ORIGINAL ARTICLE

Enhancement of Solubility and Dissolution Rate of Clopidogrel by Self-nanoemulsifying Drug Delivery System

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Abstract

Introduction: A self-nanoemulsifying drug delivery system (SNEDDS) has been explored to improve the solubility and dissolution rate of poorly water-soluble drug clopidogrel. **Materials and Methods:** Different formulations were prepared using an oil, surfactant, and cosurfactant in varying ratios. A pseudo-ternary phase diagram was constructed to identify the self-nanoemulsification region. Further, the resultant formulations were investigated for clarity, phase separation, drug content, % transmittance, globule size, freeze-thaw method, *in vitro* dissolution studies, particle size analysis, and zeta potential. **Results:** On the basis of particle size, zeta potential and dissolution profile and other studies, F6 was found to be the best formulation of clopidogrel SNEDDS. The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The particle size of the optimized SNEDDS formulation was found to be 5.2 nm and zeta potential was found to be –29 mV which comply with the requirement of the zeta potential for stability. The % release from optimized SNEDDS formulation F6 was highest (98.93%) and faster than other SNEDDS formulations and pure drug substance (32%) indicating influence of droplet size on the rate of drug dissolution. The faster dissolution from SNEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and on exposure to dissolution medium results in small droplet that can dissolve rapidly. Fourier transform infrared data revealed no physicochemical interaction between drug and excipients. **Conclusion:** Thus, clopidogrel with SNEDDS formulation may be used for the improvement of solubility and dissolution rate for the effective management of heart disease.

Key words: Acrysol K150, clopidogrel, myocardial infarction, self-nano emulsifying drug delivery system, solubility

INTRODUCTION

Drugs with poor solubility are difficult to formulate by applying conventional approaches as they pose problems such as slow onset of action, poor oral bioavailability, lack of dose proportionality, failure to achieve steady state plasma concentration, and undesirable side effects, thus resulting in over or under medication and poor patient compliance.[1] These challenges can be overcome by applying self-nanoemulsifying systems that offer benefits such as reduction in dose frequency, lowering of dose size, site-specific targeting, enhanced permeability, and improvement in oral bioavailability. Nanotechnology is a promising strategy in the development of drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility, low permeability, inadequate bioavailability, and other poor biopharmaceutical properties.[2] Self-nanoemulsifying drug delivery system (SNEDDS) formulations for poorly water-soluble drugs have shown considerable increase in solubility and bioavailability.[3] Clopidogrel, sold as the brand name Plavix among other is a medication that is used to reduce the risk of heart disease and stroke in those at high risk. The main aim of the study is to formulate and evaluate the SNEDDS clopidogrel formulation to improve its solubility and dissolution rate.

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MATERIALS AND METHODS

Materials

Clopidogrel was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad. Caproic acid, Gelucire 44/14, Transcutol P and Labrasol, Sunflower oil and Span 80, Capmul MCM C8, and Labrafil M 1944 were obtained from Strides Arcolab, Bengaluru, India. Acrysol K150 was obtained from Signet Chemicals Corporation Pvt. Ltd. Mumbai, India. Lauroglycol 90 was obtained from Ranbaxy Laboratories India. Captex 200 and Macrogol 400 were procured from Gattefosse France.

Methods

Solubility studies

The solubility study was used to find out the suitable oil, surfactant, and cosurfactant that possess good solubilizing capacity for clopidogrel. An excess amount (75 mg) of clopidogrel was added into 2 ml of each excipient oils, surfactants, cosurfactants, and kept in mechanical shaker for 24 h and centrifuged at 10,000 rpm for 20 min using a centrifuge. Supernatant was filtered through membrane filter using 0.45 µm filter disk. Filtered solution was appropriately diluted with methanol, and UV absorbance was measured at 235 nm. Concentration of dissolved drug was determined spectrophotometrically.[4]

% Transmittance

From the preliminary solubility studies, the selected vehicles were mixed at varying ratios. First, Smix (surfactant:co-surfactant) of different ratios (1:1, 2:1, 3:1, and 4:1) i prepared and later, Oil:Smix ratios ranging from 1:9 to 9:1 are prepared in 2 ml Eppendorf tubes and thoroughly shaken and kept aside. A fixed volume of water (100 ml) was added to calculated amount of vehicles (0.1 ml) and checked for phase separation and turbidity visually. Solutions with no phase separation and clear appearance were separated and used for checking transmittance using UV spectrophotometer at 235 nm. Maximum transmittance values indicate formation of small nanosize particles. Ratios showing percentage transmittance value more than 80 were considered for plotting pseudo-ternary phase diagram for representing self-emulsification region.

Construction of pseudo-ternary phase diagrams

Transmittance values more than 80 indicate formation of nanoemulsion; hence, these ratios were noted and selected for plotting pseudo-ternary phase diagram. Phase diagrams are plotted using CHEMIX software in which two ratios were fed and calculated, it shows an emulsification region which is shaded and from which 18 ratios for formulating SNEDDS were chosen.

Development of clopidogrel SNEDDS formulation

A series of SNEDDS formulations for clopidogrel were prepared based on solubility studies, %transmittance, and pseudo-ternary phase diagram. Here, Acrysol K150 was used as oil phase Captex 500 and Transcutol P were used as surfactant and cosurfactant, respectively. 75 mg of clopidogrel was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40°C. The surfactant and cosurfactant were added to the oily mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15 min and stored at room temperature until its use in subsequent studies [Table 1].

% Transmittance measurement

The percent transmittance of various SNEDDS formulations was measured at 235 nm using UV spectrophotometer keeping water as a blank.[5]

Determination of drug content

SNEDDS equivalent to 75 mg of clopidogrel was weighed accurately and dissolved in 100 ml 0.1N HCL. The solution was filtered, diluted suitable and drug content were analyzed at λ_{max} 235 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in SNEDDS}}{\text{Theoretical amount of drug in SNEDDS}} \times 100$$

Thermodynamic stability studies (freeze thawing)

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variations on SNEDDS formulations and all were subjected to freeze cycle (–20°C for 2 days followed by 40°C for 2 days) and stable formulations were further studied.[6]

Centrifugation

Centrifugation was performed at 3000 rpm for 5 min. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.[7]

Table 1: Formulation trials of clopidogrel SNEDDS

Smix (Surfactant:cosurfactant)	Oil:smix	Formulation code	Drug (mg) (Clopidogrel)	Oil (ml) (Acrysol K 150)	Surfactant (ml) (Captex 500)	Co-surfactant (ml) (Transcutol P)
1:01	1:09	F1	75	0.15	0.675	0.675
	2:08	F2	75	0.3	0.6	0.6
	3:07	F3	75	0.45	0.525	0.525
	4:06	F4	75	0.6	0.45	0.45
	5:05	F5	75	0.75	0.375	0.375
	6:04	F6	75	0.9	0.3	0.3
2:01	6:04	F7	75	0.9	0.4	0.2
	7:03	F8	75	1.05	0.3	0.15
	8:02	F9	75	1.2	0.2	0.1
	9:01	F10	75	1.35	0.1	0.05
	1:09	F11	75	0.15	0.9	0.45
	2:08	F12	75	0.3	0.8	0.4
3:01	8:02	F13	75	1.2	0.225	0.075
	9:01	F14	75	1.35	0.1125	0.0375
	1:09	F15	75	0.15	1.0125	0.3375
	2:08	F16	75	0.3	0.9	0.3
	3:07	F17	75	0.45	0.7875	0.2625
	4:06	F18	75	0.6	0.675	0.225

In vitro dissolution studies

The release of drug from liquid SNEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. SNEDDS of clopidogrel (equivalent to 75 mg of drug) was filled in size "0" hard gelatin capsules. The dissolution media are 0.1 N HCl and temperature of the dissolution medium was maintained at 37°C operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals 2, 5, 10, 15, 20, 25, 30, 45, and 60 min and filtered through 0.45 µm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh medium. The concentrations were assayed spectrophotometrically at 235 nm.

Characterization of SNEDDS

Drug-excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transform infrared (FTIR) spectroscopy method.

FTIR studies

An FTIR-8400S spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients. Analysis of pure drug, i.e., clopidogrel and physical mixtures of the drug with the excipients was carried out using diffuse reflectance spectroscopy-FTIR with KBr disk. All the samples were dried under vacuum before obtaining any spectra to remove the influence of residual moisture. For each the spectrum, eight scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 400–4000 cm^{-1}.

Determination of droplet size

The average droplet size of clopidogrel SNEDDS formulations was determined by photon correlation spectroscopy (Malvern Instrument UK) able to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement.[8]

Determination of zeta potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SNEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SNEDDS formulation was measured using a zeta meter system. The SNEDDS was diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta

potential of the resulting microemulsion was determined using a Zetasizer.[9]

Scanning electron microscopy (SEM)

Shape and surface morphology of microspheres was studied using SEM. The SNEDDS after converting to emulsion was mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument HITACHI, S-3700N.[10]

Stability studies

Stability testing was conducted at 40°C ± 2°C/75% RH ± 5% RH for 3 months using stability chamber (Thermo Lab, Mumbai). The samples were withdrawn at predetermined intervals 0, 30, 60, and 90 days period according to the ICH guidelines. Various *in vitro* parameters such as % yield, entrapment efficiency, and *in vitro* release studies were evaluated.[11]

RESULTS AND DISCUSSION

Solubility studies

Preliminary solubility analysis was carried out to select the appropriate excipient from various oils, surfactants, and cosurfactants. The solubility of pure drug was found to be 0.51 mg/ml, based on drug solubility, Acrysol K150 was used as oil phase Captex 500 and Transcutol P were used as surfactant and cosurfactant, respectively. The solubility of the drug in these vehicles was found to be highest compared to water and other vehicles [Figures 1-3].[12,13]

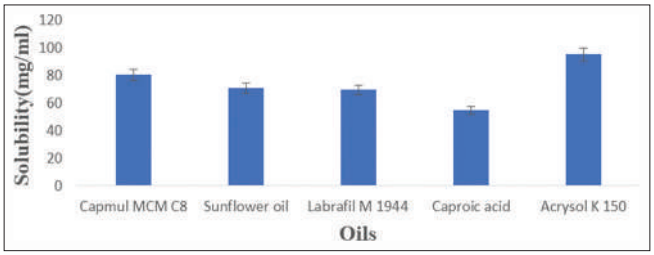

Figure 1: Solubility studies of clopidogrel in oils

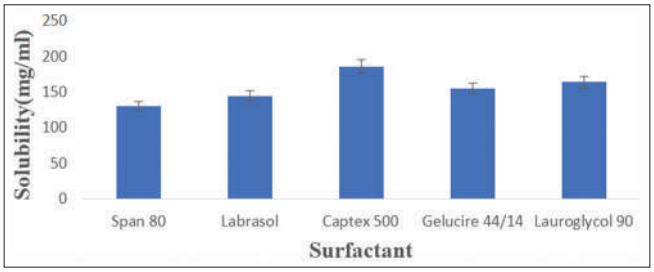

Figure 2: Solubility studies of clopidogrel in surfactant

Pseudo-ternary phase diagram

From the solubility studies, Acrysol K150, Captex 500, and Transcutol P were selected as oil, surfactant, and cosurfactant, respectively. From the phase diagram [Figure 4], it was observed that self-emulsifying region was enhanced with increasing concentrations of surfactant and cosurfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased.[14,15]

% Transmittance measurement of clopidogrel SNEDDS

The clarity of nanoemulsion was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w nanoemulsion since water is external phase formulation F6 has % transmittance value greater than 98.88%. These results indicate the high clarity of nanoemulsion. In case of other systems, %T values were less suggesting less clarity of nanoemulsion. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of nanoemulsion and thereby values of %T [Table 2].[16,17]

Drug content of clopidogrel SNEDDS

The drug content of the prepared SNEDDS was found to be in the range of 86.17–99.01% and maximum % drug content, i.e., 99.01% was found in the formulation F6 [Table 2].

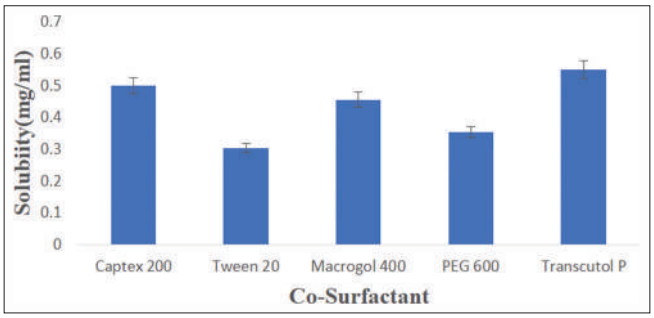

Figure 3: Solubility studies of clopidogrel in cosurfactants

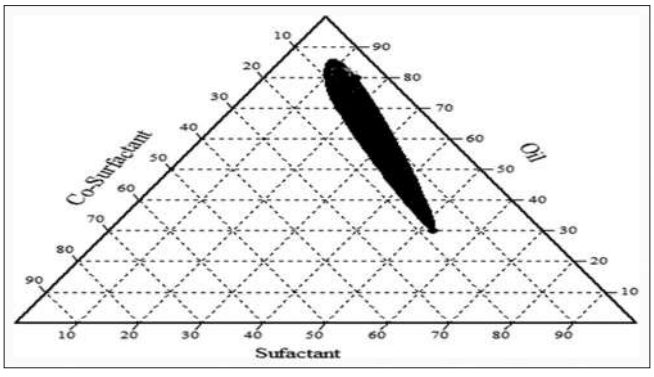

Figure 4: Ternary-phase diagram of Acrysol K 150, Captex 500, and Transcutol P

Table 2: % Transmittance and drug content of different formulations

S. No.	Formulation code	% Transmittance	% Drug content
1.	F1	73.41	90.17
2.	F2	88.14	91.47
3.	F3	90.47	88.25
4.	F4	71.14	93.14
5.	F5	80.14	94.84
6.	F6	98.88	99.01
7.	F7	90.17	91.47
8.	F8	82.47	92.17
9.	F9	72.18	96.27
10.	F10	81.27	94.22
11.	F11	89.27	96.37
12.	F12	80.14	93.12
13.	F13	83.22	88.37
14.	F14	90.1	89.37
15.	F15	91.42	95.01
16.	F16	88.77	86.17
17.	F17	94.28	91.24
18.	F18	93.21	92.88

Thermodynamic stability studies and centrifugation

No phase separation and effect of temperature variations on prepared formulations were observed during thermodynamic stability studies. There was no change in the visual description of samples after centrifugation freeze-thaw cycles. Formulations which are thermodynamically stable only those were selected for further characterization [Table 3].

In vitro dissolution studies of clopidogrel SNEDDS formulation

The faster dissolution from SNEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and on exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The release from liquid SNEDDS formulation F6 was faster and highest (98.93%) than other SNEDDS formulations and pure drug substance (32%) indicating influence of droplet size on the rate of drug dissolution [Figures 5-8].

Drug excipient compatibility studies by FTIR spectroscopy

The FTIR spectrum of pure clopidogrel [Figure 9] showed a peak at 1753 cm^{-1} due to C=O stretching vibrations and at 3012 cm^{-1} due to O–H stretching of the hydrogen sulfate moiety and due to aromatic C–H stretching vibrations

Table 3: Thermodynamic stability studies of the formulations

Formulation code	Centrifugation	Freeze thaw method	
		–20°C for 2 days	+40°C for 2 days
F1	No phase separation	No change	No change
F2	No phase separation	No change	No change
F3	No phase separation	No change	No change
F4	No phase separation	No change	No change
F5	No phase separation	No change	No change
F6	No phase separation	No change	No change
F7	No phase separation	No change	No change
F8	No phase separation	No change	No change
F9	No phase separation	No change	No change
F10	No phase separation	No change	No change
F11	No phase separation	No change	No change
F12	No phase separation	No change	No change
F13	No phase separation	No change	No change
F14	No phase separation	No change	No change
F15	No phase separation	No change	No change
F16	No phase separation	No change	No change
F17	No phase separation	No change	No change
F18	No phase separation	No change	No change

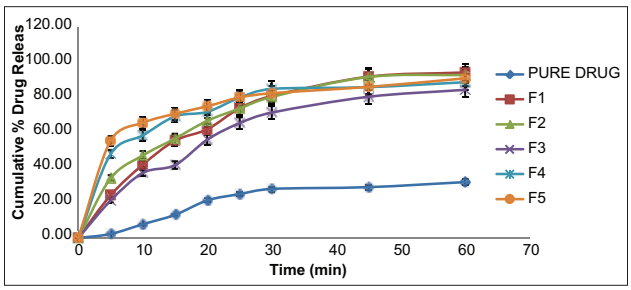

Figure 5: Dissolution profiles of clopidogrel pure drug and formulations (F1 to F5)

represented at 3414 cm^{-1} and broad absorbance band at 2343 cm^{-1} which is due to the stretching vibrations of bonded N–H resulting from salt formation between the quaternary nitrogen of clopidogrel and –OH of hydrogen sulfate and band associated with C–O stretching appeared at 1066, 1176, and 1220 cm^{-1} [Figure 10]. The FTIR spectra of clopidogrel optimized formulation [Figure 11] F6 showed similar prominent peaks pure drug and these results indicate the absence of any chemical interactions between the drug clopidogrel and used excipients in the formulation.[18,19]

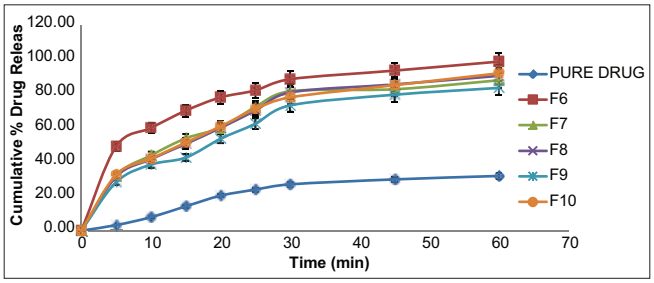

Figure 6: Dissolution profiles of clopidogrel pure drug and formulations (F6 to F10)

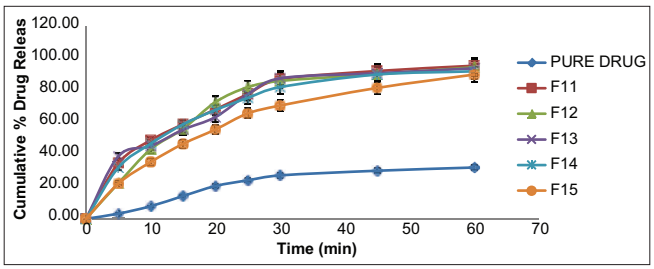

Figure 7: Dissolution profiles of clopidogrel pure drug and formulations (F11 to F15)

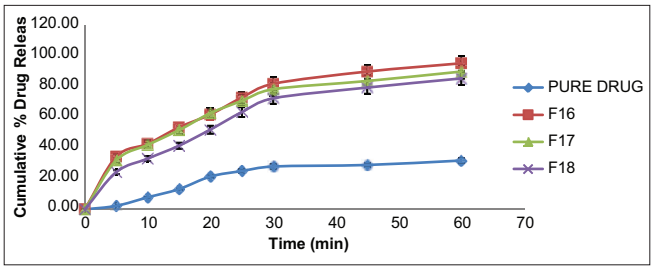

Figure 8: Dissolution profiles of clopidogrel pure drug and formulations (F16 to F18)

Particle size analysis of SNEDDS

Droplet size determines the rate and extent of drug release as well as drug absorption. Smaller the particle size, larger the interfacial surface area which may lead to more rapid absorption and improved bioavailability. SNEDDS with a mean droplet size below 200 nm exhibit excellent bioavailability. The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The particle size of the optimized SNEDDS formulation (F6) was found to be 5.2 nm and Z-Average of 4.7 nm indicating all the particles were in the nanometer range [Figure 9].

Zeta potential of SNEDDS

Zeta potential is responsible for the degree of repulsion between adjacent, similarly charged, and dispersed droplets. A zeta potential value of ±30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SNEDDS formulation (F6) was found to be -29.0 mV which comply with the requirement of the Zeta potential for stability [Figure 12].

Scanning electron microscopy (SEM) for clopidogrel SNEDDS

Scanning electron microscope studies of optimized formulation of clopidogrel (F6) revealed oval shaped

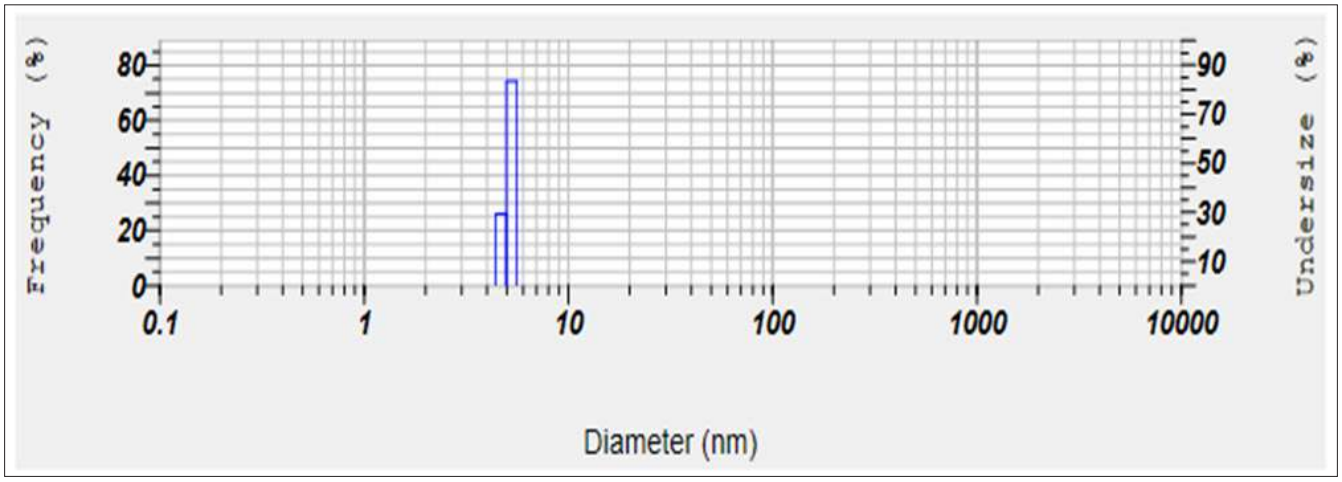

Figure 9: Particle size analysis of optimized formulation F6

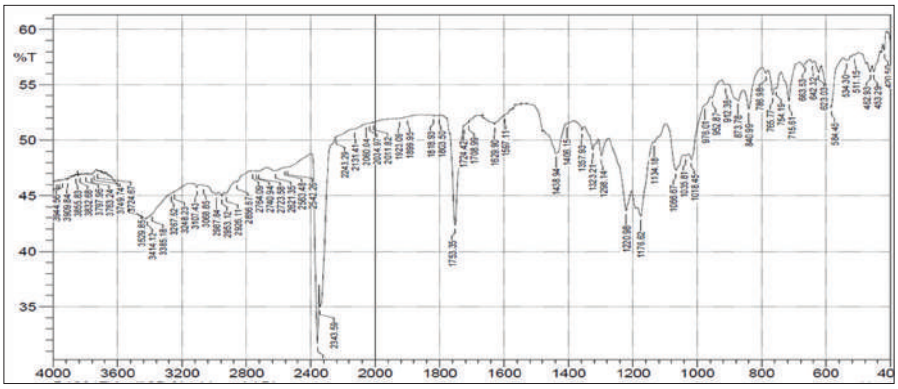

Figure 10: Fourier transform infrared spectroscopy of clopidogrel pure drug

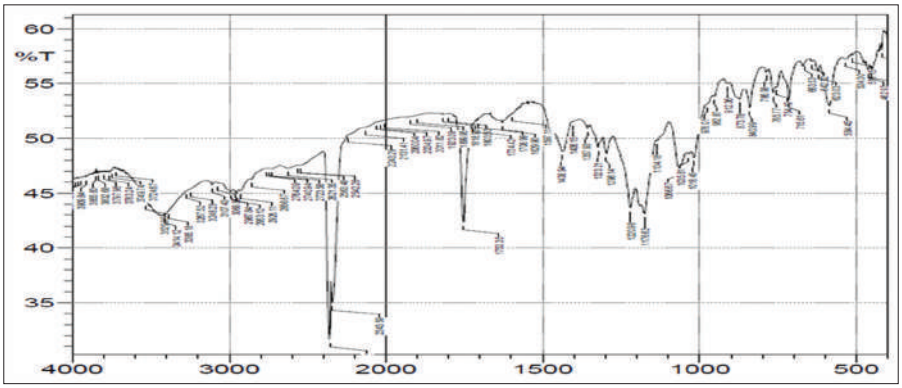

Figure 11: Fourier transform infrared spectroscopy of clopidogrel optimized formulation F6

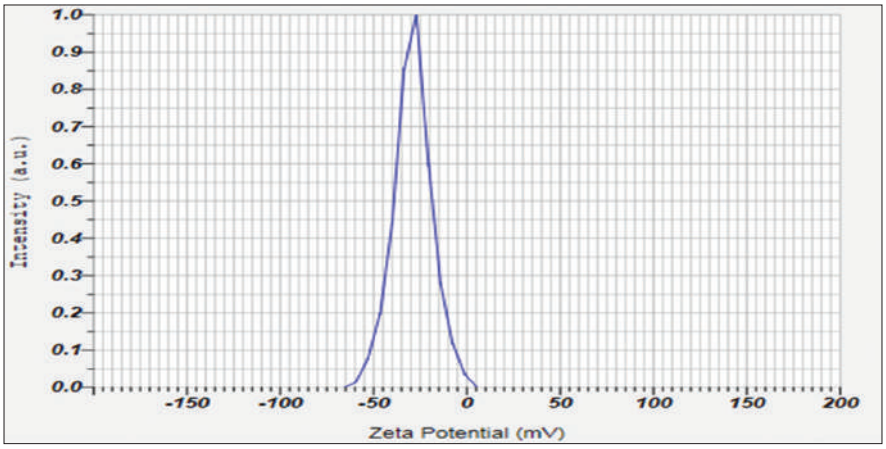

Figure 12: Zeta potential of the optimized formulation F6

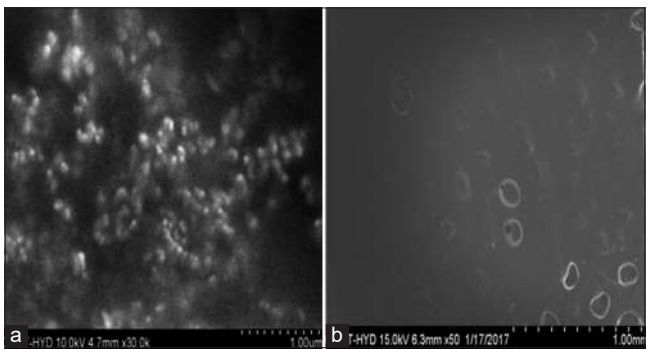

Figure 13: (a and b) Scanning electron microscopy of clopidogrel optimized formulation (F6)

globules. The size is within nanometers and there are clear liquid droplets without any pores [Figure 13a and b].

Stability studies

The clopidogrel SNEDDS F6 formulation was filled in hard gelatin capsules as the final dosage form and subjected to stability studies for 3 months. There was no significant change in the drug content and drug release. It was also seen that the formulations were compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance, or microemulsifying property [Table 4].

Table 4: Evaluation parameters of optimized formulation (F6) stored at 40 ±2°C/75 ±5% RH

Retest time for optimized formulation (F6) (days)	% Drug content	*In vitro* drug release (%)
0	99.01	98.93
30	98.76	98.12
60	98.09	97.88
90	97.67	97.06

CONCLUSION

SNEDDS of clopidogrel comprising Acrysol K150, Captex 500, and Transcutol P was prepared for enhancing the solubility and dissolution clopidogrel. SNEDDS was optimized based on the optimum globule size, increased dissolution, and drug release. Close to complete drug release was achieved from the formulation F6 which is significantly higher as compared to that of pure drug. Thus, the developed SNEDDS can be used as an effective approach for the management of heart attack with relatively low drug dose with higher solubility and dissolution rate.

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