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“Design and Development of Self Microemulsifying Drug Delivery System of Clopidogrel Bisulphate”

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Abstract:

The aim of this investigation was to design and develop SMEDDS formulation of clopidogrel for enhancing its solubility. Oral bioavailability of clopidogrel is very low (less than 50%), due to its poor water solubility. For this purpose, initially, solubility of clopidogrel was determined in various vehicles. SMEDDS was prepared with Capmul MCM NF as oil, Acrysol K140 as surfactant and Propylene Glycol as co-surfactant after preliminary screening of excipients. The system was optimized by ternary phase diagram. The prepared formulations were characterized for Drug content, % Transmittance, Visual assessment of self emulsification, particle size and *In-vitro* drug release. The optimized system contains mean particle size of 16.13 nm and zeta potential (ζ) -8.25 mV. *In-Vitro* drug release of optimized formulation carried out which gives 98.48% within 40 minute. Hence, by formulating into SMEDDS, the solubility of Clopidogrel was found to be significantly enhanced.

Keyword: Clopidogrel, SMEDDS, Solubility, Zeta potential, Phase diagrams.

Introduction:

Oral route has been the major route of drug delivery for the chronic treatment of many diseases. However, oral delivery of 50% of the drug compounds is hampered because of the high lipophilicity of the drug itself. Nearly 40% of new drug candidates exhibit low solubility in water, which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality. Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution. Various approaches should use to improve the dissolution rate for the drug. However, these methods have their

own limitations. To overcome these drawbacks, various other formulation strategies have been adopted. Among them, one formulation is self-emulsifying drug delivery systems (SEDDS).

Self-microemulsifying drug delivery systems (SMEDDS) are class of emulsion that have received particular attention as a means of enhancing oral bioavailability of poorly absorbed drugs. These systems are essentially mixes of oil and surfactant (sometimes with added co-surfactant) that form emulsion on mixing with water with little or no energy input. Self-emulsifying formulations spread readily in the GI tract, and the digestive motility of the stomach and the intestine

provide the agitation necessary for self-emulsification. SEDDS typically produce emulsions with a droplet size between 100 nm and 300 nm while SMEDDS form transparent microemulsions with a droplet size of less than 50 nm.

The main objective of the investigation is to formulate, optimize and stabilize SMEDDS containing Clopidogrel with suitable surfactants and co-surfactants. LOV, which is poorly soluble in gastric fluid through conventional dosage forms (tablet), SMEDDS are prepared to increase their solubility in gastric fluid and improve bioavailability by increasing gastrointestinal absorption through passive diffusion (droplet size < 100 nm).^[1]

MATERIALS AND METHODS

MATERIALS

Clopidogrel Bisulphate was obtained from Sun Pharma Ltd., Baroda, India. The following materials were obtained from Abitec corp., USA, and were used as received: Capmul® MCM NF (Glycerol mono-dicaprylate), Captex® 200 (Propylene Glycol Dicaprylocaprate). Transcutol® P (Highly purified diethylene glycol monoethyl ether), Labrasol (Linoleoyl macrogol-6 glycerides) were received from Gattefosse, France. Acrysol® K 140 (polyoxyl 40 hydrogenated castor oil) and Acrysol® El 135 (Polyoxyl 35 castor oil) were also gifted from Corel Pharma chem., Ahmedabad, India. Tween 80 (polyoxyethylene sorbitan

monooleate), Tween 20 (Polyoxyethylene sorbitanmonostearate), Propylene glycol were bought from Finar Chemical Limited, Ahmedabad, India. Propylene glycol (PG), Polyethylene glycol 400 (PEG 400) were bought from S. D. Fine Chemical Limited, Mumbai, India. Sunflower oil, Castor oil was purchased from Gujarat Glycol Private Limited, Ankaleshwar, India and Kush Proteins Private Limited, Anand, India. Double distilled water was used throughout the study. All other chemicals were of reagent grade.

Preliminary Work

For the preparation of SMEDDS, following parameters were optimized.

- Selection of oil phase
- Selection of surfactant
- Selection of co-surfactant

Selection of oil phase

In order to make SMEDDS systems pharmaceutically acceptable, it is necessary to prepare such systems by using nontoxic and safe components. Oil from natural sources and their derivatives, e.g. triglycerides and fatty acid methyl esters are easily degraded by microorganism and considered to be harmless to the environment. The formation of bicontinuous microemulsions with mineral oils has been intensively investigated in model experiments and for application in industrial products. Various oils were used for the formation of SMEDDS like Castor oil,

Sunflower oil, Olive oil, Capmul MCM, Capmul MCM C8 and Soyabean oil.^[1,2]

Selection of surfactants for emulsifying ability

Emulsification ability of various surfactants was screened. Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45–60°C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed and diluted with double distilled water to 50 ml to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 hrs. and their transmittance was assessed at 650 nm by UV-1800 double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank. Required HLB value to form o/w SMEDDS should be between 12-18 and the selection of surfactant was mainly based on this. Another requirement was that the surfactant to be included in formulation should be non-toxic. Surfactant selection was done on the basis of % transparency and ease of emulsification. Several surfactants were selected for screening like Tween-80, Tween-20, Span-20, Span-80, Acrysol K 140, Labrasol, Captex 100 and Captex 200.^[3]

Selection of co-surfactant

Co-surfactants were selected based on their capability to form stable SMEDDS with related surfactants at minimum concentration. Based on this, three co-surfactants were selected for screening like PEG 400, Transcutol P and PG.^[3]

Solubility method of Clopidogrel in different vehicles

3 gm. of selected vehicles was added to each cap vial containing an excess of Clopidogrel. After sealing, mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25°C for 48 hours. After reaching equilibrium, each vial was centrifuged at 2000 rpm for 15 minutes. Excess insoluble clopidogrel was discarded by filtration using 0.45µm filter disk. Filtered solution was appropriately diluted with methanol and UV absorbance was measured at 224 nm. Concentration of dissolved drug was determined using standard equation.^[4]

Physicochemical Compatibility of Drug To Polymer

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, , physical mixtures of the drug

with the excipients (Capmul MCM NF, Acrysol K 140, PG). The formulation is lyophilized using freeze dryer and FTIR studies were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each the spectrum, 8 scans were obtained at a resolution of 4 cm^{-1} from a frequency range of $4000\text{--}400\text{ cm}^{-1}$.

Ternary phase diagram study for SMEDDS

Ternary phase diagrams of microemulsion were prepared by Sigma plot version 10.0 software to decide the microemulsion zone in which at any point, microemulsion can be prepared. For the ratios of surfactant and co-surfactant were at first selected. Here three ratios of Surfactant (Acrysol k-140) and Co-surfactant (Propylene Glycol) were selected (1:1, 2:1, and 1:2). For each ratio, microemulsion were prepared by decreasing

the Oil phase (Capmul MCM NF) concentration from 90% to 10% with respect to increasing the concentration of Surfactant/Co-surfactant from 10% to 90% to decide the maximum uptake of water by microemulsion up to which they remained transparent. Optimized the concentration of oil phase, surfactant and co-surfactant was based on maximum uptake of water by microemulsion.^[4]

FORMULATION OF SMEDDS

The formulations were prepared by initially dissolving required quantity of clopidogrel in oil. Then Surfactant and Co-surfactant mixer were added and the final mixture was mixed by vortexing until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hr, and examined for signs of turbidity or phase separation prior to self-emulsification and particle size studies. Final formulation was filled in hard gelatin capsule (Size 00).

Table: Formulation of SMEDDS

Ingredient	Batch (in mg)						
	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇
Capmul MCM NF	200	100	300	200	100	200	100
Acrysol k 140	400	450	460	540	600	260	300
PG	400	450	240	260	300	540	600
Clopidogrel (mg)*	75	75	75	75	75	75	75

* Amount of drug in 1 gm SMEDDS Formulation

Evaluation of SMEDDS Formulations

1.Determination of self emulsification time

The emulsification time of SMEDDS was determined using dissolution apparatus 2. 300 mg of each formulation added drop wise to 500ml purified water at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. Emulsification time was assessed visually.^[4]

2.Transmission test

Stability of optimized microemulsion formulation with respect to dilution was checked by measuring transmittance through U.V.Spectrophotometer (U.V.1800, Shimadzu). Transmittance of samples was measured at 650nm and for each sample three replicate assays were performed.^[5]

3.Drug Content

Clopidogrel from SMEDDS formulation was extracted in methanol using sonication technique. The solutions were filtered, using wattman filter paper. The methanolic extract was analyzed for the Clopidogrel content spectrophotometrically (UV-1800, Shimadzu, Japan) at 224 nm using standard curve.

4.Visual assessment

Clopidogrel SMEDDS (approximately 0.2 ml) was diluted with purified water (100 ml) and gently stirred with magnetic stirrer. Temperature should be 37°C.^[4]

4.Droplet size measurement and Polydispersibility Index (PDI)

Formulations (F1 to F7) each of 1 ml were diluted with 100 ml of water in a volumetric flask. The volumetric flask was inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulation the droplet size of resultant Microemulsion was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a Zetasizer Nano Series (Malvern Instruments, DTS Ver.4.10, Serial No. MAL 500999). Light scattering was monitored at 25°C at 90° angle.^[6]

5.Zeta potential measurement

Zeta potential for microemulsion was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd., U.K.). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment.^[7]

6.In vitro Dissolution Studies

The *in vitro* drug release of Clopidogrel from the optimized SMEDDS was performed using USP dissolution Apparatus II (TDT-08L, Electrolab, Mumbai, India). Hard gelatin capsules, size “00” filled with preconcentrate (equivalent to 75mg Clopidogrel) were put

Table: Visual assessments of efficiency of self-microemulsification

Grade	Dispersibility and appearance	Time of Self-microemulsification
I	Rapid forming microemulsion which is clear or slightly bluish in appearance	< 1 min
II	Rapid forming, slightly less clear emulsion which has a bluish white appearance	< 2 min
III	Bright white emulsion (similar to milk)	< 3 min
IV	Dull, grayish white emulsion with a slightly oily appearance that is slow to emulsify	> 3 min
V	Exhibit poor or minimal emulsification with large oil droplets present on the surface	> 3 min

into each of 900 ml 0.1 N HCl soln. pH 1.2, at $37 \pm 0.5^\circ\text{C}$ with a 50 rpm rotating speed. Samples (5 ml) were withdrawn at regular time intervals (5, 10, 20, 30, 40, 50 and 60 min) and filtered using a $0.45 \mu\text{m}$ filter. An equal volume of the dissolution medium was added to maintain the volume constant. The drug content of the samples was assayed using UV visible spectrophotometric method at 224 nm wavelength. All measurements were done in triplicate.^[8]

8. Thermodynamic stability

- 1. Heating cooling cycle:** Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than

48h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

- 2. Centrifugation:** Passed formulations were centrifuged at 3500 rpm for 30min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.^[8]

RESULT AND DISCUSSION

Selection of Oil: Solubility studies were aimed at identifying a suitable oil phase for development of clopidogrel SMEDDS. The solubility of clopidogrel in various oil presented in figure-1.

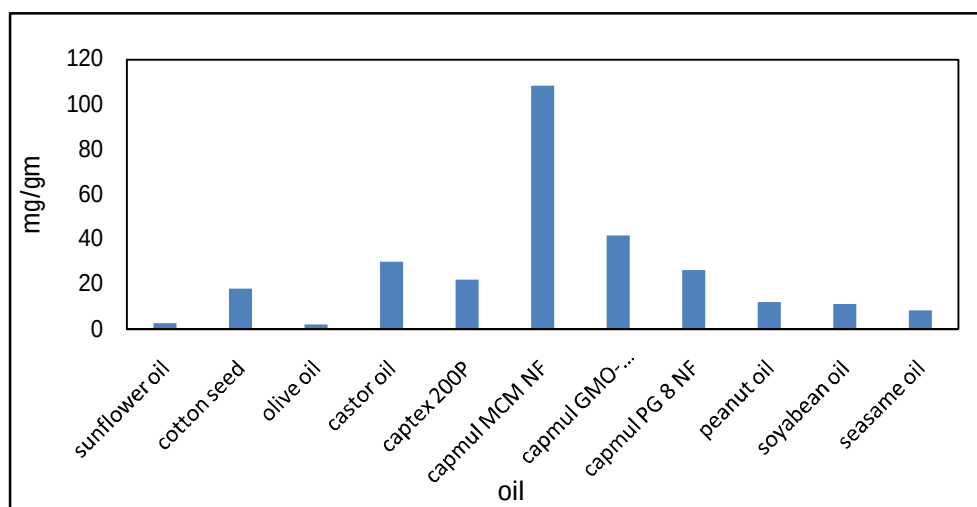


Figure-1: Schematic diagram of drug solubility in different oils

As per solubility data of clopidogrel in different oils, maximum amount of drug dissolved in Capmul MCM NF. So, we selected Capmul MCM NF (108.44 mg/gm).

Selection of surfactant

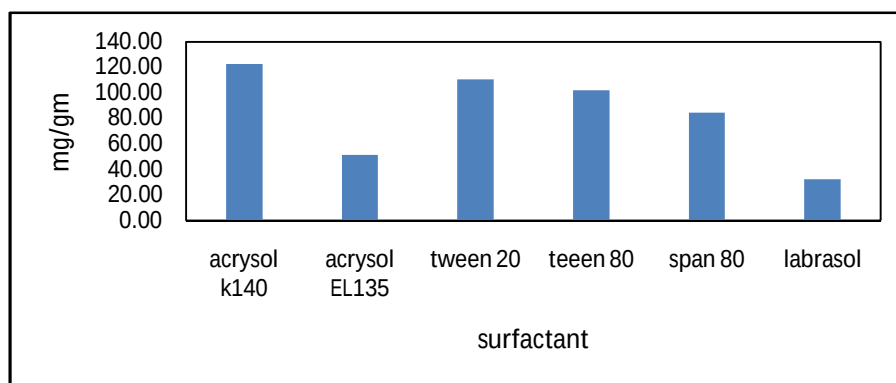


Figure-2: Schematic diagram of drug solubility in different surfactants

Among the solubility data of clopidogrel in different surfactant maximum amount of drug dissolved in Acrysol K 140 (122.95 mg/gm). Therefore this surfactant was selected for SMEDDS formulation.

Selection of co-surfactant

Co-surfactant is required with surfactant in SMEDDS for reported to improved dispersibility and drug absorption from the formulation

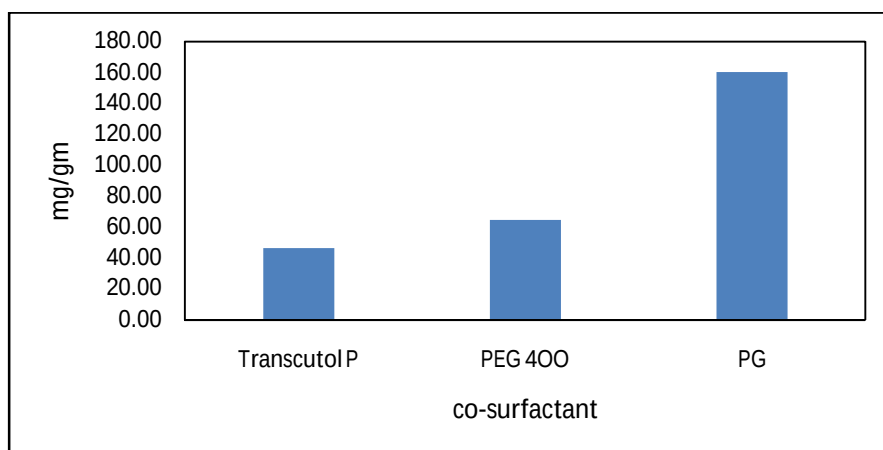


Figure-3: Schematic diagram of drug solubility in different Co-surfactants

Solubility study of clopidogrel in different Co-surfactant, maximum amount of drug dissolved in Propylene Glycol (PG) (160.58 mg/gm). Therefore this Cosurfactant was selected for SMEDDS formulation.

Drug Excipients Compatibility Study

The FTIR spectrum of clopidogrel showed absorption band at 2544 cm^{-1} ($\text{N}^+\text{-H}$), 1751 cm^{-1} (C=O , str.), 1190 cm^{-1} (C-O , str.), 1030 cm^{-1} (C-O). These peaks can be considered as characteristic peaks of clopidogrel. In the formulation absorption bands 1751 cm^{-1} (C=O , str.), 1190 cm^{-1} (C-O , str.), 1030 cm^{-1} (C-O) present so drug and excipients are compatible.

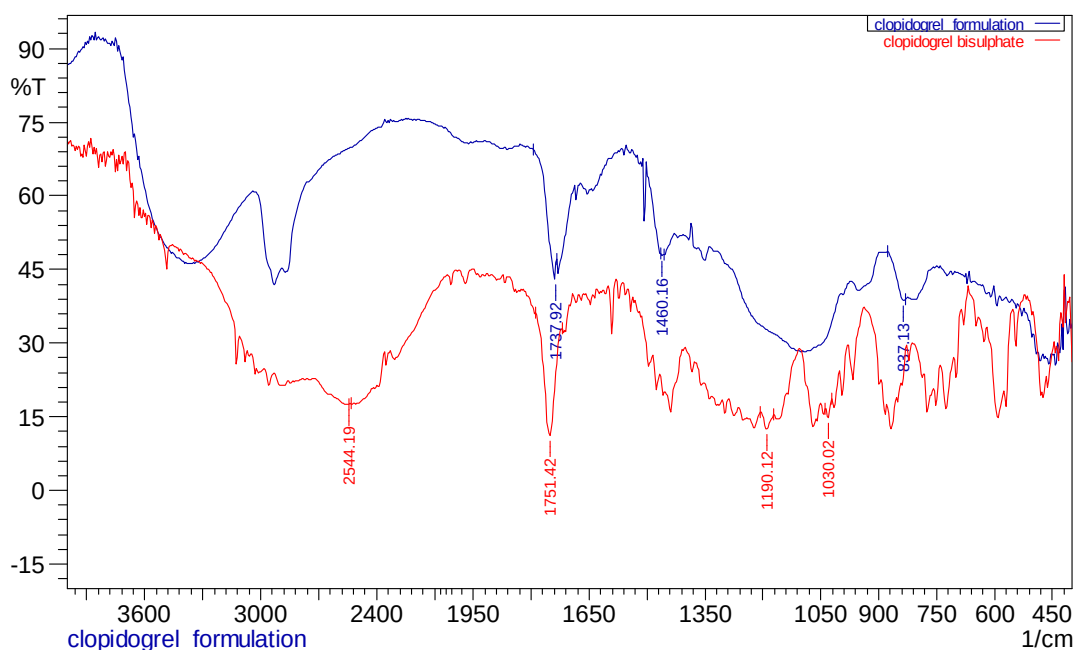


Figure-4: FTIR spectrum of Clopidogrel and excipients mixture

Ternary Phase Diagram

Formulation are prepared using Capmul MCM NF as oil phase, Acrysol K140 as surfactant and PG as co-surfactant. Formulations were prepared with surfactant/co-surfactant (S/CoS) ratio of 1:1, 2:1, 1:2. As shown in Figure, formulations 2:1 covers maximum Microemulsion region as compare to other formulations. The point where amount of oil is less than 10%, the water content is 90%. At this point Microemulsion can be diluted to infinite which fulfils requirement of SMEDDS and also particle size of this Microemulsion is less than 100nm (described in characterization of SMEDDS). The region where oil content is more than 10% and surfactant/co-surfactant is upto 50% also forms the Microemulsion but these were found to be unstable on dilution. The phase diagram shows that when S/CoS exceed to 50%, coarse emulsion forms having particle greater than 100 nm. Hence it can be predicted that concentration of S/CoS should be less than 50% to form self-Microemulsion. Further, more amount of oil also entrap less water content and thereby results in coarse emulsion.

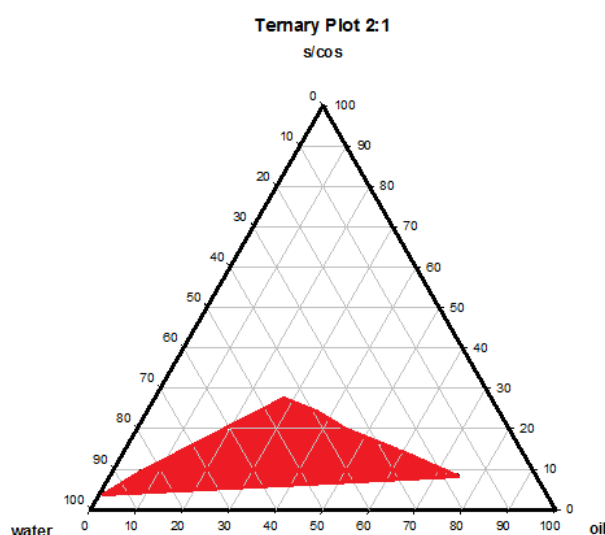


Figure-5: Ternary phase diagram of Surfactant (Acrysol K 140) /Co-surfactant (PG) ratio (2:1) and Oil (Capmul MCM NF)

Evaluation of SMEDDS Formulations

I. Emulsification Time

The emulsification time of these formulations were in the range of 14 to 26 sec.

Table: Emulsification time of formulations

Formulation Code	Emulsification Time (sec)*
F1	15 ± 1
F2	18 ± 1

F3	26 ± 2
F4	20 ± 1
F5	15 ± 1
F6	17 ± 2
F7	14 ± 1

*Values are expressed as mean ± S.D, n=3

II. Transmission test

% Transmittance was measured by directly taking the absorbance of the diluted SMEDDS. No significant difference was observed among the percentagetransmittance of formulations F1 to F7 and formulation F5 was found to have the highest percentagetransmittance. Besides signifying clarity of the formulation, a percentage transmittance closer to 100% also indicates that the size of the globules in the formulation is in the nanometer range.

Table: % Transmission test of SMEDDS formulation in water

Formulation Code	Transmittance (%)
F1	99.5
F2	98.2
F3	99.6
F4	98.5
F5	99.6
F6	99.2
F7	99.4

III. Drug Content

Irrespective of difference in composition, the drug content of formulations F1 to F7 was found in range of 95.45 – 99.56 %.

IV. Visual Assessment

Clopidogrel SMEDDS concentration (approximately 0.2 ml) was diluted with purified water (100 ml) and gently stirred with magnetic stirrer. Temperature should be 37⁰C. In this study only F3 formulation was found grade-II and all other formulation found grade-I.

V. Particle Size Determination

The droplet 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 smallest particles were observed for formulation F5 (16.13) and largest droplets were obtained for formulation F3.

Polydispersibility Index (PDI)

Polydispersibility index (PDI) (<0.3) indicates a homogenous distribution, while a PDI (>0.3) indicates a higher heterogeneous dispersion. PDI value of formulation F5 is less compare to other Formulations.

Table: Particle size and PDI of SMEDDS in water

Formulation code	Particle size in water (nm)	Polydispersibility Index
F1	29.53	0.343
F2	40.25	0.310
F3	82.10	0.268
F4	47.79	0.623
F5	16.13	0.083
F6	28.57	0.299
F7	19.97	0.235

VI. Zeta potential (ζ) Determination

If the zeta potential is reduced below a certain value (which is depends on a particular system being used), the attractive forces exceed the repulsive forces, and the particles come together leading to flocculation. The ideal zeta potential for SMEDDS is less than -30 , formulation F1 and F3 were not pass the zeta potential test. Formulation F5 has lowest zeta potential (-8.25) on this basis F5 is optimize.

Table: Zeta Potential of SMEDDS formulations

Formulation code	Zeta potential (mV)
F1	-31.9
F2	-14.9
F3	-36.8

F4	-8.42
F5	-8.25
F6	-24.3
F7	-13.8

VII. *In vitro* Dissolution studies

In vitro dissolution studies were performed to compare the release of drug from seven different SMEDDS formulations (F1-F7) is presented in Figure. Dissolution studies were performed for the SEDDS formulations in 0.1 N HCl solution. There is no any significant difference in dissolution of seven SMEDDS formulation. As the emulsification time is below 35 s, about 100% percentage of the drug is released within 50-60 min in case of SMEDDS. The dissolution studies were conducted for 1 hr. to observe the variation or occurrence of precipitation over a time. Formulation F5 give release in 40 minute due to smallest particle size (16.13) and less PDI value (0.083). Formulation F4 give release in 60 minute due to large particle size and greater PDI value. The *in vitro* dissolution studies indicates that formulation of clopidogrel in the form of SMEDDS formulation enhance the dissolution properties.

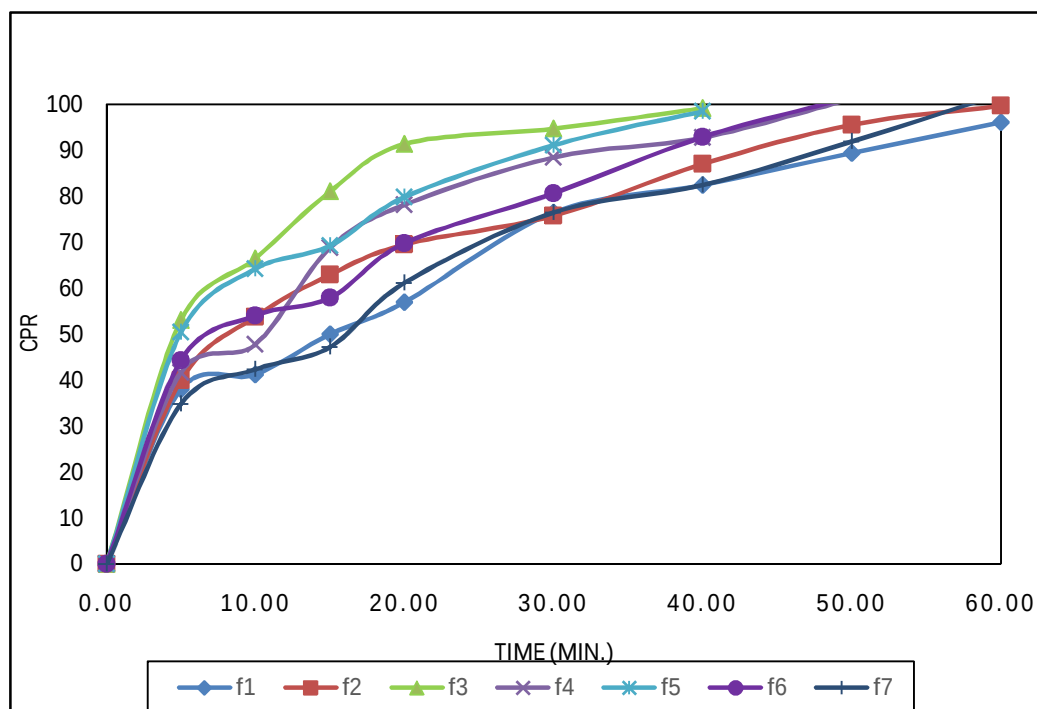


Figure-6: Dissolution Profile of Clopidogrel SMEDDS formulations.

I. Thermodynamic stability

SMEDDS are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano-or microemulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle and centrifugation stress tests.

Table: Temperature stability study

Formulation Code	Phase Separation	Flocculation	Precipitation
F1	Not Seen	Not Seen	Not Seen
F2	Not Seen	Not Seen	Not Seen
F3	Not Seen	Not Seen	Not Seen
F4	Not Seen	Not Seen	Seen
F5	Not Seen	Not Seen	Not Seen
F6	Not Seen	Not Seen	Not Seen
F7	Not Seen	Not Seen	Not Seen

Table: Centrifugation stability study

Formulation Code	Phase Separation
F1	Not Seen
F2	Not Seen
F3	Not Seen
F4	Not Seen
F5	Not Seen
F6	Not Seen
F7	Not Seen

SMEDDS are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking.

CONCLUSION

Clopidogrel was formulated as a SMEDDS in an attempt to increase its solubility and bioavailability. An optimized formulation of SMEDDS containing clopidogrel was developed through the construction of pseudo-ternary phase diagram, particle size analysis and zeta potential. SMEDDS appeared to be an interesting approach to improve problems associated with oral delivery of clopidogrel. Thus SMEDDS formulation can be regarded as a novel and commercially feasible alternative to the current clopidogrel formulations.

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