RGUHS
Journal of Pharmaceutical Sciences

ORIGINAL ARTICLE

Formulation and Evaluation of Self-Nanoemulsifying Drug Delivery System of Clofazimine

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Received date: May 7, 2022; **Accepted date:** September 12, 2022; **Published date:** September 30, 2022

Abstract

Aim: The present study aimed to formulate and evaluate Self-Nanoemulsifying Drug Delivery System (SNEDDS) for poorly water-soluble clofazimine.

Methodology: Ethyl Acetate, Captex-300, and polyethylene glycol 400 (PEG-400) were utilized for the development of SNEDDS of clofazimine. Optimization of SNEDDS was carried out using 3^2 factorial design. Evaluation parameters such as dilution study by visual observation, self-emulsification time, percentage transmission, zeta potential, particle size, and polydispersity index (PDI) were performed for formulated SNEDDS. Formulated SNEDDS batches were studied for dissolution and stability.

Results: The particle size of the formulation ranged between 288.9–918.2 nm. Zeta potential was found within the range of -1.10 to -8.25 which indicated the stability of the emulsion. Self-emulsification time of clofazimine formulations was found to be less than 1 min which confirmed its efficiency. Percentage transmission was seen at around 100% which confirmed the clarity and transparency of the nanoemulsion formulation. Dissolution of clofazimine from SNEEDS was very fast and achieved approximately 100% dissolution in 1 hour as that of the pure drug (<15%). Further stability study confirmed the stability of formulated SNEDDS.

Conclusion: Present study demonstrated a systematic approach for the development of SNEDDS which can be very useful for delivering the drug orally for many emerging hydrophobic drugs with good therapeutic potential.

Keywords: SNEDDS, Formulation, Optimization, Evaluation, Clofazimine, Oral.

Introduction

Clofazimine is a poorly soluble, broad-spectrum anti-mycobacterial agent which is mainly used in the treatment of leprosy, including dapsone-resistant leprosy and leprosy complicated by erythema nodosum leprosum. It has been recommended as an anti-leprosy medicine in the current World Health Organisation (WHO) model lists of essential medicines for adults and children.[1, 2]

Clofazimine has been in clinical use since the 1960s and it has cured more than 16 million people worldwide.[3-7] WHO has now recommended clofazimine as a second-line agent against multi-drug resistant tuberculosis, due to its potent activity against *Mycobacterium tuberculosis*.[8-12] Clofazimine is clinically efficacious for both drug-susceptible and drug-resistant strains of *M. tuberculosis*.[13-16]

Clofazimine belongs to Biopharmaceutics Classification System (BCS) class-II drug and has high permeability (log P- 7.3) and low solubility (1.51mg/ltr). The low

solubility of clofazimine creates a hurdle in its effective formulation development.[17, 18] Further, its therapeutic use is restricted due to its side effects. The solubility and toxicity issues constrained its intravenous use due to malabsorption.

Various research groups have tried different formulation approaches like nanosuspension, liposomes, etc. to enhance the solubility profile and minimize the side effects of the drug to improve therapeutic efficacy. Most of these approaches have stability issues and suffer from complexity in the manufacturing process and scale-up.[19,20]

Recently, self-nanoemulsifying drug delivery systems (SNEDDS) have emerged as a good and reliable approach for improving the oral bioavailability of lipophilic drugs like carvedilol, nimodipine, etc. A self-nanoemulsifying drug delivery system is a mixture of natural or synthetic oils, solid or liquid hydrophilic surfactant, and a co-surfactant with a solubilized drug. Hard or soft gelatine capsules can be used to encapsulate this mixture or can be converted into solid granule tablets. These SNEDDS forms fine oil-in-water (o/w) emulsion when diluted in aqueous media such as gastrointestinal fluids. They are rapidly dispersed in the gastrointestinal tract where the peristaltic movements provide gentle agitation necessary for emulsification. They are very effective in improving the absorption of hydrophobic drugs. Physically stable formulation, SNEDDS can be an effective system for improving oral bioavailability, increasing drug loading capacity, ease of manufacture, and scale-up, etc.[21, 22]

In this context, the present study was planned to develop and improve the therap eutic efficacy of clofazimine by formulating it in SNEDDS formulation.

Materials And Methods

Materials

Clofazimine was purchased from Sigma-Aldrich, Bengaluru, India. Captex-300 was obtained as a gift sample from ABITEC Corporation, Mumbai. Methanol was purchased from Loba Chemicals, Mumbai. All other chemicals and reagents used were of analytical grade.

Methods

Determination of the drug solubility in different oils, surfactants and co-surfactants

The solubility of the drug in various oils (castor oil, ethyl acetate, olive oil, coconut oil, almond oil, labrasol, labrafil, soyabean oil, isopropyl myristate), surfactants (CapmulMCM, Captex-200 P, Captex-300, transcutol, tween-20, tween-80, ethyl oleate, Brij-30) and co-surfactants [ethanol, Polyethylene glycol(PEG)-400, span-20, span-80, cremophore] was determined by the method described by Mullangi R *et al*[23]. In brief, selected vehicles were added to cap vials containing an excess of the drug. The vials were tightly closed and were stirred continuously for 72 hrs using an orbital shaker at 25°C. Once equilibrium is attained, vials were centrifuged at 3000 rpm for 10 min. The excess insoluble drug was filtered out by using a membrane filter. The samples were analyzed by ultraviolet-spectroscopy (UV-spectroscopy) and solubility was quantified with a pre-validated calibration curve (r^2=0.9966).[24]

Pseudo ternary phase diagram

Pseudo ternary phase diagrams of oil, surfactant/co-surfactant (Smix), and water were constructed using the water titration method. The oil (ethyl acetate), surfactant (Captex-300), and co-surfactant (PEG-400) were selected based on the solubility study. Smix was prepared by mixing surfactant and co-surfactant in a 1:1 ratio. In a volumetric flask, Smix and oil were mixed at a ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The resultant mixtures were diluted dropwise with distilled water till first sign of turbidity and then a clear solution. The clear solution was considered as an end point. The resultant emulsion exhibits a clear appearance. Based on the results obtained, the pseudo ternary phase diagrams were constructed to obtain a self-emulsification area by CHEMIX school software (version-3.51).[25]

Optimization study for clofazimine SNEDDS

The composition of the SNEDDS formulation was optimized by optimal mixture design. From the area of self-emulsification found in the ternary phase diagrams, the levels of independent factors were selected. The percentage of Smix (Captex-300: PEG-400) and oil (ethyl acetate) were selected as independent variables X_1 and X_2, respectively. The concentration of X_1 and X_2 were set within the range of 10-20-30% and 5-10-15%, respectively. These were coded level as -1, 0 & +1. Two response factors particle size (Y_1) and zeta potential (Y_2) were selected as independent variables. Based on 3^2 factorial designs, nine experimental runs were carried out as shown in table 1.

Table 1: Details of experimental design for clofazimine SNEDDS

Mixture number	Smix (X_1)	Oil (X_2)
C1	-1 (10%)	-1 (5%)
C2	-1	0 (10%)
C3	-1	+1 (15%)
C4	0 (20%)	-1
C5	0	0
C6	0	+1
C7	+1 (30%)	-1
C8	+1	0
C9	+1	+1

Formulation of SNEDDS

Oil, surfactant, and co-surfactant were accurately weighed and mixed by gentle stirring in a glass vial. The drug was dispersed into an oil and surfactant mixture. Gentle stirring and vortex mixing was continued at 37°C until the drug was completely dissolved. The mixture was then sealed in a glass vial and stored at room temperature until equilibrium.[26]

Evaluation of SNEDDS

Dilution study

Dilution studies were performed by diluting SNEDDS with water and buffers. The diluted SNEEDS were kept for 12 h and checked for any signs of phase separation, turbidity as well as drug precipitation.[27]

Self-emulsification time

SNEDDS formulations were mixed with purified water. Gentle mixing of the contents was carried out using a magnetic stir rer at 37°C. The time required for spontaneous emulsification and progression of emulsion droplets was observed in triplicates.[28]

Percentage transmittance test

After dilution with distilled water, the SNEDDS formulations were measured spectroscopically for their optical clarity. The percentage transmittance was measured at 650 nm using a UV spectrophotometer (Shimadzu, Japan). The value near 100% transmittance indicates clear and transparent nanoemulsion formation.[29]

Particle size, Zeta potential, and polydispersity index (PDI) analysis

Aliquots of formulations were diluted 100 times with double distilled water. Zeta potential, droplet size, size distribution (PDI) and were measured by Zetasizer (Nano ZS, Make-Malvern Instruments). All measurements were carried out at 25°C.[35]

Dissolution study

Dissolution study was performed with a paddle-type dissolution apparatus. The selected SNEDDS formulations (C3, C6, and C8) corresponding to 50 mg of the drug were filled in an empty gelatin capsule (size-00) and placed in 900 ml of phosphate buffer at pH 6.8. The dissolution study was performed at 50 rpm and 37±0.5°C. Further 50 mg of clofazimine pure drug powder was placed in an empty gelatin capsule and the dissolution study was carried out. Samples were withdrawn at pre-determined time intervals (5, 10, 15, 20, 30, 45, and 60 min). An aliquot of phosphate buffer pH 6.8 was replaced to maintain sink condition. Samples were analyzed using UV-spectrophotometer at 285 nm. The dissolution profiles of all formulations (F3, F6, and F8) were compared with the clofazimine pure drug sample.

Stability study

Stability study was performed according to the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. All the formulations were sealed tightly in a glass vial and kept in a humidity chamber maintained at 40°C for 1 month. The formulations were subjected to visual inspection, percent transmission, and self-emulsification tests and compared with earlier results.[31, 32]

Results

Solubility studies

Among the oils used, ethyl acetate and labrasol showed maximum and minimum solubility for clofazimine, respectively. Ethyl acetate (18.82 mg/ml) had more strength to dissolve clofazimine than all other selected oils. Out of the various surfactants studied Captex-300 had shown the highest drug solubility (27.64 mg/ml) and was selected for further study. The drug was most soluble in PEG-400 (31.54 mg/ml). Solubility in studied oils, surfactants, and co-surfactants are as shown in figure 1, 2, and 3, respectively.

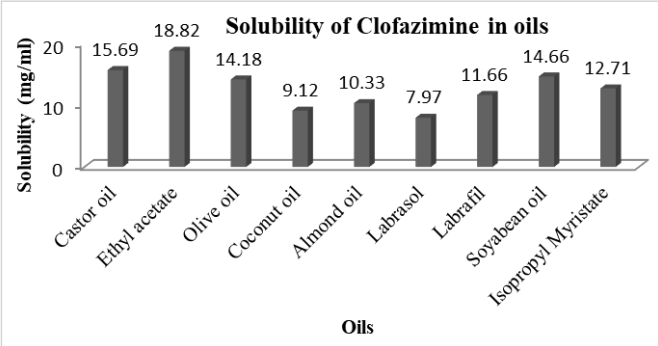

Figure 1: Solubility of clofazimine in oils

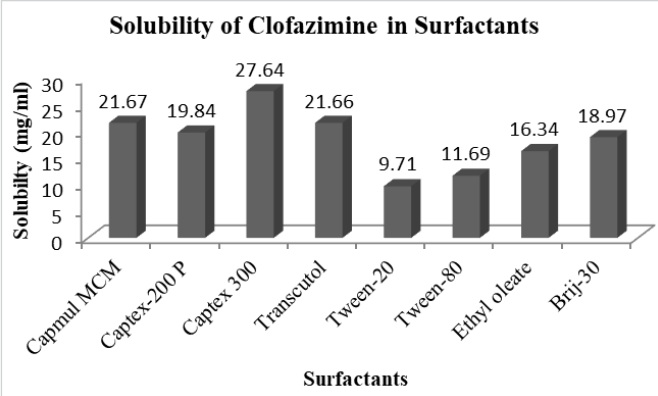

Figure 2: Solubility of clofazimine in surfactants

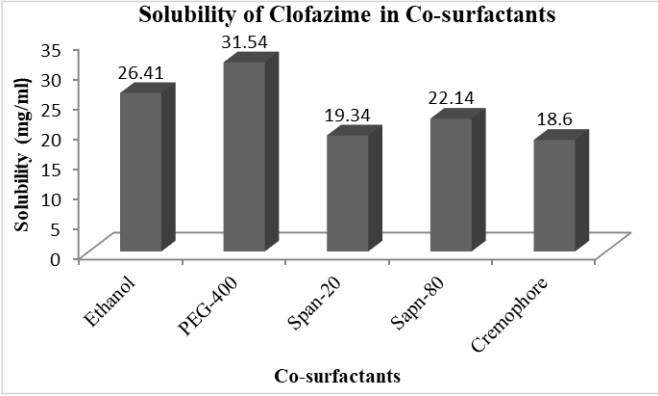

Figure 3: Solubility of clofazimine in co-surfactant

Pseudo ternary phase diagram study

Oil, surfactants, and co-surfactants were selected for SNEDDS formulation based on the solubility results. Nine potential combinations of surfactant and co-surfactant mixture to oil were used for the phase diagram study of SNEDDS. A pseudo ternary phase diagram was constructed using CHEMIX school software (version-3.51). The nine combinations used were as shown in table 2. Figure 4 depicts the emulsification area wherein emulsion was formed by gentle agitation.

Table 2: Various combinations used for phase diagram study

Composition	Smix(%)	Oil(%)	Water(%)
1.9	5.46	75.76	18.8
2.8	17.17	66.35	16.5
3.7	26.43	60.07	13.5
4.6	36.43	53.78	9.8
5.5	47.13	46.77	6.1
6.4	57.53	38.35	4.3
7.3	67.75	29.04	3.3
8.2	78.59	19.65	1.8
9.1	89.29	9.97	0.8

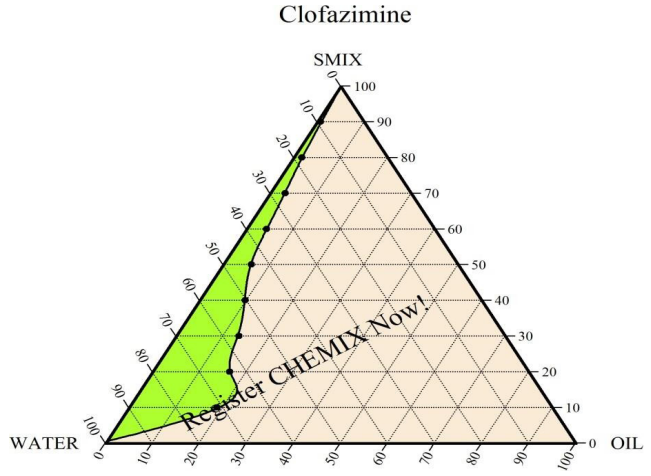

Figure 4: Pseudo ternary phase diagram for clofazimine

Formulation and Optimization study

Based on the phase diagram, 3 levels of each independent variable were selected wherein emulsion was formed. As per 3^2 factorial designs, 9 different formulations were prepared and these were further evaluated for particle size, zeta potential, and PDI. The results are summarized in table 3. The minimum particle size was found to be for C9 (288.9 nm) while the maximum was for C4 (918.2 nm). The size of the particle mainly depends on the concentration and nature of the surfactant.[36] The

PDI value ranged from 0.074 to 0.444. a low value of PDI indicates a uniform and narrow size distribution.[30] Zeta potential of formulations ranged from -1.10 to -8.25. The value of zeta potential indicates the stability of the emulsion after dilution. The higher the value of zeta potential, the greater the stability of formulation.[37] Negative value of zeta potential is usually due to the presence of free fatty acids[36] but when cationic lipid such as oleyamine is used, a positive charge develops.[39]

Table 3: Results of particle size, zeta potential, and PDI study

Mixture number	Particle size (nm) (Y1)	Zeta potential (mV) (Y2)	PDI	Self-emulsification time (Second)	% transmittance
C1	607.8	-4.30	0.173	18	98.57
C2	908.3	-3.77	0.418	21	98.31
C3	735.0	-6.67	0.178	12	97.39
C4	918.2	-5.83	0.239	17	98.85
C5	535.1	-8.25	0.074	09	99.12
C6	408.0	-1.10	0.253	15	98.16
C7	421.3	-2.02	0.285	27	99.49
C8	367.0	-5.83	0.319	14	98.12
C9	288.9	-2.21	0.444	20	99.37

PDI, polydispersity index

Based on the data obtained, 3D response plots dependent variables were plotted (Figures 5a and 5b). Further, the data was incorporated in software to get polymeric equations dependent variable. Accordingly, the final polymeric equation was computed as follows:

$$\text{Particle size } (Y_1) = 576.57 - 195.70X_1 - 85.82X_2 - 64.83\ X_1X_2$$

$$\text{Zeta potential } (Y_2) = 3.95 + 1.52X_1 + 0.4327X_2 + 0.6516X_1X_2$$

Where X_1 and X_2 represent the concentration of Smix and oil

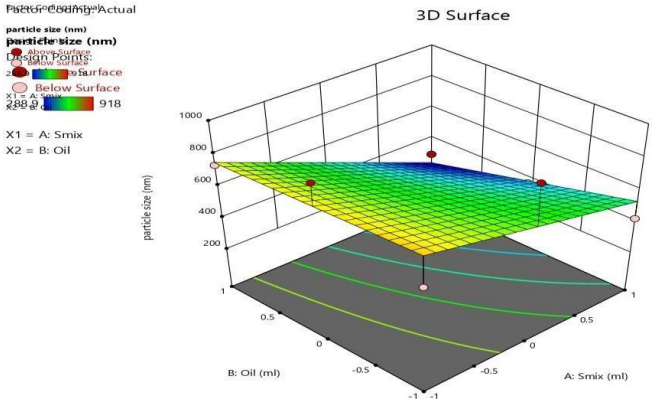

Fig. 5a: 3D response surface plot for the effect of the independent variable on particle size

(Y1)

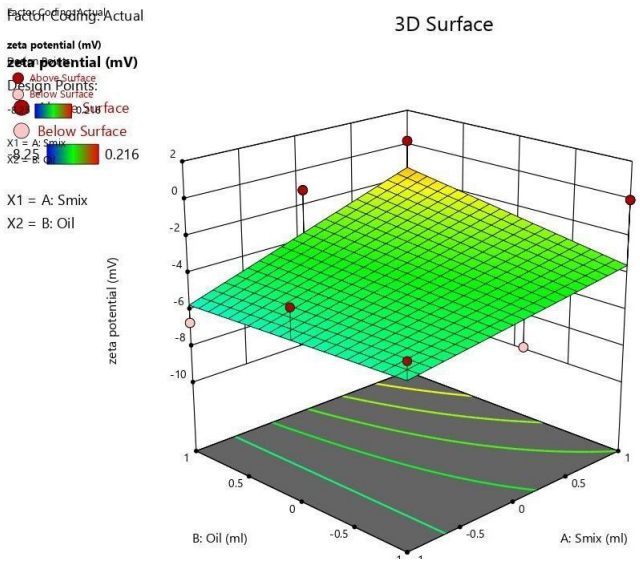

Fig. 5b: 3D response surface plot for the effect of the independent variable on zeta potential (Y2)

Evaluation of SNEDDS

Dilution study

From the results of the dilution study, no sign of phase separation or drug precipitation was observed.

Self-emulsification time

The time for self-emulsification of prepared formulations ranged from 09 to 27 seconds. The results obtained are shown in table 3.

Percentage transmittance test

The result of % transmittance is summarized in table 3. The % transmittance ranged from 97.39% to 99.49% which indicated that formulations are clear and transparent.

Dissolution study

Dissolution profiles of selected SNEDDS formulation batches are shown in Table 4 and in Figure 6. The percentage of the drug dissolved in the pure drug sample was found to be 14.26% at 60 min while all other formulations showed complete drug release at the same time.

Table 4: Dissolution profiles of API, C3, C5, and C8 batch

Sr. no.	Time	% Dissolution of API powder	% Dissolution of C3 Batch	% Dissolution of C5 Batch	% Dissolution of C8 Batch
1	5	2.57	23.18	31.26	29.64
2	10	4.43	33.88	39.55	31.37
3	15	6.61	47.09	61.61	56.14
4	20	8.47	69.69	80.82	76.81
5	30	10.54	94.14	91.96	93.67
6	45	12.65	100.25	98.62	99.14
7	60	14.26	100.80	100.47	100.94

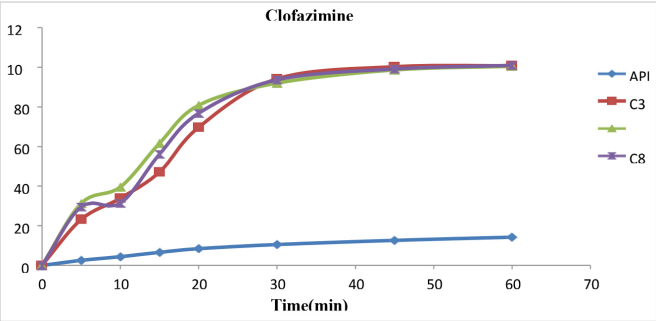

Fig. 6: Dissolution profile of Clofazimine SEDDS in Phosphate Buffer pH 6.8

Stability study

Table 5. Results of the stability study

Formulation Code	Stability condition by visual inspection	Percent transmission	Self-emulsification time (sec.)
C1	Turbid	94.48	19
C2	Clear	96.25	20
C3	Clear	94.31	11
C4	Turbid	95.31	15
C5	Clear	97.61	08
C6	Clear	96.10	18
C7	Clear	95.82	23
C8	Clear	97.21	13
C9	Clear	96.84	22

The stability studies showed that the formulations C1 and C4 were unstable. No significant variations in other formulation batches were observed.

Discussion

Solubility studies

The efficacy of SNEDDS formulation depends on the drug solubilization capacity of the oil, surfactant, and co-surfactant. Solubility studies were performed to identify suitable oil, surfactant, and co-surfactant that have good drug solubilizing capacity. Non-ionic surfactants are the most preferred in self-emulsifying systems due to their non-toxic nature. Co-surfactants help to increase the emulsification area. Further, they have a synergistic effect with surfactants in drug dispensability and dissolution. Thus ethyl acetate was selected as oil, Captex-300 as surfactant, and PEG-400 as co-surfactant because the drug showed the highest solubility in them.

The phase diagram gives hint for the selection of the optimum ratio of oil, surfactant, and co-surfactant to form stable SNEDDS. Therange of needeed for oil, surfactant, and co-surfactant were further optimized based on the rsults of the study.

Self-emulsification time is a time to form a homogenous mixture upon dilution of formulated SNEDDS. Assessment of self-emulsification determines the self-emulsifying effect of the formulation.[33] Self-emulsification is specific to the nature of oil, the nature of SA/CoSA pair, and SA/CoSA concentration. Only a very specific combination of these ingredients could result in efficient self-nanoemulsification.[34]

Percentage transmission is an indication of transparency of diluted SNEDDS formulation. Transmission near 100% confirms the clarity and transparency of nanoemulsion formation.

Dissolution data confirmed the enhanced dissolution of clofazimine SNEDDS compared to their pure form. The polarity of oil droplets affects drug release from the diluted SNEDDS. Higher polarity indicates drug release is faster into the aqueous phase from the oil droplet.[38]

Formulations C1 and C4 have been shown to become turbid during the stability study. It may be due to a reduction in repulsive forces between the two particles. Further, due to the presence of Van der Waals attractive forces, two particles come closer and hence flocculation or precipitation of the system occurs as in the case of the C1 and C4 formulation batches.

Conclusion

Self-Nanoemulsifying Drug Delivery System (SNEDDS) seems to be a promising drug delivery system to tackle the problems seen with class II drugs. It has enhanced the bioavailability of clofazimine by efficient drug delivery and by altering physiological phenomena during absorption. The present study demonstrated a systematic approach for the formulation of SNEDDS which can be very useful for the oral delivery of many emerging hydrophobic drugs with good therapeutic potential.

Conflict of interest

None

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