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Optimized self nano-emulsifying systems of ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides

Shantanu Bandyopadhyay, O.P. Katare, Bhupinder Singh*

University Institute of Pharmaceutical Sciences (UGC Centre of Advanced Studies), Panjab University, Chandigarh 160014, India

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ABSTRACT

The objective of the current work is to develop systematically optimized self-nanoemulsifying drug delivery systems (SNEDDS) using long chain triglycerides (LCT's) and medium chain triglycerides (MCT's) of ezetimibe employing Formulation by Design (FbD), and evaluate their in vitro and in vivo performance. Equilibrium solubility studies indicated the choice of Maisine 35-1 and Capryol 90 as lipids, and of Labrasol and Tween 80 as emulgents for formulating the LCT and MCT systems, respectively. Ternary phase diagrams were constructed to select the areas of nanoemulsion, and the amounts of lipid (X_1) and emulgent (X_2) as the critical factor variables. The SNEDDS were systematically optimized using 3^2 central composite design and the optimized formulations located using overlay plot. TEM studies on reconstituted SNEDDS demonstrated uniform shape and size of globules. The nanometer size range and high negative values of zeta potential depicted non-coalescent nature of the optimized SNEDDS. Thermodynamic studies, cloud point determination and accelerated stability studies ascertained the stability of optimized formulations. In situ perfusion (SPIP) studies in Sprague Dawley (SD) rats construed remarkable enhancement in the absorptivity and permeability parameters of SNEDDS vis-à-vis the conventional marketed product. In vivo pharmacodynamic studies in SD rats indicated significantly superior modification in plasma lipid levels of optimized SNEDDS vis-à-vis marketed product, inclusion complex and pure drug. The studies, therefore, indicate the successful formulation development of self-nanoemulsifying systems with distinctly improved bioavailability potential of ezetimibe.

1. Introduction

Self-nanoemulsifying drug delivery systems (SNEDDS) are newer and novel technological innovations with immense potential in oral bioavailability enhancement of lipophilic drugs. Being nano in size, such lipidic drug carrier systems are capable of surmounting the problems of low oral bioavailability of drug(s) caused owing to their poor aqueous solubility, hepatic first-pass effect, metabolism by cytochrome P450 family of enzymes present in the gut enterocytes and liver hepatocytes, P-glycoprotein (P-gp) efflux and/or restricted intestinal permeability [1].

Of the excipients employed for the formulation of SNEDDS, lipids have an immense role for the biological fate of drug. Studies have shown that the type of absorption pathway and subsequent transportation of drug is significantly influenced by the two types of lipids viz. medium chain triglycerides (MCT's) and long chain triglycerides (LCT's) [2,3]. The MCT's are directly transported by the portal blood to the systemic circulation, whereas the LCT's are transported via the intestinal lymphatics. The LCT's are likely to augment the lymphatic transport of a lipophilic drug substance leading to enhance oral bioavailability. Nevertheless, if the lipophilicity of the molecule is sufficiently high (i.e., $\log P \geq 4.5$) then the MCT-based systems are also likely to favour the lymphatic transportation.

The drug, i.e., ezetimibe, chosen in the present study is a BCS class II hypolipidemic drug with poor water-solubility and high permeability ($\log P$ of 4.56) [4]. Besides these, it undergoes rapid first-pass metabolism and P-gp efflux, leading eventually to marked reduction in the drug oral bioavailable fraction (i.e., 35%) in humans and animals like dogs, rats, etc. [5,6]. To circumvent the afore-mentioned limitations various formulation approaches of ezetimibe have been reported like, nanocrystals, cyclodextrins inclusion complex, suspensions but all with limited fruition [7–9].

Systematic optimization of such isotropic delivery systems using design of experiments (DoE), on the other hand, offers numerous advantages including high degree of precision and prognosis, and economy in terms of time, effort and money [10]. Application of such DoE techniques for the development of optimized drug

* Corresponding author at: University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies, Chandigarh 160 014, India. Tel.: +91 172 2534103; fax: +91 172 2543101.
E-mail addresses: shantanuphd78@gmail.com (S. Bandyopadhyay), drkatare@yahoo.com (O.P. Katare), bsbhoop@yahoo.com, bsbhoop@pu.ac.in (B. Singh).

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delivery products, lately termed as formulation by design (FbD), is known to provide an in-depth understanding and ability to explore and defend the ranges for varied formulation and processing factors [11].

Thus, the present research aims at studying the effect of LCT and MCT-SNEDDS of ezetimibe formulated employing systematic FbD for enhancement of its dissolution and bypassing the P-gp and first-pass effect, resulting consequently in improved oral bioavailability potential.

2. Material and methods

Ezetimibe was provided ex-gratis by M/s Ranbaxy Laboratories Ltd., Gurgaon, India. Labrafac™ Lipophile WL 1349 (medium-chain triglycerides), Capryol™ 90 (propylene glycol monocaprylate), Lauroglycol™ 90 (propylene glycol monolaurate), Labrasol® (caprylocaproyl macrogol-8 glycerides) and Maisine™ 35-1 (glycerol monolinoleate) were received as the gift samples from M/s Gattefosse, Saint-Priest, France. Cremophor® EL (polyoxyl 35 Castor Oil) was supplied ex-gratis by M/s BASF Mumbai, India. Captex® 200P (propylene Glycol Dicaprylate/Dicaprate), Capmul MCM® (medium chain fatty acids mixture, mainly of caprylic and capric) and Capmul® PG 8 (propylene glycol monocaprylate) were received as the gift samples from M/s Abitec Corp., Wisconsin, USA. Tween® 20, Tween® 60 and Tween® 80 were procured from M/s HIMEDIA Laboratories Pvt. Ltd., Mumbai, India. Ethyl Oleate and Olive Oil were procured from M/s Loba Chemie, Mumbai, India. Sesame Oil was obtained from M/s S K Oil Industries, Jalgaon, India. All other materials and chemicals procured for the studies were of analytical grade, and were used as such as obtained.

3. Initial studies for screening of excipients

3.1. Solubility studies

Equilibrium solubility of ezetimibe was determined employing various MCT's viz. Capryol™ 90, Capmul® PG 8, Captex® 200P, Labrafac™ Lipophile WL 1349 and Capmul MCM®, and various LCT's, i.e., olive oil, sesame oil, Lauroglycol™ 90, ethyl oleate, Maisine™ 35-1. An excess amount of ezetimibe was added to each of the selected lipids, and the mixture was stirred continuously for 72 h at 37 ± 1 °C. Following attainment of equilibrium, the mixture was centrifuged at 1500 rpm (88.04 × *g*) for 20 min, and the obtained supernatant was filtered through a membrane filter having pore size of 0.45 μm (M/s mdi Membrane Technologies LLC, California, USA). Spectrophotometric absorbance of the filtrate was measured using UV 3000$^+$ spectrophotometer (M/s Labindia, Mumbai, India) at a λ_{max} of 232.5 nm. Analogously, equilibrium solubility studies were also conducted in emulgents viz. Labrasol®, Cremophor® EL, Tween® 20, Tween® 60 and Tween® 80. Drug content was determined using a previously constructed standard calibration plot, taking $E^{1\%}_{1\,cm}$ as 363 and molar extinction coefficient as 14,861.22.

3.2. Ternary phase diagram

Compositions with different oil-to-emulgent ratios, within the range of 1:9 and 9:1, were selected and titrated with water at ambient temperature. The mixtures were observed visually following equilibration. Subsequently a series of ternary phase diagrams were constructed using PCP Disso software ver 3.0 (M/s Pune College of Pharmacy, Pune, India). The phase diagrams were so constructed to delineate the boundaries of various phases, i.e., nano/microemulsion, emulsion, emulgel and microgel [12]. The generated samples, clear or slightly bluish in appearance, were taken as the nanoemulsions.

3.3. Preparation of SNEDDS as per experimental design

The LCT and MCT-SNEDDS formulations were prepared by the standard admixture method, as reported by us earlier [13]. Drug was dissolved in the lipid(s) at 37 °C and the emulgent, in the predetermined ratio, was added to the lipidic drug solution, while stirring at high speed using a magnetic stirrer (M/s Perfit, Ambala, India) maintained at 37 °C. Lipidic and emulsifying excipients were chosen on the basis of the formation of maximal nanoemulsion region in the ternary phase diagram. For the preparation of LCT-SNEDDS, Maisine 35-1 as lipid (X_1) and Labrasol as emulgent (X_2), while for the preparation of MCT-SNEDDS, Capryol 90 as lipid (X_1) and Tween 80 as emulgent (X_2) were chosen and finally selected as the two critical influential factors for further formulation optimization work [10,14]. A CCD with $\alpha = 1$ was employed, where the amounts of oil and emulgent were studied at three levels each. Overall, a set of 13 experimental runs each were studied as per the experimental design matrix as depicted in Table 1. The formulation at the intermediate coded factor levels (i.e., 0,0) was studied in quintuplicate. The response variables considered for the current DoE optimization studies encompassed, amount permeated in 45 min ($Perm_{45\,min}$), globule size (D_{nm}) and %dissolution efficiency in 30 min ($\%DE_{30\,min}$).

4. Pre-optimization formulation characterization

4.1. Determination of globule size

Aliquots (1 mL) of the samples, serially diluted 100-fold with purified water, were employed to assess the globule size [15]. The emulsion globule size was determined by dynamic light scattering technique using particle size analyser (ZS 90, M/s Malvern, Worcestershire, UK) [16].

4.2. In vitro dissolution studies

Drug release studies were carried out for both LCT and MCT-SNEDDS formulation combinations, in triplicate, employing USP 31 Apparatus 2 (DS 8000A/S, M/s Labindia, Mumbai, India) using 500 mL of dissolution medium containing 0.5% (w/v) SLS as at 50 rpm and 37 ± 0.5 °C. Aliquots of sample (5 mL each) were periodically withdrawn at suitable time intervals and replaced with fresh dissolution medium. The samples, after suitable dilution(s), were analysed spectrophotometrically at a λ_{max} of 231 nm, taking $E^{1\%}_{1\,cm}$ as 260 and molar extinction coefficient as 10,644.4. Dissolution study was also conducted on pure ezetimibe in an analogous manner.

4.3. Non-everted gut sac method

Taking cognizance that the research work, involving gut sac method, in situ SPIP studies and the subsequent in vivo pharmadynamic studies, adheres to the guidelines for care and use of the laboratory animals, all the animal investigations were performed as per the requisite protocol approved by the Institutional Animal Ethics Committee of the Panjab University, Chandigarh, India [Letter no IAEC/54 dated 06/12/2010]. The Committee is duly approved for the purpose of control and supervision of experiments on the animals by the Government of India.

Ex vivo permeation studies were performed using non-everted gut sac technique in female Sprague Dawley (SD) rats, weighing between 200 and 300 g, previously made to abstain from solid