INTRODUCTION

Furosemide is a widely used high-ceiling (loop) diuretic drug. Indications of furosemide include oedematous states associated with congestive heart failure, chronic renal failure, and cirrhosis of the liver and the treatment of hypertension [1, 2]. The prime site of absorption of furosemide is stomach and upper small intestine, perhaps due to its weak acidic properties (acid dissociation constant, pKa = 3.93) [2-4]. Furosemide provides rapid onset and short duration ($t_{1/2} = 1.3\pm0.8$ hours, mean±SD) of action [4, 5]. The foremost reason for poor bioavailability of furosemide is predicted due to its poor solubility [2]. Furthermore, poor solubility leads narrow absorption window of furosemide, which in turn leads to its low bioavailability (49%±17%, mean±SD) [2, 4, 6].

Oral treatment with furosemide often becomes complicated, apparently due to its unpredictable systemic availability and due to erratic responses to a given dose [2]. Hence, the main focus of this study was to enhance the water solubility and the bioavailability of furosemide by self-emulsifying drug delivery system. For this purpose, in this experiment we have used the micro emulsion dosage forms of furosemide along with some excipients; such as, solvents (PEG 400 or Polyethylene glycol 400, PEG 600 or Polyethylene glycol 600), surfactant and emulsifier (Tween 20 or Polysorbate 20, Tween 80 or Polysorbate 80), solubilizer and lubricant (Captex 500).

A micro emulsion is considered to be a thermodynamically or kinetically stable isotropic mixture of liquid dispersion of an oil phase and a water phase, in combination with a surfactant [7]. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil or water interfacial tension [8]. As the droplet size is less than 25% of the wavelength of visible light, micro emulsions are transparent [9]. Three components are the basic requirements to form a micro emulsion: an oil phase, an aqueous phase and a surfactant [10]. The droplet size, viscosity, density, turbidity, refractive index, phase separation and pH
measurements ought to be performed to characterize the micro emulsion. The droplet size distribution of micro emulsion vesicles can be determined by either light scattering technique or electron microscopy. This technique has been advocated as the best method for predicting micro emulsion stability [11]. The physical stability of the micro emulsion must be determined under different storage conditions (4, 25 and 40°C) during 12 months [12].

Self-emulsifying drug delivery system (SEDDS) uses a micro emulsion achieved by chemical rather than mechanical means, which is defined as an isotropic mixture of natural or synthetic oils, solid or liquid surfactants or alternatively one or more hydrophilic solvents and co-solvents can be mainly used to improve the oral absorption of highly lipophilic compounds [13]. SEDDS are physically and thermodynamically stable and easy to manufacture. The factors by which SEDDS’s working ability can be determined are the nature of the oil or surfactant pair, the surfactant concentration, oil and surfactant ratio, the concentration and nature of co-surfactant or surfactant ratio, polarity of emulsion, droplet size and charge [14]. Therefore, using these modern techniques of micro emulsion and SEDDS in combination will effectively facilitate drug’s solubility and bioavailability [15]. Henceforth, the aim of this study was to investigate and develop SEDDS of furosemide and to enhance its bioavailability.

MATERIALS AND METHODS

Materials
Furosemide 40 mg (Brand name, Lasix) was purchased from Sanoﬁ, Bangladesh and used as the drug for preparing micro emulsions. The rate enhancing polymer & other excipients used in this study were purchased from various sources. PEG-400 (co-surfactant), PEG-600 (co-surfactant), tween-20 (surfactant) and tween-80 (surfactant) were purchased from Mark Pharma (India); whereas Captex-500 (oil, emulsifying agent) and distilled water (solvent) were supplied by ABITEC Corporation (USA) and Osmosia (Bangladesh) successively. All the chemicals used in this study were reagent grade. Analytical instruments used in this study were dissolution tester (Type-II USP) (Veego VDA-6DR, Germany), UV-spectrophotometer (Shimadzu, Japan), electronic balance (Ohaus LS200, Switzerland), sonicator (Power sonic 603, Korea) and water bath (CT-2000, USA).

Methods

Solubility study of furosemide
In this study, the solubility of furosemide was determined in various oils, surfactants, and co-surfactants. Mixture of oil surfactant & co-surfactant at different ratios were prepared in this experiment. Furosemide 500mg was added and dissolved in 10ml distilled water. Afterwards, the formulation was sonicated until a clear solution is achieved and subsequently the formulation was heated in water bath at 70°C for 10 minutes. After that the formulation was transferred in the shaking incubator in 2 hours. The formulation was diluted and concentration of the formulation was measured by UV-spectrophotometer. The Passed furosemide SEDDS was then centrifuged at 3000 rpm for 10 minutes. Subsequently, the supernatant was collected, filtered and measured.

Preparation of furosemide SEDDS
At first, furosemide was dissolved in surfactant and co-surfactant mixture. The mixture was then stirred well with a glass rod and then sonicated until a clear solution is attained. Then Captex-500 was added (calculated amount) & heated at 5°C above the melting point of the oil. A clear solution was achieved by this process, which was then stirred and sonicated. Finally, the calculated amount of oil filled in hard gelatin capsule shell. The data used for formulation of furosemide containing equivalent of micro emulsion has been depicted in table 1.

Table 1: Formulation of furosemide containing equivalent of micro emulsion

| Formulation | Drug (mg) | Oil (mg) | Surfactant (mg) | Co-surfactant (mg) | Drug: Polymer |
|-------------|-----------|----------|-----------------|--------------------|--------------|
| DS3         | 100       | 500      | 250             | 250                | 1:5          |
| DS4         | 100       | 300      | 350             | 350                | 1:7          |
| DS5         | 100       | 100      | 450             | 450                | 1:9          |

Preparation of dissolution media
To prepare the dissolution media, 7.65 ml of 0.1N HCl was added to 892.35 ml distilled water to produce 900 ml of 0.1N HCl.

Dissolution study of furosemide micro emulsion
First of all, 900 ml of 0.1N HCl was taken in both baskets (S1, S2) of dissolution tester type-II (paddle apparatus). Then 10 mg of the active ingredient (pure drug) of furosemide and 2 sample capsules from each formulation was inserted at 10, 20, 30, 40 and 50 minutes time intervals. 10 ml of the sample was then taken with a syringe filter and new 10 ml of fresh 0.1N HCl was provided into the basket. Finally, absorbance was measured in the UV-Visible spectrophotometer.

In vitro dissolution study
In vitro dissolution study was performed in a paddle type dissolution apparatus (USP Apparatus type II, VEGGO, India). A fixed amount of pure drug and
micro emulsion containing 10 mg equivalent of furosemide from each batch was calculated for dissolution purposes. 2000 ml of 0.1N HCl was used as dissolution media. 900 ml of 0.1N HCl was used as dissolution media in each dissolution basket at temperature of 37°C at 50 rpm speed.

The fixed amount of micro emulsion from each batch was weighed and transferred in each dissolution basket. The dissolution was carried out for 50 minutes and 10 ml sample was withdrawn at a predetermined time intervals of 10, 20, 30, 40 and 50 minutes. Each time 10 ml of fresh media was compensated into the basket. The dissolution was carried out for 50 minutes and 10 ml sample was withdrawn at predetermined time intervals of 10, 20, 30, 40 and 50 minutes. Each time 10 ml of fresh media was compensated into the basket. After that absorbance was measured in UV-Vis spectrophotometer (UV-mini-1240, Shimadzu corp., and Kyoto, Japan). Continuous tests’ results were plotted as cumulative percentage release versus time curve. Time required for 25%, 50% and 80% drug release (T25%, T50% and T80%) was used to compare the dissolution results.

Characterization of release kinetics of microemulsion

To study the release kinetics, data obtained from dissolution studies were plotted in different release kinetics models; such as: zero order as cumulative percentage of drug release versus time, first order as log cumulative percentage of drug remaining versus time [16]. To evaluate the mechanism of drug release from micro emulsions, data of the drug release were plotted in Korsmeyer–Peppas equation as log cumulative percentage of drug retard versus log time and the exponent n was calculated through the slope of the straight line [17]. In this study, if zero and first order kinetics failed to explain the drug release mechanism from polymeric formulation due to swelling and/or erosion during dissolution, in these cases, we have used the value of n obtained by fitting the data into Korsmeyer’s equation and also Higuchi’s model as cumulative percentage of drug released versus square root of time [16, 18].

Data Analysis

All the data used in this study have been analyzed by using GraphPad Prism 5.0 (GraphPad Software Inc., USA). Furthermore, all the graphs were also being produced by GraphPad Prism 5.0. Statistical (linear) regression analysis was also carried out by GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Construction of calibration curve of furosemide

Concentration of dissolved drug was determined by using the calibration curve. To prepare a calibration curve of furosemide, 5mg of furosemide was accurately weighed & dissolved in 100 ml of 0.1N HCl media. Then 1, 2, 3, 4, 5, 6, 7, 8, 9 & 10 ml of the solutions were taken in 10ml volumetric flask and 9, 8, 7, 6, 5, 4, 3, 2, 1 & 0 ml of the 0.1N HCl solutions were added to them respectively for the purpose of serial dilution. This serial dilution was carried out to obtain different concentrations of furosemide. These solutions were then analyzed by UV spectrophotometer at 274 nm. The obtained absorbance values and the concentrations used have been mentioned in table 2.

Table 2: Data for the construction of calibration curve of furosemide

| Drug+Media | Concentration | Absorbance |
|------------|---------------|------------|
| 1+9        | 1.1           | 0.07       |
| 2+8        | 2.2           | 0.132      |
| 3+7        | 3.3           | 0.244      |
| 4+6        | 4.4           | 0.331      |
| 5+5        | 5.5           | 0.486      |
| 6+4        | 6.8           | 0.583      |
| 7+3        | 7.8           | 0.658      |
| 8+2        | 8.8           | 0.727      |
| 9+1        | 10            | 0.834      |
| 10         | 11            | 0.918      |

Calibration curve of furosemide was then produced by plotting obtained absorbance values against drug concentrations (Figure 1).

![Fig 1: Calibration curve of furosemide](image-url)
Effects of different polymers on release pattern of furosemide micro emulsion

Furosemide micro emulsion containing different ratios of drug and polymers (Tween 20/80, PEG-400/600, Tween 20/80 + PEG-400/600) at 1:1 to 1:9 ratio with the formulation code DS1, DS2, DS3, DS4 and DS5 were prepared to evaluate the effects of these polymers and SEDDS. After preparation, according to the table 1 dissolution study of furosemide was carried out with paddle method at 50 rpm in 900 ml of 0.1N HCl medium at 37°C (±0.5°C). 10 mg of equivalent furosemide micro emulsions of 5 formulations were used in dissolution studies. The release profile of furosemide was monitored up to 50 minutes.

Mathematical models for furosemide release studies

Mathematical model plays pivotal role in the estimation of drug release mechanism and improvement in the formulation. Study findings also suggest that some of the successful drug delivery systems were developed based on almost random selection of components, configuration and geometries. Choosing the right mathematical model is crucial to achieve a system with optimum drug release. Currently, several models are available to describe the drug release rate from different drug delivery systems [19, 20]. In this study, the used mathematical models are zero order kinetic model, first order kinetic model, Higuchi model, Korsmeyer-peppas model and Hixson–crowell model. These models have been chosen to best describe the relationship between dissolution and release pattern of furosemide mathematically [17].

Zero order release pattern

Zero order kinetic models define the drug release system, whereby drug release rate is independent of its concentration. Mathematical equation of zero order model can be represented as:

\[ C = C_0 - K_0 t \]

Where, \( C \) = amount of drug release or dissolved (assuming that release occur rapidly after the drug dissolved), \( C_0 \) = Initial amount of drug in solution (usually, \( C_0 = 0 \)), \( K_0 \) = Zero order rate constant and \( t \) = time. This relationship of zero order kinetic model is particularly useful to define dissolution rate of modified-release (MR) dosage forms, such as transdermal drug delivery systems, coated matrix tablets of poorly water-soluble drugs, osmotic drug delivery system etc. [17, 21, 22]. In this study, we have plotted cumulative amount of drug released against time to obtain zero order release pattern of furosemide (Figure 2).

First order release pattern

First order kinetic model is typically used to describe adsorption and elimination of certain drugs. Mathematical equation of zero order kinetic model can be expressed as:

\[ \log C = \log C_0 - Kt/2.303 \]

Where, \( C_0 \) = Initial concentration of drug, \( K \) = First order rate constant, \( t \) = time, slope=K/2.303. This slope value is obtained by plotting log cumulative percentage of drug remaining versus time. The first order kinetic model is useful to describe the drug dissolved in pharmaceutical dosage forms containing water-soluble drugs in porous matrices [17, 22-24]. In this present study, we have plotted log cumulative percentages of drug remaining versus time to obtain first order release pattern of furosemide (Figure 3).
Higuchi release pattern

Higuchi model is widely used to describe drug release from matrix systems. In addition, this model is also used to describe different geometrics and porous systems. Mathematical equation of Higuchi model can be epitomized as:

\[ C = D (2qt - C_s t)^{1/2} \]

Where, \( C \) = total amount of drug release per unit area of the matrix (mg/cm\(^2\)), \( D \) = diffusion coefficient for the drug in the matrix (cm\(^2\)/hr), \( qt \) = total amount of drug in a unit volume of matrix (mg/cm\(^3\)), \( C_s \) = dimensional solubility of drug in the polymer matrix (mg/cm\(^3\)) and \( t \) = time. Higuchi model is useful to describe dissolution of drugs from a number of types of modified release pharmaceutical dosage forms, such as transdermal systems and matrix tablets with water-soluble drugs [19, 21, 22, 25]. Data obtained in this study has been illustrated as cumulative percentage of drug release versus square root of time to obtain Higuchi release pattern of furosemide (Figure 4).

Korsmeyer-Peppas release pattern

Korsmeyer-Peppas model defines drug release from a polymeric system. Usually, first 60% of the drug release data is fitted in Korsmeyer-Peppas model to explain the mechanism of drug release. Mathematical equation of Korsmeyer-Peppas model can be represented as:

\[ C_t / C_\infty = k t^n \]

Where, \( C_t / C_\infty \) = fraction of drug release at time t, k = rate constant and n = release exponent. The value of n is used
to depict different release patterns for cylindrical shaped matrices. The \( n \) value is used to characterize different release for cylindrical shaped matrices [17, 22]. Data obtained in this study were plotted as log cumulative percentage of drug release versus log time to obtain Korsmeyer-Peppas release pattern of furosemide (Figure 5).

**Korsmeyer-Peppas release pattern**

Korsmeyer-Peppas model explains the release mechanism of drug from a system, where, there is change in surface area and diameter of particle or tablet. Mathematical equation of Korsmeyer-Peppas model can be characterized as:

\[
C_0^{1/3} - C_t^{1/3} = Kt
\]

Where, \( C_t \)=amount of drug released in time \( t \), \( C_0 \)=initial amount of drug in the pharmaceutical dosage form and \( K \)=rate constant for Korsmeyer-Peppas equation.

**Hixson-Crowell release pattern**

Hixson-Crowell model explains the release mechanism of drug from a system, where, there is change in surface area and diameter of particle or tablet. Mathematical equation of Hixson-Crowell model can be characterized as:

\[
C_0^{1/3} - C_t^{1/3} = K_{HC}t
\]

Where, \( C_t \)=amount of drug released in time \( t \), \( C_0 \)=initial amount of drug in the pharmaceutical dosage form and \( K_{HC} \)=rate constant for Hixson-Crowell equation. When Hixson-Crowell is used to describe a system, it is considered that the release rate of drug particles is limited by the dissolution rate and not by the diffusion which might take place during the polymeric matrix. In addition, to describe a release pattern, this model considers that the surface of the drug particles lessen during the dissolution [17, 26]. To study the Hixson-Crowell kinetics of furosemide, we have plotted the data obtained from this study as cube root of drug percentage remaining in matrix against time (Figure 6).

**Hixson-Crowell release kinetics of furosemide**

Release profiles of furosemide micro emulsion capsules of 5 formulations were obtained from the release kinetics graphs. The total percentages of furosemide release after 50 minutes from the formulations DS1, DS2, DS3, DS4 and DS5 were 35.69%, 39.56%, 51.24%, 54.95% and 64.31% 

**Fig 5: Korsmeyer-Peppas release kinetics of furosemide**

![Korsmeyer-Peppas release kinetics of furosemide](image)

**Fig 6: Hixson-Crowell release kinetics of furosemide**

![Hixson-Crowell release kinetics of furosemide](image)
It has also been observed that the release rate was extended with the increase of polymer percentage. The highest percentage of drug release within 50 minutes was obtained from DS5, where ratio of drug and polymer was 1:9. In addition, T25%, T50%, T80% and mean dissolution time (MDT) values of the liquids were also found from the graphs. It was seen that formulation DS5 had the lowest MDT value, whereas DS1 had the highest MDT value.

The release data obtained in this study were extrapolated by the zero order, first order, Higuchi, Korsmeyer–Peppas, Hixon-Crowell equations to know the mechanism of drug release from the formulations. In this experiment, the in vitro release profiles of furosemide out of all the mathematical models, was best expressed by Higuchi equation as the plots showed highest linearity (Coefficient of determination, $R^2 = 0.94$). The formulations showed good linearity when plotted according to Higuchi equation, the formulation DS3 showed highest linearity ($R^2 = 0.94$). It can be inferred that the release was dependent on both motility and polymer relaxation. The poor correlation coefficient ($R^2 = 0.66$ to 0.71) was found in first order release kinetic model. Moreover, zero order kinetic model also showed poor linearity ($R^2 = 0.68$ to 0.76).

**CONCLUSION**

SEDDS is a crucial tool to overcome the formulation difficulties and to improve the oral bioavailability of hydrophobic or lipophilic drugs. Furosemide is a poorly water-soluble drug. To increase its solubility in water, we have used several ratios of polymers (Tween-20/80, PEG-400/600, Tween-20/80+PEG-400/600) which were 1:5 to 1:9 with the formulation code DS3, DS4 and DS5 were prepared to evaluate the effects of the micro emulsions. The formulations showed significant improvements in solubility and dissolution than the pure drug. The total percentages of furosemide micro emulsion release after 50 minutes from the formulations DS3, DS4 and DS5 were 51.24%, 54.95%, 64.31% respectively. It was observed that the release rate was extended with the increase of percentage of the polymer. The highest percentage of drug release was within 50 minutes and was obtained from DS5, where the ratio was 1:9. From this present study, it is clear that there is a good chance to modulate the rate and to extend the drug release with these polymers and formulations, which could be useful for the preparation comprised with the combination of drug and release modifiers. Furthermore, the results of this study strongly recommend the successful use of SEDDS to increase solubility, dissolution and in turn bioavailability of poorly soluble drugs like furosemide, which ultimately can decrease the dose and side effects of the drug. A future study in the in vitro condition can justify the release pattern observed from this current study and a standard drug release profile of furosemide micro emulsion could be commercially explained and evaluated.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**

1. Castaneda-Hernandez G, Verges J, Pichette V, Heroux L, Caille G; Input Rate as a Major Determinant of Furosemide Pharmacodynamics: Influence of Fluid Replacement and Hypoalbuminemia. Drug Metab Dispos, 2000; 28(3): 323-328.

2. Ponto L, Schoenwald R; Furosemide (frusemide). A pharmacokinetic/pharmacodynamic review (Part I). ClinPharmacokinet, 1990; 18(5): 381-408.

3. Staib A, Woodcock B, Loew D, Schuster O; Remote control of gastrointestinal drug delivery in man, in: Prescott LF, Nimmo WS (eds.). In L. Prescott, & W. Nimmo, Novel Drug Delivery and Its Therapeutic Application Chichester, UK: John Wiley, 1999; 79-88.

4. Klausner E, Lavy E, Stepensky D, Cserepes E, Barta M, Friedman M, et al.; Furosemide pharmacokinetics and pharmacodynamics following gastro retentive dosage form administration to healthy volunteers. J Clin Pharmacol., 2003; 43(7): 711-20.

5. Jackson E; Diuretics. In J. Hardman, & L. Limbird, Goodman & Gilman’s The Pharmacological Basis of Therapeutics, 10th edition, New York, USA: McGraw-Hill, 2001; 757-787.

6. Murray M, Haag K, Black P, Hall S, Brater D; Variable furosemide absorption and poor predictability of response in elderly patients. Pharmacotherapy, 1997; 17(1): 98-106.

7. Slomkowski S.E; Terminology of polymers and polymerization processes in dispersed systems (IUPAC Recommendations 2011). Pure Appl. Chem., 2001; 83(12): 2229–2259.

8. Kumar S, Singh V; Nano emulsification - A Novel Targeted Drug Delivery Tool. Journal of Drug Delivery & Therapeutics, 2012; 2(4): 40-45.

9. Thakur A, Walia M, Kumar S; Nano emulsion in Enhancement of Bioavailability of Poorly Soluble Drugs: A Review. Pharmacophore, 2013; 4(1): 15-25.

10. Deshmukh P, Salunkhe K, Patil W, Chaudhari S, Davange R, Varpe U; Micro emulsion: A Novel Approach for Drug Delivery System. Journal of Advanced Drug Delivery, 2016; 3(2): 1-2.

11. Patel R, Joshi J; An Overview on Nano emulsion: A Novel Approach. International Journal of Pharmaceutical Sciences and Research, 2012; 3(12): 4640-4650.

12. Türkylımaz A, Çelebi N, Gönül B, Alkan-Önyüksel H; Physical Characterization and Stability of a Micro emulsion for Potential Oral Administration of a Peptide. In A. Hincal, & H. Kaş, Biomedical Science and Technology, New York, USA: Springer Dordrecht, 1998; 65-72.

13. Sallam M, Boscá M; Optimization, ex vivo permeation, and stability study of lipid nanocarrier
loaded gelatin capsules for treatment of intermittent claudication. Int J Nanomedicine, 2015; 10: 4459–4478.
14. Singh A, Singh V, Juyal D, G R; Self-emulsifying systems: A review. Asian Journal of Pharmaceutics, 2015; 9(1): 13-18.
15. Suresh R, Bansal B, Sharma A, Singh C; Review On: Self Dispersing Formulations & Characterization. International Journal of Current Research in Chemistry and Pharmaceutical Sciences, 2014; 1(9): 52-62.
16. Jaiswal P, Aggarwal G, Harikumar S, Singh K; Development of self-micro emulsifying drug delivery system and solid-self-micro emulsifying drug delivery system of telmisartan. International Journal of Pharmaceutical Investigation, 2014; 4(4): 195–206.
17. Dash S, Murthy P, Nath L, Chowdhury P; Kinetic modelling on drug release from controlled drug delivery systems. Acta Pol Pharm., 2010; 67(3): 217-23.
18. Alany R, Tucker I, Davies N, Rades T; Characterizing colloidal structures of pseudo ternary phase diagrams formed by oil/water/amphiphile systems. Drug Dev Ind Pharm, 2001; 27: 31–8.
19. Siepmann J, Siepmann F; Mathematical modelling of drug dissolution. International Journal of Pharmaceutics, 2013; 453(1): 12-24.
20. Hopfenberg H; In D. Paul, & F. Haris, in controlled release polymeric formulation Washington: American Chemical Society, 1976; 222.
21. Singhavi G; In vitro drug release characterization models. International journal of Pharmaceutical studies and research, 2011; 2: 77-84.
22. Lokhandwala H; Kinetic modeling and dissolution profiles comparison: An overview. Int.J.Pha Bio Sci., 2013; 4(1): 728-737.
23. Bravo S, Lamas M, Salomón C; In-vitro studies of diclofenac sodium controlled-release from bio polymeric hydrophilic matrices. J Pharm Pharmaceut Sci, 2002; 5(3): 213-219.
24. Costa P, Sousa Lobo J; Modeling and comparison of dissolution profiles. Eur J Pharm Sci., 2001; 13(2): 123-33.
25. Grassi M.G; Mathematical modeling and controlled drug delivery; matrix systems. Curr Drug Deliv, 2005; 2: 97-116.
26. Ramteke K; Mathematical models of drug dissolution: A review. Sch.Acad.J.Pharm, 2014; 3(5): 388-396.