Research Article

Development of a BCS Class II Drug Microemulsion for Oral Delivery: Design, Optimization, and Evaluation

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Our work is aimed at exploring the composition and the properties of microemulsion (ME), as a drug delivery system, to enhance the permeability across the gastrointestinal (GI) barrier of fenofibrate, a BCS class II drug. It is a prodrug that is converted rapidly after oral administration into a major active metabolite which is the fenofibric acid. It undergoes a nearly complete presystemic metabolism. Its main drawback is the low bioavailability of the metabolite. A quick selection of excipients was made based on the capacity of solubilization and the value of hydrophilic-lipophilic balance. The classical method of ME development was coupled with the factorial design in order to minimize the droplet size using a low concentration of surfactant. The optimized ME showed a droplet size of 48.5 nm and physical stability. The passive permeability evaluated using Sartorius was 1.6 times higher than that of the free drug. The ex vivo technique, performed using the everted gut sac model, showed a 2.5-fold higher permeability. This suggests that the carrier-mediated uptake/efflux may present the dominant transport mechanism of fenofibrate. The use of the excipients that inhibit GI P-glycoprotein may be a new perspective. Thus, this paper shows that the composition and the characteristics of ME may be explored to increase the permeability of fenofibrate across the GI membrane.

1. Introduction

Fenofibrate is a poorly water soluble drug, and it is neutral and lipophilic (log P = 5.2) [1]. It is a class II drug according to the Biopharmaceutical Classification System (BCS) since it has a high permeability. It is a lipid-lowering agent used to treat high levels of cholesterol and triglyceride. As a prodrug, fenofibrate will be rapidly converted after oral administration by hydrolysis of the ester into an active and major metabolite, the fenofibric acid [2]. This active metabolite presents a poor bioavailability which has been considered as its main drawback [3]. Various formulation strategies have tried to overcome this problem such as solid dispersion, complexations with cyclodextrins, and coprecipitates [4]. Microemulsions (MEs) are a lipid-based formulation. They are systems of water, oil, and surfactant frequently combined with a cosurfactant. They are transparent, isotropic, and thermodynamically stable with a droplet size usually in the range of 20-200 nm [5]. These characteristics seem ideal for an oral formulation

of a poorly aqueous soluble drug [6]. However, in order to provide a very low interfacial tension (≤10^{-3} mN/m), the surface charge properties are studied through the zeta potential. The value of the particle surface charges indicates the stability of ME at the macroscopic level [7]. To microemulsify the entire oil and water phases, ME requires high surfactant concentrations [8]. In addition, ME has shown a high solubilization capacity of lipophilic drugs with a droplet size of the order of nm which leads to more efficiency for a large area upon dispersion. This can be an interesting strategy to enhance the drug permeability across the gastrointestinal (GI) barrier, where high surfactant levels are often not acceptable. The reasons may be bio incompatibility, economy, or performance [9]. To formulate a ME, the classical and recognized approaches are phase titration and phase inversion [10]. The two approaches require a large number of experiments and, consequently, a lot of work time and a high cost.

The technique of factorial design indicates the relative significance of a number of variables and their interactions

[11]. It is an efficient method that has been used to optimize the development of ME [12].

The main purpose of this study is to enhance the permeability of fenofibrate by formulating a ME for oral administration. The selection of excipients was based on the solubilization capacity of fenofibrate and its permeability improvement across the GI membrane. The realization of ternary phase diagrams was coupled with the factorial design. The objective of the experimental design is to minimize the droplet size and to maximize the zeta potential using a low concentration of surfactant. Two methods were used to investigate the permeability of the optimized ME across the GI membrane, and the results were compared with those of the free drug powder.

2. Material and Methods

2.1. Material. Fenofibrate was kindly provided by Galpharma laboratories (Sfax, Tunisia). Miglyol 812, Tween 80, and Transcutol P were purchased from Prolabo (France). Lauric alcohol, caprylic acid, monopotassium phosphate, and disodic phosphate were purchased from Sigma Aldrich Laborchemikalien GmbH (Germany). NaOH, HCl, citric acid, NaCl, KCl, HCO3Na, and CaCl2 were acquired from Chemi-Pharma Laboratories (Tunis, Tunisia).

2.2. Construction of Phase Diagrams. In order to find the concentration range of components corresponding to the area of microemulsion, pseudo-ternary phase diagrams of oils, surfactants/cosurfactants, and water were plotted using the method of water titration at 25°C. The surfactant mixture was prepared by blending the surfactant and the cosurfactant into each tube at specific weight ratios of $1:1$, $2:1$, and $3:1$ and stirring vigorously for 1 min. Three-phase diagrams were developed by varying the ratios of the oil phase and the mixture of surfactant/cosurfactant from $9.5:0.5$ to $0.5:9.5$ (w/w). Under gentle magnetic stirring, distilled water was added to the mixture of surfactant/cosurfactant and oil, drop by drop. The water titration was continued, and the appearances from clear to turbid and from turbid to clear were investigated, respectively, by visual observation [3]. For the phase diagram, ME was the region of clear transparent and isotropic solution [13]. Based on the largest ME area, the phase diagram was selected. It corresponds to the appropriate ratio of oil and surfactant/cosurfactant mixture, and it was selected for the application of the factorial design and the preparation of fenofibrate's ME.

2.3. Optimization of Microemulsion Formulation. Among the three-phase diagrams that were produced, the most robust (i.e., with the widest microemulsion area) was chosen. The experimental design was used to identify the points that give a stable and isotropic microemulsion. A three-level experimental design (3^2) was applied in the formulation of a microemulsion by varying the concentrations/levels of Smix and oil and by measuring the globule size (GS) and zeta potential (ZP) as responses. The domain that covers the ME in the selected phase diagram ($3:1$) was used to determine the high and low levels of surfactant/cosurfactant (S/CoS) mixture and oil.

According to the experimental design, nine batches of ME were prepared by the titration method. These batches were evaluated for GS and ZP. The independent factors and the dependent variables used in this design are listed in Table 1.

A design matrix comprising 9 experimental runs was constructed. The polynomial equation is given as follows: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 + E$ [1], where Y is the dependent variable, b_0 is the intercept, b_1 to b_5 are the regression coefficients, and X_1 and X_2 are the independent variables (factors). Y is the response resulting from different combinations of factor levels. The average resulting from changing one factor from the low level to the high level represents the major response. When the two factors change simultaneously, the average response shows the interaction terms. The following equations were derived by the best-fit method to describe the relationship between the globule size (Y_1) and ZP (Y_2) with the oil concentration (X_1) and surfactant concentration (X_2). Optimization was performed using a desirability function to obtain the levels of X_1 and X_2, which minimized (Y_1) and maximized (Y_2).

Table 2 shows the experimental domain. A formulation plan was generated using the levels of X_1 and X_2.

2.4. Preparation of Fenofibrate Microemulsions. We added fenofibrate (2% w/w, of the vehicle) to the blank formulations, prepared with the various proportions of oil, surfactant, and cosurfactant. The powder of fenofibrate was dissolved in the mixture of oil and S/CoS by constant stirring; then, the required volume of distilled water was added.

2.5. Characterization and Evaluation of Fenofibrate ME

2.5.1. Droplet Size Analysis. The droplet size of ME was determined by dynamic light scattering using Zetasizer Nano S (Malvern Instruments, UK). The formulation was diluted at 25°C in a concentration of 1% (v/v) with distilled water in a conical flask and sonicated for five minutes. All the studies were carried out in triplicate ($n = 3$).

2.5.2. Zeta Potential. The zeta potential of the samples was measured by Zetasizer (Malvern Instruments, UK). The samples were placed in clear disposable zeta cells, and the results were recorded [14].

2.5.3. Freeze-Thaw Cycle. To evaluate the stability of the formulations, they were subjected to 3 freeze-thaw cycles. Each cycle consisted of 24 hours at -4°C followed by 24 hours at 40°C. After the freeze-thaw cycles, the formulations were centrifuged at 3000 rpm for 5 minutes and then observed for any phase separation.

2.5.4. Stability. The optimum loaded ME was stored at 25°C for 3 months. In addition, it was diluted 100 times with distilled water. After centrifugation, the occurrence of crystal or the dispersed phase was investigated [3].

TABLE 1: Variables used in the factorial design.

Independent variables (factors)	Levels used, actuel (coded)		
	Low (-1)	Medium (0)	High (+1)
X_1 = %S/CoS mixture	8.6	24.3	40.0
X_2 = %oil	0.4	5.0	2.7
Dependent variables (responses)	Goals		
	Low	High	Goal
Y_1 = droplet size (nm)	51	324	Minimize
Y_2 = zeta potential (mV)	-8	-17.9	Maximize (absolute value)

TABLE 2: Experimental domain of the factorial design.

Experimental runs	Experimental matrix		Experimental domain	
	X_1	X_2	Y_1	Y_2
1	-1	-1	8.6	0.4
2	1	-1	40	0.4
3	-1	1	8.6	5
4	1	1	40	5
5	-1	0	8.6	2.7
6	1	0	40	2.7
7	0	-1	24.3	0.4
8	0	1	24.3	5
9	0	0	24.3	2.7
10	0	0	24.3	2.7
11	0	0	24.3	2.7
12	0	0	24.3	2.7

2.5.5. In Vitro Drug Release Study. The quantitative in vitro release of fenofibrate (67 mg) from the optimized ME and the free drug was determined according to USP dissolution apparatus type 2 at 100 rpm. The ME formulations were placed into hard gelatin capsules (00 sizes); the results were compared with those of the free drug. To maintain the sink condition, Tween 80 was added to the dissolution medium (equivalent to the amount used in the formulation). Dissolution studies were performed in various media (buffer pH of 1.2, 4.5, and 6.8) to examine the effect of pH on the drug release. Aliquots of 3 mL were removed at 5, 10, 15, 20, 30, and 45 min and analyzed by UV-visible spectrophotometer (Thermo Scientific UV EVO 60) at 300 nm. An equivalent sample volume of fresh medium was added in the dissolution medium each time. The release from the drug and from the conventional capsule was compared with the optimized formulation of ME.

The dissolution studies were repeated three times ($n = 3$) to express the results as mean ± SD.

2.5.6. Drug Permeability Studies. Permeability studies were performed using two models, the biomimetic artificial membrane and the everted gut sac (EGS) technique.

The Sartorius Absorption Simulator (an in vitro model) used in our study is the Sartorius SM 16750 Absorption Simulator (Sartorius Membrane filter GmbH, Germany) [15].

This apparatus consists of a donor compartment (A) and a receiver compartment (B). Both media were maintained at $37 \pm 0.5°C$. Compartment A is filled with a buffer solution of pH 6.8 and B with a phosphate buffer solution of pH 7.4. The liquid circulation is continuous on the two sides of the diffusion cell with a peristaltic pump at a rate of 9.5 ml/min. The diffusion cell contains an artificial nitrocellulose membrane (OSMONICS Micronsep® model, Bioblock, France, diameter = 90 mm and pore size = 0.45 μm). A lipidic mixture of caprylic acid and lauric alcohol (50 : 50 w/w) was used for the immersion of the membrane for 1 hour. Then, an absorbing paper was used to eliminate the excess of lipidic mixture. The calculated percentage of lipidic mixture absorption, obtained by measuring the weight of the membrane before and after the lipidic immersion, ranged between 90 and 110% [16]. The tested drug (67 mg of fenofibrate free drug or its equivalent of optimized ME) was added to the donor compartment, and samples were withdrawn from the receptor compartment at 5, 10, 15, 20, and 30 minutes. They were assayed spectrophotometrically at 300 nm and immediately put back in the medium. The experiments were conducted 3 times for each drug. Drug absorption was expressed as a percentage. No interference was observed with the components of the membrane during the diffusion assays.

The EGS technique is an ex vivo model used to carry out experiments on male Wistar rats (250–300 g, 6-8 weeks old) purchased from the Pasteur Institute (Tunis, Tunisia). The animals were hosted in polypropylene cages at constant room temperature of $25 \pm 2°C$ and subjected to 12 h light/12 h dark cycles with free access to water and standard pellets ad libitum. The experimental procedures involving animals and their care were performed in conformity with the guidelines established by the European Union regarding the Use and the Animal Care (CCE Council 86/609) adopted in our laboratory.

The employed method was modified from experimental procedures well described in the literature [17]. The rats were kept fasting 24 hours before the experiments, with free access to water. On the day of the experiment, the animals were sacrificed by cervical dislocation. The jejunum portions were immediately isolated and cleaned and then placed in a Petri dish with Tyrode's solution at pH = 7.4 (NaCl 136.9, KCl 2.68, CaCl2 1.8, MgCl2 1.05, NaHCO3 11.9, NaH2PO4 0.42, and glucose 5.55 mmol/L), oxygenated with 95% O_2 and 5% CO_2 and maintained at 37°C. Segments of approximately 6 cm long were immediately placed into a tissue bath

filled with Tyrode's solution, kept at a normal body temperature of 37°C, and constantly bubbled with the mixture 95% O_2+5% CO_2.

The segments were gently everted and filled with fresh Tyrode's solution. Each portion was fixed in the recipient of dissolution apparatus type I. At the defined time points, the segments were removed and dried. They were then opened, and the fluid was drained into small Eppendorf vials. The vials were centrifuged for 10 min at 3000 rpm. The supernatant was filtered with a 0.45 μm filter to determine the drug concentration by UV spectrophotometer.

2.6. Calculation of the Apparent Permeability Coefficients. The permeability coefficients (Papp) obtained with the Sartorius SM 16750 Absorption Simulator and the EGS method were calculated according to Eq. (1).

$$\text{Papp} = \frac{dQ}{dt} \times \frac{1}{AC_0} \tag{1}$$

Papp is the apparent permeability coefficient (cm/s). dQ/qt is the amount of drug penetrated per unit of time. It is determined from the sampling time points and presents the regression line. A is the available area for permeation (cm^2), and C_0 is expressed in μg/ml [16, 18].

3. Results and Discussion

3.1. Selection of the Excipients and Construction of the Pseudo-Ternary Phase Diagrams. Lipid excipients are widely used and largely available from excipient suppliers. It is essential to control the characteristics of these various lipid excipients since they are involved in the process of absorption [19]. Many factors determine the choice of excipients for lipid-based formulations like purity and chemical stability, capsule compatibility, miscibility, self-dispersibility, abilities of solubilization and promoting self-dispersion, digestion properties, toxicity, and cost [20].

The choice of excipients is essential to obtain the desired formulation. Each surfactant and oil has a specific hydrophilic-lipophilic balance (HLB). The lowest interfacial tension between oil and water phases is obtained if the corrected HLB of the mixture surfactant/cosurfactant is similar to the HLB of the oil. This reflects the stability of the system.

In our study, the oil of choice was Miglyol 812 with an HLB value of 15.36 [21] and the surfactant was Tween 80 with an HLB value of 15 [22]. Miglyol 812 is a medium chain triglyceride (MCT) composed of a mixture of caprylic triglyceride acid (55% of C8) and capric fatty acid (45% of triglycerides C10). It is derived entirely from plant resources and is a stable substitute for mineral or vegetable oil [23]. It is a type of lipid, and because it is completely digested and absorbed, it does not cause safety issues [20]. Medium chain fatty acids (C6-C12), monoglycerides, diglycerides, and triglycerides, especially C8/C10 monoglycerides/diglycerides, have been used in mixed micelles and emulsion formulations for the solubility and absorption enhancement of several drugs.

The ability of the drug solvent depends mainly on the effective concentration of the ester group [24]. MCT is the most common oil choice for ME due to its solvent capacity ratio and its higher resistance to oxidation [25, 26]. Labrafac CM 10 is an MCT that shows excellent solubility for fenofibrate and has a wider production range of the ME zone for all surfactant/cosurfactant combinations. Therefore, it is longer than LCT Maisine 35 [27].

Tween 80 was selected as a surfactant. It is a nonionic surfactant with high stability, low toxicity, low irritation, and possible biodegradability [8]. It is a surfactant with an average HLB value of 11, mainly used with water-insoluble molecules [20, 28]. The hydroxyl group on the sorbitan ring of Tween 80 is substituted with a bulky polyoxyethylene group which makes it hydrophilic. This substitution leads to greater water solubility and therefore to an ease in forming oil-in-water emulsions. In addition, Tween 80 is generally considered safe and approved for use in many medicines, cosmetics, and foods because it is nonirritant and has low toxicity [29]. However, due to biological incompatibility, performance, and/or economic reasons, high surfactant levels are often unacceptable. Therefore, formulators are very interested in surfactants and their concentrations.

It is well known that the addition of another amphiphilic excipient as a cosurfactant can adjust the efficiency and the concentration of the surfactant required to form a single-phase ME [8]. The reason for their use can be attributed to the increased solvent capacity of the formulation [20].

Transcutol P (diethylene glycol monoethyl ether) was chosen as the cosolvent. It is widely used in formulations for oral, transdermal, and topical use to increase the solubility of other substances (solubilizer) and to enhance their absorption (absorption enhancer) [30]. It is a C6 alcohol with a low content of molecules at the interface, but it provides some other interactions on the oil side. According to the traditional Winsor R reasoning [31], an increase in the interaction between the two parts (oil and water) will increase the solubility.

Figure 1 shows the pseudo-ternary phase diagram of the studied quaternary system, water/Miglyol 812/Tween 80/Transcutol P. The formation of the ME system is observed at ambient temperature (shaded area). Phase studies have shown that the maximum amount of ME appears when the ratio of surfactant to cosurfactant is equal to 3 : 1. This can be explained by the important role of surfactants and cosurfactants in ME formulations.

In fact, the selected surfactant must be able to decrease the interfacial tension to a very low value. This reduction facilitates the dispersion process during the preparation of ME and the formation of a flexible film around the droplets. This film has the appropriate lipophilic characteristics to provide an interface with the correct curvature. The role of the cosurfactant is to provide the required flexibility to the film to adapt the different curvatures in a wide range of compositions [5].

3.2. Optimization of the Formulation and Preparation of the Loaded ME. The construction of the phase diagram facilitates the determination of the maximum oil ratio in the existing range of ME [30]. It is an important tool for screening formulation components and evaluating their influence on the
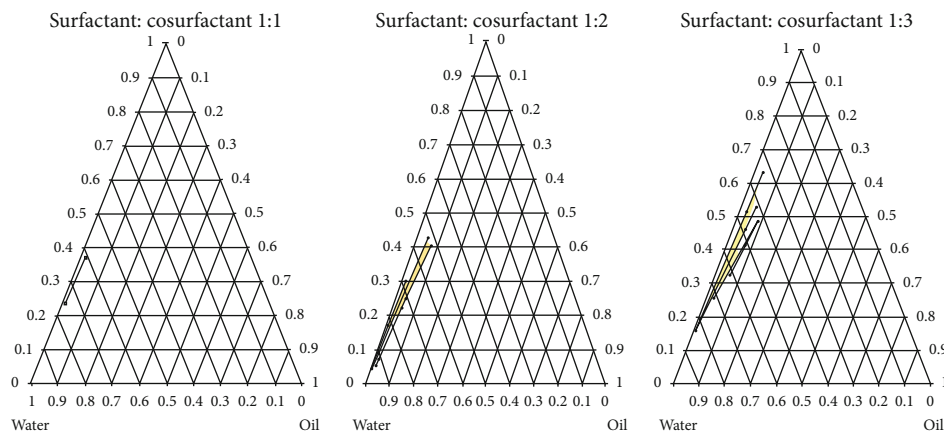
FIGURE 1: Pseudo-ternary phase diagrams consisting of the following components: oil (Miglyol 812) and Smix (Tween 80 and Transcutol P) prepared, respectively, at 1 : 1, 2 : 1, and 3 : 1. Shaded region: microemulsion.

in vitro performance of the FD formulation [32]. However, it may take a lot of time to find the best formula.

To improve this conventional method, FD was exploited in the pseudo-ternary phase diagram that revealed the largest region of ME. The particle size and zeta potential were chosen as dependent variables (responses). They are the two most commonly mentioned properties that are responsible for a range of biological effects of nanoparticles. Emerging data indicate the influence of these two factors on the release profile of ME as a drug delivery system [33]. The range of the three independent factors was determined from the experimental points of the ME area in the selected pseudo-ternary phase. Twelve experiences were required for the experimental design. On this basis, the combinations of factors produced different responses (Table 3).

The particle size range of all preparations was 51 nm to 324 nm. The droplet size (Y_1) of the nine formulations was less than 200 nm. In addition, the range of the zeta potential (Y_2) was -8 to -17.65 mV (Figure 2). For a colloidal droplet moving under an electric field, the zeta potential (Y_2) reflects the electrokinetic potential at the slipping plane. This potential is the difference between the electric double layer of electrophoretically mobile particles and the layer of dispersant around them at the slipping plane. It is the potential at the interface particle-"dispersing liquid medium" and is often used as an indicator of droplet stability. The greater the ZP, the more likely the droplets will resist aggregation. Guidelines classifying nanoparticle dispersions with ZP values of ±0-10 mV, ±10-20 mV, ±20-30 mV, and ±30 mV as highly unstable, relatively stable, moderately stable, and highly stable, respectively, are reported in drug delivery studies [7]. The literature reported that a value of ±10-15 mV may be enough to ensure the stability of a dispersed system [34].

Formulations with a reduced droplet size and a high zeta potential resulted all from a synchronized increase of S/CoS ratio. These batches were chosen according to the highest ZP in the range of ±15 mV and to GS = 50 nm. When the two responses are optimized at the same time, in our case, Y_1 is set to have the lowest value and Y_2 is set to have the highest value.

TABLE 3: Formulation plan.

Run	Formulation plan				Responses	
	% S	% CoS	% oil	% water	Y_1	Y_2
1	6.45	2.15	0.40	91	228	-9.78
2	30	10	0.40	59.60	324	-16.16
3	6.45	2.15	5.00	86.40	123	-10.6
4	30	10	5.00	55.00	221	-8
5	6.45	2.15	2.70	88.70	105	-11.94
6	30	10	2.70	57.30	170	-11.32
7	18.225	6.075	0.40	75.30	200	-14.33
8	18.225	6.075	5.00	70.70	51	-8.67
9	18.225	6.075	2.70	73.00	55	-17.6
10	18.225	6.075	2.70	73.00	56	-17.9
11	18.225	6.075	2.70	73.00	54	-17.5
12	18.225	6.075	2.70	73.00	53.00	-17.65

FIGURE 2: Nine batches of microemulsion prepared according to the experimental design.

The desirability function is the parameter that helps to obtain the appropriate combination in the area of ME. It covers the experimental design and is measured by the Statgraphics Centurion software (Version 15.1.02, USA). The scale of the desirability function (D) varies between 0 and 1, with $D = 0$ when the response is totally undesirable and $D = 1$ when the response is most suitable.

The total desirability was determined by incorporating the single desirability function for each factor (Figure 3).
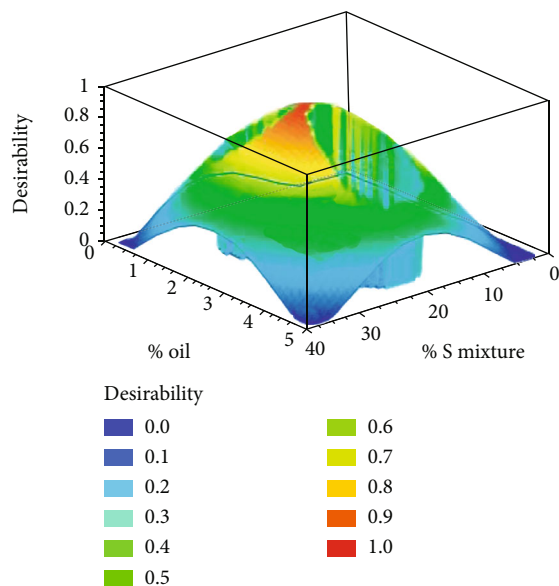
Figure 3: Global desirability as a function of % Smix and % oil.

Table 4: Comparison of predicted and observed experimental values of fenofibrate-loaded ME prepared under optimum conditions.

	Y_1 droplet size (nm)	Y_2 zeta potential (mV)
Predicted value	51	-16.71
Observed value	48.5	-13.92
% bias	0.051	0.200

%bias = (predicted value − observed value)/observed value.

The highest function value was attained at X_1 (%Smix) = 24.3 and X_2 (%oil) = 2.7 (w/w) with the associated D value of 0.968.

To confirm whether the model is sufficiently predictive, a batch of formulation with the best composition was prepared and the response was evaluated. The results are shown in Table 4.

To prove the validation of the model, the predicted values were compared to the experimental values. There was a suitable agreement which suggests the success of the optimization of the ME formulation by coupling the FD and the desirability function.

The best loaded ME (2% w/w) was prepared according to the following percentages: 2.72, 24.3, and 73, and the characteristics are listed in Table 5.

3.3. In Vitro Drug Release Study.
The release of fenofibrate from the ME formulation was significantly higher and faster than the release from free powder (Figure 4). This can be an important factor to enhance the absorption and improve the bioavailability by increasing the release rate of the drug into the aqueous phase [35]. It can be considered that ME preparations can spontaneously form ME with liquid crystal regions of small droplet size containing fenofibrate, so that

Table 5: Characterization of the optimum loaded ME.

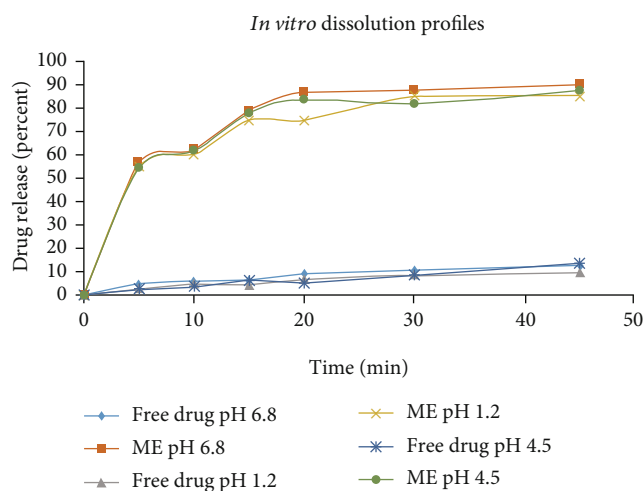| Visual aspect	Clear
Droplet size (nm)	48.5
Zeta potential (mV)	-13.92
Freeze-thaw cycle	Stable
Stability	Stable
Transmittance (%)	98

Figure 4: Dissolution profiles of fenofibrate release from free drug and the optimum ME in different dissolution media.

the drug is released into the water phase faster than ordinary fenofibrate.

Therefore, this enhanced availability of dissolved fenofibrate from ME formulations can increase the absorption and the oral bioavailability. It can also be seen that the variation of pH (pH 1.2, pH 4.5, and pH 6.8) of the dissolution medium did not affect the drug release of fenofibrate or ME reparations. This can be explained by the absence of ionizable groups so that the solubility is independent of pH [27].

3.4. In Vitro and Ex Vivo Intestinal Permeability Studies.
Different pathways are possible for the dissolved drug after oral administration, to reach the systemic circulation. The intestinal barrier may be crossed by one or multiple pathways which are passive paracellular and/or transcellular diffusion and/or carrier-mediated uptake/efflux [36]. Various models are currently used to characterize the permeability of the drug such as the partition coefficient octanol-water, artificial membrane permeability, in vitro/in vitro models based on cultured cells or tissues, in situ and in vivo intestinal perfusion, and computer-simulated methods [37].

In our study, in order to evaluate the effect of ME preparations on the permeability of fenofibrate and to study the possible permeability pathways of fenofibrate API, we used two models, Sartorius SM 16750 and the EGS models. Sartorius SM 16750 Absorption Simulator is an in vitro model that uses a biomimetic artificial lipid membrane to simulate passive diffusion. We find that the oil phase ratio can simulate
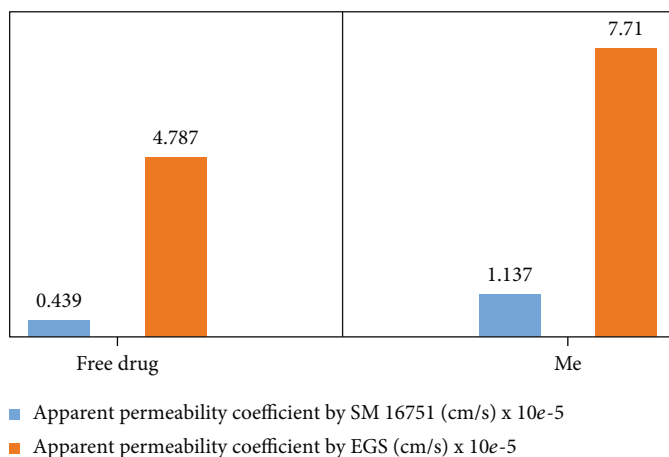
FIGURE 5: Comparative results of the apparent permeability coefficient of fenofibrate free drug and optimized ME using Sartorius SM 16751 and EGS models.

the intestinal barrier and give suitable results. The $0.45 \, \mu$m membrane pores are filled with the lipid phase, so that the fat-soluble drugs can dissolve in the membrane and then diffuse. This is due to the fact that the concentration gradient on the lipid pores is significantly greater than that of the fenofibrate molecules [16].

The second tested model is EGS which is an ex vivo model using the rat small intestine. This technique is frequently used for in vitro studies. It estimates the effect of substances in the enhancement of drug absorption from different formulations and measures the weight of paracellular transport of hydrophilic molecules. EGS has many advantages compared to the Sartorius model, such as the presence of a mucus layer and the large surface area available for absorption. EGS is reproducible which shows its efficiency as a screening tool for evaluating the transport of P-glycoprotein (Pgp) substrates and molecules that can modify the Pgp properties.

Therefore, the EGS model can be efficient in assessing the properties of potential absorption enhancers, their mechanism of action, and their eventual toxicity [16, 38].

By comparing the permeability coefficients of fenofibrate obtained from the two in vitro models, it is found that the apparent permeability coefficient Papp of EGS is lower than that of the Sartorius model. These results indicate that passive transcellular diffusion is not the only mechanism for fenofibrate to cross the gastrointestinal barrier, and other processes may be related to its transport.

Nevertheless, the ME formulation can still enhance the permeability of fenofibrate in both models (Figure 5). The passive diffusion of free drug powder provided by the Sartorius model was increased 1.6-fold with fenofibrate ME. This can be attributed to the following reasons: Fenofibrate is a BCS II drug, and the micronized drug cannot be dissolved quickly in the donor compartment. Reducing the particle size in the ME formulation to 48 nm is a key factor to improve the dissolution rate and then to improve the passive diffusion.

On the other hand, the apparent permeability coefficient (Papp) of the best ME was 2.5-fold higher than Papp of the free drug. This significant enhancement may be attributed

to the interaction with the efflux transporter; fenofibrate is lipophilic and neutral, indicating a high affinity for Pgp.

Previous studies have shown that fenofibrate might inhibit the activity of Pgp when the Pgp level is conceivable [39]. Nevertheless, if these interactions occur, they are limited to the gastrointestinal Pgp, because fenofibrate undergoes almost complete presystemic metabolism, and the main active metabolite (fenofibrate acid) does not interact with Pgp [40].

The presence of the PGP inhibitor Tween 80 may cause competition with the free part of fenofibrate. This competition can explain the increase in fenofibrate solute fraction and then the increase in permeability.

Selection of excipients for ME preparations may increase the permeability of the intestinal membrane or improve the affinity between ME and the intestinal membrane. Therefore, Miglyol 812 (medium chain triglycerides) may also be transformed to monoglycerides and improve the intracellular drug delivery in the presence of lipase [41].

Surfactants like Tween 80 can increase permeability by interfering with the lipid bilayer of epithelial cell membranes [42]. In addition, the residence time in the gastrointestinal tract may be prolonged. This can be explained by the adherence of the small droplets of ME to the intestinal membrane [3].

Despite its low drug content (2% w/w), the optimized ME required a low surfactant concentration (18% w/w). It showed a physical stability and an in vitro permeability enhancement by the two models used in this study. Further studies are needed to investigate in vivo absorption.

4. Conclusion

The formulation of ME is optimized by the combination with the classical development approach and therefore the factorial design. The domain of the pseudo-ternary diagram, which provides the most important area of ME, was used to determine the range of the three independent factors. The optimum loaded ME (2% fenofibrate w/w), composed of Miglyol 812 (2.72%), Tween 80 (18.24%), Transcutol P

(6.08%), and 73% of water (w/w), includes a particle size in the order of 50 nm. The formulation was stable after the freeze-thaw cycles, long-term storage for 3 months at 25°C, and self-emulsification for 3 days. An in vitro model and an ex vivo diffusion model show a remarkable increase of the permeability of fenofibrate by the optimized ME compared to the free drug. The outcomes acquired from the EGS study suggest a new perspective in the use of the excipients that inhibit gastrointestinal Pgp in the formulation of fenofibrate ME. Finally, the optimized design of fenofibrate ME for oral use can be used as an appropriate drug delivery system.

Data Availability

Raw data were generated at Laboratory of Chemical, Galenic and Pharmacological Development of Drugs (LR12ES09), Faculty of Pharmacy of Monastir, University of Monastir, 5000 Monastir, Tunisia. Derived data supporting the findings of this study are available from the corresponding author on request.

Conflicts of Interest

The authors declare that they have no competing interests.

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