Journal of Drug Delivery Science and Technology 40 (2017) 35–44

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Quality by design based silymarin nanoemulsion for enhancement of oral bioavailability

Amrita Nagi [a], Babar Iqbal [a], Shobhit Kumar [a], Shrestha Sharma [a], Javed Ali [b], Sanjula Baboota [b, *]

[a] Research Scholar, Pharmaceutical Research Laboratory, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India
[b] Associate Professor, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India

ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form
27 May 2017
Accepted 30 May 2017
Available online 31 May 2017

Keywords:
Nanoemulsion
Oral bioavailability
Silymarin
Experimental design
Box-behnken design

ABSTRACT

Silymarin has been approved as a safe herbal hepatoprotective drug as well as drug of choice for several hepatic disorders. However it suffers from the problem of poor oral bioavailability. In current work silymarin loaded nanoemulsions were prepared using high pressure homogenization (HPH) technique. Capryol 90, Solutol HS 15 and Transcutol HP were selected as oil phase, surfactant and co-surfactant, respectively. Quality by design was employed to optimize nanoemulsion in terms of amount of surfactant/co-surfactant mixture (S_{mix}), processing pressure and number of cycles. Globule size, polydispersity index (PDI), zeta potential, transmittance and percentage *in vitro* drug release of optimized formulation were found as 50.02 ± 4.5 nm, 0.45 ± 0.02, −31.49 mV, 100.00 ± 2.21% and 90.00 ± 1.83%, respectively. The everted gut sac studies showed that the nanoemulsion facilitated the improvement of the apparent permeability coefficient (P_{app}). P_{app} of silymarin in nanoemulsion and oral suspension was 1.00×10^{-5} cm/h with flux of 0.422 μg/cm^2/h and 6.30×10^{-6} cm/h with flux of 0.254 μg/cm^2/h at 2 h, respectively. Pharmacokinetic study showed significantly ($p < 0.05$) enhanced bioavailability of silymarin in nanoemulsion as compared to oral suspension thus nanoemulsion can be a promising oral delivery system for silymarin with enhanced oral bioavailability.

1. Introduction

Nowadays, more attention has been given to lipid based preparations to enhance the oral bioavailability of poorly aqueous soluble drugs. Actually, the mainly accepted techniques include the incorporation of the lipophilic drug in hydrophobic carrier like oils, emulsifier dispersions, emulsions, liposomes, micro- or nanoemulsions and self-emulsifying formulations. All these enhance the surface area of the lipophilic drugs to increase solubilisation performance. From this perspective of oral drug delivery, formulations based on lipids are considered to enhance solubility and absorption of lipophilic drugs.

Various approaches like self-microemulsifying drug delivery system (SMEDDS) [1], liposomes [2] and proliposome [3] have been investigated by different scientists to improve the bioavailability of silymarin. Amongst the different lipid based formulations nanoemulsions have gained an edge and have been exploited vastly for improving the aqueous solubility thereby bioavailability. They are capable of delivering the drug in its molecular form. Moreover, these are kinetically stable systems and their nanosize globules are responsible for their stability as these are insensitive to gravitational force. Nanoemulsions have been used for a wide variety of poorly aqueous soluble drugs such as mebudipine [4], curcumin [5], breviscapine [6] and itraconazole [7] for improvement of oral bioavailability. The advantage of preparing nanoemulsion include their utilization to incorporate lipophilic drug molecules into the oil phase hence increasing their solubility. Generally high energy emulsification and low energy emulsification are the two basic techniques employed for the production of nanoemulsions and both methods have capability to produce stable nanoemulsions. High energy emulsification technique is widely employed and accepted technique for the production of nanoemulsions in which mechanical energy (disruptive force) is used to disrupt the phases resulting in generation of small size droplets. The main benefits of

* Corresponding author.
E-mail addresses: sbaboota@jamiahamdard.ac.in, sbaboota@rediffmail.com (S. Baboota).

http://dx.doi.org/10.1016/j.jddst.2017.05.019
1773-2247/

this technique are its simplicity and easy production mechanism.

Silymarin is a naturally occurring flavonoid compound extracted from the seeds of *Silybum marianum* [8]. It has been approved as a safe herbal hepatoprotective drug as well as drug of choice for several hepatic disorders [9]. Silymarin acts as an antioxidant by scavenging of free radicals and inhibit various oxidase enzymes [10,11]. However, after oral administration, it is only 23–47% absorbed and the peak plasma concentration is reached at approximately 6–8 h [12].

In the present investigation an attempt has been made to develop silymarin loaded nanoemulsion using Capryol 90 as oil phase, Solutol HS 15 as surfactant and Transcutol HP as co-surfactant with an aim to increase silymarin bioavailability. Nanoemulsions have been prepared by subjecting a coarse emulsion to high pressure homogenization (HPH) [13]. A box behnken design (BBD) has been employed to investigate the effect of surfactant/co-surfactant mixture (S_{mix}) concentration, processing pressure and number of cycles on globule size, polydispersity index (PDI), transmittance and drug release.

2. Material and methods

2.1. Materials

Silymarin was obtained from DD Nutrition Pvt. Ltd, India. Labrafac Lipophile WL1349, Capryol 90 and Transcutol HP were received as gift sample from Gattefosse, Mumbai, India. Captex GTO and Capmul MCM were obtained from Abitec Corporation, Janesville. Tween 20, Tween 80 and Solutol HS 15 were bought from Signet Chemicals, India. Unitop FFT 40 was procured as gift sample from Unitop East West Estate, India. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Screening and selection of oils

Oils with different properties were screened for the solubility of silymarin. Solubility of silymarin in oils was estimated by adding an excess quantity of silymarin in 1 ml of oil (placed in vial). These samples were kept at 25 ± 0.5 °C in an isothermal shaker. After 72 h, the samples were collected and centrifuged [14]. Supernatant was carefully taken out and the concentration of silymarin was determined using UV spectrophotometer at 288 nm.

2.2.2. Screening and selection of surfactants and co-surfactants

Solubility of silymarin in various surfactants was determined in the same way as mentioned in screening of oils. Surfactant selection was also based on miscibility studies with the selected oil. For this the surfactant was mixed with oil in a 1:1 ratio using vortex shaker and was observed visually. The basic criterion for selection of co-surfactant was the miscibility of co-surfactant with selected oil in 1:1 ratio. The mixture that appeared clear was considered for the preparation of nanoemulsion.

2.2.3. Formulation and optimization of nanoemulsion by experimental design

A three factors, four levels BBD statistical design was selected to optimize silymarin loaded nanoemulsion. We have selected range of 30–40% for S_{mix} and 1000–2500 bar for homogenization pressure. The range of homogenization cycles was taken as 5–15. The constraints for globule size and PDI was set at minimum whereas for percentage transmittance and drug release the constraints set at maximum. Table 1 summarizes an account of the independent and dependent variables. BBD software (Design Expert® 9.0.4.1, State-Ease Inc., Minneapolis, USA) was employed to evaluate the effects of S_{mix} concentration, processing pressure and number of cycles on the globule size, PDI, % transmittance and percentage drug release. Several nanoemulsion formulations were prepared as per the design described in Table 2 for 17 runs generated and were investigated for globule size, PDI, transmittance and % drug release as the response variables.

Oil in water nanoemulsions loaded with silymarin were prepared by high energy emulsification technique. Briefly, coarse emulsions (20 ml) were formulated by mixing oil, S_{mix}, water and silymarin using magnetic stirrer and bath sonicator. Nanoemulsions were prepared by passing coarse emulsions through a high pressure homogeniser (STANSTED® Pressure Cell Homogeniser, Harlow, Essex CM19 5FN, UK) [13,14].

The results for each of the response factors were fitted to non-linear polynomial model. Furthermore, effect of independent variables on the dependent variables was predicted by employing response surface methodology. The experimental data was statistically analyzed to determine the best fit model for the three independent variables. The equations for independent variables were produced by putting values of coefficients in equation as shown below,

$$\text{Response (R)} = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2$$

where, A, B, and C indicated S_{mix} (%) content, homogenization pressure (bar) and number of homogenization cycles, respectively. β_0 represented the intercept coefficient; β_1, β_2 and β_3 represented the linear coefficients; β_4, β_5 and β_6 were the interaction coefficients. The quadratic coefficients were represented by β_7, β_8 and β_9. On the basis of constraint of response, optimized formulation was selected by numerical technique with higher desirability factor. This optimized formulation was further subjected to *in vitro* and *in vivo* characterization.

2.2.4. Characterization of nanoemulsions

2.2.4.1. Globule size and PDI. Globule size and size distribution of nanoemulsion formulation was determined using photon correlation spectrometer (PCS, Zetasizer 1000 HAS, Malvern Instruments, Worcestershire, UK). In order to determine the globule size, all formulations were diluted to about 200 times with distilled water followed by vigorous shaking to minimize multiple scattering effects prior to each measurement. Each sample was then analyzed by zetasizer for globule size distribution and average globule diameter. Light scattering was measured at 25 °C using 90° angle. PDI, a measure of globule size distribution in the sample was measured in triplicate [15–17].

2.2.4.2. Transmittance. Transmittance of silymarin nanoemulsion formulations was determined spectrophotometrically using Shimadzu UV-VIS spectrophotometer (Shimadzu, Japan). The 1 ml of each formulation was diluted 100 times using water and analyzed at 630 nm for % transmittance [15].

2.2.4.3. Viscosity and refractive index. Viscosity was measured without dilution using a Brookfield DV III ultra V6.0 RV cone and plate viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA). All experiments were carried out at a temperature of 25 ± 0.5 °C using Rheocale V2.6 software. Refractive index of the formulation was determined using an Abbe type refractrometer (Precision Standard Testing Equipment Corp., Germany) which was standardized using castor oil. Few drops of the formulation was placed on the slide and refractive index was measured thrice [15].