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# FORMULATION AND IN-VITRO EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM OF FUROSEMIDE

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## ABSTRACT

The aim of the present study was to enhance the solubility and dissolution of poor water soluble drug Furosemide by formulating into Self Emulsifying Drug Delivery System. Self-Emulsifying Drug Delivery System (SEDDS) possess unparalleled potential in improving oral bioavailability of poorly water soluble drugs. Self-emulsifying formulations are isotropic mixtures of drug, lipids (natural or synthetic oils) and emulsifiers (solid or liquid), usually with one or more of hydrophilic co-solvents/co-emulsifiers. Following their oral administration, these systems rapidly disperse in gastrointestinal fluids, yielding micro or nano emulsions containing the solubilized drug. The solubility of Furosemide was determined in various vehicles such as Water, Tween 80, Tween 20, Oleic Acid, Sunflower Oil, PEG 400 and Glycerol. Furosemide was found to be more soluble in Tween 80 (Emulsifier), PEG 400 (Co-surfactant) and Oleic Acid (Oil) and was selected to formulate SEDDS. Sixteen different formulations varying the proportion of Oleic Acid, Tween 80 and PEG 400 were prepared. Effects of lipids and surfactants on physical properties of SEDDS such as *in vitro* emulsification efficiency in terms of self-emulsification time, emulsion droplet size, percent transmittance and dissolution profile were measured. From the evaluations it was observed that the higher proportion of surfactant (Tween 80) significantly increased dissolution of Furosemide while decreasing emulsion droplet size and emulsification time. The SEDDS formulation of Furosemide showed faster and better release profile than market conventional tablets of Furosemide.

**KEYWORDS**: Self-emulsifying drug delivery system, Furosemide, Bioavailability enhancement, Emulsion droplet size, *in-vitro* drug release

## INTRODUCTION

Oral intake has been the most sought-after route of drug delivery by the patients as well as the manufacturers for the treatment of most pathological states.[1] Low oral bioavailability as a consequence of low water solubility of drugs is a growing challenge to development of new pharmaceutical products. To triumph over these problems, various formulation strategies are exploited such as use of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins, nanoparticles and solid dispersions. Recently, much attention has been paid to lipid-based formulations with particular emphasis on Self-Emulsifying Drug Delivery Systems (SEDDS) to improve the oral bioavailability of lipophilic drugs.[2] Recent advances in these formulation technologies have led to the successful commercialization of lipid-based formulations.[3] Mechanisms underlying enhancement of drug absorption by SEDDS are In vivo solublization of drugs, Prolongation of gastric residence time, Promotion of intestinal lymphatic transport, Affecting intestinal permeability, Reduced metabolism and efflux activity. Lipid based formulations offer a potential platform for improving oral bioavailability of drugs especially those belonging to Biopharmaceutical Classification System (BCS) class II and class IV.[4] Advantages of SEDDS are Improvement in oral bioavailability, Ease of manufacture and scale-up, Reduction in inter-subject and intra-subject variability and food effects, Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT, No influence of lipid digestion process and Increased drug loading capacity. One of the obstacles for the development of SMEDDS and other lipid-based formulations is the lack of good predicative in vitro models for assessment of the formulations. The drawbacks of this system include chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30-60%) which irritate GIT. Formulations containing several components become more challenging to validate. SEDDS is commonly composed of the following components are oils/lipids, Surfactants (Emulsifiers) and Co-Surfactants (Co-solvents).[5] Furosemide displays poor solubility and low bioavailability when administered orally in the form of tablets. Hence to enhance its bioavailability, we can change its formulation and increase its aqueous solubility without altering its permeability. One of the approaches is the preparation of Self Emulsifying Drug Delivery System which is a lipid based technology.

## MATERIALS AND METHODS

**Materials:** Furosemide and its reference standard (Potency: 99.19 %) were received from Lomus Pharmaceutical Pvt ltd, Gothatar, Bhaktapur, Nepal as a gift sample. Market

Furosemide Tablets, Tween 80, Tween 20, Polyethylene Glycol 400 (PEG 400), Glycerol, Oleic Acid, Sunflower Oil, Hard Gelatin Capsules and other chemicals and reagents were provided by National Model College for Advance Learning, Khusibu, Kathmandu, Nepal.

## Methods

### Analytical method development

UV-visible spectrophotometric method of analysis was developed by preparing 10 mcg/ml solution of Furosemide in 0.1M NaOH and distilled water. The prepared solution was scanned from 400-200nm wavelength and $\lambda_{max}$ was determined.

### Analytical Method Validation

UV visible-spectrophotometric method validation was done in terms of Linearity, Specificity, Accuracy and Precision, Limit of Detection (LOD) and Limit of Quantification (LOQ).[6]

### Solubility Screening

A solubility test of Furosemide was performed in various Oils (Oleic Acid, Sunflower oil), Surfactants (Tween 80 and Tween 20) and Co-surfactants (Glycerol and PEG 400) and distilled water. For this excess of Furosemide (i.e. 1000mg) was added mixed in 10 ml of the selected vehicles. The mixtures were then shaken for 48 hours in an orbital shaker at 50 rpm maintained at 37±1°C. Mixtures were equilibrated for 24 hours at room temperature and then centrifuged at 8000 rpm for 10 min. The supernatant was collected and excess insoluble furosemide was discarded. The concentration of solubilized Furosemide was quantified by UV spectrophotometer at 271 nm.[2,7,8] For this, 1ml of the supernatant liquid was pipetted out and necessary dilution was made with 0.1M NaOH. Solubility of Furosemide in each vehicle was calculated from the calibration curve of furosemide in 0.1M NaOH.

### Preparation of SEDDS

Sixteen different SEDDS formulations were prepared using Oleic Acid as the Oil, Tween 80 as surfactant and Polyethylene Glycol 400 as co-surfactant. For the preparation of systems, amount of surfactant was varied form 10% to 80% (v/v), co-surfactant form 10% to 80% (v/v) and oil was used to adjust remaining mixture proportion. In all formulations, the level of Furosemide was kept constant (i.e. 20mg per 0.5ml). SEDDS formulations were prepared by dissolving accurately weight furosemide in surfactant-cosurfactant mixture by gentle stirring on a magnetic stirrer at 50°C until furosemide was completely dissolved and then adding oil to it. The mixture was stored at room temperature until further use.

**Table 1: Composition of SEDDS of Furosemide containing 20mg/ml furosemide**

| Formulation Name | Surfactant (% v/v) | Co-surfactant (% V/V) | Oil (% v/v) |
|---|---|---|---|
| **F1** | 20 | 20 | 60 |
| **F2** | 20 | 30 | 50 |
| **F3** | 20 | 40 | 40 |
| **F4** | 30 | 20 | 50 |
| **F5** | 30 | 30 | 40 |
| **F6** | 30 | 40 | 30 |
| **F7** | 40 | 20 | 40 |
| **F8** | 40 | 40 | 20 |
| **F9** | 20 | 60 | 20 |
| **F10** | 60 | 20 | 20 |
| **F11** | 80 | 10 | 10 |
| **F12** | 30 | 60 | 10 |
| **F13** | 50 | 40 | 10 |
| **F14** | 30 | 10 | 60 |
| **F15** | 60 | 10 | 30 |
| **F16** | 10 | 10 | 80 |

**In vitro Characterization and Evaluation of the prepared formulations**

**Assessment of Emulsification Time**

The emulsification time of SEDDS formulations were determined in a USP dissolution tester. The SEDDS formulation equivalent to 20 mg of Furosemide i.e. 500 µL was added drop wise to 500 mL of distilled water maintained at 37±0.5°C. Gentle agitation was provided by a paddle rotating at 50 rpm. Time taken by the formulation for emulsion formation as observed visually was noted with the help of stop watch.[2,7]

**Emulsion Droplet Size Determination**

Droplet size of SEDDS diluted with water was determined using an optical microscope with an aid of reticule and stage micrometer. Briefly, SEDDS formulations (equivalent to 20mg Furosemide) were diluted with 500 mL distilled water and thereafter, the droplet size was immediately determined after 2 min stirring. Each determination was done in triplicate.[2,7]

**Spectroscopic Characterization of Optical Clarity**

Each formulation equivalent to 20 mg furosemide was diluted with 500 mL of distilled water. The transmittance values of each emulsion post-dilution were measured by a UV spectrophotometer at 723nm.[7]

**Determination of Specific Gravity of SEDDS formulations**

Specific Gravity of each formulation was determined using a Picometer.

$$\text{Specific Gravity} = \frac{\text{Density of Formulation}}{\text{Density of Water}} \quad \text{.....1.1}$$

**Drug Content**

Prepared SEDDS containing Furosemide equivalent to about 50 mg was weighed accurately and transferred to a 100.0 ml volumetric flask and was shaken with 50 ml of 0.1M NaOH and then sufficient 0.1M NaOH was added to produce 100.0 ml and then filtered. 5.0 ml of the filtrate was diluted to produce 50.0 ml with 0.1M NaOH. Again, 10.0 ml of this solution was diluted to 50.0 ml with 0.1M NaOH. The absorbance of the resulting solution was measured by a UV spectrophotometer at 271 nm. The content of furosemide was calculated taking 580 as the value of A(1%, 1cm) at the maximum at about 271 nm.[9]

Drug Content was calculated using the formula:

$$\text{Drug Content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{standard weight}}{\frac{\text{sample weight}}{\text{specific gravity}}} \times \frac{\text{sample dilution}}{\text{Standard Dilution}} \times 0.5 \times \text{Purity} \quad \text{.....1.2}$$

$$\%\ \text{of label claim} = \frac{\text{observed value}}{\text{label claim}} \times 100 \quad \text{.....1.3}$$

**Preparation of SEDDS formulation or dosage form**

After performing assay, 0.5ml of prepared formulations were filled into hard gelatin capsules, size ‘0’ using a micropipette such that each hard gelatin capsule contains 20 mg of furosemide.

***In vitro* Dissolution Studies**

Dissolution of SEDDS of Furosemide was performed by USP type II apparatus in 900ml of distilled water. The temperature of the medium was maintained at 37±0.5°C and the paddle was set at the rotation speed of 50 rpm. 0.5ml of SEDDS formulations were filled into size ‘0’ capsules and used for drug release studies. Capsules were made to sink using capsule sinkers. 15mL of samples were removed from each basket at 5, 10, 15, 20 and 30 minutes respectively and immediately the removed volume was replaced with same volume of distilled water respectively. The samples were filtered and necessary dilutions with distilled water were made to give a solution expected to contain about 0.001% w/v of furosemide. Then the absorbance of this solution was measured at 277nm using UV-visible spectrophotometer. Then the drug release was calculated using the formula

$$\%\ \text{Drug release} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{Standard Dilution}}{\text{Sample dilution}} \times \text{purity} \times 100 \quad \text{.....1.4}$$

## RESULT AND DISCUSSION

**Analytical Method Development:** The absorption maxima of Furosemide in water and 0.1M NaOH was observed to be 277nm and 271nm respectively.

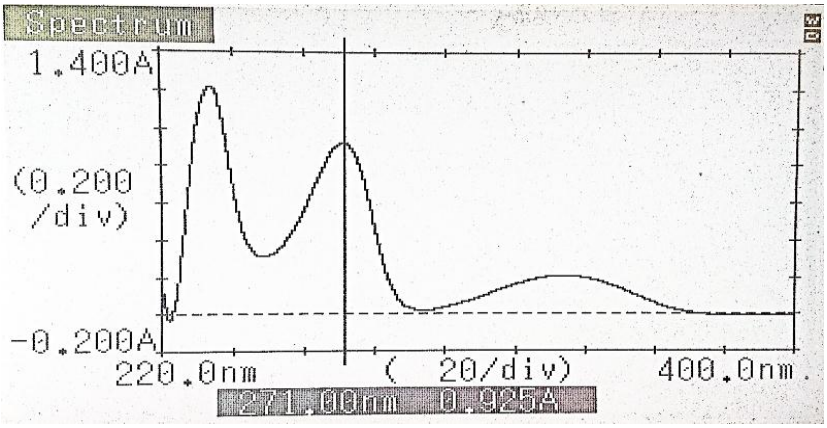


**Figure 1: UV-spectrophotometric scan of Furosemide in 0.1M NaOH**

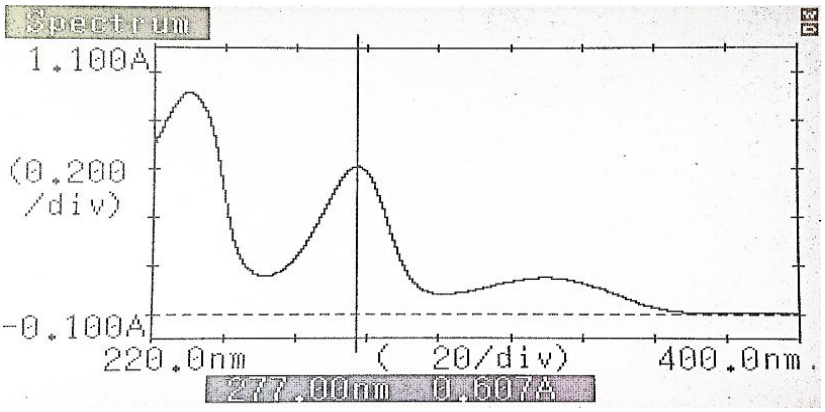


**Figure 2: UV-spectrophotometric scan of Furosemide in Water**

**Analytical Method Validation:** The absorbance showed a linear relationship with the concentration of furosemide having a correlation coefficient ($R^2$) 0.9986 and regression equation y = 0.0606x - 0.0734 ……1.5

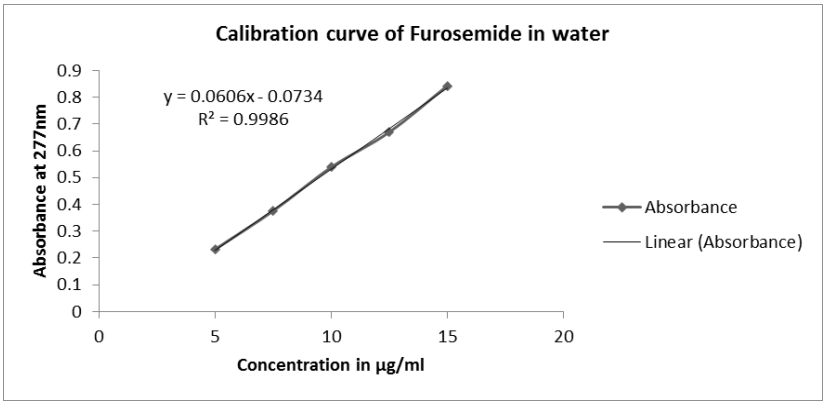


**Figure 3: Calibration curve of Furosemide in water**

The absorbance showed a linear relationship with the concentration of furosemide having a correlation coefficient ($R^2$) 0.9981 and regression equation

y = 0.0548x + 0.02……..1.6

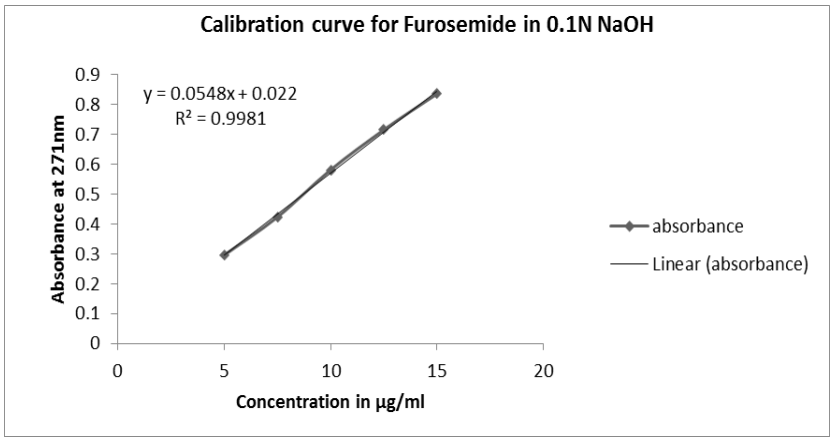


**Figure 4: Calibration curve for Furosemide in 0.1N NaOH**

The method was found to be specific to furosemide with negligible interference. The method also holds good accuracy with % recovery of 100.58% - 100.77% and good precision with % RSD of 0.73 % which is NMT 2 %. The detection limit was 0.06997µg/ml and quantification limit was found to be 0.212µg/ml.

**Solubility Study**

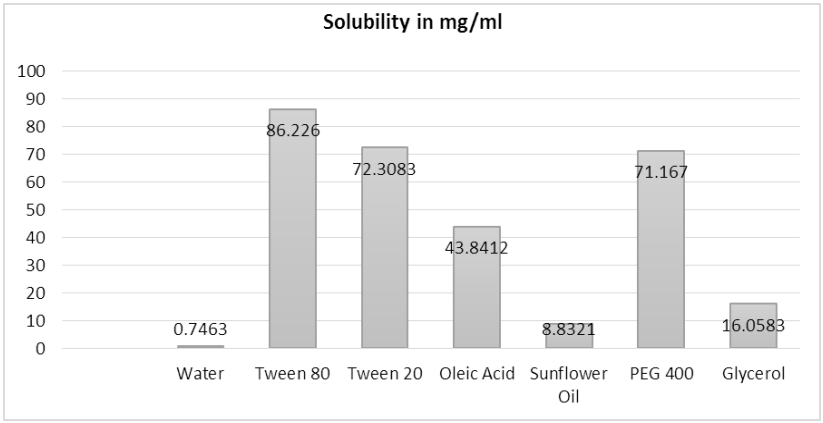


**Figure 5: Solubility of Furosemide in different vehicle**

Aqueous solubility of Furosemide was very poor in water (0.7463 mg/ml). The solubility was improved in surfactants, co-surfactants and lipid vehicles. Oleic acid, Tween 80 and PEG 400 exhibited higher solubility than other vehicles. The solubility of furosemide in these vehicles were 43.8412 mg/ml, 86.226 mg/ml and 71.167 mg/ml respectively. These three excipients were selected for further studies, where Oleic acid was chosen as the oil phase, Tween 80 as the surfactant and PEG 400 as the cosurfactant.

## *In vitro* Characterization and Evaluation of the prepared formulations

### Characterization on the basis of Emulsification Time

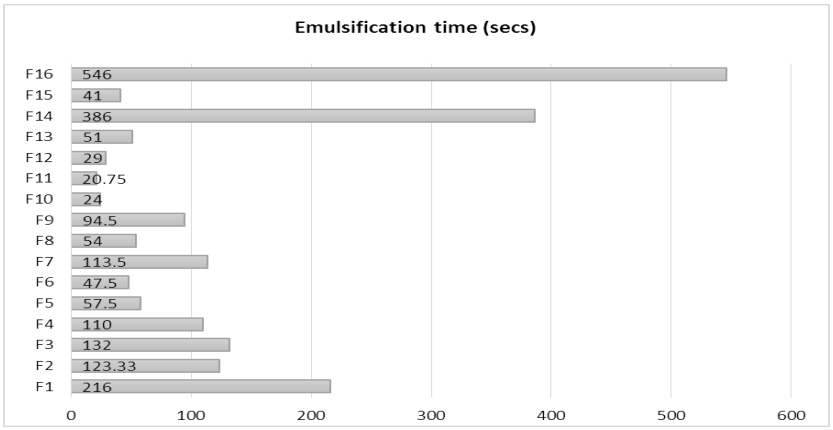


**Figure 6: Emulsification time of various SEDDS formulation**

The formulation containing a higher amount of surfactant or cosurfactant took less time to be emulsified. Rapid emulsification (≤30 seconds) was observed in the formulations F10 (24 sec), F11 (20.75 sec) and F12 (29 sec). Formulation F11 took the least time for emulsification. It might be due to the presence of a higher concentration of surfactant (i.e. 80%), which facilitated the self-emulsification process that eventually led to a high emulsification rate. Co-surfactant also had the same effect as of surfactant as evident by the fact the formulation F12 which contain 60% percent of co-surfactant took only 29 seconds for emulsification.

Those formulations which contain less amount of surfactant and/or co-surfactant showed greater time required for emulsification. Formulation F16 had greatest emulsification time i.e. 546 seconds (9 min 6 sec) and had only 20% of surfactant-cosurfactant concentration.

### Characterization on the basis of Emulsion Droplet Size

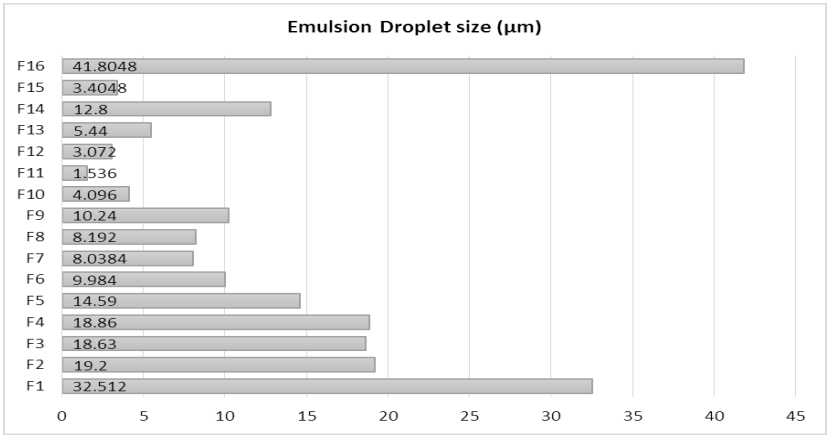


**Figure 7: Emulsion Droplet size of various SEDDS formulation**

Microemulsion droplet size was within the range of 1.536 to 41.8048 µm. Emulsion globule size analysis showed that the amount of oil and surfactant affects the globule size of the emulsion. The result showed that on increasing in surfactant concentration (10 to 80%), globule size decreased from 41.8048 µm (F16) to 1.536 µm (F11). Similarly, when the proportional amount of the oil was increased, there was simultaneous increase in globule size. The presence of 80% surfactant in F11 resulted in finer emulsion droplets. On the other hand, F16 produced larger emulsion droplets, as it was comprised of only 10% of surfactant. This may be explained by the fact that stabilization of the oil droplets is a result of the localization of the surfactant molecules at the oil-water interface.[7]

**Spectroscopic Characterization of Optical Clarity**

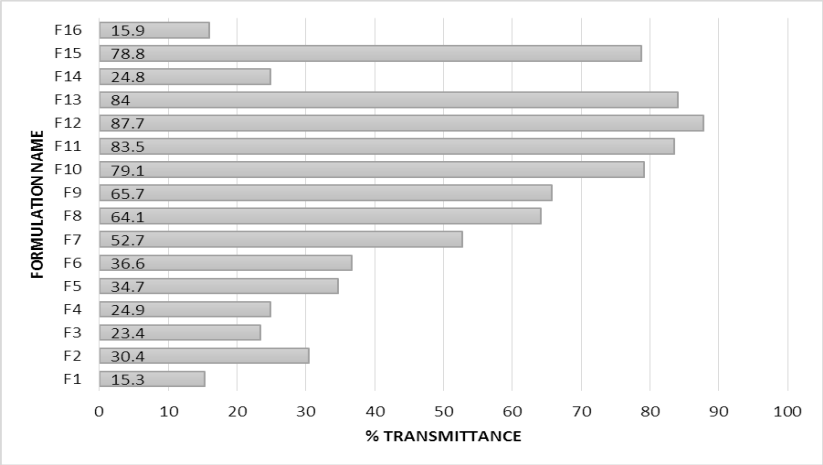


**Figure 8: Percentage Transmittance of various SEDDS formulations**

The value of % transmittance varied from 15.3% to 87.7%. Higher % transmittance values were observed in formulations which contained higher concentration of surfactant. Formulation F12 had the greatest value of % transmittance.

**Table 2: Specific gravity and assay of prepared SEDDS formulation**

| Formulation | Density (kg/m$^3$) | Specific Gravity | Assay percentage |
|---|---|---|---|
| **F1** | 0.975 | 0.9720 | 92.45 ± 0.752 |
| **F2** | 1.003 | 1.0000 | 92.35 ± 0.824 |
| **F3** | 1.042 | 1.0388 | 97.84 ± 0.369 |
| **F4** | 1.003 | 1.0000 | 98.82 ± 0.345 |
| **F5** | 1.04 | 1.0368 | 99.17 ± 0.215 |
| **F6** | 1.049 | 1.0458 | 99.24 ± 0.365 |
| **F7** | 1.024 | 1.0200 | 98.31 ± 0.425 |
| **F8** | 1.07 | 1.0660 | 100.02 ± 0.236 |
| **F9** | 1.072 | 1.0680 | 99.34 ± 0.102 |

| | | | |
|---|---|---|---|
| **F10** | 1.053 | 1.0490 | 93.25 ± 0.452 |
| **F11** | 1.078 | 1.0740 | 94.17 ± 0.324 |
| **F12** | 1.098 | 1.0940 | 96.75 ± 0.275 |
| **F13** | 1.083 | 1.0790 | 98.55 ± 0.814 |
| **F14** | 0.974 | 0.9710 | 94.13 ± 1.022 |
| **F15** | 1.038 | 1.0348 | 97.92 ± 0.371 |
| **F16** | 0.951 | 0.9481 | 93.08 ± 0.124 |

Density of water =1.003 kg/$m^3$

**In vitro Dissolution Studies**

When *in vitro* dissolution study of marketed tablet of Furosemide (20mg) was compared with SEDDS of Furosemide (20mg) in distilled water, marketed tablet showed only 54.2% drug release in 30 min and complete release in almost all formulation respectively. While almost all SEDDS formulation showed complete drug release within 30 min or less, out of all SEDDS formulation, formulation F11 showed fastest release compared to others. It could be suggested that the SEDDS formulation (F11) resulted in spontaneous formation of a micro emulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of marketed Furosemide tablets. The dramatic increase in the rate of release of Furosemide from SEDDS compared to marketed formulation can be attributed to its quick dispersability and ability to keep drug in solubilized state.

Thus, this greater availability of dissolved Furosemide from the SEDDS formulation could lead to higher absorption and higher oral bioavailability.

**Table 3: Dissolution profile of various SEDDS formulations and Market product**

| **Percentage release of drug** | | | | | |
|---|---|---|---|---|---|
| **Formulation** | **5** | **10** | **15** | **20** | **30** |
| **F1** | 63.98 | 70.09 | 73.56 | 79.47 | 78.85 |
| **F2** | 37.9 | 55.01 | 56.44 | 64.39 | 81.71 |
| **F3** | 42.99 | 46.45 | 81.1 | 99.03 | 94.34 |
| **F4** | 63.78 | 69.07 | 79.47 | 84.36 | 91.9 |
| **F5** | 55.2 | 80.48 | 85.58 | 97.4 | 101.06 |
| **F6** | 62.76 | 95.56 | 95.36 | 105.34 | 103.36 |
| **F7** | 66.83 | 71.72 | 83.95 | 89.65 | 104.94 |
| **F8** | 69.28 | 92.3 | 98.42 | 105.34 | 109.83 |
| **F9** | 62.76 | 76.01 | 88.63 | 92.71 | 97.6 |
| **F10** | 78.24 | 81.71 | 89.86 | 101.06 | 106.97 |
| **F11** | 91.49 | 98.42 | 100.45 | 104.73 | 104.12 |
| **F12** | 79.67 | 87.82 | 93.73 | 100.25 | 103.51 |
| **F13** | 79.47 | 97.6 | 102.9 | 105.14 | 106.16 |

| | | | | | |
|---|---|---|---|---|---|
| **F14** | 44.82 | 51.14 | 62.76 | 95.56 | 95.36 |
| **F15** | 74.17 | 80.69 | 94.14 | 99.03 | 106.16 |
| **F16** | 43.81 | 48.29 | 54.2 | 57.05 | 75.19 |
| **Market Product** | 35.25 | 41.36 | 47.27 | 49.92 | 54.2 |

## CONCLUSION

From this study it can be concluded that dissolution of Furosemide can successfully be enhanced by incorporating it in SEDDS. Oleic acid, Tween 80 and PEG 400 can be choices of oil, surfactant and co-surfactant respectively. In order to achieve maximum drug release, minimum droplet size along with minimum emulsification time is desired. And to achieve these, oleic acid should be kept in its lower values, whereas the surfactant (Tween 80) should be kept in its highest value.

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## REFRENCES

1. Singh, B., Bandopadhyay, S., Kapil, R., Singh, R., Katare, O.P. “Self-Emulsifying Drug Delivery Systems (SEDDS)”: Formulation Development, Characterization, and Applications. Critical Reviews™ in Therapeutic Drug Carrier Systems, 2009; 26(5): 427–521.
2. Kadu, P.J., Kushare, S.S., Thacker, D.D., Gattani, S.G. "Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS)." Pharmaceutical Development and Technology, 2010; 16(1): 65-74.
3. Kalepu, S., Manthina, M., Padavala, V. “Orallipid-baseddrug delivery systems—an overview,” Acta Pharmaceutica Sinica B, 2013; 3: 361–372.
4. Sarpal, K., Pawar, BY and Bansal, KA. Self -Emulsifying Drug Delivery systems: A Strategy to improve Oral Bioavailability. CRIPS 2010; 11(3): 42-49.
5. Shukla, J.B., Koli, A.R., Ranch, K.M., and Rarikh, R.K. SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEM. Pharma Science Monitor: An International Journal Of Pharmaceutical Sciences, 2010; 1(2): 13-33.
6. Guideline, I.H.T., Validation of analytical procedures: Text and Methodology. Q2 (R1), 2005. 1.

7. Khan, F., Islam, M. S., Roni, M. A., & Jalil, R. Systematic Development of Self-Emulsifying Drug Delivery Systems of Atorvastatin with Improved Bioavailability Potential. Scientia Pharmaceutica, 2012; 80(4): 1027–1043.
8. Saifee, M. M., Zarekar, S., Rao, V. U., Zaheer, Z., Soni, M. R., & Burande, S. Formulation and In vitro evaluation of Solid-Self-Emulsifying Drug Delivery System (SEDDS) of Glibenclamide. American Journal of Advanced Drug Delivery 2013; 1(3): 323-340.
9. India. (1985). Pharmacopoeia of India (the Indian pharmacopoeia). Delhi: Controller of Publications.