, F15 formulation was found that the drug release is best and the cumulative % of drug release was 98.75% respectively. The promising formulation F15 was found by evaluation studies were compared with Marketed product (Lofibra 50mg), the F15 formulation gave 98.75% of the drug release and the Marketed product gave 47.56 % of drug release in 1 hr of dissolution study. The in-vitro intestinal permeability results exhibits the drug diffused at a faster rate from the self nanoemulsion system than from the capsule dosage form. After 1 hour of diffusion, 75.45% of drug was diffused from the self microemulsion system, as compared with 33.38% diffused from the capsule. Our studies indicate that the use of SNEDDS for the delivery of fenofibrate can improve its bioavailability.

Key Words:- Fenofibrate, SNEDDS, Zeta potential, In vitro release studies and Stability studies.

INTRODUCTION

Fenofibrate is a fibric acid derivative whose lipid modifying effects reported in humans are mediated via activation of Peroxisome Proliferator Activated Receptor type alpha (PPARα). Through activation of PPARα feno fibrate increases the lipolysis and elimination of atherogenic triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein CIII. Activation of PPARα also induces an increase in the synthesis of apoproteins AI and AII, which leads to a reduction in very low - and low density fractions (VLDL and LDL) containing apoprotein B and an increase in the high density lipoprotein fraction (HDL) containing apoprotein AI and AII. In addition, through modulation of the synthesis and catabolism of VLDL fractions,

Corresponding Author

A. Madhu Babu
Email:- ananthmadhu@gmail.com

fenofibrate increases the LDL clearance and reduces small and dense LDL, the levels of which are elevated in the atherogenic lipoprotein phenotype, a common disorder in patients at risk for coronary heart disease (Horter D *et al.,* 2011; Amidon GL *et al.,* 1995).

Many studies has been focused on enhancing the solubility of poorly water soluble drugs and improving bioavailability to administer them through oral route resulting in increasing their clinical efficacy. One of the most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles, such as oils, surfactant dispersions, self-emulsifying formulations, emulsions, and liposomes (Dressman JB *et al.,* 1998; Charman S *et al.,* 1992; Constantinides P *et al.,* 1994; Kovarik J *et al.,* 1994; Garti N *et al.,* 2000; Bhatt PP *et al.,* 2004; Aungust B *et al.,* 1993; Burcham D L *et al.,* 1997; Charman S A *et al.,* 1992; Constantinides P P *et al.,* 1995; Craig D Q M *et al.,* 1993).

In the present study, a SNEDDS was prepared using the non-ionic Tween 80 (as surfactant), PEG-400 (as cosurfactant) and Meglyoil. Pseudoternary phase diagrams were constructed to find out the zone of self-microemulsion at different ratios of surfactant to cosurfactant (1:1, 2:1, 3:1).

MATERIALS AND METHODS

Fenofibrate was obtained as a gift samples from (Dr Reddys laboratories, Hyderabad, India. Meglyoil, Tween 80, PEG 400 were purchased from Merck specialities pvt limited, Mumbai, India. HPLC Grade Acetonitrile and all other buffering agents of analytical grade were purchased from Sd fine chemicals, ltd, Mumbai, India. HPLC grade water prepared by using SG-LABOSTAR^{TM} 3 TWF-UV ultrapure water system.

Solubility Studies

The solubility of fenofibrate in various oils, surfactants, and cosurfactants was determined. An excess amount of fenofibrate was added into each vial containing 10 mL of selected vehicle. Then, the mixture was heated at 40°C in a water bath to facilitate the solubilization. Mixing of the systems was performed using a cyclo mixer (CM 101, Remi, India) for 10 min in order to facilitate proper mixing of drug with the vehicles. Then, the formed suspensions were shaken for 48 h in a mechanical shaker (Remi, India). After reaching equilibrium, the mixtures were centrifuged at 2500g for 20 min to remove undissolved fenofibrate, followed by filtration through a 0.45-µm millipore membrane filter paper. The concentration of fenofibrate was quantified by HPLC (Palin K J *et al.,* 1986). The solubility of fenofibrate in various oils and surfactants were represented in graph.

Construction of Phase Diagrams

The pseudo-ternary phase diagrams of oil, surfactant: cosurfactant, and water were developed using surfactant titration method: the mixtures of oil and water at certain weight ratios were titrated with surfactant/co-surfactant mix in a drop wise manner. Three types of surfactant phases were prepared Tween 80 + PEG 400 (1:1, 2:1, 3:1) for each phase diagrams at a specific ratio of surfactant/cosurfactant transparent and homogenous mixture of oil and drug was formed under the mixing by magnetic stirring. Then, visually observed for phase clarity and flow ability. After the identification of self-microemulsion region in the phase diagrams, the SMEDDS formulations were selected at desired component ratios. In order to form the self-microemulsion (Schwendener R A *et al.,* 1996; Pradip Kumar G *et al.,* 2006).

Preparation of SNEDDS formulations

On the basis of the "Solubility studies" section, the oil (Meglyoil), surfactant (Tween 80), and cosurfactants (PEG 400) were selected due to their greater solubility enhancement effect on Fenofibrate. Various formulations were tried as shown in Table-1. The formulations were prepared by dissolving Fenofibrate in the mixture of oil, surfactant, and cosurfactant and were heated at 50°C in an isothermal water bath. This mixture was mixed well and subjected to vortexing using cyclomixer (Remi, India), until a transparent preparation was obtained. All the mixtures were stored at ambient temperature for further use.

CHARACTERIZATION AND EVALUATION OF SNEDDS

Self-emulsification and precipitation assessment

In brief, various compositions were categorized on the basis of clarity and apparent stability of the resultant emulsion. Visual assessment was performed by drop wise addition of the preconcentrate (SNEDDS) into 250 mL of distilled water taken in a glass beaker at room temperature. The contents were gently stirred either using glass rod or magnetically at ~100 rpm. They were observed immediately after dilution for assessment for self-nanoemulsification efficiency, appearance (transparency),phase separation, and precipitation of drug. Precipitation was evaluated by visual inspection of the resultant nanoemulsion after 24 h. The formulation were then categorized as clear (transparent or transparent with bluish tinge), non clear (turbid), stable (no precipitation at the end of 24 h), or unstable (showing precipitation within 24 h).

Emulsion droplet size analysis/particle size determination

The droplet size and surface charge of the emulsions was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using Nano Zeta sizer (Horiba Instruments, Japan) able to measure sizes between 10-3000 nm. Light scattering was monitored at 25^{o}C at a 90^{o} angle. The dispersed formulations were measured after dilution (1:100) to produce the required count rate (50-200) to enable the accurate measurement (Pradip Kumar G *et al.,* 2006).

Percent drug content estimation

Fenofibrate from preweighed SNEDDS was extracted by dissolving in 20 mL of The mobile phase prepared by using Phosphate buffer (pH 7.5) combined with HPLC grade Ace-tonitrile in the ratio of 40: 60 v/v. Fenofibrate content in the mobile phase extract was analyzed using HPLC (Shimadzu) at 287 nm.

Zeta potential determination

The zeta potential of the diluted SNEDDS formulations was measured using a Nano Zeta sizer (Horiba Instruments, Japan). The SNEDDS were diluted with a ratio of 1:2500 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. Zeta potential of each SNEDDS was determined in triplicate.

Viscosity

The rheological property of the self-nanoemulsion was evaluated by BROOKFIELD-DV-II+pro viscometer using spindle 00 UL adaptor at 25+_0.5 °C, at 5 rpm. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation (Bennett K E *et al.,* 1982).

FTIR studies

FTIR spectrums of fenofibrate and drug–self-nanoemulsion formulation were obtained by means of a FTIR spectrophotometer (Bruker-Alpha T). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 cm−1 over the range of 400–4000cm−1. After running the spectra, significant peaks relating to major functional groups were identified; spectra of the subsequent sample of the same compound were compared with the original (Shah N *et al.,* 1994).

Thermodynamic Stability Studies

The self-nanoemulsion formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 months for intermediate and accelerated conditions and 6 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating HPLC method (Matuszewska B *et al.,* 1996).

In vitro drug release studies

The release of Fenofibrate from the optimized SNEDDS and marketed capsule was determined according to USP dissolution apparatus type-II. To permit the quantitative drug release from SNEDDS and marketed capsule, 900 ml of phosphate buffer PH-5.5 was placed in the dissolution vessel and then the SNEDDS formulation filled in hard gelatin capsule and capsule was placed in the dissolution medium and was agitated at 50 rpm at 37oC. At predetermined time intervals of 5min (up to 1 hour), 5 ml of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 287nm. The volume withdrawn was replaced each time with fresh dissolution medium. Cumulated released amounts were plotted as a function of time (Bok K K *et al.,* 2004).

In Vitro Intestinal Permeation Studies

The methods employed were modified from experimental procedures well described in the literature. Male Sprague-Dawley rats (250-300g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intra duodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The SNEDDS sample was diluted with 1 mL of distilled water (outside mixing for 1 minute by vortex mixer), and for the capsule sample a suspension of powder inside the capsule was made in distilled water. The resultant sample (1 mg/mL) was injected into the lumen of the duodenum using a syringe, and the 2 sides of the intestine were tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37^{o}C. The receiver compartment was filled with 30mL of phosphate-buffered saline (pH 5.5). At predetermined time intervals of 5min (up to 1 hour), 2 ml of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 287nm the percent

diffusion of drug was calculated against time and plotted on a graph (Bok K K *et al.*, 2004).

RESULTS AND DISCUSSION

Solubility studies

Solubility studies were performed to identify suitable oily phase, surfactants, and cosurfactants for the development of SNEDDS of Fenofibrate. Because an important consideration when formulating a self-emulsifying formulation is avoiding precipitation of the drug on dilution in the gut lumen *in vivo*. The components used in the system should have high-solubilization capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion.

The results of solubility studies are reported in figure-1. It is evident from the results that, among surfactants Tween 80 and PEG 400 provided higher solubility than other vehicles and Meglyiol as oil was selected respectively, for the optimal self-nanoemulsion formulation resulting in improved drug loading capabilities. Hence, for the preparation of SNEDDS, Meglyoil, Tween 80, and PEG 400 were chosen as an oil, surfactant, and cosurfactant.

Pseudoternary phase diagram

A pseudoternary phase diagram of the investigated quaternary system water/Meglyoil/Tween 80/PEG 400, is presented in Figure 2. Formation of nanoemulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, one-phase low-viscous self- nanoemulsion system was formed. The phase study revealed that the maximum proportion of oil was incorporated in self- nanoemulsion systems when the surfactant-to-cosurfactant ratio was 1:1. From a formulation viewpoint, the increased oil content in self-nanoemulsions may provide a greater opportunity for the solubilization of fenofibrate. Moreover, when the composition (% w/w) of surfactant mixture (Smix) in a self-nanoemulsion preparation was <50%, the formulation was less viscous. The optimum formulation of self-nanoemulsion contained fenofibrate (50 mg), Meglyoil (15.2% w/w), Tween 80 (53.09% w/w), and PEG 400 (31.88% w/w).

Preparation of SNEDDS for Fenofibrate

Several SNEDDS systems with the ability to dissolve 50 mg of Fenofibrate were prepared and compared. During preliminary study, some SNEDDS were eliminated due to detection of oil droplets on the surface of the diluted SNEDDS, which translates to an incomplete emulsification. SNEDDS that were not able to self-emulsify upon mixing with water under mild-agitation or yielded an unstable emulsions were rejected. A few SNEDDS formulations were eliminated due to the formation of milky emulsions upon dilution. The transparency of the diluted SNEDDS reflects the proximity of the droplet size to that of the nanoemulsion range. Formulations, F1, F12, F15, F16, F20 which were obtained transparent were given in Table-1, and they were subjected to test for self-emulsification and precipitation assessment.

Self-emulsification and precipitation assessment

Evaluation of self-microemulsifying properties of SNEDDS formulations was performed by visual assessment as reported. These studies were carried out on various SNEDDS formulations. During the study, it was found that some formulations, F12 and F15 showed turbidity, precipitation and thus was not stable, due to the relative decrease in surfactant concentration and the presence of PEG 400. Hence, F1, F16, and F20 were prepared with increased concentrations of surfactant. Formulation F16 could be mixed with Meglyoill, Tween 80, and PEG 400 and hence was selected as good formulation and subjected to further investigation regarding droplet size, Zeta potential, etc.

Evaluation of SNEDDS for droplet size analysis, zeta potential, drug content determination and viscosity

Droplet size distribution following self-nanoemulsification is a critical factor to evaluate a self-nanoemulsion system. The mean globule size of selected SNEDDS formulation F16, of Fenofibrate was 104.6 nm Table 2 is indicated the ability of the present technology to produce nanoemulsion that offers larger interfacial surface area required for drug absorption (Pradip Kumar G *et al.*, 2006; Bennett K E *et al.*, 1982). An increase in the ratio of the oily phase (Meglyoil) resulted in a proportional increase in particle size, because of the simultaneous decrease in the s/cos proportion. Increasing the s/cos (surfactant to cosurfactant) ratio led to decrease in mean droplet size. The optimized SNEDDS, with the highest proportion of surfactant (56.91% w/w Tween 80) at a fixed amount of oil (25.2% w/w), was produced lowest mean particle diameter of 104.6 nm. This could be attributed to an increased surfactant proportion relative to cosurfactant.

The optimized SNEDDS showed high absolute zeta potential value of -54.78 mv. The emulsion stability is directly related to the magnitude of the surface charge (Shah N *et al.*, 1994; Matuszewska B *et al.*, 1996; Bok K

K *et al.*, 2004). Generally, an increase of electrostatic repulsive forces between microemulsion droplets prevents the coalescence of droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. The results of zeta potential and drug content estimation are indicated in Table 3. The percent drug content (99.95 ± 3.32) of SNEDDS of fenofibrate was found satisfactory.

FTIR studies

The compatibility of drug and excipients used in the SNEDDS were characterized by their FTIR spectra. The FTIR spectrum of pure fenofibrate has four characteristic peaks at, 2997, 1746cm−1, 1658 and 1597 for O–H stretching vibration, C-H vibration and ester stretching vibration and lactone carbonyl functional group respectively. The FTIR spectrum of pure Formulation has four characteristic peaks at 2990cm−1, 1740cm−1, 1660cm−1, and at 1600cm-1. The FTIR spectrum of pure Fenofibrate and self-microemulsion formulation were almost similar because of the same functional groups. It indicates that there was no interaction between Fenofibrate and excipients used in the formulation. Depicted on figure 5.

Thermodynamic stability

The developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. Table 3 gives the results of the evaluation test conducted on stability sample. The formulation was found to be stable for 3 months at intermediate and accelerated conditions and 6 months at long-term conditions. There was no significant change in the drug content, or particle size of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. Furthermore, the formulation was found to show no phase separation, drug precipitation, or capsule leaks. Thus, these studies confirmed the stability of the developed formulation and its compatibility with hard gelatin capsules.

In-vitro drug release studies

The in-vitro drug release studies for capsule (Lofibra 50mg) and SNEDDS was determined in USP dissolution medium pH 5.5. The results are shown in Figure 6. At the end of 1 h, the release of fenofibrate from the microemulsion was significantly greater (98.75%) than that for marketed capsule (47.56%). This may be the result of surfactant molecules which leads to the enhancement of solubility of the drug in dissolution medium.

In Vitro Intestinal Permeability Study

The drug concentration was determined by High performance liquid chromatography at maximum wavelength 287nm and the percent diffusion of drug was calculated against time and plotted on a graph. The in-vitro intestinal permeability results exhibits the drug diffused at a faster rate from the nanoemulsion system than from the capsule dosage form. After 1 hour of diffusion, 75.45% of drug was diffused from the nanoemulsion system, as compared with 33.38% diffused from the capsule.

Fig 1. Graph showing solubility of Fenofibrate in various Oils and Surfactants, The solubility of Fenofibrate was determined in various vehicles by HPLC. The solubility of Fenofibrate in surfactant was found to be high in Tween 80 & PEG 400, among oils Meglyoil exhibited the highest solubility

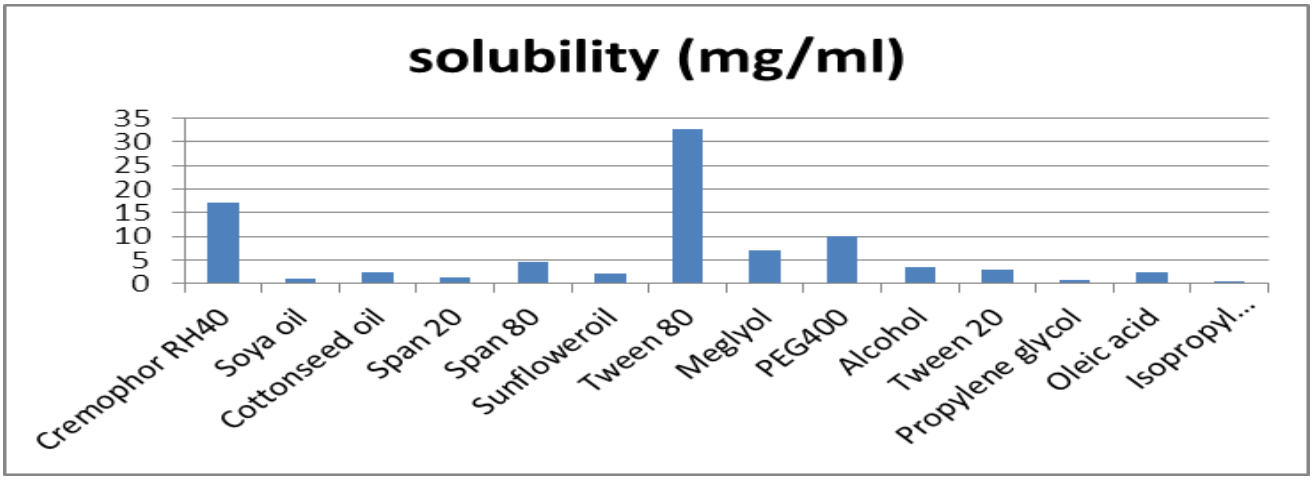
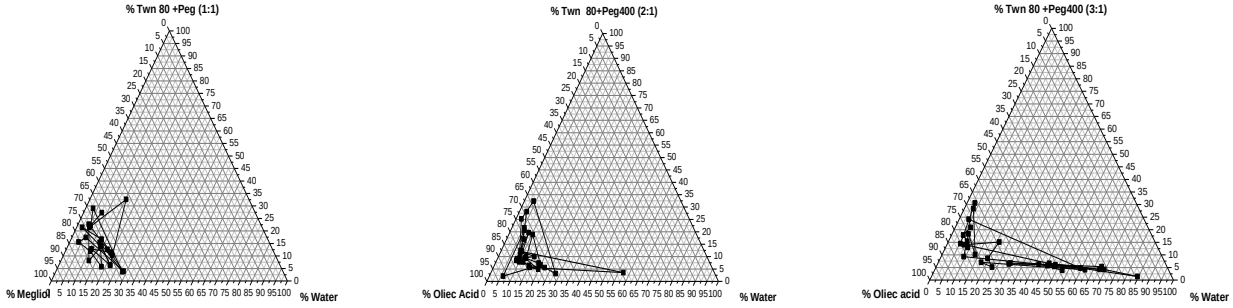


Fig 2. Pseudo-ternary phase diagrams indicating the efficient self-nanoemulsion region containing (Tween 80/PEG 400) = (a) 1:1 (w/w), (b) 2:1 (w/w), (c) 3:1(w/w)



(A) (B) (C)

Fig 3. Droplet size graph

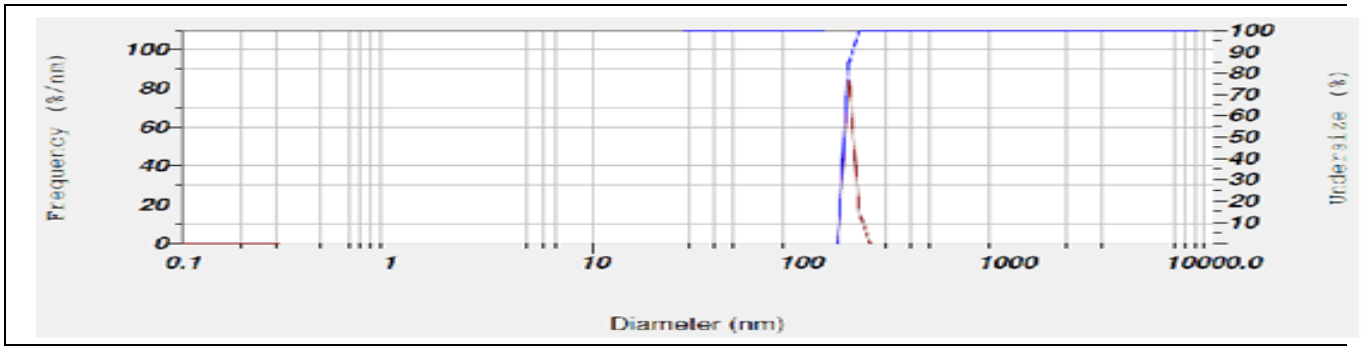


Fig 4. Zeta potential graph

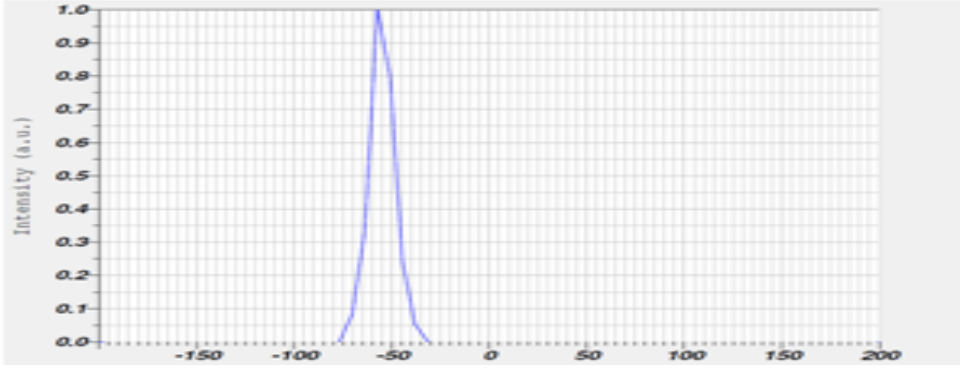


Fig 5. FTIR Spectra of Fenofibrate self-nanoemulsion with excipients

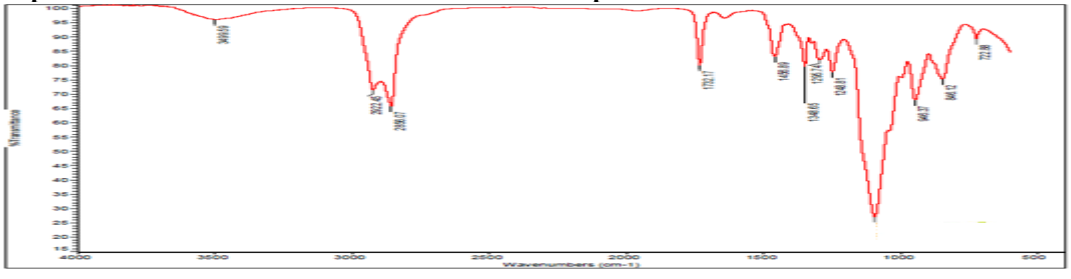


Fig 4. Comparative in vitro dissolution profile of fenofibrate (–♦–) SNEDDS (F16) and (–■–) capsule.

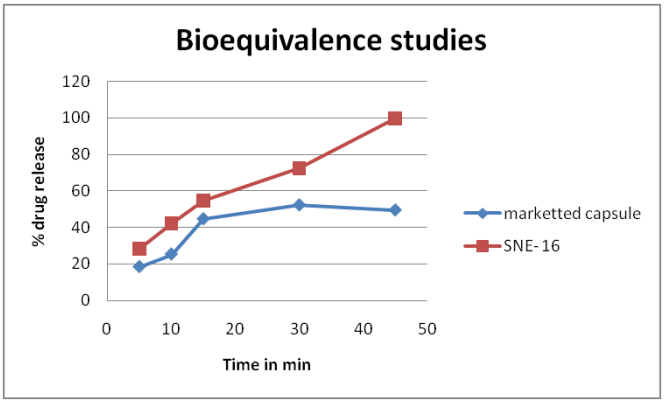


Fig 5. Comparative in vitro diffusion profile of fenofibrate through rat duodenum (–♦–) for SNEDDS (F16) and (–■–) for capsule.

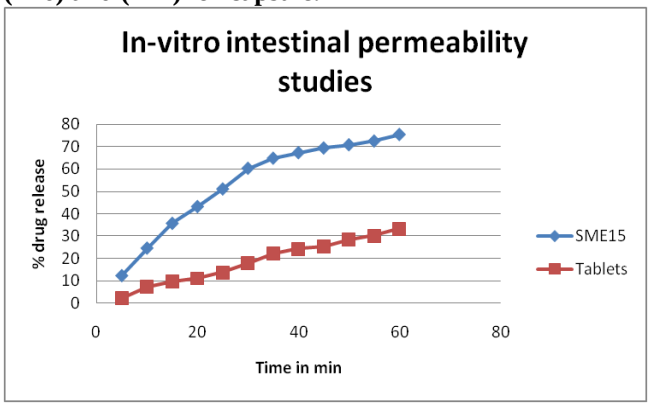


Table 1. Composition of self-nanoemulsifying drug delivery systems formulations of fenofibrate

Ingredients (%w/w)	F1	F12	F15	F16	F20
Fenofibrate	50mg	50mg	50mg	50mg	50mg
Meglyoil	28.46	12.88	27.13	15.04	25.2
Tween 80	60.26	65.97	54.26	53.09	56.91
PEG 400	10.26	21.13	18.60	31.85	17.88

Table 2. Evaluation parameters of self-nanoemulsifying drug delivery systems formulation of fenofibrate, F16 (n = 3)

Evaluation Parameter	Result
Mean droplet size (nm)	104±3.85
Mean Zeta potential (mv)	-30.5±4.26
% Drug found (mg/ml^{-1})	99.95±3.32
Viscosity(cp)	104.68

Table 3. Evaluation data of formulation subjected to stability studies

Condition	Sampling point	Droplet size(nm)	% drug content
A= (25°C/60% RH)	0 days	45.4±0.54	97.82±0.94
	45 days	45.2±0.24	96.56±0.42
	3 months	44.6±0.64	95.63±0.24
	6 months	43.3±0.34	94.75±0.61
B= (30°C/65% RH)	0 days	45.4±0.66	97.82±0.24
	45 days	44.7±0.14	96.55±0.65
	3 months	43.3±0.24	95.46±0.45
C=(40°C/75% RH)	0 days	45.4±0.24	97.82±0.75
	45 days	44.1±0.65	96.24±0.65
	3 months	43.7±0.88	95.65±0.25

CONCLUSION

An optimized SNEDDS formulation of Fenofibrate consisting of fenofibrate (50mg), Meglyoil (25.2% w/w), Tween 80 (56.91% w/w), and PEG 400 (17.88% w/w) was successfully developed with an increased solubility and dissolution rate. The SNEDDS of fenofibrate possessed mean microparticle size of 104+3.6 nm and other ideal characteristics required for enhanced dissolution rate. Thus, our study confirmed that the SNEDDS formulation can be used as a possible alternative to traditional oral formulations of fenofibrate to improve its dissolution rate leading to enhanced bioavailability.

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