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Research Article

Formulation and *In-vivo* Studies of Clopidogrel by Self-nanoemulsifying Drug Delivery System

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ABSTRACT

A self-nano emulsifying drug delivery system (SNEDDS) has been explored to improve poorly water-soluble drug clopidogrel's solubility and dissolution rate. Different formulations were prepared using oil, surfactant, and co-surfactant in varying ratios. From the ternary phase diagram resultant formulations were investigated for clarity, phase separation, drug content, % transmittance, globule size, freeze-thaw, in vitro dissolution studies, particle size analysis, and zeta potential. Based on particle size, zeta potential and dissolution profile, and other studies, F6 was the best formulation of clopidogrel SNEDDS. 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 complies 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 the influence of droplet size on the drug dissolution rate. FTIR data revealed no physicochemical interaction between drug and excipients. In vivo bioavailability studies were carried out on the optimized formulation (F6), mean time to attain peak drug concentration (T_{max}) was 0.5 ± 0.53 and 1.5 ± 0.72 minutes for the optimized and pure drug, respectively, while means maximum drug concentration (C_{max}) was 6.77 ± 1.73 ng/mL and 2.10 ± 0.39 ng/mL respectively. $AUC_{0-\alpha}$ and AUC_{0-t} for the optimized formulation were significantly higher ($p<0.05$) 20.5 ± 2.48 ng.h/mL than the pure drug 6.34 ± 1.73 ng h/mL, respectively. Thus, the results indicate clopidogrel with SNEDDS formulation may be used to improve solubility and dissolution rate for the effective management of heart disease.

Introduction

Drugs with poor solubility are difficult to formulate by applying conventional approaches as they pose problems such as the 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-nano emulsifying systems that offer benefits like reduction in dose frequency, lowering of dose size, site-specific targeting, enhanced permeability, and improvement in oral bioavailability.[2] Nanotechnology is a promising strategy in drug delivery systems, especially for those potent drugs whose clinical development failed due to poor solubility, low permeability, inadequate bioavailability, and other poor biopharmaceutical properties. SNEDDS formulations for poorly water-soluble drugs have shown a considerable increase in solubility and bioavailability. Clopidogrel, sold as the brand name Plavix among others, is used to reduce the risk of heart disease and stroke in those at high risk.[3-5] The study's main aim is to formulate and evaluate the SNEDDS clopidogrel formulation to improve its solubility and dissolution rate.

Materials and Methods

Clopidogrel was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad. Caproic acid, gelucire 44/14, transcutol p and labrasol, sunflower oil and

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span 80, capmul MCM C8 and labrafil M 1944 were obtained from Strides Arcolab, Bangalore, 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 Gattefose France.

Solubility Studies

The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for clopidogrel. An excess amount (75 mg) of clopidogrel was added to 2 mL of each excipient oil (capmul MCM C8, sunflower oil, labrafil M 1944, caproic acid acrysol K 150). Surfactants (span 80, captex 500, gelucire 44/14, labrasol and lauroglycol 90). Co-surfactants (captex 200, PEG 600, tween 20, macrogol 400, and transcotlol P) and kept in mechanical shaker for 24 hours and centrifuged at 10,000 rpm for 20 minutes using a centrifuge. The 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. The concentration of dissolved drug was determined spectrophotometrically.[6]

Pseudo Ternary Phase Diagram

To determine the concentration of components for the existing range of SNEDDS, pseudo ternary phase diagram was constructed using the water titration method at ambient temperature (25°C). Surfactant and co-surfactant (S_{mix}) in each group were mixed in different volume ratio (1:1, 2:1, 3:1). Oil and surfactant/co-surfactant mixture (S_{mix}) were mixed thoroughly in different volume ratios 1:9 to 9:1 for all the three Smix ratios 1:1, 2:1, 3:1. Under gentle agitation, the mixture of oil, surfactant, and co-surfactant at certain ratios was titrated with water by dropwise addition. Deionized water was used as diluting medium and added to the formulation. The proper ratio of one excipient to another in the SNEDDS formulation was analyzed. Pseudo ternary plots were constructed using Chemix software.[7]

Visual Observation

A visual test to assess the self-emulsification properties was modified and used in the present study. Using this method, a predetermined volume of the mixture (0.2 mL) was added to 300 mL of water in a glass beaker under stirring, and the temperature was maintained at 37°C using a magnetic stirrer. The tendency of formation of the emulsion was observed. If the droplet spreads easily in water was judged as 'good' and judged as 'bad' when there was milky or no emulsion or presence of oil droplets (Tables 1 to 3).[8]

Development of SNEDDS Formulation

A series of SNEDDS formulations for clopidogrel were prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Here, acrysol K150was used as oil phase captex 500 and transcutol P as surfactant and co-surfactant, respectively. 75 mg of clopidogrel was added in an accurately weighed amount of oil into a screw-capped glass vial and heated in a water bath at 37°C. The surfactant and co-surfactant were added to the oily mixture using a positive displacement pipette and stirred with a magnetic bar. The formulation was further sonicated for 15mins and stored at room temperature until its use in subsequent studies (Table 4).[9,10]

%Transmittance Measurement

The percent transmittance of various SNEDDS formulations was measured at 235 nm using UV spectrophotometer, keeping water as a blank.[11]

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 was analyzed at λ_{max} 235 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

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

Table 1: Visual observation test for S_{mix} (Surfactant: Co-surfactant) ratio 1:1

Oil: S_{mix}	*Time of emulsification (Min)*	*Grade*
01:09	<1	I
02:08	<1	I
03:07	<2	I/ II
04:06	<2	III
05:05	<2	III
06:04	<1	I
07:03	<1	I
08:02	<1	I
09:01	<1	I

Table 2: Visual observation test for S_{mix} (surfactant: C o-surfactant) ratio 2:1

Oil: S_{mix}	*Time of emulsification (Min)*	*Grade*
01:09	<1	I
02:08	<1	I
03:07	<2	I/ II
04:06	<2	III
05:05	<2	III
06:04	<1	I
07:03	<1	I
08:02	<1	I
09:01	<1	I

Freeze Thawing

The main objective of this study was to evaluate the phase separation and effect of temperature variations on SNEDDS formulations and were subjected to freeze cycle (-20°C for 2 days followed by 40°C for 2days) and stable formulations were further studied.[12]

Centrifugation

Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.[13]

Table 3: Visual observation test for S_{mix} (surfactant: co-surfactant) ratio 3:1

Oil: S_{mix}	*Time of emulsification (Min)*	*Grade*
01:09	<1	I
02:08	<1	I
03:07	<2	I/ II
04:06	<2	III
05:05	<2	III
06:04	<1	I
07:03	<1	I
08:02	<1	I
09:01	<1	I

In-vitro Dissolution Studies

The release of the drug from liquid SNEDDS formulations and the 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 is 0.1N HCl, and the 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 minutes 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.[14]

Characterization of SNEDDS

Drug-excipient Compatibility Studies

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

FTIR Studies

An FTIR-8400S Spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) 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

Table 4: Formulation Trials Of Liquid SNEDDS

Smix (Surfactant: Co-surfactant)	*Oil: Smix*	*Formulation Code*	*Drug (Clopidogrel)*	*Oil (Acrysol K 150)*	*Surfactant (Captex 500)*	*Co-surfactant (Transcutol P)*
			(mg)	*(mL)*	*(mL)*	*(mL)*
01:01	01:09	F1	10	0.15	0.675	0.675
	02:08	F2	10	0.3	0.6	0.6
	03:07	F3	10	0.45	0.525	0.525
	04:06	F4	10	0.6	0.45	0.45
	05:05	F5	10	0.75	0.375	0.375
	06:04	F6	10	0.9	0.3	0.3
	06:04	F7	10	0.9	0.4	0.2
02:01	07:03	F8	10	1.05	0.3	0.15
	08:02	F9	10	1.2	0.2	0.1
	09:01	F10	10	1.35	0.1	0.05
	01:09	F11	10	0.15	0.9	0.45
	02:08	F12	10	0.3	0.8	0.4
03:01	08:02	F13	10	1.2	0.225	0.075
	09:01	F14	10	1.35	0.1125	0.0375
	01:09	F15	10	0.15	1.0125	0.3375
	02:08	F16	10	0.3	0.9	0.3
	03:07	F17	10	0.45	0.7875	0.2625
	04:06	F18	10	0.6	0.675	0.225

drug with the excipients, were conducted using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum before obtaining any spectra to remove the influence of residual moisture. For each spectrum, 8 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) to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement.[15]

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 (Malvern Zetasizer ZS90). The SNEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with a magnetic stirrer. The Zeta-potential of the resulting microemulsion was determined using a Zetasizer.[16]

Scanning Electron Microscopy

The shape and surface morphology of microspheres was studied using scanning electron microscopy (SEM). The SNEDDS, after converting to emulsion were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument HITACHI, S-3700N.[17]

Stability Studies

Stability testing was conducted at 40 ± 2°C / 75% RH ± 5% RH for 3 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0-, 30-, 60-, and 90-days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated.[18]

Pharmacokinetic Studies of Clopidogrel

Healthy Wistar rats were (weighing 150–180 g) selected for this study, all the animals were healthy during the experiment period. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25°C, Relative Humidity 45% and 12 hours alternate light and dark cycle) with 100% fresh air exchange in animal rooms, uninterrupted power, and water supply. Rats were fed with a standard diet and water *ad libitum.* The protocol of the animal study was approved by the institutional animal ethics committee (IAEC) with reference No: 1292/ac/09/CPCSEA/47.

Study Design

Rats were divided into two groups at random, with six rats each. The treatments as given below were administered to the rabbits.

The rats fasted for 24 hours before the experiments. After 4 hours of dosing, foods were reoffered. The first group was administered with pure clopidogrel (as such) made suspension with 0.5% methocel, and second group was administered prepared clopidogrel optimized SNEDDS diluted in 0.5% methocel by oral route at a dose of 1.17 mg.[19]

HPLC Determination of Clopidogrel in Rat Plasma

Acetonitrile: phosphoric acid (70:30, v/v), methanol: water (60:40), methanol: acetonitrile: phosphoric acid (50:30:20, v/v/v), and methanol: 25 mM aqueous Phosphoric acid (80:20, v/v) (pH 3.0) were used as isocratic mobile phases pumped at different flow rates in the range of 1–2.5 $mL.min^{-1}$. The injection volume was kept 50 mL, and the eluents were monitored at various wavelengths in the range of 210–250 nm. The column oven temperature was studied in the range of 15–40°C. Diclofenac sodium (at the level of 1.0 mg mL^{-1}) was used as internal standards (IS).[20]

Pharmacokinetic Analysis

The pharmacokinetic parameters evaluate C_{max} (maximum plasma concentration), T_{max} (time to attain C_{max}), $AUC_{0\text{-}t}$ (area under plasma concentration-time curve from zero to the last sampling time), $AUC_{0\text{-}\infty}$ (area under plasma concentration-time curve from zero to infinity).

The $AUC_{0\text{-}t}$ was calculated formula

$$AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + \frac{C_t}{K_E}$$

Results and Discussion

Solubility Studies

Preliminary solubility analysis was carried out to select the appropriate excipient from various (Oils - capmul MCM C8, sunflower oil, labrafil M 1944, caproic acid, and acrysol K 150). Surfactants – (span 80, captex 500, gelucire 44/14, labrasol and lauroglycol 90). Co-surfactants (captex 200, PEG 600, tween 20, macrogol 400 and transcotlol P). The solubility of the pure drug was 0.51 mg/mL, based on drug solubility, Acrysol K150 was used as oil phase Captex 500and Transcutol P was used as surfactant and co-surfactant, respectively. The solubility of the drug in these vehicles was highest compared to water and other vehicles. (Fig. 1, 2 & 3).

Pseudo Ternary Phase Diagram

From the solubility studies, acrysol K150, captex 500, and transcutol P were selected as oil, surfactant, and co-surfactant. The phase diagram (Fig. 4) observed that the self-emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil.

The efficiency of self-emulsification was good when the surfactant concentration increased.

Visual Observation

With the use of visual observation method, the tendency of formation of emulsion was observed. Visual observation test was performed for different ratios by keeping the surfactant and co-surfactant ratio (S_{mix}) as 1:1, 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of nanoemulsion. Ratios 1:9, 2:8, 3:7, 4:6, 5:5and 6:4 of S_{mix} 1:1 and 6:4,7:3,8:2,9:1,1:9 and 2:8 of S_{mix} 2:1 and 8:2,9:1,1:9,2:8,3:7 and 4:6 of S_{mix} 3:1 showed rapid formation of microemulsion within a minute having a clear appearance. Therefore, these ratios were selected for the formulation of SNEDDS. (Tables 4 to 6 and Fig. 5)

% Transmittance Measurement

The clarity of nanoemulsion was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w nanoemulsion since water is an external phase. Formulation F6 has %transmittance value greater than 98.88%. These results indicate the high clarity of nanoemulsion. In the case of other systems %T values were less, suggesting less clarity of nanoemulsion. This may be due to the greater particle size of the formulation. Due to larger particle size, oil globules may reduce the transparency of nanoemulsion and thereby values of %T. (Table 6)

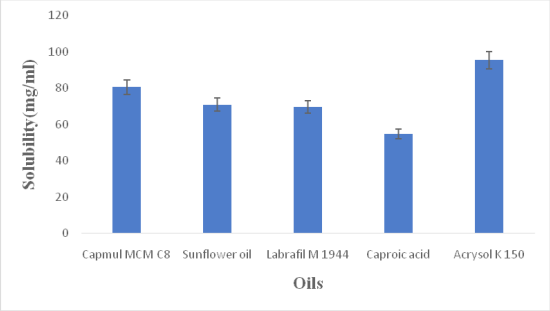

Fig. 1: Solubility studies of clopidogrel in oils

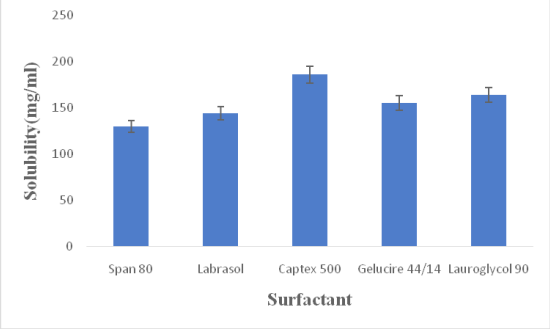

Fig. 2: Solubility studies of Clopidogrel in surfactant

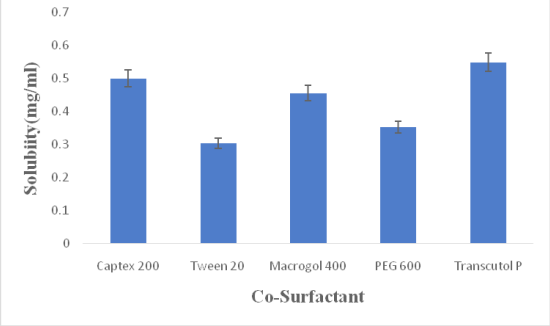

Fig. 3: Solubility studies of Clopidogrel in co-surfactants

Drug Content of 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 5).

Thermodynamic Stability Studies

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. Thermodynamically stable formulations only were selected for further characterization (Table 6).

In-vitro Dissolution Studies of SNEDDS

The faster dissolution from SNEDDS may be attributed to the fact that the drug is a solubilized form in this formulation and, upon exposure to dissolution medium, results in small droplets 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 substances (32%), indicating the influence of droplet size on the rate of drug dissolution (Fig 6 to 9).

Drug Excipient Compatibility Studies by FTIR Spectroscopy

The FTIR spectrum of pure Clopidogrel (Fig. 11) 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 represented at 3414 cm^{-1} and broad absorbance band at 2343 cm^{-1} which is due to the stretching vibrations of

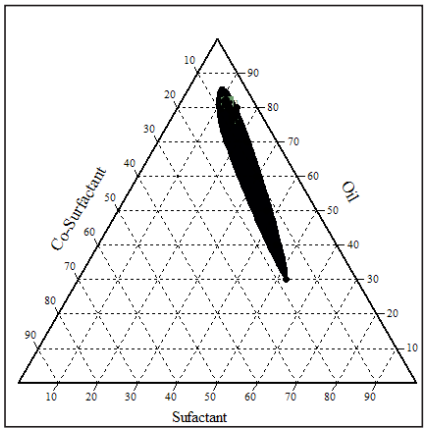

Fig. 4: Ternary phase diagram of Acrysol K 150, Captex 500 and Transcutol P

Table 5: %Transmittance of different formulations

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

Table 6: Thermodynamic stability studies of the formulations

		Freeze thaw method	
Formulation code	*Centrifugation*	*-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

bonded N–H resulting from salt formation between the quaternary nitrogen of clopidogrel and –OH of hydrogen sulfate. The band associated with C–O stretching

Fig. 5: Visual observation test

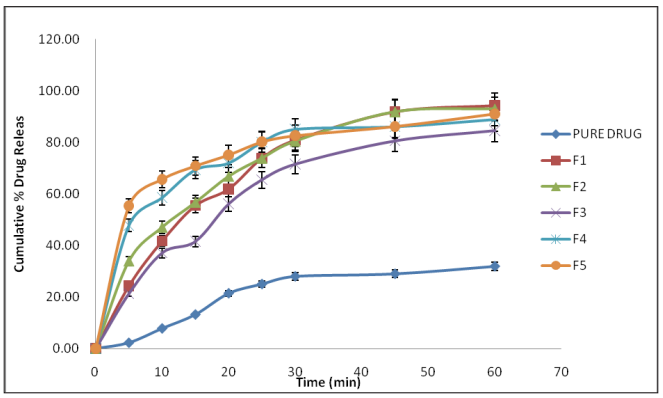

Fig. 6: Dissolution profiles of Clopidogrel pure drug and formulations (F1 to F5)

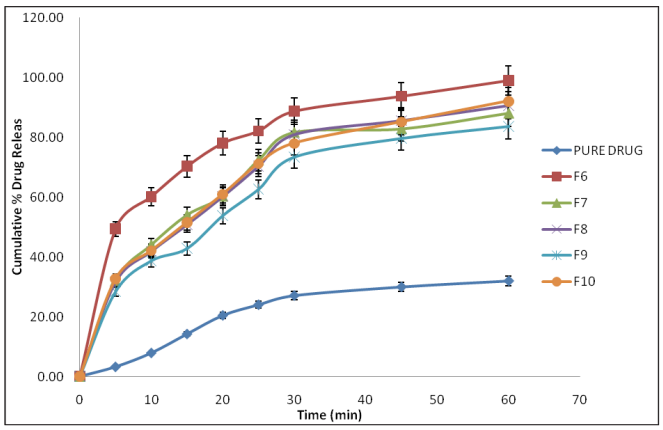

Fig. 7: Dissolution profiles of Clopidogrel pure drug and formulations (F6 to F10)

appeared at 1066, 1176 and 1220 cm^{-1}. The FTIR spectra of Clopidogrel pure drug (Fig. 10) F6 showed similar prominent peaks of optimized formulation (Fig. 11), and these results indicate the absence of any chemical interactions between the drug clopidogrel and used excipients in the formulation.

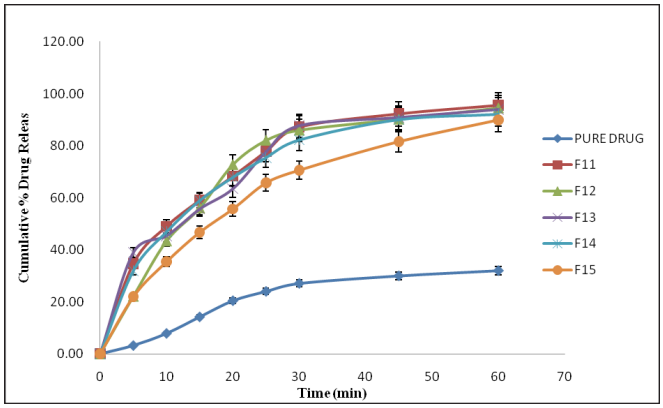

Fig. 8: Dissolution profiles of Clopidogrel pure drug and formulations (F11 to F15)

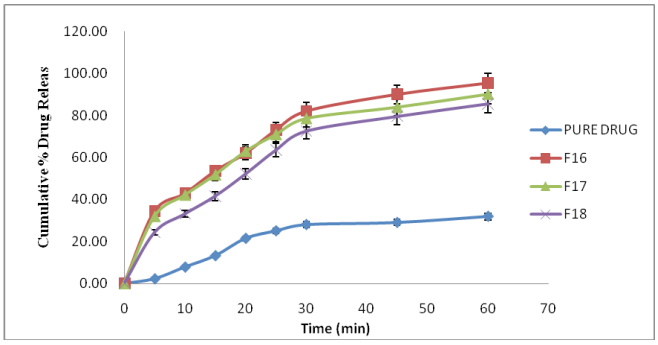

Fig. 9: Dissolution profiles of Clopidogrel pure drug and formulations (F16 to F18)

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, leading 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 and absorption. The particle size of the optimized SNEDDS formulation (F6) was 5.2 nm & Z-Average of 4.7 nm, indicating all the particles were in the nanometer range (Fig. 12).

Zeta Potential of SNEDDS

Zeta potential is responsible for the degree of repulsion between adjacent, similarly charged, dispersed droplets. A zeta potential value of ± 30 mV is sufficient for the stability of a microemulsion. The zeta potential of the optimized SNEDDS formulation (F6) was -29.0 mV, which complies with the zeta potential for stability (Fig. 13).

Scanning Electron Microscopy (SEM) for Clopidogrel SNEDDS

Scanning electron microscope studies of optimized formulation of clopidogrel (F6) revealed oval-shaped globules. The size is within nanometers and there are clear liquid droplets without any pores (Fig 14A, 14B).

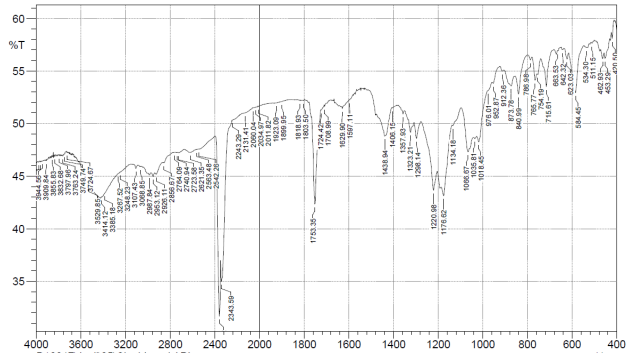

Fig. 10: FTIR Spectroscopy of Clopidogrel pure drug

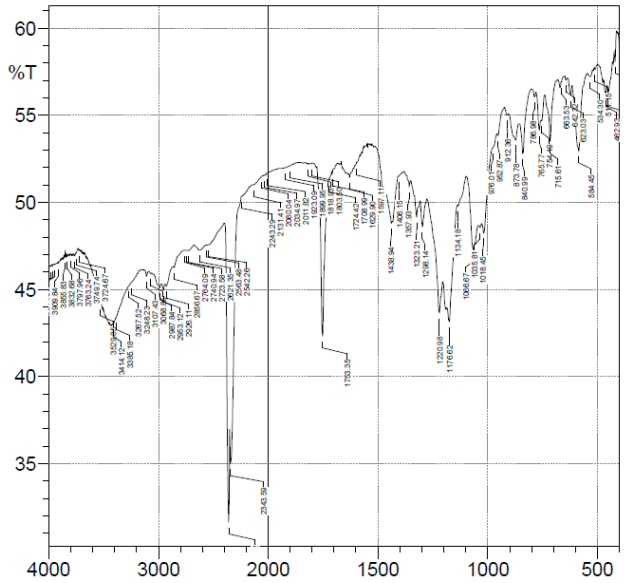

Fig. 11: FTIR Spectroscopy of Clopidogrel optimized formulation F6

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 micro emulsifying property (Table 7).

Pharmacokinetic Data of Clopidogrel

Clopidogrel concentrations in plasma following oral administration of pure drug, optimized clopidogrel SNEDDS administered oral route, and respective plasma concentration-time curves are shown in Fig. 15 to 17. The pharmacokinetic parameters were calculated as per the equations explained earlier, and the results are shown in Table 8.

Fig. 16 shows the plasma concentration-time curve in Wister rats after a single oral dose of clopidogrel SNEDDS formulation compared to clopidogrel pure. At all the indicated time points, the clopidogrel plasma

concentrations in rats treated with SNEDDS formulation were significantly higher than those treated with pure drug. Pharmacokinetic parameters of clopidogrel after oral administration of the two formulations in Wister rats are shown in Table 8.

C_{max} of the SNEDDS 6.77 ± 1.73 ng/mL was significant ($p < 0.05$) as compared to the pure drug 2.10 ± 0.39 ng/mL. T_{max} of both SNEDDS formulation and pure drug was 0.5 ± 0.53 and 1.5 ± 0.72 minutes, respectively. AUC is an important parameter in evaluating drug bioavailability from the dosage form. It represents the total integrated area under the blood concentration-time profile and the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for SNEDDS

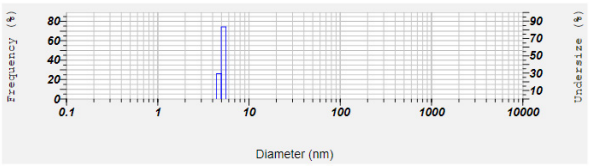

Fig. 12: Particle size analysis of optimized formulation F6

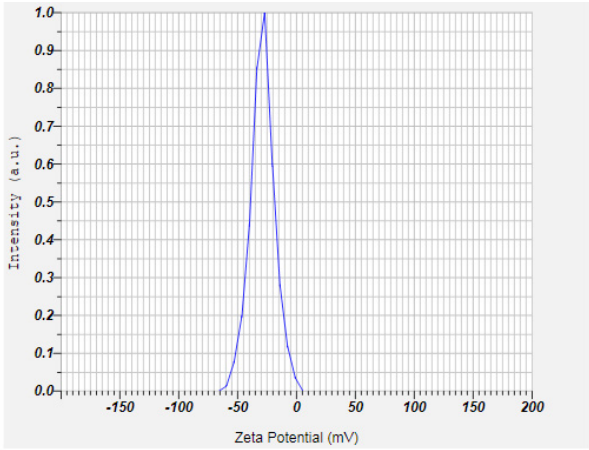

Fig. 13: Zeta potential of the optimized formulation F6

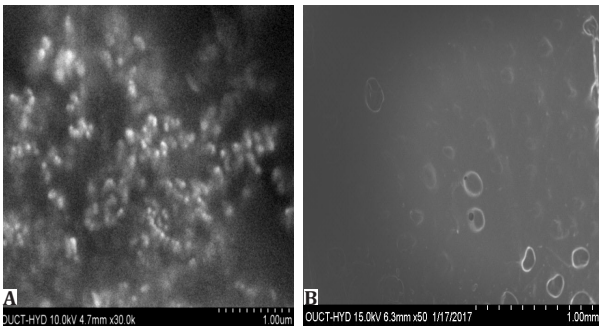

Fig. 14: Scanning Electron Microscopy of Clopidogrel optimized formulation (F6)

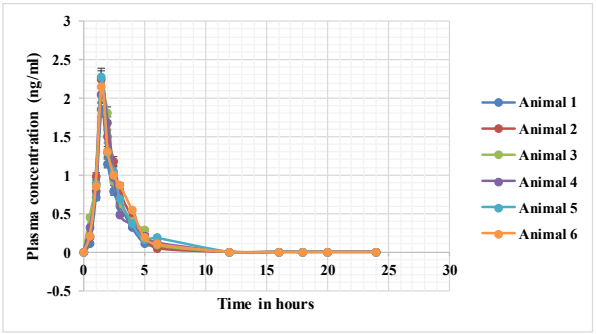

Fig.15: Plasma concentration-time profile of clopidogrel pure drug in rat plasma

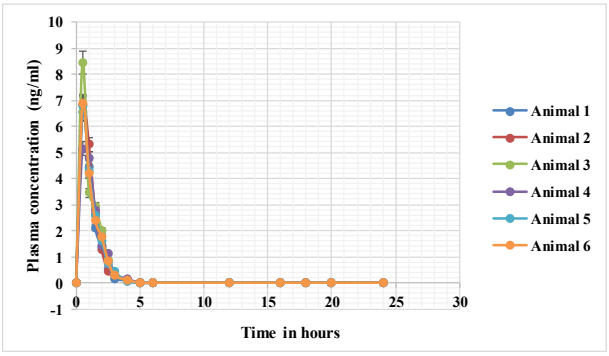

Fig. 16: Plasma concentration-time profile of clopidogrel optimized SNEDDS in rat plasma

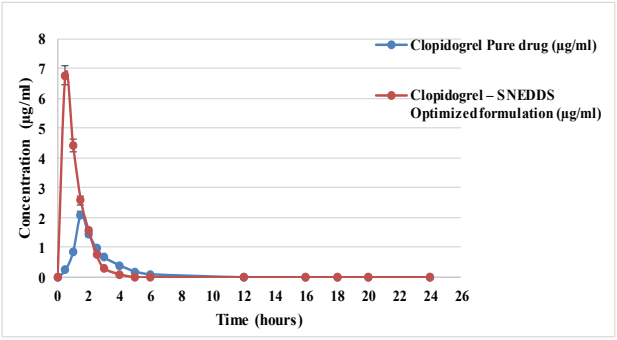

Fig. 17: Mean plasma concentration-time profiles for clopidogrel pure drug and clopidogrel optimized SNEDDS formulation in rats (n = 6)

Table 7: Evaluation parameters of optimized formulation (F6) stored at 40 ± 2°C /75 ± 5%RH

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

Table 8: Mean pharmacokinetic parameters of clopidogrel pure drug and clopidogrel optimised SNEDDS formulation

Pharmacokinetic parameters	*Clopidogrel Pure drug*	*Clopidogrel optimised SNEDDS*
C_{max} (ng/mL)	2.10 ± 0.39	6.77 ± 1.73
AUC_{0-t} (ng.h/mL)	5.11 ± 1.02	16.81 ± 0.21
AUC_{0-inf} (ng.h/mL)	6.34 ± 1.73	20.5 ± 2.48
T_{max} (h)	1.5 ± 0.72	0.5 ± 0.53
$t_{1/2}$ (h)	1.8 ± 0.02	0.8 ± 0.05

formulation was higher (20.5 ± 2.48 ng.h/mL) than the pure drug 6.34 ± 1.73 ng.h/mL. Statistically, AUC_{0-t} of the SNEDDS formulation (16.81 ± 0.21 ng.h/mL) was significantly higher ($p<0.05$) as compared to the pure drug (5.11 ± 1.02 ng.h/mL). A higher amount of drug concentration in blood indicated better systemic absorption of clopidogrel from SNEDDS formulation than the pure drug.

Conclusion

SNEDDS of clopidogrel comprising of acrysol K150, captex 500 and transcutol P were prepared for enhancing the solubility and dissolution clopidogrel. SNEDDS were 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 than that of pure drug. Thus, the developed SNEDDS can be used as an effective approach for managing heart attacks with relatively low drug dose with higher solubility and bioavailability.

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