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# DESIGN AND EVALUATION OF LIPID BASED DRUG DELIVERY SYSTEM OF CLOFAZIMINE FOR LEPROSY TREATMENT

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## ABSTRACT

The aim of the study was to design and evaluate a lipid based drug delivery system particularly self microemulsifying drug delivery system in order to improve solubility, dissolution and enhance systemic exposure of a BCS class II anti-leprotic drug, Clofazimine (CLZ). In the present study, solubility of CLZ was determined in various oils, surfactants and cosurfactants to identify the microemulsion components. Pseudoternary phase diagrams were plotted to identify the micro-emulsification existence area. SMEDDS formulation of CLZ was prepared using oil (Ethyl oleate) and a surfactant/ co-surfactant (Tween 80/PEG 400) mixture and was characterized by appropriate studies, *viz.*,self emulsification, thermodynamic stability test, droplet size measurement, zeta potential, *in vitro* dissolution, *etc.* The optimized formulation of SMEDDS comprised of 31.2% oil (ethyl oleate), 51.6% surfactant (Tween 80), and 17.2% co-surfactant (PEG 400). The solubility of CLZ (35 mcg/ml) significantly increased in SMEDDS which in water being 0.3 mcg/ml), which indicates approx. 106 fold enhancement in solubility. The average particle size and zeta potential of SMEDDS containing CLZ was about 82.46nm and -23.3 mV when diluted in water. All the SMEDDS were found to form clear dispersion and none of the formulation showed any drug precipitation, suggesting that developed SMEDDS was stable. The dissolution study *in-vitro* showed that about 99% of CLZ in SMEDDS could be dissolved in pH 1.2 or pH 6.8 buffer solutions in 30 min, however, less than 30% for crude CLZ in 60 min. Thus, the study illustrated that the developed SMEDDS formulation held great potential as a possible alternative to traditional oral formulations of CLZ.

**KEYWORDS:** SMEDDS, Phase diagram, Clofazimine

## INTRODUCTION

Two-thirds of compounds emerging from the drug discovery channel in recent years have an aqueous solubility <100 µg/mL (0.1 mg/mL).[1] Owing to their poor aqueous solubility, these drugs lead to poor absorption following oral administration. A lipid based drug delivery system, in which the drug is solubilised in lipids or lipid-like excipients, has been recognized as an attractive approach for increasing the bioavailability of these compounds.[2, 3] Lipid based systems consist of delivering a drug dissolved in a mixture of one or more excipients which may be a mono, di and tri-glyceride, lipophilic and hydrophilic surfactants and a cosurfactant. The primary mechanism by which lipid excipients based formulations enhance bioavailability is through solubilization of the drug, although other mechanisms of absorption enhancement have been implicated and include reduction of P-glycoprotein-mediated efflux, mitigation of hepatic first pass metabolism through enhanced lymphatic transport, alteration in enterocyte based drug transport and disposition.[4] Recently, due to good and reliable result, there is a great emphasis on self emulsifying drug delivery systems (SEDDS) to improve the oral bioavailability of lipophilic drugs. Clofazimine, a fat-soluble riminophenazine, lipophillic antibiotic dye used for the treatment of leprosy, possesses both antimycobacterial and anti-inflammatory activities. It is used as part of multidrug regimens for the treatment of multibacillary leprosy, including dapsone-resistant lepromatous leprosy and leprosy complicated by erythema nodosum leprosum. Owing to its poor water solubility (0.225 mg/ml) low oral bioavailability its formulation into a lipid based delivery system gives better results with improved drug characteristics.

## MATERIALS AND METHODS

Clofazimine was gifted from Sangrose Pvt. Ltd. (kerela, India). Castor oil, Oleic acid, ethyl oleate, Tween® 80, PEG 400 were purchased from central drug house, New Delhi, India. Caproyl 90, Capmul MCM, Labrafil M 2125 CS, Captex 355 were gifted from Gattefosse and Abitec corporation, Mumbai. All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

**Solubility studies:** To find out appropriate oils and surfactants as compositions of SMEDDS, the solubility of Clofazimine in various oils and various surfactants was analyzed. An excess amount of clofazimine was added to 5 ml of oil or 20% (w/w) surfactant aqueous solutions. The resultant mixture was shaken reciprocally at 37$^{o}$C for 72 h, followed by centrifugation at

12000 rpm for 10min. The supernatant was filtered through a membrane filter (0.45µm) to remove the remaining insoluble clofazimine. After the appropriate dilution with benzene, the drug concentration in the filtrate was quantified by UV spectrophotometer.

**Pseudoternary phase diagrams**[5, 6]

Surfactant (Tween 80) and cosurfactant (PEG 400) were mixed (Smix) in different weight ratios (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1, etc). Ethyl oleate was optimized as an oil phase based on the solubility study. For each phase diagram, oil (ethyl oleate) and specific Smix ratio were mixed thoroughly in different weight ratios viz., 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2, 1:2.2, 1:1.5, 1:1, 1:1.66, 1:2.33, 1:0.43, 1:0.25, 1:0.11.

Each ratio of oil and Smix was taken and titrated with water at 5% intervals and then mixed on a vortex mixer. The solutions were observed visually and were categorized into different phases

1. Transparent with good flow: oil/water microemulsions (ME)
2. Transparent gel with medium flow: microemulsion gel (G)
3. Milky with good flow: emulsion (E)
4. Milky gel with good flow: emulgel (M)

Based on the observation, phase diagram was constructed using TRIPLOT version 4.12 software with point A as oil, point B as Smix and point C as water. For each Smix ratio, a separate phase diagram was constructed and the area of microemulsion was shaded.

**Preparation of SMEDDS formulations**[5, 7]

Based on the area of microemulsification from the phase diagrams, Smix ratio of 3:1 was selected for the formulation development studies. SMEDDS formulations were prepared using Tween 80 and PEG 400 as surfactant and co-surfactant with Smix ratio of 3:1. The weight of the formulation was kept approx. 1000 mg. Level of Clofazimine in all the formulation was kept constant (50 mg). Clofazimine was accurately weighed and placed in an ependrof tube with the respective required quantity of ethyl oleate, heated at 40$^{o}$C-50$^{o}$C under vortex. Surfactant/co-surfactant were mixed together in separate test tube vortexed and heated at 50$^{o}$C-60$^{o}$C. The mixture in ependrof tube was transferred into test tube and heated at 50$^{o}$C in a sonicator and Clofazimine was dissolved, the mixture was then stored at room temperature.

**Table No. 1 Developed formulations with their composition**

| Formulation code/ Components (mg) | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| **Clofazimine** | 50 | 50 | 50 | 50 | 50 | 50 |
| **Smix ratio** | 3:1 | 3:1 | 3:1 | 3:1 | 3:1 | 3:1 |
| **Oil: Smix** | 1:7 | 1:4.33 | 1:2.2 | 1:1.66 | 1:4.66 | 1:2.33 |
| Ethyl oleate | 125 | 187 | 312 | 375 | 176 | 300 |
| Tween 80 | 656 | 609 | 516 | 469 | 618 | 525 |
| PEG 400 | 218 | 203 | 172 | 156 | 206 | 175 |

**Thermodynamic stability studies**[8]

**Heating cooling cycle**: Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

**Centrifugation:** Passed formulations were centrifuged at 3500 rpm for 30 min. Diluted to 1:10, 1:50, 1:100. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

**Freeze thaw cycle**: Three freeze thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 h was done for the formulations.

**Emulsion droplet size analysis and polydispersity index**[6]

The formulation (0.1 mL) was dispersed in 50 mL of water in volumetric flask and gently mixed by inverting the flask. Globule size of the formulation was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer Ver. 6.01 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle**.**

**Zeta potential determination**[6, 7]

SMEDDS (1 ml) was diluted 10 times with distilled water in beaker with constant stirring on a magnetic stirrer. Zeta-potential and electrophoretic mobility of the resulting microemulsion was then determined using the Zetasizer (Malvern Instruments, UK).

**Robustness to dilution**[5]

SMEDDS were diluted to 10, 100 times with water in a beaker. After storing for 12 h, the diluted micro-emulsions were observed for any signs of phase separation or drug. Diluted SMEDDS was prepared by diluting the SMEDDS formulation with 25 ml of water.

**Determination of Drug Content:** 1000 mg of the developed SMEDDS formulation (containing 50 mg of clofazimine) was diluted with 10 ml of benzene. Then further diluted with benzene and content was analyzed spectrophotometrically using UV/Visible spectrophotometer (Labindia 3200) at 452 nm against the corresponding blank.

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

***In vitro* dissolution studies** [7,8, 9]: SMEDDS of Clofazimine was filled in size "0"capsules. *In vitro* release profiles from SMEDDS of clofazimine, pure clofazimine powder, were studied using USP XXIV apparatus II at 37±0.5 °C with a rotating speed of 50 rpm in phosphate buffer, pH 6.8 and 0.1 N HCl, pH 1.2 as the dissolution media. 5 ml of aliquots were removed at predetermined time intervals (0, 5, 10, 15, 30, 45, 60 min) from the dissolution medium and replaced with fresh media. The amount of clofazimine released in the dissolution medium was determined by UV- spectrophotometer.

## RESULTS AND DISCUSSION

**Table No. 2 Results of solubility studies**

| S.No. | Vehicle | Solubility (µg/ml) |
|---|---|---|
| 1. | Labrafil M 2125 CS | 20.4692 |
| 2. | Capryol 90 | 21.3851 |
| 3. | Castor oil | 18.2535 |
| 4. | Oleic acid | 19.1356 |
| 5. | Capmul MCM | 20.8064 |
| 6. | Transcutol CG | 15.5754 |
| 7. | Cremophor RH 40 | 23.1088 |
| 8. | Tween 80 | 30.2729 |
| 9. | Labrasol | 08.6781 |
| 10. | Captex 355 | 07.3500 |
| 11. | Isopropyl myristate | 18.5966 |
| 12. | Olive oil | 12.6167 |
| 13. | Isopropyl alcohol | 11.0685 |
| 14. | Ethyl oleate | 32.7942 |
| 15. | Polyethylene glycol 400 | 29.3890 |

**Solubility Studies:** While formulating a self-emulsifying formulation it is important to avoid precipitation of the drug on dilution in the gut lumen *in vivo.*[10] Therefore, the components used in formulating SMEDDS should have high solubilizing capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion. Ethyl oleate showed the highest solubility from various oils screened for solubilization of clofazimine. The formulations

prepared with ethyl oleate yielded clear and transparent emulsion, while those prepared with other oils yielded turbid or unstable emulsions leading to phase separation, as observed visually. To obtain optimum drug solubility and emulsion quality, ethyl oleate was selected as oil phase. Tween 80 and PEG 400 showed the highest solubilizing potential for clofazimine among the various surfactants, cosurfactants screened.

**Pseudo-ternary phase diagram:** Selection of oil, surfactant, and cosurfactant and mixing ratio to S/CoS plays an important role in formation of SMEDDS.[7,12]

Ethyl oleate, Tween 80 and PEG 400 are mixed in seven different Smix ratios, with decreasing order of surfactant, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4 and then titrated with water to get microemulsion regions. microemulsion regions were observed visually and graded as transparent with good flow: oil/water micro-emulsions (ME), transparent gel with medium flow: micro-emulsion gel (G), milky with good flow: emulsion (E), milky with medium flow: emulgel (M). The visual observations were tabulated. From Fig.1 (a-c), it is evident that in the different Smix ratios the only 3:1, 2:1, 1:1 ratio show microemulsion region out of which 3:1 showed the largest emulsification region followed by Smix ratio of 2:1 and 1:1. Larger the size of microemulsion region in ternary phase diagram, greater is the self-emulsification efficiency. Also Smix ratio of 3:1 and 2:1 showed greater micro-emulsification region with infinite dilutions. In addition both these Smix ratio exhibited a greater range for oil incorporation (approx. 9% to 45%). In contrast Smix ratio of 2:1, 1:1, 1:2, 1:3, 1:4 showed a small micro-emulsification region and did not form any microemulsion with infinite dilution. So, depending on the above results, Smix ratio of 3:1 was selected for further studies.

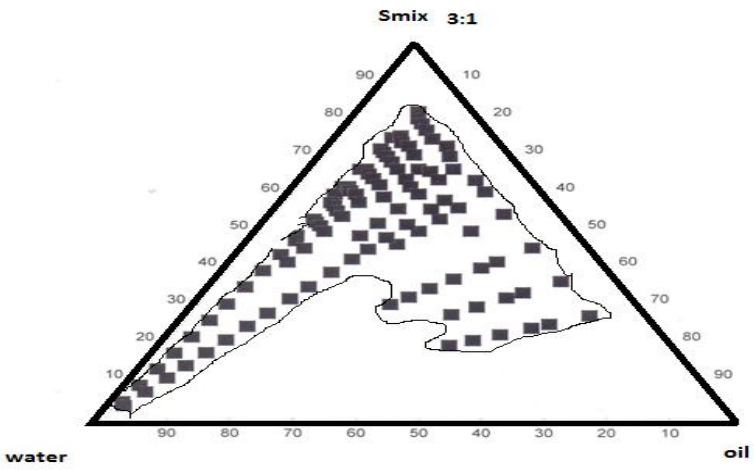


**Fig. 1(a): Pseudo-ternary phase diagram of Ethyl oleate -Tween 80-PEG 400 systems indicating microemulsion existence region with Smix ratio (3:1)**

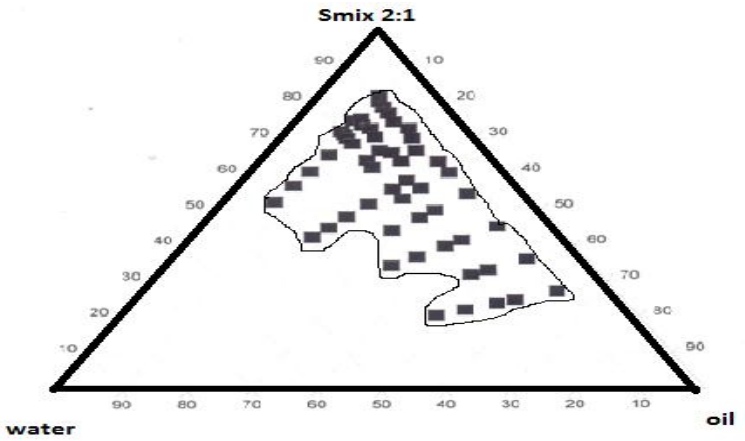


**Fig. 1(b): Pseudo-ternary phase diagram of Ethyl oleate -Tween 80-PEG 400 systems indicating microemulsion existence region with Smix ratio (2:1)**

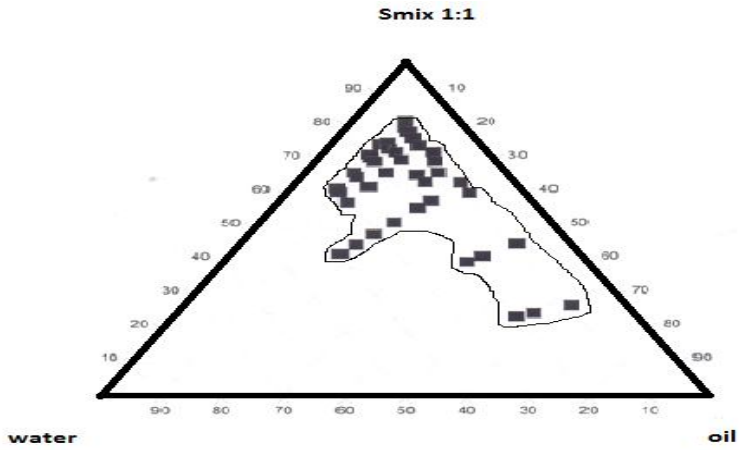


**Fig. 1(c): Pseudo-ternary phase diagram of Ethyl oleate -Tween 80-PEG 400 systems indicating microemulsion existence region with Smix ratio (1:1)**

**Thermodynamic stability studies:** Thermodynamic stability tests were performed to eliminate the metastable systems. Formulations were subjected to different stress tests, such as centrifugation, heating-cooling cycle, and freeze-thaw cycle tests. Formulations F3 to F6 (Smix ratio 3:1) did not show any signs of phase separation but F1 and F2 did not passed the test. Results have been shown in table 3.

**Table No.3 Thermodynamic stability test of the formulations**

| Formulation code | Heating/cooling cycle | Centrifuge cycle | Freeze thaw. | Inference |
|---|---|---|---|---|
| F1 | √ | × | × | Failed |
| F2 | √ | × | × | Failed |
| F3 | √ | √ | √ | Passed |
| F4 | √ | √ | √ | Passed |
| F5 | √ | √ | √ | Passed |
| F6 | √ | √ | √ | Passed |

**Emulsion droplet size analysis and Zeta potential determination**

The droplet size of SMEDDS is an important factor in self-emulsification performance as it determines the rate and extent of drug release as well as absorption. It is known that the particle size distribution is one of the most important characteristics of emulsion for the evaluation of its stability and also *in vivo* fate of emulsion.[13] The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption.[10,14] Globule size, PDI and zeta potential for all the SMEDDS have been summarized in Table 4. Formulations F3 and F4 showed particle size (less than 100nm), PDI (<1) and Zeta potential (<±30 mV) within the range owing to the fact that particle size is lower when surfactant concentration is high and also the emulsification time is also high. So, formulations F3 and F4 were used for *in vitro* drug release studies.

**Table No. 4 Emulsion globule size, polydispersity index and zeta potential of SMEDDS**

| Formulations | F3 | F4 | F5 | F6 |
|---|---|---|---|---|
| **Emulsion globule size (nm)** | 82.46 | 93.78 | 178.4 | 193.2 |
| **PDI** | 0.151 | 0.208 | 0.464 | 0.353 |
| **Zeta potential (mV)** | -23.3 | -17.8 | -14.6 | -29.1 |

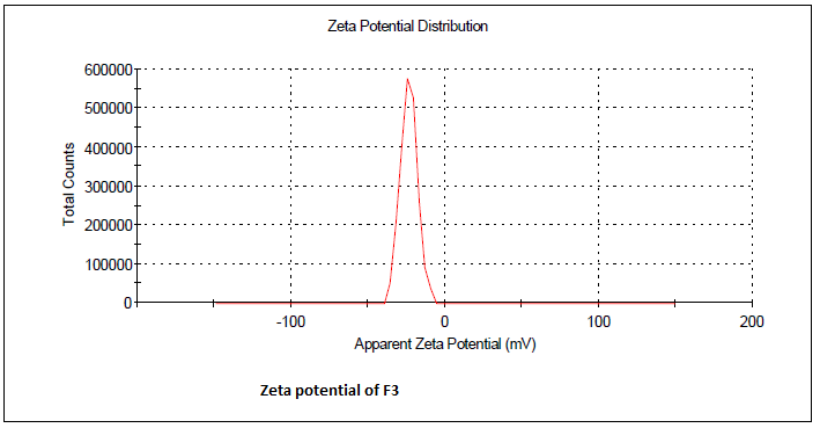


**Fig. 2: Zeta potential of F3 formulation**

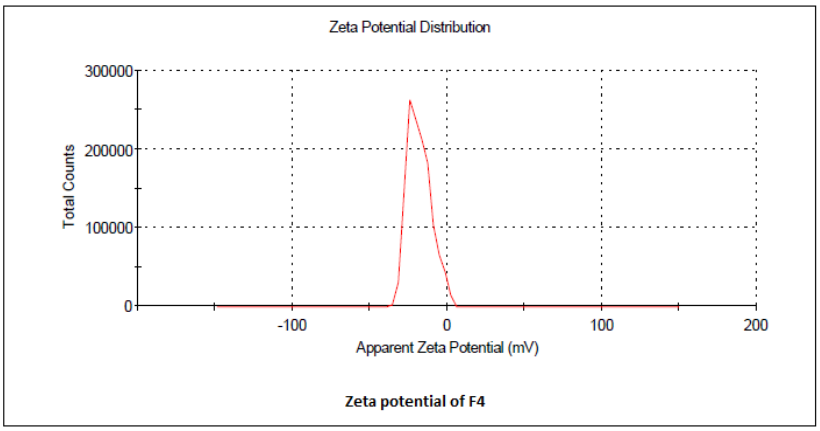


**Fig. 3: Zeta potential of F4 formulation**

**Robustness to dilution**[5]

Formulation F3 to F6 showed no signs of drug precipitation or phase separation on dilution of 10, 100 times. This implies that all the developed formulations were robust to dilution in the aqueous medium.

**Table No. 5 Robustness to dilution and effect of pH of dilution media data of formulations.**

| Formulations | | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|
| Dilution with water | 10 times | No ppt | No ppt | No ppt | No ppt |
| | 100 times | No ppt | No ppt | No ppt | No ppt |
| Dilution with HCl pH 1.2 | 10 times | No ppt | No ppt | No ppt | No ppt |
| | 100 times | No ppt | No ppt | No ppt | No ppt |
| Dilution with phosphate buffer 6.8 | 10 times | No ppt | No ppt | No ppt | No ppt |
| | 100 times | No ppt | No ppt | No ppt | No ppt |

**Determination of Drug content**

Drug content of the optimized SMEDDS formulation batch F3 and F4 were found to be 99.6%, 99.85% respectively which is within the acceptable range (98.5%-101.5%).[11]

***In-vitro* dissolution studies** [7]

Dissolution profile of capsule filled with developed self-microemulsifying formulation of clofazimine (F3 and F4) was compared with that of the pure drug (clofazimine 50 mg) filled in “zero” size capsule employing following experimental conditions

| | | |
|---|---|---|
| Apparatus | : | USP 24 Type II (Rotating paddle) |
| Dissolution medium | : | 0.1 N HCl Solution, phosphate buffer 6.8 |
| Volume | : | 900 ml |
| Paddle speed | : | 50 rpm |
| Temperature | : | 37± 0.5ºC |
| Sampling intervals | : | 5, 10, 15, 30, 45, and 60 mins |

**Table No. 6 Dissolution profile of developed SMEDDS capsule formulation of clofazimine (F3, F4), capsule containing pure drug (clofazimine 50 mg) in 0.1 N HCl 1.2**

| S. No. | Time (min) | % Cumulative drug release | | |
|---|---|---|---|---|
| | | Capsule of optimized SMEDDS formulation F3, F4 | | Capsule containing pure drug clofazimine (50 mg) |
| 1 | 0 | **F3** | **F4** | 0 |
| 2 | 5 | 11.19±1.22 | 8.11±0.15 | -0.24±0.15 |
| 3 | 10 | 38.80±1.24 | 34.65±1.3 | 3.5±0.78 |
| 4 | 15 | 54.52±1.16 | 67.008±2.75 | 5.64±0.96 |

| | | | | |
|---|---|---|---|---|
| 5 | 30 | 99.841.23 | 92.71±1.76 | 9±0.44 |
| 6 | 45 | - | 99.83±1.32 | 12.21±1.59 |
| 7 | 60 | - | - | 23.16±2.22 |

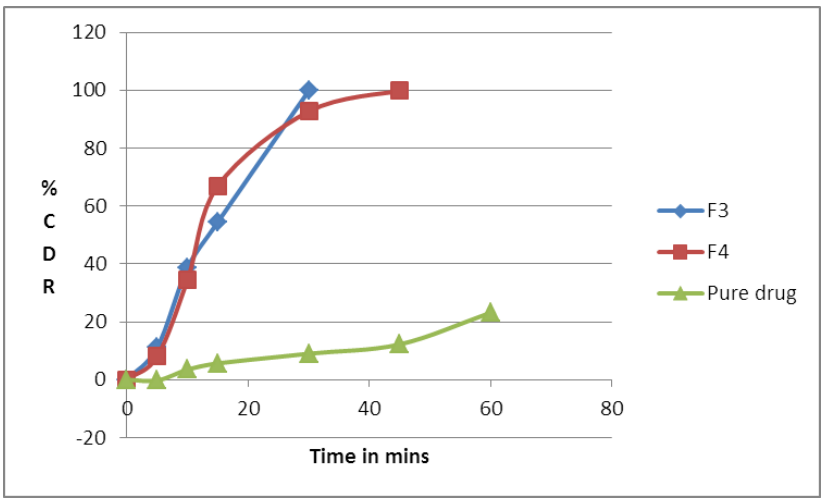


**Fig.4: Comparison of % cumulative drug release of F3,F4 and pure drug in 0.1 N HCl.**

**Table No. 7 Dissolution profile of developed SMEDDS capsule formulation of clofazimine (F3, F4), capsule containing pure drug (clofazimine 50 mg) in phosphate buffer 6.8**

| S. No. | Time (min) | % Cumulative drug release | | |
|---|---|---|---|---|
| | | Capsule of optimized SMEDDS formulation F3, F4 | | Capsule containing pure drug clofazimine (50 mg) |
| 1 | 0 | F3 | F4 | 0 |
| 2 | 5 | 11.95±1.65 | 8.78±1.06 | 0.0864±0.28 |
| 3 | 10 | 44.26±1.34 | 29.31±2.51 | 1.81±0.67 |
| 4 | 15 | 71.07±0.50 | 69.09±1.32 | 5.702±0.50 |
| 5 | 30 | 99.82±0.59 | 99.73±0.56 | 9.79±1.3 |
| 6 | 45 | - | - | 14.65±1.26 |
| 7 | 60 | - | - | 28.45±0.85 |

## CONCLUSION

A SMEDDS formulation of a poorly water soluble drug, clofazimine was formulated for directly filling in hard gelatin capsules for oral administartaion. The formulation F3 was found to be the optimized formulation on the base of results of pseudoternary phase diagram, *in vitro* drug release, droplet size and zeta potential. The optimized formulation showed rapid self emulsification in an aqueous media. The results from the study demonstrated successful preparation of self-microemulsifying drug delivery systems of clofazimine with increased solubility approx. 106 fold enhancement and dissolution rate.

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