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Nanoemulsion as a tool for improvement of Cilostazol oral bioavailability

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ABSTRACT

Cilostazol (CLZ) is a well known therapeutic agent which is used for antithrombotic activity but suffers from the drawback of poor oral bioavailability. Research studies have explored the utilization of various lipid based formulations like nanoemulsions to overcome such limitations. CLZ nanoemulsions were produced by aqueous titration technique to improve the oral bioavailability. CLZ solubility in oils and emulsifiers was estimated to select the excipients for the formulation. Triacetin and Capryol 90 (1:1) were used as the hydrophobic phase. Amongst various surfactants and co-surfactants, CLZ showed solubility of 4.0 ± 0.2 mg/ml and 8.0 ± 0.04 mg/ml in tween 80 and transcutol P, respectively and therefore they were selected for the formulation of nanoemulsions. Results of droplet size for optimized batch were found to be 93.72 nm. CLZ nanoemulsions exhibited polydispersity index (PDI) of 0.278. Enhanced CLZ release was obtained with nanoemulsions. Pharmacokinetic studies showed that the optimized nanoemulsion (X1) showed 3.29 times improvement in bioavailability in comparison to CLZ suspension.

1. Introduction

CLZ is 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3, 4-dihydro-2(1*H*)-quinolinone and has been used as antiplatelet, vasodilatory and antithrombotic agent. According to the BCS classification, CLZ is a BCS class II drug and shows low oral bioavailability due to poor aqueous solubility [1]. Different approaches like micronization [2], complexation [1] and nanoemulsifying system [3] have been researched by different scientists to improve the aqueous solubility of CLZ thereby improving its bioavailability. Amongst the various lipid based approaches that have been explored till now to improve the aqueous solubility of poorly soluble drugs, nanoemulsions have gained an edge and have been exploited vastly for improving the aqueous solubility and hence the bioavailability. They are capable of delivering the drug in its molecular form [4,5]. They are kinetically stable systems [6–9] and nano droplets are responsible for their stability as these are insensitive to gravitational force [8–12]. This size is also responsible for lowering the attractive forces acting between the droplets. Thus the formulation does not destabilize by flocculation of droplets [6,13–17]. All these benefits of nanoemulsions help in improvement of bioavailability of the loaded drugs [18–20]. Nanoemulsions composed of non-ionic surfactants show non-toxic nature due to low surfactant level, making them more effective [7,8,21]. The present study deals with preparation of CLZ nanoemulsions and their testing to analyse the improvement in CLZ bioavailability.

2. Materials and methods

2.1. Drug and reagents

CLZ was provided by Zydus Cadila Pharmaceutical limited (Ahmedabad, India). Tween 80 and Triacetin were procured from Merck (Mumbai, India). Transcutol P and Capryol 90 were gift samples from Gattefosse (Saint Priest, Cedex, France).

2.2. Screening of components

Maximum solubility of CLZ in various components required for the formulation of nanoemulsion like oil, surfactant and co-surfactant was the major criteria for their selection. Different components were screened out by checking CLZ solubility in them. The excipients showing highest CLZ solubility were selected as this ensured high CLZ loading in the formulations and prevented precipitation of CLZ on long term storage. About 1 ml of excipient was taken into the small tubes and CLZ in an excess quantity was added to it and subsequently shaking was done. The various excipients screened are shown in Table 1. Throughout the analysis the temperature was maintained at 25 °C, because any fluctuation could have hindered the solubility determination [5,22]. After centrifuging the tubes at 5000 rpm, the samples were analysed for CLZ amount by spectrophotometer at 258 nm.

2.3. Construction of pseudoternary phase diagrams

These diagrams were plotted as previously described by Sharma and co-workers, to identify the regions where O/W nanoemulsion area exists. It was drawn by formulating a range of S_{mix} by taking different

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Table 1
Solubility of CLZ in oils, surfactants and co-surfactants.

Solubility of CLZ in oils		Solubility of CLZ in surfactants		Solubility of CLZ in co-surfactants	
Oil	Solubility ± S.D. (n = 3)(mg/ml)	Surfactant	Solubility ± S.D. (n = 3) (mg/ml)	Co-surfactant	Solubility ± S.D. (n = 3) (mg/ml)
Oleic acid	3.5 ± 0.36	Tween 20	3.5 ± 0.1	PEG	4.0 ± 0.1
Capryol 90	5.0 ± 0.20	Tween 80	4.0 ± 0.2	PEG 400	5.0 ± 0.15
Capryol PGMC	4.5 ± 0.31	Labrasol	2.9 ± 0.2	Transcutol P	8.0 ± 0.04
Sefsol 218:Capryol 90 (1:1)	11 ± 0.03				
Triacetin:Capryol 90 (1:1)	14.9 ± 0.56				

fractions of surfactants and co-surfactants. In the present work ratios ranging from 1:0 to 4:1 were tried. Additionally a decreasing 1:2 and 1:3 amounts of surfactant with respect to co-surfactant were also tried. Measured volume of hydrophobic phase was taken and mixed with S_{mix}. Finally, it was gradually titrated with hydrophilic medium. After each successful addition, the systems were observed for any phase transitions. These observations were applied to plot a phase diagram [22]. The region where combinations of components showed clarity/transparency were selected and considered as nanoemulsion area. The main reason for these combinations was to cover maximum ratio. This helped to cover the entire boundaries of phase diagram.

2.4. Selection of formulation

The formulas comprising of oil percentage that solubilized CLZ (without showing precipitation) were chosen from phase diagrams. Priority was given to the formulas comprising less quantity of emulsifiers and high volume of water and these selected formulations were subjected to various physical stability tests.

2.5. Physical stability studies

2.5.1. Heating cooling cycle

This test was aimed to reject the physically unstable formulations. As described by Shakeel and co-workers, heating cooling cycle is an important test to assess the stability of formulation. Briefly, the preparations were stored between 4 ± 2 °C and 40 ± 2 °C and observed for any phase transition [23].

2.5.2. Freeze thaw test

The preparation which remained stable at these above mentioned storage conditions were further checked by second test known as freeze thaw test. In this test, preparations were stored at −20 ± 2 °C for 1 day. For this purpose deep freezer was employed. The preparations were also kept at 21 ± 2 °C. Only those preparations which restored to their initial form (in less than 3 min) were considered as stable [22,23].

2.5.3. Centrifugation test

To examine the creaming and phase separation, preparations were centrifuged. Centrifugation was done for about 1 min at 5000 rpm. Preparations that remained stable after centrifugation were considered for CLZ loading [22,23].

2.6. Dispersibility test

This test was carried out on selected stable nanoemulsions. The test was performed by USP apparatus (type II) using 500 ml of 0.3% SLS solution. Throughout analysis the temperature was maintained at 37 ± 0.5 °C. One millilitre of nanoemulsion was dispersed in solution. This test was carried out to verify the homogeneity of the formulation. As represented in Table 2, different grading was given to the formulation [22].

2.7. Drug loading

Placebo showing stability against stress conditions were selected for drug loading. Specified amount of CLZ was solubilised in hydrophobic phase and mixed with required percentage of S_{mix}. Finally this mixture was titrated with water, followed by sonication for 20 min. All stress tests were again conducted on CLZ nanoemulsions.

3. Characterization of formulations

3.1. Droplet size and PDI

To assess the droplet size of nanoemulsions, Nano ZS90, Malvern Instrument, U.K was employed. Prior to determination, the formulation was suitably diluted with continuous phase in ratio of 1:400. Samples were analysed at refractive index of 1.41; scattering angle = 90° using He–Ne laser (633 nm) [24]. The value of PDI represents the uniformity of droplet size distribution within the formulation. Uniformly sized droplets distribution ensures better nanoemulsion stability. More the PDI, lower is uniformity of droplet size in nanoemulsion formulation and *vice-versa*. Generally, the value of PDI can range from 0.00 (for mono-dispersed system) to 1.00 (for highly dispersed systems).

3.2. Percentage transmittance

It was measured spectrophotometrically (Shimadzu, Kyoto, Japan) at 630 nm by diluting the sample with methanol [22].

3.3. Viscosity

Brookfield DV III cone and plate rheometer (Middleboro, MA, USA) was used. Four millilitre of nanoemulsion was used for rheological characterization. Spindle speed was 50 rpm throughout the analysis [22].

3.4. Refractive index

It was evaluated by making use of Abbe-type refractometer. Each sample was repeated 3 times and standard deviation was calculated [22].

Table 2
Observations for dispersibility test.

Grade	Observation
A	Rapidly forming (within a minute) nanoemulsion, having a clear appearance
B	Rapidly forming, slightly less clear emulsion
C	Fine milky emulsion formed within 2 min of dilution
D	Dull white emulsion having slightly oily appearance that was slow to emulsify (longer than 2 min)
E	Formulation which exhibited either poor or minimal emulsification with large oil droplet present on the surface