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Furosemide self nano emulsifying drug delivery system (SNEDDS) formulation comprising of capryol-90, polysorbate-80, and peg-400 with *simplex-lattice-design*

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ABSTRACT

Preparation of SNEDDS aims to improve solubility and absorption of furosemide in the body to reduce the dosage and minimize the side effects of drugs. Ternary diagram constructed from composition mixture produced nanoemulsion in the range of 20-40% of capryol-90, 20-40% polysorbate-80 and 40-60% PEG-400. Formulations of SNEDDS using Design-Expert®10 with simplex-lattice-design method in the study was aimed to investigate the effect of SNEDDS each component's proportions towards test responses. Emulsification time, drug content and viscosity were best demonstrated by run-7 with consecutive values of 131.68±2.14 seconds, 99.89±2.68% and 0.87±0.0043 mm^2/s. The optimum formula was obtained through entering test response parameter data of all thirteen formula. Drug content and emulsification time was 107.0 ± 1.44% and 155.59±1.56 seconds with viscosity value 0.91±0.00 mm^2/s. From the physical stability studies, SNEDDS formulas were stable and did not show phase separation when exposed to temparature stress testing.

Keywords: Furosemide, SNEDDS, Capryol-90, Polysorbate-80, PEG-400

1. INTRODUCTION

Diuretics are edema therapy used due to heart failure, renal failure, and high blood pressure-lowering therapy. Diuretics meshes Henle, such as furosemide, bumetanide, and torsemid are diuretic commonly used in the treatment of heart failure. Furosemide has been formulated in various dosage preparations including tablets and injection dosage. Absorption from oral use of furosemide erratic ranging between 11-90% depends on the formulation and effectiveness (Chungi et al., 1979; Akbuga et al., 1988; Jackson, 2006).

According to Ozdmir and Ordu (1998) furosemide is clasified into the biopharmaceutical classification system (BCS) Group IV that has a problem in solubility and low permeability. Nanoparticulate form of self-emulsifying drug delivery system (SNEDDS) furosemide is expected to resolve the issue of its low solubility. SNEDDS is an isotropic mixtures of oil, surfactant, co-surfactant and drug in a dosage of nanoemulsion oil in water (o/w). SNEDDS nanoemulsion is formed due to the mild agitation motility of the gastrointestinal tract (Hiral et al., 2013). Furosemide is dissolved in the oil phase into the gastrointestinal tract that contains the water phase, nanoemulsion droplet is formed and come in contact with the gastric mucosal membrane. Droplet will release furosemide and be absorbed into the blood vessels surrounding mucosa thus improving the bioavailability of furosemide (Jyoti et al., 2012; Balakumar et al., 2013).

SNEDDS constituent component of the manufacturing process comprise of: an oil phase in the form of capryol-90 a monoglycerides compound that has the ability to dissolve lipophilic drugs well, polysorbate (tween) 80 as a surfactant because of its high emulsifying properties and can increase the permeability of furosemide and polyethylene glycol (PEG) 400 as a co-surfactant to increase the loading dose, accelerate emulsification, and minimize droplet size (Anton and Vandamme, 2009; Rowe et al., 2009; Kumar and Rajeshwarrao, 2011; Wulandari, 2013).

The simplex lattice design (SLD) was employed during formula optimization to observe effect of main components concentration variation towards furosemide SNEDDS physicochemical characteristics (Bolton, 1997). Software DX®10 used was able to facilitate data analysis and determine the most optimum composition ratio with minimal number of experiments compared to trial and error.

2. EXPERIMENTAL SECTION

2.1. Materials and Equipments

Furosemide was kindly gifted by PT. Ifars Pharmaceutical Laboratories. Capryol-90 was purchased from Gattefose. The other materials used in this study are pharmaceutical and analytical grade products supplied by Merck such as PEG-400, polysorbate-80 fil-

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ter Whatmann size 0.22 µm, KH_2PO_4, and NaOH pellets.

Equipments used in this research are laboratory staple equipments and spectrophotometer UV to evaluate furosemide SNEDDS drug content.

2.2. Furosemide solubility testing in Capryol-90

The maximum solubility of furosemide (mg) in capryol-90 was measured by the method of trial and error. Pure furosemide was weighed as much as 1, 2, 3, 4, and 5 mg and dissolved respectively in 0.5 mL capryol-90. The mixture was stirred for 15 minutes at a speed of 240 rpm. The highest concentrations (mg) to dissolve furosemide in 0.5 mL capryol-90 is recorded as the highest concentration that will be used in the formulation process.

2.3. Construction of the Ternary Diagram

Ternary diagram was made by mixing each component of SNEDDS furosemide (capryol-90, polysorbate-80, and PEG-400) in different proportions according to the diagram. Testing of nanoemulsion region was conducted by mixing a solution of 40 mL capryol-90, polysorbate-80, and PEG-400 in 25 mL of distilled water and stirred at 100 rpm (Soni et al., 2014). Evaluation of results was done organoleptically by its dispersibility and colour.

2.4. Formula Determination with Simplex-Lattice-Design (SLD*)*

Determining the composition of formula was done by the simplex-lattice-design. Low and high value of each component was determined based on the ternary diagram result. Order used in the SLD method is quadratic with three times formula replication to obtain 13 formulas tested.

2.5. SNEDDS Furosemide Preparation

Furosemide put in each formula was each tailored to preliminary test results of solubility and put in a glass beaker. The oil phase (capryol-90) was stirred with a hot plate stirrer for 15 minutes and sonicated for 45 minutes. PEG-400 is inserted into the solution above, while the solution is constantly stirred for 15 minutes with a hot plate stirrer. In the next stage, the mixture is sonicated and mixed with polysorbate-80 dropwise using a micro pipette into the glass beaker. The mixture was sonicated for 45 minutes until it becomes a light yellow clear solution. The amount of each composition for the formula can be seen in Table 1.

Table 1. SNEDDS furosemide formulation composition with SLD method

Formula	Component			
	Furosemide (mg)	Capryol-90 (mL)	Polysorbate-80 (mL)	PEG-400 (mL)
Run 1	16	33,33	23,33	43,33
Run 2	16	40	20	40
Run 3	16	20	20	60
Run 4	16	23,33	23,33	53,33
Run 5	16	30	30	40
Run 6	16	23,33	33,33	43,33
Run 7	16	20	40	40
Run 8	16	20	30	50
Run 9	16	26,67	26,67	46,67
Run 10	16	40	20	40
Run 11	16	30	20	50
Run 12	16	20	20	60
Run 13	16	20	40	40

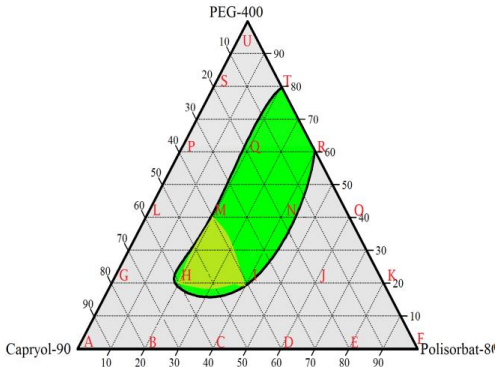

Figure 1 Nanoemulsion Area Determination Result (Green and Yellow)

2.6. Drug Content Test

The quantity of furosemide contained in SNEDDS preparation was measured by mixing 25 mL of the solution with 5 mL methanol for analysis. Absorbance of the mixture was measured by UV-Vis spectrophotometer at a wavelength of 275 nm (Yadav et al.. 2014).

2.7. SNEDDS pH Test

Furosemide was checked for pH level to determine whether it stable in the pH range of 4-9 (Kenneth et al., 1986). pH strip indicators were used in the pH test.

2.8. Viscosity Test

SNEDDS furosemide viscosity testing is done by diluting SNEDDS furosemide at a1: 100 scale with distilled water and then put into Oswald viscometer. Take note of the time required by the solution to cross the first line and the second line.

2.9. Emulsification Time Test

Nanoemulsion formation time was determined by dropping every 20 mL of each formula in 12.5 mL of distilled water and stirred 100 rpm at room temperature. Record the time required to make the mixture becomes clear (Soni et al.. 2014).

2.10. Physical Stability Test

Physical stability of the SNEDDS formula is tested by heating-cooling method performed on the formula for three cycles. Viscosity changes. deposition of furosemide. and clarity of solution were observed in each cycle. A cycle is a combination of 0°C temperature for 1x24 hour and a temperature of 40°C for 1x24 hours (Kaur et al.. 2013).

3. RESULTS AND DISCUSSION

Furosemide solubility testing aims to determine how much a

Table 2. SNEDDS Furosemide 13 Formulas Response Test Results

Formula	Drug Content (%) ± SD	Emulsification Time (second) ± SD	Viscosity Response (mm^2/s) ± SD	pH	Physical Stability
1	97.774 ± 4.093	900.00 ± 0.00	0.8291 ± 0.0007	5 – 6	Stable
2	92.937 ± 0.817	900.00 ± 0.00	0.8101 ± 0.0027	5 – 6	Stable
3	91.531 ± 1.326	568.50 ± 4.50	0.8474 ± 0.0021	5 – 6	Stable
4	86.128 ± 2.096	727.56 ± 5.29	0.8402 ± 0.0074	5 – 6	Stable
5	90.564 ± 3.150	900.00 ± 0.00	0.8410 ± 0.0039	5 – 6	Stable
6	95.818 ± 0.327	900.00 ± 0.00	0.8561 ± 0.0033	5 – 6	Stable
7	99.899 ± 2.681	131.68 ± 2.14	0.8767 ± 0.0043	5 – 6	Stable
8	93.306 ± 1.810	272.84 ± 1.81	0.8743 ± 0.0024	5 – 6	Stable
9	90.523 ± 0.791	900.00 ± 0.00	0.8696 ± 0.0063	5 – 6	Stable
10	95.604 ± 2.705	900.00 ± 0.00	0.8252 ± 0.0014	5 – 6	Stable
11	89.825 ± 2.136	900.00 ± 0.00	0.8280 ± 0.0022	5 – 6	Stable
12	91.742 ± 0.610	553.63 ± 3.30	0.8433 ± 0.0008	5 – 6	Stable
13	94.777 ± 1.908	130.39 ± 2.13	0.8727 ± 0.0061	5 – 6	Stable

capryol-90 (mL) can dissolve furosemide (mg). The results obtained from this preliminary test is 8 mg of furosemide which can be dissolved in 1 mL capryol-90 and was used as a reference to the addition of the furosemide active substance to the SNEDDS solution formulation

The construction the ternary diagram was intended to determine nanoemulsion area that can be formed with a mixture of components capryol-90, polysorbate-80, and PEG-400. Observation result of the nanoemulsion region produced can be seen in Figure 1. Nanoemulsion area indicated by the shading colored green or yellow is marked by a clear colloidal white milk solution (droplet of oil is not visible), while the area in gray is an area that contained the separation phase of oil and water or produces oil globules that were still visible to the eye. Capryol-90 in the range of 20-40%, polysorbate-80 20-80% and PEG-400 0-80% were found to produce the nanoemulsion mixture.

The use of stirrer in the preparation was intended to mix all substances evenly in capryol-90 with a constant speed and power. Sonication process aims to homogenize and shrink the size of furosemide which had not been dissolved after the stirrer. The solution of the SNEDDS furosemide produced for all 13 formulas with different proportions of components is in the form of clear yellow solution.

Results of the drug content thirteen formula testing still qualifies the USP requirements range (80-120%) as shown in Table 2. Formula 7 is a formula with the biggest drug response value among other formulas with ANOVA test analysis showing no significant differences between the formula with a p-value (> 0.05).

The equation obtained after entering the drug content response value into DX®10 program is as follows:

$$Y = 94{,}35\ A + 97{,}42\ B + 91{,}72\ C - 19{,}99\ AB - 11{,}53\ AC - 3{,}75\ BC + 607{,}64\ A^2BC + 160{,}66\ AB^2C - 654{,}05\ ABC^2$$

From the equation polysorbate-80 concentration (B) had a bigger contribution towards drug content compared to capryol-90 (A) and PEG-400 (C).

PH test results from the 13 SNEDDS formulas can be seen in Table 2. The pH of SNEDDS was measured using a pH indicator showing results between pH value of 5 - 6. Capryol-90 pH is between 4.0 to 6.0, while the pH of PEG-400 (5% w/v) is 4.0 to 7.0 and polysorbate-80 having a pH of 6.0 to 8.0 (Rowe et al.. 2009). Pure furosemide is stable in the pH 4-9 so SNEDDS preparations are made to maintain the stability of the active substance in the absence of hydrolysis-mediated due to excessive acid or alkaline (Kenneth et al.. 1986).

Viscosity can affect the stability of liquid solution. The higher the viscosity of the preparation, the more stable the substances that are in it because there is less tendency of the particles to collide with each other. Viscosity data from the 13 formulas of SNEDDS furosemide can be seen in Table 2 with the analysis of ANOVA p-value (<0.05). which means there is a significant difference among the SNEDDS formula. Formula 7 has the highest viscosity among the thirteen other formulas. The equation that states the relationship of response and proportion of constituent components SNEDDS furosemide is as follows:

$$Y = 0.82\ A + 0.88\ B + 0.85\ C$$

Polysorbate-80 (B) accounts for higher viscosity level compared to capryol-90 (A) or PEG-400 (C) due to the viscosity of polysorbate-80, which is bigger than the other components.

SNEDDS solution emulsification time observation was performed to determine how quickly it would form an emulsion of nanometer-size when it is inside the body. The faster the solution turns into nanometer-sized oil droplet. the faster the drug can be dissolved and be absorbed into the blood vessels (Hiral et al.. 2013). Emulsification time test results are shown in Table 2. The best emulsification time value is shown by formula 7 and 13 which are replication formula. Emulsification time response on the proportion of each component can be seen in the following equation:

$$Y = 903.28\ A + 134.32\ B + 564.35\ C + 1577.26\ AB + 717\ AC - 253.5\ BC - 16785.81\ A^2BC + 33474\ AB^2C - 630\ ABC^2$$

ANOVA tests of thirteen formula produces a p-value of (<0.05), which means that there are significant differences in each formula.

SNEDDS furosemide turned into solid form (frozen) when placed in 0ºC temperature, with yellowish muddy color. SNEDDS frozen state when left to sit at room temperature within minutes transformed back into liquid formwith the same physical appearance as before it is inserted into the freezing temperature. Temperature can affect the activation energy of a molecule drugs. the greater the activation energy then the drug will be slower to decompose (Anderson and Scott, 1991). This result showed that temperature can affect the physical appearance of SNEDDS but it did not affect the emulsified state of dosage form.

CONCLUSION

SNEDDS of furosemide can be prepared using the mixture of capryol-90, polysorbate-80 and PEG-400 with favorable results. The proportion of capryol-90, polysorbate-80, and PEG-400 in forming nanoemulsion regions are 20-40%, 20-40% and 40-60%. Capryol-90 in the mix will increase emulsification time, in contrast to the proportion of polysorbate-80 which if added will reduce the time of emulsification and increase viscosity. Drug content response was not influenced by differences in the composition of SNEDDS.

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