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Design and optimization of self-nanoemulsifying drug delivery systems (SNEDDS) for enhanced dissolution of gemfibrozil

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ABSTRACT

Self-nanoemulsifying drug delivery systems of gemfibrozil were developed under Quality by Design approach for improvement of dissolution and oral absorption. Preliminary screening was performed to select proper components combination. Box–Behnken experimental design was employed as statistical tool to optimize the formulation variables, X_1 (Cremophor® EL), X_2 (Capmul® MCM-C8), and X_3 (lemon essential oil). Systems were assessed for visual characteristics (emulsification efficacy), turbidity, droplet size, polydispersity index and drug release. Different pH media were also assayed for optimization. Following optimization, the values of formulation components (X_1, X_2, and X_3) were 32.43%, 29.73% and 21.62%, respectively (16.22% of gemfibrozil). Transmission electron microscopy demonstrated spherical droplet morphology. SNEEDS release study was compared to commercial tablets. Optimized SNEDDS formulation of gemfibrozil showed a significant increase in dissolution rate compared to conventional tablets. Both formulations followed Weibull mathematical model release with a significant difference in t_d parameter in favor of the SNEDDS. Equally amodelistic parameters were calculated being the dissolution efficiency significantly higher for SNEDDS, confirming that the developed SNEDDS formulation was superior to commercial formulation with respect to in vitro dissolution profile. This paper provides an overview of the SNEDDS of the gemfibrozil as a promising alternative to improve oral absorption.

1. Introduction

Gemfibrozil (Gem), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a benzene derivative of valeric acid (Fig. 1). It has been the clinical choice for the hyperlipidemia (type III) and hypertriglyceridemia (type IV) (Sweetman, 2005). It has been found to decrease serum triglycerides and very low density lipoprotein-cholesterol (VLDL) and to increase high density lipoprotein-cholesterol (HDL) (Kersten et al., 2000) by activating the peroxisome proliferator activated receptors (PPARs), acting mainly on the PPARα isoform (Loomba and Arora, 2009; Fruchart and Duriez, 2006). Additional effects have been noted including their anti-inflammation effects (Takano et al., 2000; Delerive et al., 2001; Marx et al., 2002), inflammatory process plays an important role in the pathogenesis of coronary heart disease (Zhao et al., 2003). Gemfibrozil has been previously shown to reduce some indexes of thrombin generation, without affecting or even raising plasma fibrinogen levels (Wilkes et al., 1992; Broijersen et al., 1996; Kockx et al., 1997). Some gastrointestinal symptoms and rash were observed as side effects of its treatment.

Gemfibrozil is a small molecule with a poor water solubility, around 0.01 mg/mL (Kasim et al., 2004) and low dissolution rate in the gastrointestinal tract (GIT), which limits its effective absorption and bioavailability after oral administration (Ghebre-Sellassie and Fawzi, 1994).

It has been classified as a class II drug in the biopharmaceutical classification system with a high dose number (240). Thus, it can be assumed that the low oral bioavailability of Gem is due to its solubility and dissolution limitations (Kasim et al., 2004).

An increasingly important area of pharmaceutical research is in finding safe and effective methods of solubilizing poorly soluble drugs (Croy and Kwon, 2004). In this context several technological trials have been adopted to conquer this challenge as micronization of Gem (Huang et al., 2008), delivery systems as microspheres and macromolecular conjugates also have been assayed (Martinac et al., 2002). Lipid based formulations represent a solution to delivery poorly soluble actives, among these lipid based systems, the self-nanoemulsifying drug delivery systems (SNEDDS) are considered as promising technology to improve the rate and extent of absorption of poorly water soluble drugs (Singh et al., 2010; Hong

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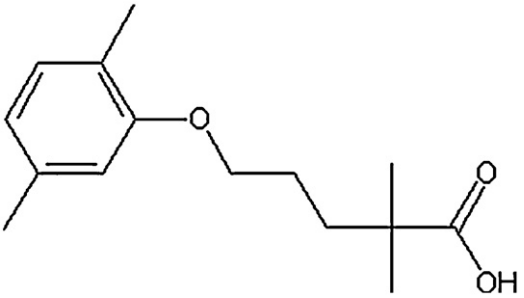

Fig. 1. Chemical structure of gemfibrozil.

et al., 2006). This poorly water-soluble active behaves differently in similar vehicles, thus highlighting the need to assess candidate compounds on an individual basis (Kohli et al., 2010).

Self-nanoemulsifying drug delivery systems are isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers that form fine oil in water nanoemulsions upon mild agitation in an aqueous medium with a droplet sizes ranging 20–200 nm (Mou et al., 2008; Porter et al., 2008). In the gastrointestinal tract environment (fluids and motility) this systems spontaneously emulsify (Nazzal et al., 2002; Devani et al., 2004; Patel and Vavia, 2007). They can improve oral bioavailability by the increase in the drug solubilization (the water-insoluble drug is usually dissolved in the oil phase), enhancing permeation across the intestinal membrane through a wide distribution in the GIT (due to the small droplet size), and decreasing food effect (Wang et al., 2010; Ke et al., 2005). On the other hand, SNEDDS formulations containing bioenhancers with certain types of surfactants such as Cremophor® improves the bioavailability of absorbed compounds by facilitating transcellular and paracellular absorption (Basalious et al., 2010).

Selection of a suitable self-emulsifying formulation depends upon the assessment of the solubility of the drug in various media, the area of the self-emulsifying region as obtained in the pseudoternary phase diagrams, and droplet size distribution of the resultant emulsion following self-emulsification (Kommuru et al., 2001).

The digestion of lipid-based formulations induces changes in lipid composition. It plays an important role in the solubilization capacity and consequently the absorption of co-administered drugs (Kossena et al., 2005; Porter et al., 2007). Equally an adequate dosage form must be chosen for the administration of these lipid systems, because liquids produce some disadvantages; the large quantity of surfactants in the formulations can induce gastrointestinal irritation (Tang et al., 2008). Optimal alternative to tackle these disadvantages are hard gelatine capsules (Wang et al., 2009; Halbaut et al., 1996), pellets (Abdalla et al., 2008), tablets (Attama et al., 2003), gelatine hollow type suppositories (Kim and Ku, 2000), and so on. There have been relatively few studies that introduce SNEEDS in a systemic way, especially with respect to dosage form development, preparation techniques (Tang et al., 2008) and quantification of the release patterns of these emulsions from their dosage forms (Nazzal et al., 2002).

Extensive survey of literature and patent databases did not reveal any SNEDDS formulation developed of gemfibrozil for improvement of the dissolution and oral absorption. The aim of this study was the development of self-nanoemulsifying drug delivery system in hard gelatine capsule dosage forms for the oral delivery of gemfribozil to augment the in vitro release by improving solubility. These gemfribozil-loaded SNEDDS were compared with commercially available tablets of gemfibrozil.

2. Material and methods

2.1. Materials

The active gemfibrozil was obtained as a gift from Menarini Lab. (Badalona, Spain), and the commercial gemfibrozil tablet (equivalent to 600 mg gemfibrozil) was purchased from STADA (Barcelona, Spain). Oils: lemon essential oil, anise essential oil, peppermint essential oil, soybean oil, as well as, polyoxy 35 castor oil (Cremophor® EL), were purchased from Fagron Iberica S.A.U. (Terrassa, Spain). Mono/diglycerides of caprylic acid (Capmul® MCM-C8) was kindly supplied by Abitec Corp. (Jamesville, USA). Lauroyl macrogol-6 glycerides EP/lauroyl polyoxyl-6 glycerides NF (Labrafil® M2130CS) from Gattefossé (Saint-Priest Cedex, France). Potassium chloride, potassium hydrogen phthalate, potassium dihydrogen phosphate, sodium hydroxide 0.2 M, hydrochloric acid 0.2 M, used to prepare buffer solutions, methanol and glacial acetic acid (both of HPLC grade) were purchased from Panreac Química S.A.U. (Barcelona, Spain). Double-distilled water was used after filtration in a Milli-Q® Gradinet A10 system apparatus (Millipore Iberica S.A.U., Madrid, Spain). Methacrylic acid-methylmethacrylate copolymer (Eudragit® L) was generously provided by Evonik Degussa Int. AG (Barcelona, Spain), and 00-size hydroxypropyl methylcellulose capsules (HPMC) were purchased from Fagron Iberica, S.A.U. (Terrassa, Spain)

2.2. Methods

2.2.1. Solubility studies for oil selection

Four commonly used oils, lemon essential oil, anise essential oil, peppermint essential oil and soybean oil, were screened for their properties to dissolve maximum amount of Gem. An excess amount of Gem was added into clear screw thread glass vials that contained 2 g of each oil followed by vortex-mixing in a MELB1719 vortex (Merck Eurolab; Lutterworth, UK). Mixtures were shaken for 20 min at 40 °C (50 °C for soybean oil) in a thermostatically controlled shaking water bath MP-5 (Julabo Labortechnik GmbH; Seelbach, Germany), followed by equilibrium for 12 h at 37° C (50 °C for soybean oil samples). Mixtures were then centrifuged at 3000 rpm for 10 min in a microcentrifuge Millifuge CT10 (Millipore Iberica S.A.U.; Madrid, Spain). Aliquots of supernatant were filtered trough 0.45 μm membrane filter (Millipore Iberica S.A.U.; Madrid, Spain) and the excess of Gem was discarded. The filtered sample was then diluted with glacial acetic acid:methanol (1:99 v/v) (mobile phase) and the amount of Gem solubilized was analyzed using a validated high-pressure liquid chromatography (HPLC) method spectrophotometrically at 276 nm using automated injection system Waters 717 plus HPLC autosampler (Waters; Milford, USA) and a detector instrument UV-1750 (Jasco; Dunmow, UK). This assay was performed in triplicate.

2.2.2. Differential scanning calorimetry studies

Based on solubility studies. Thermal technique were use to detect any interaction between drug and oil. A differential scanning calorimeter (DSC) method was used to observe thermal changes of Gem, lemon essential oil and an oily mixture of Gem and lemon essential oil (1:1; w/w). Approximately 5 mg of sample was sealed in the aluminum pan and analyzed using a differential scanning calorimeter DSC822e (Mettler-Toledo; Barcelona, Spain). Thermal analyses were carried out between 30 and 300 °C under nitrogen gas flow (50 mL/min) against an empty reference pan at a heating rate of 10 °C min^{-1}. Equally DSC of SNEDDS was performed later, once the experimental design were concluded (Section 2.3).

2.2.3. Selection of surfactants for emulsifying ability

Self-nanoemulsifying properties of SNEDDS strongly depend upon the selected lipids, surfactants, and their relative amounts. This mixture gives the possibility to optimize the SNEDDS for a particular drug. A total of 135 self-nanoemulsifying systems in five series without active substance were prepared by varying percentages of the essential oils (90–10%), surfactant Cremophor® EL (5–60%) and co-surfactant Capmul® MCM-C8 (5–30%). "A" series containing lemon essential oil, "B" series containing anise essential oil, "C" series containing peppermint essential oil, "D" series containing soybean oil, and "E" series containing soybean oil, surfactant, and as co-surfactants a mixture Capmul® MCM-C8: Labrafil® M2130CS (1:1; w:w) (5–30%) Table 1.

Their self-emulsifying properties were studied using a modified and adopted method of visual examination reported by Craig et al. (1995) and utilized in others investigations (Kommuru et al., 2001). Briefly, 1 g of formulation was introduced into 4 mL of distilled water in a glass beaker at 25 °C and the contents were mixed gently with a magnetic stir bar. The tendency to spontaneously form a transparent emulsion was judged as "good", and it was judged "bad" when there was poor or no emulsion formation. "Good" were nanoemulsions quickly forming, with a clear, transparent or milky-white appearance. "Bad" were nanoemulsion with large oil globules on the surface of dilution medium with signs of phase separation.

2.2.4. Phase diagrams studies

For each series (A, B, C, D, and E) of self-nanoemulsifying systems, ternary diagrams of surfactant, co-surfactant and oil, each of them representing an apex of the triangle, were constructed to recognize the zone of nanoemulsion formation. Twenty-seven samples were prepared with varying percentage of excipients. For E series the third phase was a mixture of co-surfactants as shows Table 1. Surfactant and co-surfactant were mixed in different volume ratios (1:1, 1:2 and 2:1). For every phase diagram, oil and specific surfactant:co-surfactant ratio were mixed in ratios ranging from 1:9% to 9:1% (w/w) in nine ratios like 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1.

2.2.5. Emulsion droplet size analysis and turbidity measurements of self-emulsifying systems

The droplet size of 49 nanoemulsions with the best self-emulsify capacity was determined by dynamic light scattering (DLS), with a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). DLS is a non-invasive, well-established technique for measuring the size of molecules and particles typically in the submicron region, providing in parallel the width of the size distribution expressed as polydispersity index (PDI) (Luykx et al., 2008). Samples were directly placed into the module and the data were collected at room temperature. All studies were repeated, with good agreement among measurements.

On the other hand, turbidity of these nanoemulsion systems given in nephlometric turbidity units (NTU) was measured using a turbidimeter HACH 2100P (HACH Co.; Düsseldorf, Germany), with approximated accuracy of ±0.001 NTU with stray light ≤0.01 NTU as specified by the manufacturer. The measure was performed on 30 mL of sample stored in a clear screw-capped sample vials. Previously the device was calibrated with formazin standards. The largest source of error at low turbidities is the stray light, that is, the light that reaches the detector due to sources other than sample turbidity (Sadar, 1998).

2.3. Experimental design optimization of Gem-loaded SNEDDS

After selecting the best suitable oil, surfactant and co-surfactant in accordance with studies performed, and taking into account the utility of the experimental design methodology as a very good tool for studying preparation of nano-emulsions (Gutiérrez et al., 2008). Response surface methodology (RSM) is a collection of techniques useful when only a few significant factors are involved in optimization (Ragonese et al., 2002), such as Box–Behnken and Central Composite design models. The Box–Behnken design was specifically selected since it requires fewer treatment combinations than a Central Composite design in case involving three or four factors. This design is an independent, rotatable or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design), in which the treatment combinations are at the midpoints of the edges of the process space and at the center (Govender et al., 2005).

Firstly, a Box–Behnken design was constructed to estimate the best amount of Gem in SNEDDS, with combinations from three factors (independent variables) which were the amounts (mg) of the component system: surfactant X_1 (Cremophor® EL), co-surfactant X_2 (Capmul® MCM-C8) and the oily phase X_3 (lemon essential oil:Gem; 1:1) and three levels and replicas of one of these combinations three times (in random run order) to estimate pure error, the replicas contained equal amounts of all three ingredients. High, medium, and low levels were selected from the preliminary experimentation. Visual characterization (Y_1), turbidity (Y_2), mean droplet size (Y_3), polydispersity index (Y_4). The responses of quadratic model formulations were treated by Design-Expert® version 7.1.6 software (Stat-Ease Inc., Minneapolis, USA) resulting in fifteen mixtures.

For Gem-loaded SNEDDS manufacturing process, accurately weighed gemfibrozil was mixed with lemon essential oil in a clear screw thread glass vial. Then, the vial was placed into a water bath using open bath circulators MP-5 (Julabo Labortechnik GmbH; Seelbach, Germany) at 40 °C for 20 min with gentle stirring to melt the oily mixture and facilitate the solubilization of Gem. Cremophor® EL and Capmul® MCM-C8 were accurately weighed and added to the oily mixture using a positive displacement pipette. All weighs were measured using PJ 360 Delta Range analytical balance (Mettler-Toledo; Barcelona, Spain). Formulations were stirred gently using a magnetic stir bar and a magnetic stir plate MELB1719 (Merck Eurolab; Lutterworth, UK) until reaching homogeneous solution. Formulations were then labeled and equilibrated into a water bath at 37 °C for 12 h to detect possible precipitation risk of drug before their use in subsequent studies.

Secondly, based on the results of the former design, a new Box–Behnken design was performed to optimize pH conditions medium in which Gem-loaded SNEDDS would possess an efficient emulsification properties. This design follows the same approach in factors and levels, but a new dependent variable was added, cumulative amount of Gem dissolved after 30 min in the selected medium (Y_5). USP buffer solutions pH 1.2, pH 4.5 and pH 7.5 were prepared according to the directions in The United States Pharmacopoeia, 2006 (USP 29), the fourth assayed medium was distilled water pH 6.8. The responses of quadratic model formulations were treated by Design-Expert® version 7.1.6 software (Stat-Ease Inc., Minneapolis, USA) resulting others fifteen mixtures.

These Gem-loaded SNEDDS were manufactured in the same way as it is related in the previous paragraph.

While molten, these fifteen Gem-loaded SNEDDS with different concentrations of surfactant, co-surfactant, and oil, but containing gemfibrozil at a final loading of 150 mg, were filled into size 00 HPMC hard gelatine capsules required for release studies. Then the capsules were coated with Eudragit® L by successive layer coating process. A gastro-resistance test was performed in HCl 0.1 N solution at 37 ± 0.5 °C for 2 h and immediately introduced in the selected buffer solution for 1 h at the same temperature.

Filled capsules were stored at room temperature for 24 h before their use in subsequent studies.

Table 1
Composition of formulations series.

Component	A series	B series	C series	D series	E series
Oil	Lemon essential oil	Anise essential oil	Peppermint essential oil	Soybean oil	Soybean oil
Surfactant	Cremophor® EL	Cremophor® EL	Cremophor® EL	Cremophor® EL	Cremophor® EL
Co-surfactant	Capmul® MCM-C8	Capmul® MCM-C8	Capmul® MCM-C8	Capmul® MCM-C8	Capmul® MCM-C8/Labrafil® M2130CS

2.4. Characterization of Gem-loaded SNEEDS

2.4.1. Visual characterization and turbidimetry

For each assayed medium a batch with samples of the fifteen developed Gem-loaded SNEDDS formulations was prepared. Briefly, 60 mg of each Gem-loaded SNEEDS formulation was accurately weighed and placed into 100 mL of selected aqueous solution medium. The visual characteristics of the emulsification process were observed in the resultant nanoemulsion. The appearance of the nanoemulsions after gentle agitation was noted and classified as: very cloudy, cloudy or clear.

The turbidity of the resultant nanoemulsions given in nephlometric turbidity units was determined using a HACH 2100P turbidimeter (HACH Co.; Düsseldorf, Germany). Experiments were repeated in sixplicate for each formulation at room temperature.

2.4.2. Droplet size measurements

Similarly, the droplet size analyses were preformed to the resultant Gem-loaded SNEDDS previously homogenized. Droplet size and its PDI were measured by photon correlation spectroscopy using Zetasizer Nano ZS (Malvern Instrument; Worcestershire, UK) and disposable cells 10 mm × 10 mm × 48 mm (Sartedt ref. 67.754) with dynamic light scattering particle size analyzer at a wavelength of 635 nm and at a scattering angle of 90° at 25 °C. Tests were repeated six times for each formulation at room temperature.

2.4.3. Freeze thawing

Freeze thawing was employed to evaluate the stability of Gem-loaded SNEEDS. Formulations were subjected to 3 freeze-thaw cycles, which included freezing at −4 °C for 24 h followed by thawing at 40 °C for 24 h. Centrifugation was performed at 3000 rpm for 5 min. The formulations were then observed for phase separation.

2.4.4. Transmission electron microscopy

Morphological and structural examination of Gem-loaded SNEDDS was carried out using transmission electron microscopy (TEM) with a Zeiss 902 CEM microscope (Zeiss, Barcelona, Spain). After sample dilution with distilled water (1:200) and mixed by slightly shaking, one drop of sample was deposited on copper grids covered with a layer of Formvar standing for 4 min. After, the excess was removed by absorbing on a filter paper. The grids were later stained with one drop of 2% uranyl acetate solution and allowed to dry for 5 min before examination under the electron microscope (Hayat, 1989).

2.5. In vitro drug release studies

In vitro dissolution tests were performed using a USP XXIV paddle apparatus SR8 PLUS (Hanson Research, Canada) at 37 ± 0.5 °C and rotating speed of 50 rpm in 900 mL of phosphate buffer solution pH 7.5. The coated capsules with SNEDDS containing 150 mg of Gem and commercially available tablets containing 600 mg of Gem were held to the bottom of the vessel using copper sinkers. For the coated capsules of Gem, 5 mL aliquots were removed at predetermined time intervals; 1, 3, 5, 10, 15, 30 and 45 min from the dissolution medium and replaced with fresh buffer and were tested for Gem using a spectrofluorometric method. Commercial tablets of Gem samples (5 mL) were withdrawn after 1, 3, 5, 10, 15, 30 and 45 min and tested for Gem using an ultraviolet spectroscopy method.

Analysis of variance (ANOVA) and *t*-tests were performed to evaluate the significant differences between release profiles of two formulations. Data were considered statistically significant at $p < 0.05$.

Data obtained from the in vitro release studies were measured, as detailed in the following section, and values reported as the mean ± SD of the six replicates. Four different kinetic models (zero order, first order, Higuchi and Weibull function) were used to fit the experimental data obtained in the drug release experiment (Costa and Sousa, 2001).

$$\frac{\%R_t}{\%R_\infty} = k \times t \quad \text{Zero order} \tag{1}$$

$$\frac{\%R_t}{\%R_\infty} = 1 - e^{-K \times t} \quad \text{First-order} \tag{2}$$

$$\frac{\%R_t}{\%R_\infty} = k \times t^{1/2} \quad \text{Higuchi's equation} \tag{3}$$

$$\frac{\%R_t}{\%R_\infty} = 1 - e^{-(t/t_d)\beta} \quad \text{Weibull's equation} \tag{4}$$

where $\%R_t$ is the percentage drug released at time t, $\%R_\infty$ is the total percentage drug released, $\%R_t/\%R_\infty$ is the fraction of drug released at time t, k is the release rate constant, t_d is the time in which the 63.2% of the drug is released and β is the shape parameter.

A nonlinear least-squares regression was performed using the WinNonLin® Professional edition version 3.3 software (Pharsight Corporation, USA), and the model parameters calculated. Also the Akaike's information criterion (AIC) was determined for each model as it is an indicator of the model's suitability for a given dataset (Yamaoka et al., 1978). The smaller the value of AIC, the better the model adjusts the data.

Besides, some additional amodelistic parameters as dissolution efficiency (DE) and mean dissolution time (MDT) were also calculated from the in vitro release data. These parameters are very useful to compare different profiles types because the evaluation of dissolution characteristics of the different formulations is not conditioned by the goodness of the fitting to a theoretical model of our data. The DE (%) was calculated by mean of the following equation:

$$\text{DE}(\%) = \frac{\int_0^t M \times dt}{C_{100} \times t} \times 100 \tag{5}$$

where the numerator represents the area under the dissolution curve up to a certain time, t, and the denominator is the rectangular area between the total amount of drug released and the last experimental time point, t. For area under dissolution curve (AUC) calculation, a continuous plot of the evolution of released quantities across the whole of the experimental time was calculated. Afterward, respective AUC values were calculated by means of a trapezoidal rule for the whole time values interval.

MDT is defined as the mean residence time of a drug in the formulation. It is a useful parameter to characterize the drug release rate from a dosage form. It was calculated using the equation:

$$\text{MDT} = \frac{\sum_{i=1}^{n} \bar{t}_i \times \Delta M_i}{M_\infty} \tag{6}$$

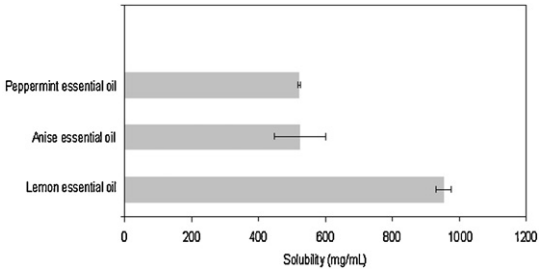

Fig. 2. Solubility of gemfibrozil in various oils. Data are expressed as mean ± SEM ($n = 3$).

where i is the sample number, n is the number of dissolution sample times, t_i is the time at midpoint between t_i and t_{i-1} (calculated with the expression $(t_i + t_{i-1})/2$), ΔM_i is the increase in the amount of drug released at each time interval, and M_∞ is the asymptote of the dissolved amount of drug.

Both amodelistic parameters were reliably calculated because the maximum percentage of drug released was 90% (Khan, 1975).

2.6. Spectrofluorometric measurements

Spectorofluorometric measurements were performed in six replicates using a SFM25 Kontron spectrofluorometer (Kontron Instruments, Basel, Switzerland). A xenon lamp with excitation and emission wavelengths of 276 and 304 nm, respectively was used. The analysis was carried out at room temperature. Quartz cells for the spectrofluorometry were used. Solution mixture of methanol and glacial acetic acid 1% was used as mobile phase. The instrument was calibrated before the study with good results.

3. Results and discussions

3.1. Solubility study

Results of the solubility of gemfibrozil in various essential oils are reported in Fig. 2. This study allows us to identify the suitable oil to load gemfibrozil into the formulations. In this case, gemfibrozil solubility was highest in lemon essential oil and was the selected essential oil to the experiments.

Screening of appropriate oil is primary requirement of SNEDDS development. Solubility studies were aimed at identifying suitable oil having maximal solubilizing potential for the development of SNEDDS. Among the selected oils, lemon essential oil was found to solubilize maximum amount of Gem 0.953 ± 0.022 mg/mL, peppermint and anise essential oils exhibited minor solubilizing capacity but very similar between them, 0.521 ± 0.003 mg/mL and 0.523 ± 0.077 mg/mL, respectively. Hence lemon essential oil would be preferred for further study to achieve optimum drug loading and avoiding precipitation of the drug on dilution in the gut lumen in vivo (Pouton, 2006), but final selection among these oils would secondly be confirmed according to emulsification properties with other ingredients (Date and Nagarsenker, 2007). The selection of surfactant or co-surfactant in the further study was governed by their emulsification efficiency rather than their ability to solubilize Gem. Soybean essential oil was discarded as the saturated mixture of Gem in oil resulted in a semisolid state without supernatant.

3.2. Differential scanning calorimetry studies

Fig. 3 shows a narrow endothermic peak in the dynamic DSC thermogram of gemfibrozil at 61.93 °C, which corresponds to the melting point of the material. This endothermic peak of Gem is in agreement with recent report (Aignera et al., 2012). The peak temperature is shifted at lower temperatures and its melting enthalpy was 114.21 J g^{-1}. The dynamic DSC thermogram of lemon essential oil shows a wide endothermic peak at 173.22 °C, the peak temperature is shifted at higher temperatures and its melting enthalpy was 371.43 J g^{-1}. DSC thermogram for the 1:1 oily mixture Gem-oil shows two endothermic peaks. The first peak at 40.14 °C (enthalpy of 38.23 J g^{-1}) and the second peak at 166.23 °C (enthalpy of 86.88 J g^{-1}) those peaks are related with the solubilization of Gem into lemon essential oil. The first peak reflects slightly the melting point of Gem present in the oily mixture. Dynamic DSC of Gem-loaded SNEDDS were performed after preparation. Three endothermic peaks were observed. These peaks are broader with smaller areas related to endothermic processes; there are some

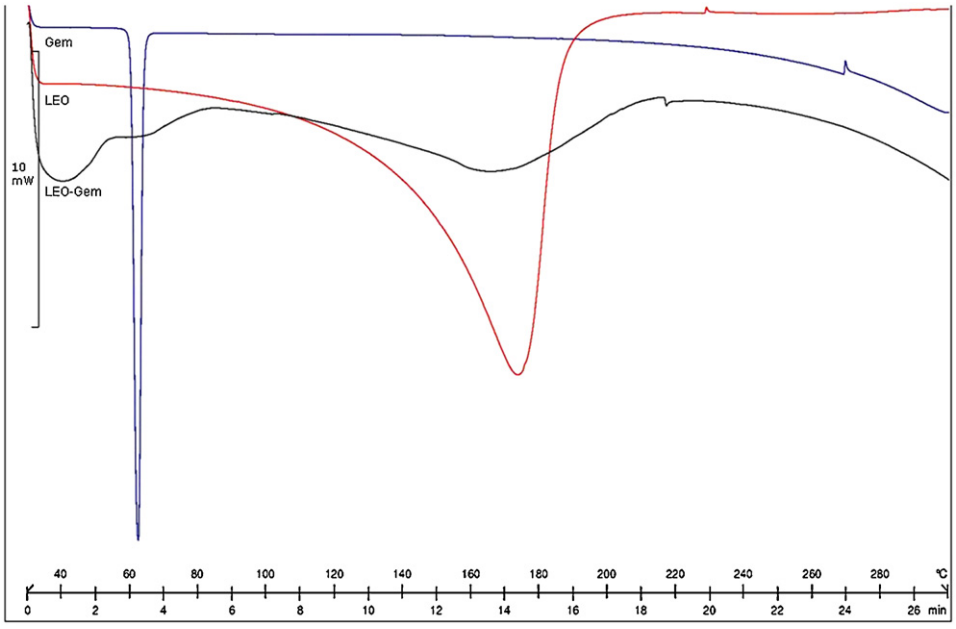

Fig. 3. Differential scanning calorimetry curves of gemfibrozil (Gem), lemon essential oil (LEO) and mixture lemon essential oil–gemfibrozil (LEO–Gem).

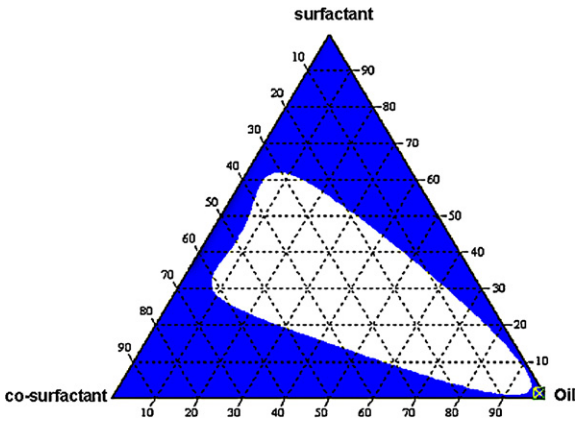

Fig. 4. Ternary phase diagrams of the selected system dispersed in water at 25 °C. The white area represents self-nanoemulsion region.

fluctuations among thermograms but no obvious peak for Gem was found for the SNEDDS indicating that the drug must be present in amorphous or molecularly dissolved state in SNEDDS.

3.3. Selection of surfactants for emulsifying ability

Emulsification studies clearly distinguished the ability of various surfactants to emulsify Gem. For the development of a self-emulsified formulation, a right blend of low and high HLB (hydrophilelipophile balance) surfactant is necessary for the formation of a stable nanoemulsion (Craig et al., 1995; Pouton, 2000). Cremophor® EL with an average HLB of 13 with inhibitory effects on p-gp and CYP enzymes (enzymes incorporated in dimensioned bioavailability of many drug substrates) (Chen, 2008) and Capmul® MCM-C8 with an average HLB of 3.5 were utilized. Capmul® is likely to increase the interfacial fluidity of surfactant boundaries in the micelles because of the entrapment of Capmul® in the high HLB surfactant enhancing the emulsification process upon dilution with aqueous medium (Taha et al., 2004). These excipients had been reported for the manufacture of eutectic mixtures (Nazzal et al., 2002; Date and Nagarsenker, 2007; Birdi, 2009) and are listed as generally regarded as safe (Rowe et al., 2006) and accepted for oral ingestion. They are also reported to provide better stability to emulsion over a wider range of pH and ionic strength. In addition, they can produce reversible changes in intestinal mucosal permeability (Swenson et al., 1994.), further facilitating absorption of the co-administered drug. Moreover, Labrafil® M2130CS also was used in a mixture with Capmul® MCM-C8 (1:1). The graphical representation of a phase diagram with the assayed percentages let visualize the area of nanoemulsion formation by adding water (Fig. 4).

The whole experiment required 135 formulations to be assayed and grouped in five series: A, B, C, D and E (Table 1) depending on composition and excipients rate. Only 49 formulations presented "Good" emulsifying ability. Among these, 18 were from A series, 11 were from B series, 8 from C series, 5 from D series and 7 from E series. In Fig. 5 are showed four formulations, panels 2A and 2B correspond with self-nanoemulsifying systems showing "Good" emulsification capacity, contrary panels 2C and 2D shows systems with "Bad" emulsification capacity.

Equally these formulations were stored for one year at 4 ± 2 °C in refrigerator. There was a change from semisolid to solid and opaque. Color changes were also observed in B series, C series and D series whereas A and D series uniform color were maintained.

3.4. Construction of phase diagrams

Based on the results of preliminary studies, ternary phase diagrams of the five systems were constructed with the objective to study the relationship between the phase behavior and the composition also help to determine the concentration range of components for the formation of a nanoemulsion (Fig. 6). All the components were converted to weight/weight percent (w/w%) before constructing the phase diagrams. The white area enclosed in the triangle represents the region of self-emulsification. Within this area the SNEDDS form fine oil in water emulsion with only gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence then improves the thermodynamic stability of the nanoemulsion formulation (Reiss, 1975). Furthermore, co-surfactants increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (Constantinides and Scalart, 1997).

As seen from the ternary phase diagrams systems containing essential oils show wider emulsification region than systems containing soybean oil (Fig. 7). Among essential oils, lemon essential oil exhibits the best region. With the addition of the co-surfactant Labrafil® M2130CS the soybean oil system improves the area of self-emulsifying properties, but does not reach a sufficiently wide region.

In view of current investigation, due to larger nanoemulsion region and greater capacity for incorporation of oily phase, which is most desirable for Gem, lemon essential oil: Cremophore® EL: Capmul® MCM-C8 system (A) was selected for further studies.

3.5. Droplet size analysis and turbidity measurements of self-emulsifying systems

The concentration of oil, surfactant and co-surfactant of the 49 nanoemulsions with the best self-emulsify capacity were assayed to be optimized on the basis of droplet size and turbidity. Decreasing turbidity values are shown with decreasing droplet sizes. The finest fourteen formulations possessing droplet sizes <300 nm and turbidity values ≤245 NTU are shown in Table 2.

An increase in oil concentration led to decrease in nanoemulsion droplet size, especially with lemon essential oil. Minor droplet sizes were observed when the proportion of Cremophore® EL: Capmul® MCM-C8 was 2:1 for C, D and E systems. For A and B systems this fact was indifferent. At lower surfactant concentration and higher oil content, larger droplet size was observed. Contrary effect exhibited in C, D and E. The least size was obtained with 10% oil, 60% surfactant and 30% co-surfactant, however, gastric irritation may occur at higher surfactant concentration (Lawrence and Rees, 2000). The size was found to be less than 150 nm, and then most consistent with oil:surfactant:co-surfactant of 90:6.66:3.33; 80:13.33:6.66 and 50:16.66:33.33. Addition of Labrafil® M2130CS as co-surfactant involves a decrease in droplet size, but this event does not reach a sufficient improvement as forming fine nanoemulsions with droplet size minor than 300 nm.

3.6. Design of Gem-loaded SNEDDS

Once lemon essential oil was selected and delimited the percentage of surfactant and co-surfactant for SNEDDS, a Box–Behnken design was performed with constraints on visual characterizations (appearance), turbidity, mean droplet size and polydispersity index. In this case, a total of 15 experiments were performed and the observed responsed for 15 formulations are given in Table 3.

As turbidity values increase, mean droplet size and PDI also raise. Only in run 1, 4 and 6 was observed as very cloudy with the higest turbidity and mean droplet sizes values (250–635 NTU;

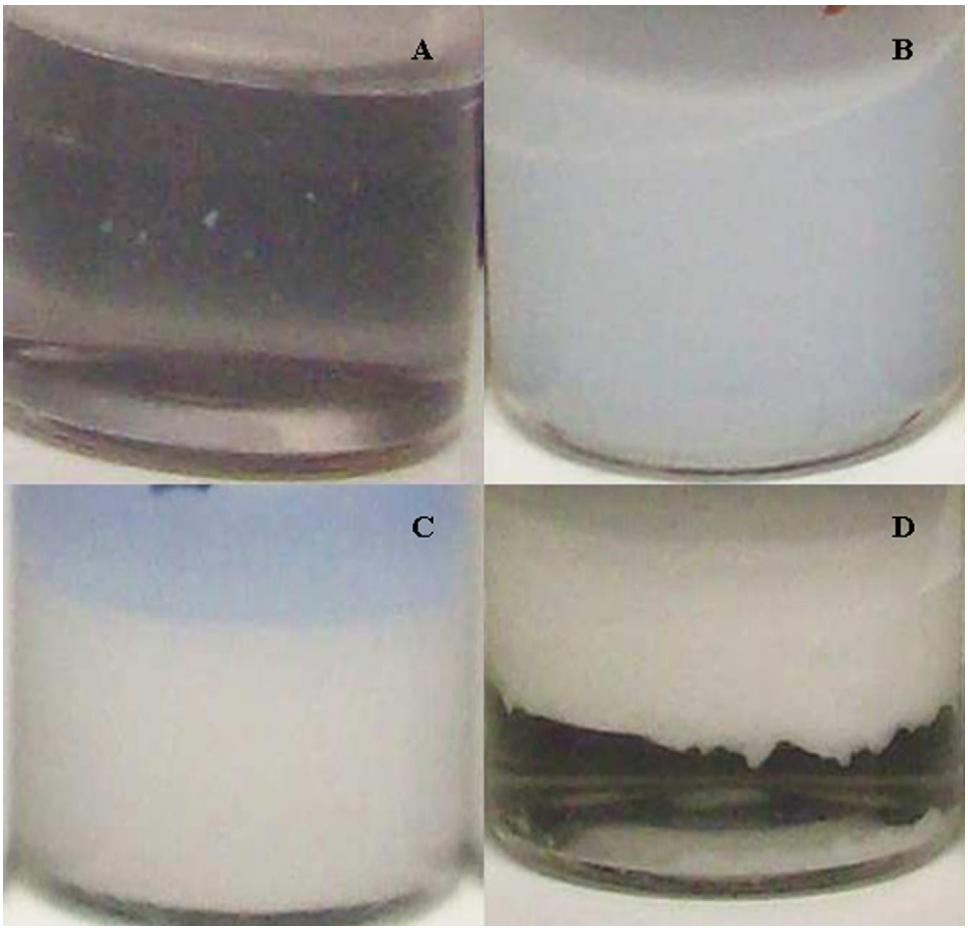

Fig. 5. Formulations classified as "Good" for emulsifying ability (A and B) and formulations classified as "Bad" for emulsifying ability (C and D).

155–430 nm), PDI ranging from 0.35 to 0.73 the amount of Gem was 200 mg, 175 mg and 175 mg, respectively. Run 3, 11 and 13 which were cloudy the amout of Gem was 175 mg, 175 mg and 200 mg, respectively with high mean droplet size and PDI ranging between 0.21 and 0.55. Run 5 although was cloudy his mean droplet size was good (13.6 nm). Among all the samples, were run 2 and 12 which exhibited the best responses: PDI 0.7 and 0.1, respectively with mean size droplet minor than 85 nm and turbidity values of 17.8 and 23.17 NTU. Moreover these mesasurement was performed with the smallest experimental error 0.22 and 0.16, respectively. The quantity of gemfibrozil loaded in these was 150 mg, this value was the less level for the factor X_3 defined as the quantity of oily phase (lemon essential oil:Gem; 1:1).

A second Box–Behnken design was performed with constraints on visual characterizations (appearance), turbidity, mean droplet size, and polydispersity index. This time the main goal was to determine the levels of factors which yielded optimal pH medium in which Gem-loaded SNEDDS had the best responses assayed, and consequently to evaluate the effect of pH in the dependent variables. In accordance with the results of the previous design, the quantity of Gem loaded in SNEDDS was 150 mg. Others 15 experiments were performed and the observed responses are shown in Table 4.

The effects and interactions with respect to appearance are shown in Table 5 in the range of pH 1.2; 4.5; 6.8 and 7.5. Three categories were used to evaluate, clear, cloudy and very

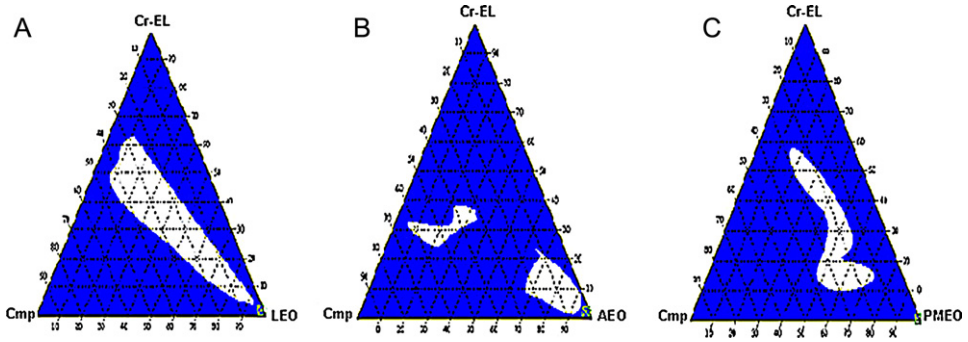

Fig. 6. Ternary phase diagrams of different selected systems. Cremophore® EL (Cr-EL), Capmul® MCM-C8 (Cmp), lemon essential oil (LEO) (A), anise essential oil (AEO) (B) and peppermint essential oil (PEO) (C). The white area represents self-nanoemulsion region.

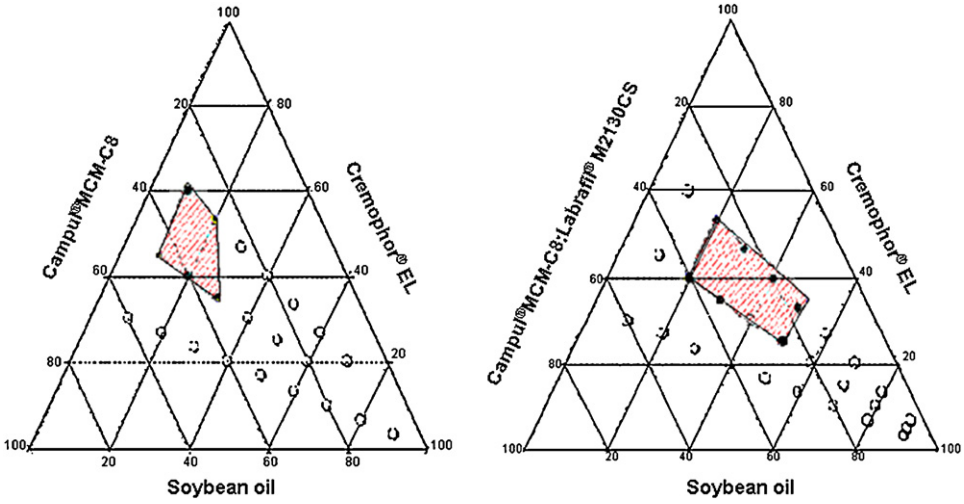

Fig. 7. Ternary phase diagrams of selected systems. The dark area represents self-nanoemulsion region.

Table 2
Oil, surfactant and co-surfactant percentage of fourteen nanoemulsions with the best self-emulsify capacity. *Cosurfactant mixture (Capmul® MCM-C8:Labrafil® M2130CS; 1:1).

Batch	Oil (%)	Surfactant (%)	Co-surfactant (%)	Droplet size (nm) ± SD (n = 3)	Turbidity (NTU) ± SD (n = 3)
A(1:1)1:9	90	5	5	229.6 ± 4.61	133 ± 22
A(1:1)2:8	80	10	10	257.2 ± 4.97	245 ± 33
A(1:1)3:7	70	15	15	298.8 ± 9.23	136 ± 28
A(2:1)1:9	90	6.66	3.33	148.3 ± 10.0	87 ± 9
A(2:1)2:8	80	6.66	6.66	252.1 ± 4.83	132 ± 7
B(1:1)3:7	70	15	15	219.9 ± 7.6	165 ± 0.89
B(2:1)2:8	80	13.33	6.66	35.64 ± 10.0	10.4 ± 0.8
C(1:1)5:5	50	25	25	179.2 ± 5.91	89 ± 8
C(1:2)5:5	50	33.33	16.66	164.4 ± 4.30	98 ± 0.9
C(2:1)5:5	50	16.66	33.33	53.99 ± 3.83	34 ± 9
C(2:1)6:4	40	40	20	192.5 ± 10.0	98 ± 6
C(2:1)8:2	20	53.33	26.66	238.4 ± 9.57	189 ± 0.9
D(2:1)9:1	10	60	30	22.7 ± 0.9	13 ± 0.3
E(2:1)8:2*	20	53.33	26.66	269.8 ± 0.3	167 ± 37

Table 3
Observed responses for the 15 formulations of Box–Behnken design. Y_1: appearance; Y_2: turbidity; Y_3: mean droplet size and Y_4: poly dispersity index. *Center points of the experimental design.

Run	Y_1	Y_2 (NTU) ± SD (n = 6)	Y_3 (nm) ± SD (n = 6)	Y_4
1	Very cloudy	253.9 ± 20.87	164.5 ± 31.8	0.62
2	Clear	17.8 ± 1.14	54.1 ± 0.4	0.07
3*	Cloudy	38.9 ± 0.13	68.32 ± 5.3	0.21
4	Very cloudy	257.4 ± 0.96	156.51 ± 71.5	0.35
5	Cloudy	13.6 ± 0.27	81.5 ± 1.6	0.43
6	Very Cloudy	631.5 ± 1.05	431.3 ± 371.6	0.73
7	Clear	12.3 ± 0.83	40.19 ± 2.2	0.28
8	Clear	10.8 ± 0.77	43.64 ± 1.9	0.20
9*	Clear	11.5 ± 0.16	43.89 ± 2.9	0.12
10	Clear	7.9 ± 0.17	39.75 ± 1.3	0.29
11	Cloudy	182.0 ± 0.58	124.17 ± 4.4	0.31
12	Clear	23.17 ± 0.64	84.24 ± 0.3	0.10
13	Cloudy	88.5 ± 0.12	71.39 ± 12.2	0.55
14	Clear	18.69 ± 0.17	62.57 ± 1.2	0.31
15*	Clear	11.75 ± 1.24	42.75 ± 0.8	0.23

Table 4
Variables in Box–Behnken design.

Factor	Independent variables	Levels (mg)		
		Low (−1)	Middle (0)	High (+1)
X_1	Cremophor® EL	150	225	300
X_2	Capmul® MCM C8	200	275	350
X_3	Lemon essential oil	150	175	200

Dependent variables
Y_1: visual characterization of the preformed nanoemulsion in each solution medium (appereance)
Y_2: turbidity (NTU)
Y_3: mean droplet size (nm)
Y_4: polydispersity index

Table 5
Observed responses for the dependent variable appearance at different pH media.

Run	Appearance			
	pH 1.2	pH 4.5	pH 6.8	pH 7.5
1	Clear	Clear	Clear	Clear
2	Very cloudy	Cloudy	Cloudy	Clear
3	Very cloudy	Very cloudy	Cloudy	Clear
4	Cloudy	Cloudy	Cloudy	Clear
5	Very cloudy	Clear	Clear	Clear
6	Cloudy	Turbia	Cloudy	Clear
7	Cloudy	Cloudy	Very cloudy	Clear
8	Cloudy	Cloudy	Cloudy	Clear
9	Cloudy	Cloudy	Cloudy	Clear
10	Cloudy	Very cloudy	Clear	Clear
11	Cloudy	Cloudy	Cloudy	Cloudy
12	Very cloudy	Very cloudy	Very cloudy	Clear
13*	Cloudy	Cloudy	Cloudy	Clear
14*	Cloudy	Cloudy	Cloudy	Clear
15*	Cloudy	Cloudy	Cloudy	Clear

* Centre points of the experimental design.

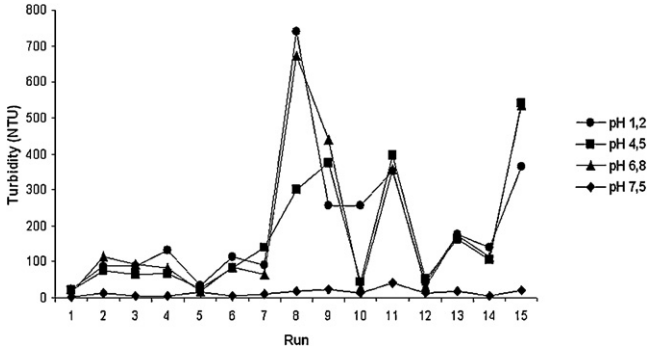

Fig. 8. Effect of pH medium on turbidity.

cloudy. Samples were all cloudy or very cloudy for pH 1.2, 4.5. Equally in pH 6.8, were all cloudy and very cloudy except 3 runs which were clear, in these the proportions of excipients were 22.22:29.63:25.93; 20.69:37.93:20.69 and 25.71:40.00:17.14 (Cremophore® EL: Capmul® MCM-C8: lemon essential oil). For pH 7.5 were all clear except one. Then solubility of Gem increases for pH 7.5 and improve the nanoemulsion generation, this fact is in accordance with results reported by Ghebre-Sellasie et al. (1989).

Turbidity study revealed important variation of turbidity values among the media assayed it can be due to the different solubility of Gem in the aqueous media. Values higher than 100 NTU were obtained in pH 1.2 and 4.5 media even in water. In contrast in pH 7.5 medium turbidity values ranged from 3 to 41 NTU. Then influence of pH in turbidity is globally highlighted with decrease of turbidity as pH increases, e.g. run 8 values decreased from 257.0 NTU (pH 1.2) to 4.0 NTU (pH 7.5). Fig. 8 shows influence of pH in turbidity. Also it can be observed that results exhibit the lowest variability for pH 7.5.

Variations in droplet size and PDI were also evaluated as a function of pH. The mean droplet size of the developed formulations ranged between 86 and 337.7 nm in pH 1.2 medium, only one run was <100 nm. From 87.1 to 556.3 nm in pH 4.5 medium, only 3 runs were <100 nm. Between 55.3 and 221.7 in pH 6.8 medium, where 5 runs were <100 nm, and between 53.2 and 103.1 nm in pH 7.5 medium with 14 runs <100 nm only one with 103.1 nm. Equally the lowest values of PDI and DSD error were observed for pH 7.5 indicating better quality. In general, buffer pH 1.2 and buffer pH 4.5 show a higher value of PDI and DSD error compared to water and buffer pH 7.5 medium.

Four pseudo-diagrams were constructed to observe the effect that the pH had on the preformed nanoemulsions (Fig. 9). SNEDDS with droplet size <100 nm were identified in the pseudo-diagram for each medium. At pH 7.5 SNEDDS of gemfibrozil shows best results, where 14 preformed nanoemulsions show droplet size smaller than of 100 nm. Fig. 10 shows the effect of pH in mean droplet size on nanoemulsions. These behaviors in different pH media can be explained by the fact that Gem has an ionizable group.

The Gem-loaded SNEDDS behavior in different pH values, and concretely in acid media, was the main reason why capsules containing these, were coated with Eudragit® L to provide a gastro-resistance barrier. In accordance with the USP 29 for gastro-resistance test, the observed amounts of Gem released from these capsules in acid medium were observed to be minor than 10% of the total quantity of drug contained.

pH 7.5 was the medium in which observed properties of SNEEDS were better. On the basis of these pH assays results, the amount of Gem dissolved after 30 min was calculated for pH 7.5 medium due to its dissolution is pH dependent. The fifteen formulations were filled into 00 hard gelatine capsules coated with Eudragit® L each of them containing 150 mg of Gem. All runs reached cumulative amounts of Gem higher than 79.8% in 30 min, the range of values were 79.8–107%.

3.7. Optimization of Gem-loaded SNEDDS

The aim of the optimization of pharmaceutical formulations is generally to determine the levels of the variables from which a robust product with high quality characteristics may be produced (Basalious et al., 2010). By means of the response surface methodology the influence of two independent variables (factors) in a response (dependent variable) keeping constant the third factor can be represented. In order to obtain the best Gem-loaded SNEDDS, based on the previous experimental design, data from the characterization of these Gem-loaded SNEDDS (pH 7.5) were used to perform a RSM optimization and determine the levels and interactions of these independent and response variables after generating mathematical relationships between dependent and independent variables by using Design-Expert® version 7.1.6 software (Stat-Ease Inc., Minneapolis, USA). The responses represented were turbidity values, mean droplet size and cumulative amount of Gem released after 30 min. Observed responses are sowed in Table 6.

The approximation of responses were fitted to three mathematical models, quadratic, linear and two factors interactions (2FI). The mathematical relationship in the form of factors coefficients and

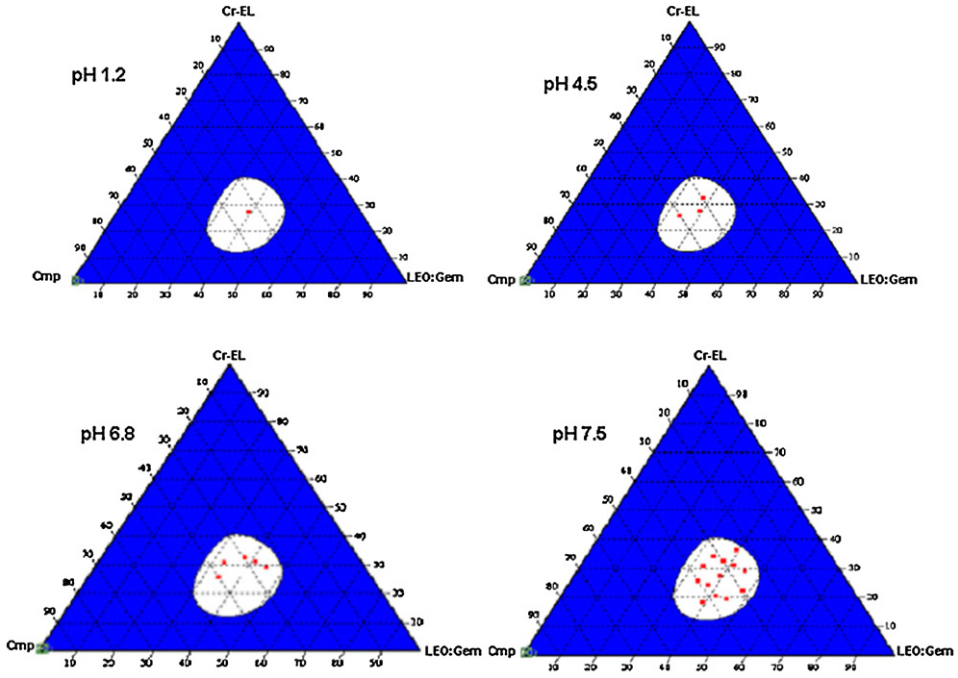

Fig. 9. Ternary phase diagrams of different selected systems. Cremophore® EL (Cr-EL), Capmul® MCM-C8 (Cmp), lemon essential oil–gemfibrozil (LEO–Gem) at different pH media. Points in the white area represents SNEDDS formation with droplet size <100 nm.

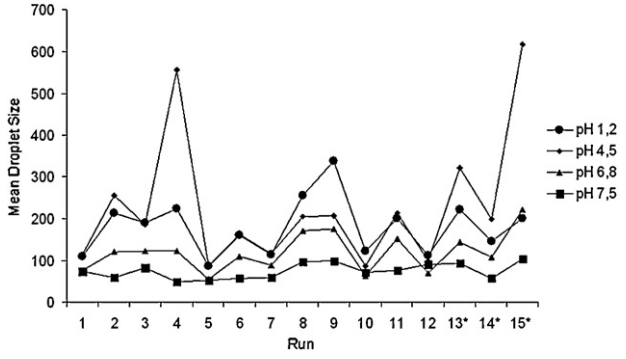

Fig. 10. Effect of pH medium on mean droplet size.

its corresponding *p*-values for the measured responses is listed in Table 7. Coefficients with R^2 closest to 1 and *p*-value less than 0.05 had a significant effect on the prediction efficacy of the model for the measured response. The approximation of response values of Y_1, Y_2 and Y_3 based of the quadratic model was the most suitable.

The values of the coefficients X_1, X_2 and X_3 are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Huang et al., 2005) (Table 7) The larger coefficient means the independent variable has more potent influence on the response. To identify the significance of the effects and interactions between them, analysis of variance was performed for each parameter.

Turbidimetry was used to monitor the process of self-emulsification by measuring the turbidity of the solution during dissolution as the emulsification process takes place. On turbidity a main effect was statistically significant (*p*-values <0.05), amount of Cremophor® EL. As shown in Fig. 11A this effect is negative (−5.95) suggesting an inversely proportional relationship with turbidity of the Gem-loaded SNEDDS. A trend toward smaller turbidity values was observed with increasing the concentration of surfactant. It could be attributed to the decrease in surface tension and thus smaller droplet size of the SNEDDS. This identifies efficient self-emulsification by establishing whether the dispersion reaches equilibrium rapidly.

Equally on mean droplet size there are two statistically significant effects (Fig. 11B). The droplet size of the emulsion is important factor in SNEDDS formulation, as this determines the rate and extent of drug release as well as absorption. Quantity of Cremophor® EL has influence providing negative effect (−10.825), suggesting an inversely proportional relationship with the mean droplet size of the surfactant, meaning that on increasing Cremophor® EL a higher tendency to produce smaller SNEDDS is observed. Contrary quantity of Capmul® MCM-C8 has influence providing positive effect (10.1875), suggesting a directly proportional relationship with the mean droplet size of the surfactant, meaning that on increasing Capmul® MCM-C8 a higher tendency to produce higher SNEDDS is observed. This fact is in accordance with the results reported by Nazzal et al. (2002), Taha et al. (2004) and Gao et al. (1998), it was reported that the addition of surfactants to the microemulsion systems causes the interfacial film to stabilize and condense, while the addition of co-surfactant causes the film to expand.

Similarly, *p*-values reveal the significance of effects and interactions on the amounts of the drug release after 30 min. A main effect, quantity of Cremophor® EL, has an statistically significant positive effect on the drug release profiles after 30 min (8.5875) suggesting an directly proportional relationship Also the an interaction (Cremophor® EL, lemon essential oil) with a positive effect (6.35) was statistically significant, so with a directly proportional relationship (Fig. 11C).

The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance. In ANOVA, the "Prob > *F*" parameter is the observed significance probability (*p*-value) of obtaining a greater *F*-value by chance alone if the specified model fits no better than the overall response mean. Observed significance probabilities of 0.05 or less are often considered evidence of a regression effect. A Prob > *F* of 0.0452, 0.0495 and

Table 6
Observed responses for the 15 formulations of Box–Behnken design.

Run	Cremophor® EL (mg) X_1	Capmul® MCM-C28 (mg) X_2	Lemon essential oil (mg) X_3	Turbidity (NTU) Y_1	Mean droplet size (nm) Y_2	(%) Released after 30 min Y_3
1	150	200	175	3.0 ± 0.0	73.4 ± 0.29	96.8 ± 3.7
2	300	200	175	12.3 ± 0.6	59.9 ± 0.19	96.1 ± 3.4
3	150	350	175	6.3 ± 1.2	81.8 ± 0.35	103.6 ± 6.0
4	300	350	175	5.0 ± 0.0	48.4 ± 0.16	92.1 ± 5.3
5	150	275	150	18.3 ± 0.6	96.6 ± 0.42	75.7 ± 14.4
6	300	275	150	4.3 ± 0.6	57.8 ± 0.35	99.1 ± 7.7
7	150	275	200	9.7 ± 0.6	58.6 ± 0.22	86.2 ± 6.4
8	300	275	200	15.7 ± 0.6	53.2 ± 1.27	101.2 ± 4.1
9	225	200	150	23.0 ± 0.0	99.4 ± 0.74	79.8 ± 4.0
10	225	350	150	4.0 ± 0.0	72.2 ± 0.32	107.0 ± 27.0
11	225	200	200	41.0 ± 0.0	76.5 ± 0.41	101.2 ± 12.8
12	225	350	200	12.0 ± 0.0	90.2 ± 11.08	102.0 ± 4.4
13	225	275	175	19.0 ± 0.0	92.7 ± 0.39	105.5 ± 6.1
14	225	275	175	5.0 ± 0.0	57.1 ± 0.43	92.8 ± 1.7
15	225	275	175	20.0 ± 0.0	103.1 ± 0.89	89.6 ± 2.6

Table 7
Mathematical relationship in the form of factors coefficients and its corresponding P-values for the measured responses.

Model	Coefficient	Y_1	Y_2	Y_3
	X_1	−5.95	−10.825	8.5875
	X_2	−0.6375	10.1875	3.6125
	X_3	−3.0875	5.7875	0.875
	X_1X_2	0.925	4.9	2.325
	X_1X_3	4.825	−1.9	6.35
	X_2X_3	1.85	5.275	−1.75
	X_1^2	3.608333	18.45417	−1.8875
	X_2^2	−1.56667	12.57917	−7.5375
	X_3^2	4.533333	−1.07083	5.6375
Linear	SD	5.475	15.53158	7.931734
	R^2	0.524	0.434121	0.503034
	p-Value	0.0044	0.27979	−0.05409
2FI	SD	5.239	17.43469	7.880942
	R^2	0.683	0.481417	0.643185
	p-Value	0.0045	0.09248	−0.8573
Quadratic	SD	4.171	11.58787	5.139407
	R^2	0.874	0.856822	0.90516
	p-Value	0.0050	0.049101	−0.49711

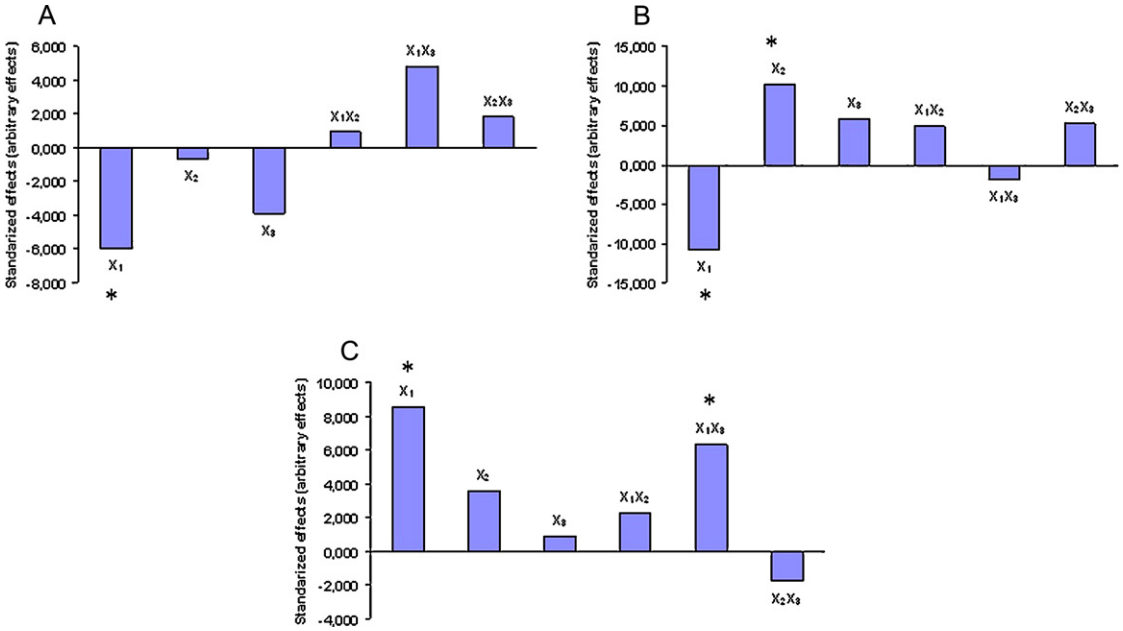

Fig. 11. Evaluation of the standardized effects of the Cremophor EL® (X_1), Capmul® MCM-C8 (X_2) and lemon essential oil (X_3), and their interactions on (A) turbidity, (B) mean droplet size, (C) cumulative amount Gem released. *Considered statistically significant for p-value <0.05.

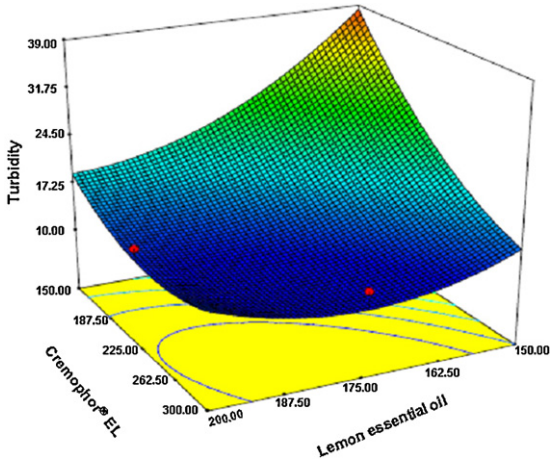

Fig. 12. Response surface plots showing the effect of the variable Cremophore® EL and lemon essential oil on the response turbidity.

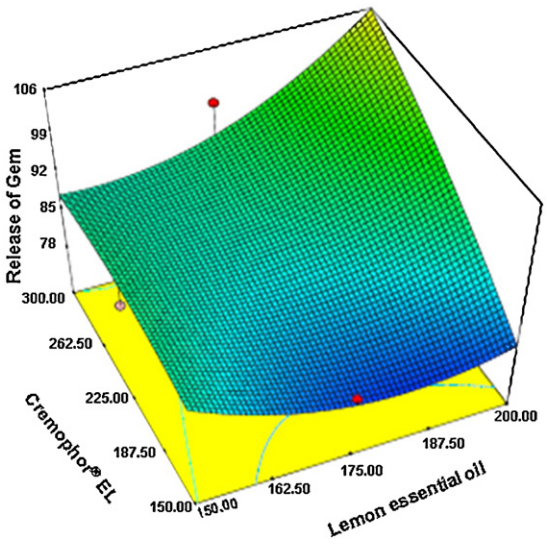

Fig. 14. Response surface plots showing the effect of the variable Cremophore® EL and lemon essential oil on the response on the response release of gemfibrozil in 30 min.

0.0404 indicated a significant effect of the independent factors on the responses Y_1, Y_2 and Y_3, respectively.

Response surface plots based on ANOVA results were constructed to elucidate the statistically significant relationship between the dependent and independent variables previously reported. The effect of X_1 on Y_1 is showed in Fig. 12. The amounts of surfactant and lemon essential oil (X_3) are represented at fixed low level (200 mg) of co-surfactant (X_2). The influence on turbidity is revealed appearing the lowest values in the area (darker) where the quantity of surfactant is higher and the lemon essential oil middle.

In the same way Fig. 13 shows the response surface diagram from the effect of surfactant (X_1) and co-surfactant when mean droplet size (Y_2) is considered to be the response. As can be seen increasing the Cremophor® EL content reduced the surface tension to form smaller particles, simultaneously Capmul® MCM-C8 content is decreased. The smallest droplet size of the plot corresponds to the blue area in which the highest surfactant values (300 mg) and the smallest co-surfactant values (200 mg) are represented.

Finally Fig. 14 shows the response surface plot from the interaction of surfactant (X_1) and lemon essential oil (X_3) when cumulative amount of Gem released after 30 min from SNEDDS (Y_3) is considered to be response. Cremophor® EL and lemon essential oil contents are represented at fixed low level (200 mg) of co-surfactant. The highest values of Gem released are reached when the lemon essential oil and surfactant contents are higher.

From the obtained results it can be concluded that an optimal Gem-loaded SNEDDS formulation may be composed of Cremophor® EL 300 mg, Capmul® MCM-C8 275 mg, and lemon essential oil 200 mg. This surfactant to co-surfactant rate is close to 1. In accordance with the studies reported by Nazzal et al. (2002) this rate showed the best emulsification rate probably due to an optimum HLB of the mixture. Equally the oil loading is 25.8%. At higher concentrations of the oily phase, proportion of the surfactant mix that facilitates water penetration decreases and the mixture becomes more lipophilic with increasing difficulty of emulsification (Halbaut et al., 1996).

After evaluation of the selected formulation for appearance, turbidity, mean droplet size and PDI in six replicates. The resulting Gem-loaded SNEDDS were transparent, with a mean turbidity value of 14.1 ± 0.87 NTU, mean droplet size of 56.5 ± 1.8 nm and mean PDI value of 0.26 ± 0.19.

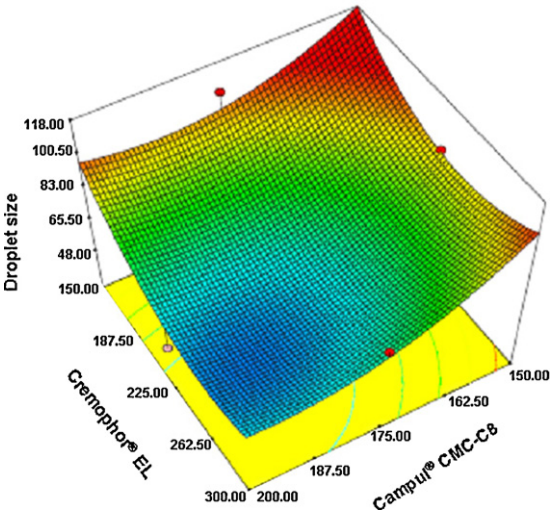

Fig. 13. Response surface plots showing the effect of the variable Cremophore® EL and Capmul® MCM-C8 on the response mean droplet size.

3.8. Freeze thawing

Therefore to check the stability, Gem-loaded SNEDDS were exposed to centrifugation study, and freeze thawing cycle. All formulations except run 11, in which phase separation signs were observed, passed the test.

3.9. Transmission electron microscopy

Morphological and structural examination of the optimized Gem-loaded SNEDDS formulation was carried out using transmission electron microscopy. TEM images post dilution showed that spherical micelles were formed with sizes ranging from 50 to 60 nm (Fig. 15). These results were according to DLS results with no signs of coalescence confirming the efficiency of the nanoemulsion preparation method used. The nanoemulsion droplets emerged as dark and the surroundings were found to be bright. No signs of drug precipitation were observed inferring the stability of the formed nanoemulsion. Closer analysis of TEM images reveals that each globule is surrounded by a thick layer indicating the formation of monolayer around the emulsion droplets, reducing the interfacial energy, and forming a barrier to coalescence.

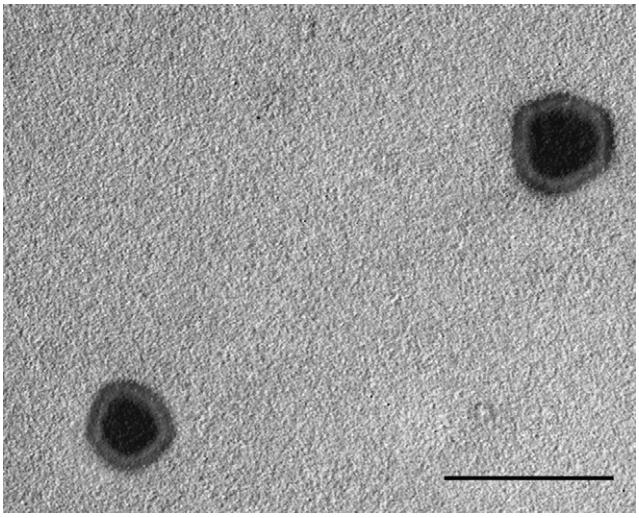

Fig. 15. TEM of optimized Gem-loaded SNEDDS formulation (50,000×). Bar length 100 nm.

3.10. Release drug study

The in vitro release study was performed by spectrofluorometric method with the developed Eudragit®L coated capsules filled with the optimized Gem-loaded SNEEDS formulation. Buffer pH 7.5 medium was selected as it showed the best conditions of NTU, Intensity DSD and PDI. Also, this medium had lower values of intensity DSD error. SNEEDS formulation was compared with the commercially available tablets (Gemfibrozilo Stada® EFG) which contain 600 mg of Gem, in order to evaluate this new formulation.

Fig. 16 shows the release patterns of Gem (%) for 45 min using 6 replicates in both formulations. The quantities of Gem in all replicates were higher than 85% of the total amount of Gem in the capsules containing SNEDDS and the conventional Gem tablets. This result is in accordance with the established requirements of the USP 29.

The release profiles indicate a two-step process for SNEDDS. The initial step shows a burst release which can be attributed to the surface associated drug, followed by a slower sustained release phase. The phenomenon exhibits that the release of Gem is controlled by diffusion. In contrast, the release from the commercial tablets indicates only one step, this is much slower. It was observed that the release of the drug was enhanced from SNEDDS, as 90% drug was released within 15 min in comparison to 30% from conventional tablets. From the Student's *t*-test, *p*-values showed significant differences ($p < 0.005$) for times 10, 15, 30, and 45 min between both

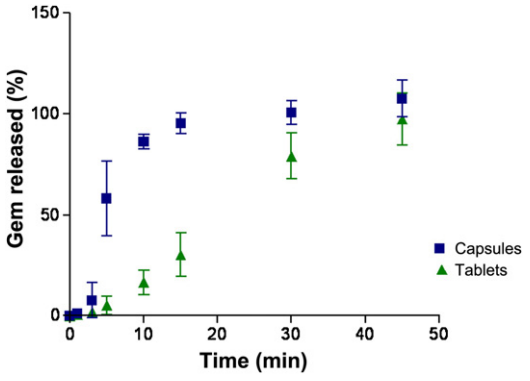

Fig. 16. In vitro dissolution profiles of gemfibrozil from optimized SNEDDS in coated Eudragit® L capsules and commercially available tablets (mean% ± SD).

formulations. Thus, this greater availability of dissolved Gem from the SNEDDS formulation could lead to higher absorption and therefore higher oral bioavailability, and also supports the hypothesis that nano-sized droplets of emulsion can enhance the release of poorly soluble drugs (Nielsen et al., 2008).

Further, in order to study the mechanism of Gem release from SNEDDS and conventional tablets, data obtained from the in vitro release study was fitted to various kinetic equations. The kinetic models used were a zero-order equation, first-order equation, Higuchi's square root of time equation and Weibull's equation (Table 8).

A criterion for selecting the most appropriate drug release model was based on the best fitting model which had the smaller value of AIC and higher correlation coefficient of the linear regressions (r^2) thus, statistically described best the drug release mechanism.

It has been reported the advantages of using empirical equations, they can be fitted very simply to experimental data (Siepmann and Peppas, 2001). However, as main disadvantage it has been proposed that the constants in the equations lack physical meaning and are therefore dependent upon how the data are measured (Costa and Sousa, 2001). Then it would be possible to extract the diffusion coefficients from the empirical constants, as long as the latter are calibrated.

The drug release data for the kinetics of Gem-SNEDDS in coated capsules fits well to the Weibull function with a Q_∞ value of 99.4 (%Gem). In the case of the conventional tablets, the kinetic model that best seems to fit is also the Weibull function, with a Q_∞ value of 96.7 (%Gem), thus as showed Student's *t*-test no significant differences were observed between values ($p = 0.7623$). In accordance with other references, in which in vitro release studies results were also fitted to Weibull model (Chen et al., 2010).

On the use of the Weibull function for the discernment of drug release mechanisms, estimates for $\beta \leq 0.75$ indicate Fickian diffusion in either fractal or Euclidian spaces while a combined mechanism (Fickian diffusion and swelling controlled release) is associated with β values in the range $0.75 < \beta < 1$. For values of β higher than 1, the drug transport follows a complex release mechanism (Papadopoulou et al., 2006). For Gem-SNEDDS in coated capsules β value is 2.05 and 3.45 for conventional tablets, neither significant differences were observed between values ($p = 0.1099$). These β values has been described as a sigmoid curve indicative of complex release mechanism, the rate of release does not change monotonically. In fact, the release rate initially increases nonlinearly up to the inflection point and thereafter decreases asymptotically (Papadopoulou et al., 2006).

Only for t_d values which were 6.44 for Gem-SNEDDS in coated capsules and 26.5 for conventional tablets, significant differences were observed ($p = 0.0001$), t_d represents the time interval necessary to dissolve or release 63.2% of the drug present in the pharmaceutical dosage form, meaning that in 45 min study, 63.2% of Gem was released in 6.44 min from SNEDDS, and conventional tablets took 26.5 min. The measured release rate from SNEDDS was significantly faster than that of Gem from the conventional tablet. This suggests that larger interfacial areas present in emulsions with smaller drops promote rapid drug release (Tarr and Yalkowsky, 1989).

Equally amodelistic pharmacokinetic parameters independent of the release model such as EF and MDT were calculated with the experimental values ($n = 6$) for both studied formulations.

The Student's *t*-test for EF and MDT indicated significant differences between the conventional tablets and the coated capsules of Gem-SNEDDS. It could be observed that 83.4% EF value from the coated capsules of SNEDDS. This value was greater than from the conventional tablets whose EF percentage was 53.2%. Importantly, the drug dissolution from a dosage form plays an important role in the development of new drug formulations being in this case much

Table 8
Discriminatory parameters obtained after fitting the release data from the gemfibrozil SNEDDS and the gemfibrozil conventional tablets to different kinetic model equations.

Function	Conventional tablets of gemfibrozil			Coated capsules gemfibrozil SNEDDS		
	AIC	Line parameters	Kinetic parameters	AIC	Line parameters	Kinetic parameters
Zero order	65.21	$y = -14.04x + 597.4$ $r^2 = 0.9553$	$K = 14.04$ mg/min $Q_\infty = 597.4$ mg	41.69	$y = -3.963x + 137.8$ $r^2 = 0.5656$	$K = 3.96$ mg/min $Q_\infty = 137.8$ mg
Weibull	16.92	$y = 1.651x - 5.512$ $r^2 = 0.8284$	$Q_\infty = 596.6$ mg $t_d = 28.18$ min $\beta = 1.651$	34.16	$y = 1.273x - 3.22$ $r^2 = 0.7219$	$Q_\infty = 206.3$ mg $t_d = 12.5$ min $\beta = 1.273$
First order	50.53	$y = -0.0574 + 6.597$ $r^2 = 0.9090$	$K = 0.057$ min^{-1} $Q_\infty = 732.9$ mg	43.85	$y = -0.1141 + 4.95$ $r^2 = 0.5871$	$K = 0.11$ min^{-1} $Q_\infty = 142$ mg
Higuchi	56.23	$y = 122.1x - 229.9$ $r^2 = 0.9671$	$K = 122.1$ mg/min$^{1/2}$	57.37	$y = 36.54x + 2.223$ $r^2 = 0.7569$	$K = 36.54$ mg/min$^{1/2}$

better for SNEDDS, this value is similar to the maximum percentage of drug release (Q_∞ close to 100%). On the other hand, there was a little but significant difference for MDT, just 2 min more for coated capsules with the SNEDDS than conventional tablets.

This results could lead to better absorption of Gem in the G.IT which can be attributed to the fact that when Gem is enclosed in the oil phase of the nanoemulsion, the oil droplet is absorbed by various lipid absorption mechanism: passive diffusion, pinocytosis or endocytosis (Georgakopoulos et al., 1992), whereas Gem in aqueous medium environment of the GIT minimal absorption would occur due to its macro-hydrophilic molecule nature as well as other actives studied (Rao et al., 2008). Besides their small droplets size also provides a large interfacial surface area for drug release and absorption and hence bioavailability (Wang et al., 2009). For this reason it has been developed self-nanoemulsifying drug delivery system in which Gem is loaded in the oily phase.

4. Conclusion

This study reports an approach on the use of a Box–Behnken design and response surface methodology in the optimization of self-nanoemulsifying drug delivery systems of Gem for in vitro evaluation. Following optimization, gemfibrozil loaded SNEDD system composed of Gem (16.22%), lemon essential oil (21.62%), Cremophor® EL 300 (32.43%) and Capmul® MCM-C8 (29.73%) was selected. Visual characterizations, turbidity, mean droplet size, polydispersity index, and Gem amount dissolved after 30 min assays showed differences at various pH media being the best results for pH 7.5 medium. The quantity of surfactant and co-surfactant was found to significantly impact the droplet size of SNEDDS. Turbidity and dissolved amount of Gem were also influenced by the surfactant. In vitro release studies revealed a two-step release pattern (burst followed by a slower sustained release) with 100% release at 45 min, compared to conventional tablets with slower pattern release. SNEDDS of Gem showed a significant increase in release rate compared to conventional tablets under the same conditions, 90% drug was released within 15 min in comparison to 30% from conventional tablets. Both formulations followed a Weibull mathematical model of release with significant difference in t_d (time interval necessary to dissolve or release 63.2% of the drug) tablets were about four-fold slower than for SNEDDS, also amodelistic dissolution efficiency parameter was significantly higher for SNEDDS, confirming that the developed SNEDDS formulation was superior to commercial formulation with respect to in vitro dissolution profile, and would enhance bioavailability because of droplet size in nanometers. It is concluded that the proposed SNEDDS containing Gem for the oral administration would be a promising dosage form with good in vitro pharmaceutical results.

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