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**Research Article**

# THE DISSOLUTION AND DIFFUSION OF FUROSEMIDE ON SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS)

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## ABSTRACT

Furosemide a diuretic exhibits low solubility in water and low bioavailability. The purpose of this study was to determine the effect SNEDDS formation to dissolution and diffusion of furosemide. SNEDDS was made with a mixture of 66% tween 80, 26% propylene glycol, 8% oleic acid and furosemide 40mg/mL. Test for SNEDDS dissolution of the capsules was developed using USP dissolution apparatus I and compared to market products, furosemide suspenssion and furosemide powder. The medium consists of 900mL of *Artificial Gastric Fluid* (AGF) , phosphate buffer pH 5.8 at 37±0.5°C and stirred with a speed of 100rpm. Diffusion test of SNEDDS furosemide was conducted by using reversed rat intestinal bowel and compared to furosemide suspension and furosemide solution. The SNEDDS formulation could enhance the dissolution and diffusion of furosemide compared to the non-SNEDDS formulation.

**Keywords**: furosemide, SNEDDS, dissolution, diffusion

## INTRODUCTION

Furosemide is a loop diuretic which is used in the treatment of edema, congestive heart failure and hypertension (Berkó *et al*., 2002). Furosemide has poor and variable bioavailability due to poor solubility in gastric fluid (5-20μg/mL) (Lindenberg *et al*., 2004). Furosemide is a drug belonging to Biopharmaceutical Class System (BCS) class IV (Custodio *et al*., 2008), with the low dissolution rate. This low dissolution rate represents the first factor responsible for poor bioavailability. A technological approaches are able to improve this property, can represent an important starting point for enhancing drug pharmaceutical use.

Furosemide dosage forms development is being undertaken to increase solubility which eventually is expected to improve bioavailability. One such development is to create SNEDDS. SNEDDS are isotropic mixtures of oil, surfactant, cosurfactant and drugs that form an oil nanoemulsion in water when added to the water phase under slowly stirring (Nazzal *et al*., 2002). These systems spontaneously form an nanoemulsion when exposed to Gastro Intestinal Tract (GIT) liquids to form oil droplet in water with the size in the range of 20-200nm (Porter *et al*., 2008). SNEDDS able to improve the bioavailability of poorly soluble drugs by improving dissolution and permeability through biological membranes due to the present of lipid and surfactant (O'Driscoll, 2002). Small globule size of SNEEDS also provides a large surface area interface for drug release and absorption (Wang *et al*., 2009). Other advantags are of high stability, entrapment efficiency of 100%, a potential decrease in the dose and dose frequency (due to increase bioavailability), the potential to provide protection for the drug against degradation in the environment of the GIT and ease of manufacturing and scale-up (Kale and Patravale, 2008; Shaji and Jadhav, 2010). SNEDDS is preferred because small

total volume are allowed being filled into hard or soft gelatin capsules. SNEDDS also contain surfactants which are in lower amount than self-micro emulsifying drug delivery system (SMEDDS), thereby reducing the risk of surfactants to cause stomach irritation and other toxicity (Gupta *et al*., 2011).

In previous studies had been obtained the optimum formula SNEDDS furosemide (Wahyuningsih *et al*., 2016) but has yet been determined the impact of the dissolution and diffusion of furosemide. The purpose of this study was to determine the effect of SNEDDS against dissolution and diffusion of furosemide.

## MATERIAL AND METHODS

### Preparation of SNEDDS

SNEDDS was made with a mixture of 66% tween 80, 26% propilene glycol, 8% oleic acid and furosemide 40mg/mL. Preparation of SNEDDS furosemide formula was carried out by mixing tween 80 and Propylene Glycol (PG) in vortex mixer for 1min, then added oleic acid and vortex again for 2.5min.

### Physical characteristics

The SNEDDS of furosemide was characterized for transmittance, emusification time and particle size. The % transmittance of SNEDDS furosemide was measured at 630nm using UV-Vis spectrophotometer against distilled water as the blank. Emusification time for SNEDDS furosemide was performed using USP dissolution apparatus II by agitation at 100rpm. One hundred μL of SNEDDS furosemide was added of water (500mL) at temperature 37ºC. The mean droplet size was determined by using Particle Size Analyzer (Horiba Scientific SZ-100).

### Dissolution test

SNEDDS furosemide was filled into soft gelatin capsules and stored at room temperature for 24h (Basalious *et al*., 2010). Test for furosemide release of the capsules was developed using USP dissolution apparatus I /basket and compared to market products, furosemide suspenssion and furosemide powder. The medium consists of 900mL of AGF, phosphate buffer pH 5.8 at 37±0.5°C and stirred with a speed of 100rpm. At regular intervals, 5mL samples were taken and replaced with an equal volume of fresh medium to maintain the volume. Sample was then filtered using a membrane filter and the drug concentration was determined by mean of UV spectrophotometer.

### Diffusion test *in vitro*

Diffusion test was performed using reverse intestine taken from Wistar male rats that were fasted for 20-24h prior to the test. Mice were sacrificed using chloroform and dissected the stomach along the center line of the body and the intestinal was separated. The intestine was taken 15cm from the pylorus. The intestinal length of 20cm, was taken then the intestine divided into 2 equal lengths approximately 10cm each. Intestinal contents was purged with NaCl 0.9% w/v and then gut was reversed with a rod diameter of 2mm. The intestine was attached to the cannula tube of Crane & Wilson then was tied to the aeration cannula with an effective length of 7cm. The study protocol had been approved by the Ethics Committee of Gadjah Mada University No. 426/KEC-LPPT/II/2016.

Each of furosemide and furosemide SNEDDS solution that has been prepared was used as a mucosal fluid and placed in a tube test as much as 75mL. The tube was is then placed into a water bath with the temperature of 37°C. The serosal fluid with pH 6.2 phosphate buffer solution was incorporated into the intestinal sac mounted on a cannula, was then inserted into the tube which has been conditioned in a waterbath. The system was aerated with oxygen with the velocities of approximately 100bubbles/min. During the experiments must be confirmed that the entire portion of the intestine has been submerged in mucosal fluids.

One mL aliquot was taken at 20, 40, 60 and 80min. Each of the sample was placed into a test tube and then was added 2mL of zinc sulfate 5% w/v and 2mL of 0.3N barium hydroxide and was centrifuged for 25min. The supernatant was separated and the furosemide contains was measured by spectrophotometry method.

## RESULT AND DISSCUSION

Some physical properties of the furosemide SNEDDS obtained (Table I).

Table I. Physical properties of the furosemide SNEDDS

| Parameters | Average | SE |
|---|---|---|
| Transmittan (%) | 95.773 | 0.0315 |
| Emusification time (s) | 28.5 | 1.5 |
| Particle size (nm) | 88.9 | 4.9 |

The transparancy of the SNEDDS was expressed in percent transmittance. This is one of the characteristics of SNEDDS to be determined because of the effect on particle size. Observations clarity visually represent qualitative parameters spontaneity of dispersion (Xia *et al.*, 2010), while the transmittance is close to 100% indicates that SNEDDS produce a clear and transparent dispersion with a droplet size estimated at the nanometer (Bali *et al.*, 2010). When the SNEDDS formula was mix with water produced clear water-emulsion with the transmittance of more than 95%, indicating that the size of the droplets produced has met the criteria of nanoemulsion.

Emulsification time test was performed to determine how fast formula SNEDDS form an emulsion (Zhao, 2015). The test results showed emulsification time less than 1min. The SNEDDS formula capable of forming the emulsion after direct contact with gastric fluid, to produce an emulsion system is quite clear (Makadia *et al.*, 2013).

Determining the size of the droplet is made to ensure that the nanometer-sized emulsion formed. Droplet categorized nanoemulsion if the file size is below 100nm (Doh *et al.*, 2013). The results obtained showed that the droplet size was of 88.9nm.

The release of furosemide from SNEDDS, suspension and other formulation (Figure 1). The SNEDDS showed different dissolution profile when compared with powders, suspensions or tablets on the market, especially at pH 1.2. *In vitro* release test was carried out in Artificial Gatro Fluid (AGF) solution pH 1.2 and pH 5.8 phosphate buffer (Figure 1). Drug release at pH 5.8 and at pH 1.2 as shown in the dissolution efficiency (DE) (Table II). At pH 5.8, the drug is released quickly enough and complete in 2h. The release of furosemide lower at pH 1.2, it is because furosemide has pKa ~3.9. At pH 1.2, furosemide, particularly in the form of molecule whereas at pH 5.8 while keeping in ionized form furosemide is more soluble in water.

Table II. Dissolution efficiency value

| Formulation | DE Value | |
|---|---|---|
| | AGF | Buffer phosphat 5.8 |
| Furosemide powder | 5.37±0.047 | 28.04±0.009 |
| Furosemide SNEDDS | 36.46±0.030* | 40.70±0.127* |
| Furosemide Suspension | 4.55±0.010 | 46.44±0.340 |
| Marketed dosage form | 9.91±0.118 | 43.80±0.272 |

Specification: *significant difference in all formulations ($p<0.05$)

At pH 1.2 media, Dissolution Efficiency (DE) furosemide produced most of SNEDDS furosemide, at the pH 5.8 furosemide was not significantly different between the SNEDDS and the suspension. The DE SNEDDS furosemide was largest because in part is affected by SNEDDS components, namely oleic acid, tween 80 and PG. Oleic acid was chosen as the oil phase in the formulation SNEDDS for self-emulsifying capabilities of its high-capacity and large drug dissolution (Miryala and Kurakula, 2013).

PG can increase solubility of a hydrophilic surfactant such as tween 80 and the solubility of the drug in the oil base (Amrutkar *et al.*, 2014). The results are consistent with the results of research conducted by Swaroopa *et al.*, (2014), oleic acid formulation, water, tween 80 and PG can increase the release of the drug than the formula contains oleic acid, water, Cremophor RH 40 and ethanol.

From (Figure 2) that the number of furosemide that diffuses from SNEDDS furosemide greater than furosemide suspension and furosemide solution . The same thing can also (Table III) parameters of diffusion permeability and flux SNEDDS furosemide have the greatest value. Increased furosemide

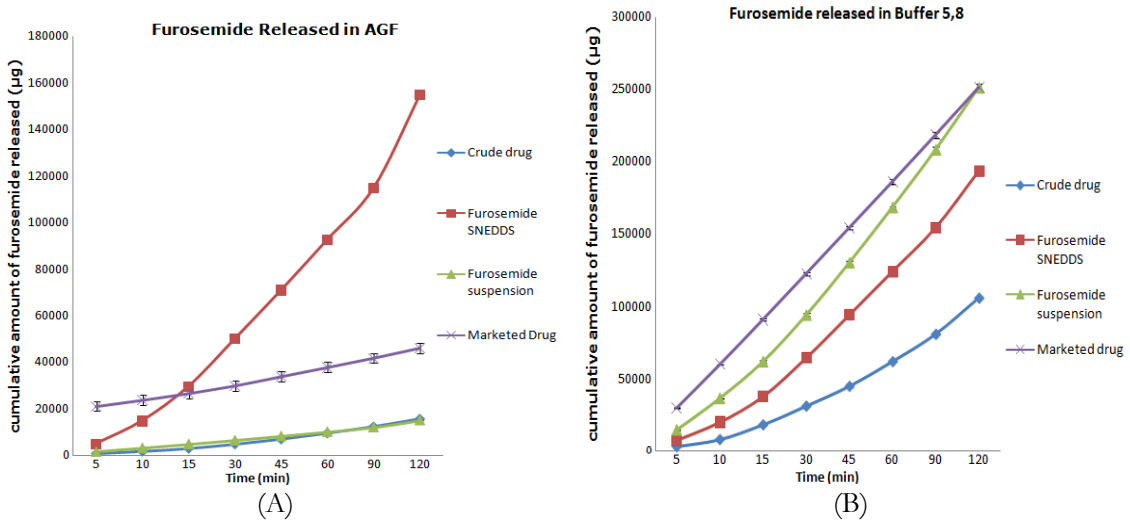


Figure 1. The profile of furosemide release in(A) AGF(pH1.2) and in (B) phosphate buffer (pH 5.8) at 37ºC ± 0.5 of furosemide powder, furosemide SNEDDS and furosemide suspension. Data represent the average of five independent determinations ± SE and furosemide suspension. Data represent the average of five independent determinations ± SE.

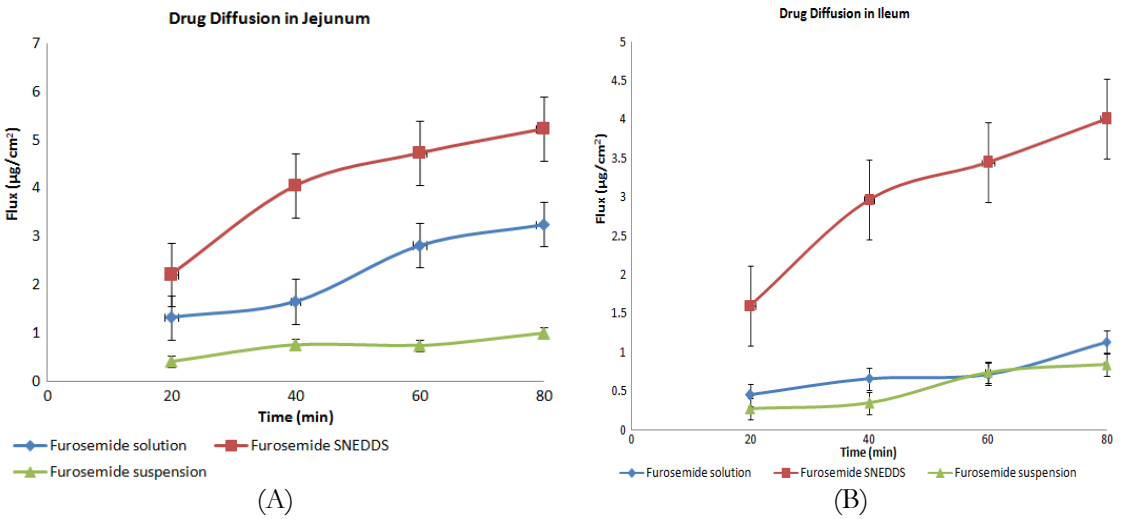


Figure 2. *In vitro* drug diffusion of crude drug, furosemide SNEDDS and furosemide suspension in (A) jejunum (B) ileum. Data represent the average of three independent determinations ± SE

diffusion due to the influence of each of the excipients used in SNEDDS which are oleic acid, tween 80 and PG.

Oleic acid can act as an enhancer with localizes hydrophobic drug to the multilamellar lipid membrane (Yu *et al*., 2003). Oleic acid, the class of fatty acids, is included enhancers to increase the rate of diffusion by increasing membrane fluidity (Hadgraft and Walters, 1993).

The absorption of furosemide was inhibited significantly by P-gp, whereas Tween 80 showed to inhibit P-gp pump. As a result, It will inhibit furosemide efflux, which the concentration of furosemide inside cells is remain high (Al-Mohizea, 2010). Tween 80 has also the ability to inhibit P-gp of intestines and has been widely used to increase the permeability of various drugs in *in vitro* study (Prabhakar *et al*., 2013). Surfactants can increase

Table III. Diffusion parameters of solution, suspension, SNEDDS furosemide in jejunum and ileum.

| Formulation | Jejunum | | Ileum | |
|---|---|---|---|---|
| | Permeability ($min^{-1}.cm^{-2}$) | Flux ($\mu g.min^{-1}.cm^{-2}$) | Permeability ($min^{-1}.cm^{-2}$) | Flux ($\mu g.min^{-1}.cm^{-2}$) |
| Furosemide solution | $1.74x10^{-4}\pm5.28x10^{-5}$ | $3.47x10^{-2}\pm1.05x10^{-2}$ | $5.27x10^{-5}\pm1.44x10^{-5}$ | $1.05x10^{-2}\pm2.87x10^{-3}$ |
| Furosemide SNEDDS | $2.44x10^{-4}\pm5.20x10^{-5}$ | $4.87x10^{-2}\pm1.04x10^{-2}$ | $1.92x10^{-4}\pm6.00x10^{-5}$ | $3.84x10^{-2}\pm1.20x10^{-2}$ |
| Furosemide suspension | $9.63x10^{-5}\pm1.46x10^{-5}$ | $1.93x10^{-2}\pm2.93x10^{-3}$ | $1.05x10^{-4}\pm1.57x10^{-5}$ | $2.11x10^{-2}\pm3.14x10^{-3}$ |

fluidity of the intestinal membrane and open the tight junctions which resulted in an increase of membrane permeability (Mohsin *et al*., 2012; Porter *et al*., 2008; Rajesh *et al*., 2010). On the other hand, tween 80 can also act as an enhancer to improve membrane permeation (Acharya *et al*., 2013).

PG is one of the compounds to facilitate drug transportation to the polar lipids of membrane (Trommer and Neubert, 2006). It will increase the penetration of drugs across the membrane. PG is as cosolvent to produce a saturated solution to maximize the drug thermodynamic activity. PG can be used as a penetration enhancer at a concentration of 1% to 10% (Williams and Barry, 2004).

The finding of this study consistent with that of Acharya *et al*., (2013) which proved that the combination among oleic acid, tween 80 and PG can increase the diffusion of carbamazepine.

## CONCLUSION

SNEDDS formulation can enhance the dissolution and diffusion of furosemide.

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