Research Article

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Enhanced diuretic action of furosemide by complexation with β-cyclodextrin in the presence of sodium lauryl sulfate

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Abstract: Preparation of inclusion complex using cyclodextrins is a well-known formulation strategy to elevate the solubility of drugs. However, often cyclodextrins alone may not bring a considerable improvement in the solubility of low solubility drugs. In this study, the inclusion complexation of furosemide (FSM) was tried with β-cyclodextrin (β-CD) either with the use or without the use of sodium lauryl sulfate (SLS), which is a surfactant. By using the kneading method, the binary complex of FSM/β-CD in the equal molar ratio was used. FSM and β-CD were kneaded continuously until a thick past was achieved, which was evaporated for a period of about 24 h. The solid complexed product was then crushed and stored in airtight container until use. Phase solubility studies confirmed a stoichiometric ratio of 1:1 (FSM/β-CD and FSM/β-CD with SLS). The apparent stability constant and complexation efficiencies of significantly enhanced in the presence of SLS. The prepared complexes were evaluated for DSC, PXRD, ^{1}H NMR, and *in vitro* release studies. The results exhibited a significant enhancement in diuresis in rats. It is evident that the addition of SLS with β-CD significantly enhances the solubilizing efficiencies and hence bioavailability of FSM.

Keywords: furosemide, cyclodextrin, complex, solubility, diuretic activity

1 Introduction

Furosemide (FSM) is one of the potent sulfonamide diuretics; it belongs to a loop diuretic group. FSM is frequently used in the management of edema, renal diseases, and hypertension [1–6]. It blocks reabsorption of specific ions, including sodium, potassium, and chloride in the loop and ascending limb of Henle, resulting in the loss of these ions and water into the urine, hence the diuretic effect [7–9].

FSM, 4-chloro-*N*-furfuryl-5-sulphamoylanthranillic acid (Figure 1), is a weak acid with three functional groups capable of forming H-bonding, and the free acid portion has been found to appear in seven different polymorphic forms (four polymorphs [I, II, III, IV], two solvates [IV-DMS and V-dioxane], and one amorphous form) [10,11]. According to the BCS (Biopharmaceutics Classification System), FSM is labeled in Class IV drug because of both its limited aqueous solubility (5–20 μg/mL) and low permeability; therefore, it has limited bioavailability (60–65%) and an erratic pharmacokinetic profile [12–14].

β-Cyclodextrin (β-CD) belongs to a group of natural cyclic oligosaccharides. Many studies reported the ability of CD complexation to improve water solubility, especially for hydrophobic drugs [15]. Cyclodextrins consists of either 6, 7, or 8 glucopyranose subunits (α,β,γ-cyclodextrin, respectively) connected in a 1,4-configuration with several diameters [16]. The exterior of the CD ring is hydrophilic, and the interior appears to be a lipophilic core in which suitable sized organic molecules can establish a noncovalent binding with the CD (i.e., inclusion complexes) [17]. Inclusion complexation with β-CD is a well-known method to improve the water solubility of water-limited solubility drugs and, therefore, its stability, the kinetics of dissolution, and bioavailability [18].

Surfactants are amphiphilic molecules that depress the surface tension between a gas and a liquid or interfacial tension happening between two liquids or between a liquid and a solid, allowing for easy spreading of a droplet on the surface. With the increase in surfactant concentration in the aqueous phase, the surfactant that will be undergoing adsorption at the surface is increased, resulting in a decrease in surface or interfacial tension [19]. One crucial characteristic of surfactants is the ability to

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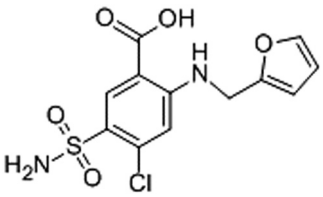

Figure 1: Chemical structure of FSM.

aggregate, forming micelle in solution, which have particular significance in medicine because of their ability to elevate the aqueous solubility of limited aqueous-soluble drugs [20].

Sodium lauryl sulfate (SLS) is one of the anionic surfactants that has the chemical formula of $CH_3(CH_2)_{11}SO_4Na$. SLS is widely used as an emulsifier, wetting agent, and detergent in cosmetics and pharmaceutical products. SLS is one of the synthetic surfactants of an amphiphilic identity, which consists of an anionic organosulfate (tail – chain consists of 12-atoms of carbons) coupled to a sulfate group since it has a negative charge [21].

This study is developed to evaluate the complexation of FSM with β-CD and confirm the presence of complex formation, increase solubility, and enhance diuretic action of FSM. The prepared complexes were evaluated by phase solubility studies and characterized for various physicochemical investigations such as differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), ^{1}H NMR, and *in vitro* release studies. Besides, the *in vivo* diuretic activity of FSM β-CD in rats is to be evaluated in this study.

2 Materials and methods

2.1 Materials

FSM β-CD and SLS were obtained from Sigma, Aldrich, USA. All chemicals and solvents used in the study were of A.R. grade. Milli Q water (Millipore, Germany) is the water we used for all the studies.

2.2 Phase solubility studies for FSM in binary and ternary systems

Phase solubility studies of FSM/β-CD and FSM/β-CD with 0.5% SLS were used to evaluate complexation [22]. In this study, extra quantities of FSM were added to 10 mL of distilled water containing increment amounts of β-CD and β-CD with 0.5% SLS (5–30 mm). The mixed suspensions were shaken at 100 rpm and 37°C for 72 h in a water bath. The resultant solutions were filtered using a 0.45 µm membrane filter and then analyzed by UV at 272 nm [23]. The binding strength of FSM/β-CD and FSM/β-CD with 0.5% SLS was calculated in terms of apparent stability constant (K_s) using the following equation:

$$K_s = \frac{\text{slope}}{S_o \times (1 - \text{slope})}, \quad (1)$$

where S_o is the intrinsic solubility of FSM in water and slope is the slope of phase solubility diagram.

The complexation efficiency (CE) is the ratio of complex formed to free β-CD concentration, which is an excellent method to calculate the solubilizing effect of β-CD. The CE was estimated using the following equation:

$$\text{CE} = \frac{\text{Slope}}{1 - \text{Slope}}. \quad (2)$$

2.3 Preparation of complexes in binary and ternary systems

Inclusion complexes containing FSM/β-CD (1:1) were established in preliminary phase solubility studies. The inclusion complexes of FSM/β-CD and FSM/β-CD with 1% (w/v) SLS in 1:1 molar ratio were prepared by utilizing kneading, which is considered as a simple, universal, and cheap technique for preparing complexes. The binary complex of FSM/β-CD in an equal molar ratio was prepared by mixing one mole of FSM and β-CD each in a mortar followed by the addition of about 4 mL of water–ethanol solution (50% v/v). The mixture was continuously kneaded for 15 min until a thick paste was achieved, which was evaporated at 50°C for 24 h. The solid product was then crushed, passed through a No. 80 sieve, and stored in airtight container until use. The ternary complex of FSM/β-CD with SLS was prepared by the same aforementioned procedure, and only 1% SLS was kneaded with FSM/β-CD complex.

2.4 Differential scanning calorimetric studies

DSC studies of pure drug FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS were performed using a DSC instrument

"(Scinco, DSC N-650, Seoul, Korea)." Precisely weighted samples (5 mg) were pressed in an aluminum pan by pressing with a hand press; the pressed pan was kept on the DSC sample holder and heated to a range of 50–350°C at a warming rate of 20°C/min. The samples were continuously supplied with nitrogen gas with a flow rate of 20 mL/min.

2.5 PXRD studies

PXRD style of pure drug FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS were analyzed using "Ultima IV diffractometer" (Rigaku Inc. Tokyo, Japan) at college of Pharmacy, Prince Sattam Bin Abdulaziz University)" in the range of 2–60° (2θ) at a scan speed 0.5 deg min^{-1}. The diffraction pattern of each sample was scanned at voltage/current (40 kV/40 mA) using Cu tube anode. The recorded diffraction patterns were evaluated for crystallinity and amorphic of powder.

2.6 ^{1}H NMR spectral analysis

^{1}H NMR spectra of FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS were recorded in the DMSO solvent using "Bruker program on UltraShield Plus 500 MHz (Bruker)" instrument that works at 500 MHz. The values of chemical shifts of β-CD in the inclusion complexes were calculated that confirmed the inclusion of FSM in the β-CD cavity.

2.7 *In vitro* drug release

The release studies of FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS were performed using the USP II paddle-type method. The apparatus was set with a paddle speed of 75 rpm at 37 ± 0.5°C, and 0.1 N HCl (pH 1.2) used as a dissolution medium. The aliquot of 5 mL of the sample was withdrawn at each time point (0, 10, 20, 30, 40, 50, and 60 min) and compensated with new media. The samples were filtered, suitably diluted, and analyzed by UV at 272 nm [23].

2.8 Diuretic activity

The diuretic activity of the complexes was evaluated in male Wistar rats by dividing the animals into four groups of four rats each. Group I composed of vehicle-treated rats of 2 mL/100 g of body weight as the control. Group II composed of rats treated with standard FSM (10 mg/kg) orally. Binary (FSM/β-CD) and ternary (FSM/β-CD with 0.5% SLS) complexes were given orally (10 mg/kg equivalent to FSM) in group III and IV, respectively. Each animal was kept isolated in a metabolic cage 24 h before the start of the experiment and then fasted overnight with free access to water. The total urine output (mL) of each rat was measured after 24 h. The pH, conductivity, and total dissolved solids (TDS) were measured for every treated group. The diuretic activity of the prepared complexes was calculated from the ratio of the mean urine output in the test group and in the reference group (FSM treated group). The diuretic activity is considered nil, little, moderate, and good for values <7.2, 0.72–1.00, 1.00–1.5, and

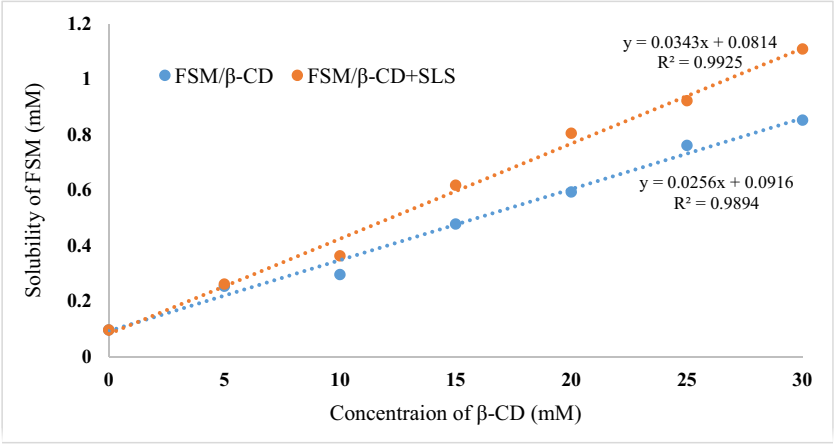

Figure 2: Phase solubility curves of FSM/β-CD and FSM/β-CD with SLS.

Table 1: Complexation parameters of FSM/β-CD and FSM/β-CD with SLS

Complexes	Slope	Stability constant (K_s)	Complexation efficiency (CE)
FSM/β-CD	0.0256	276 M^{-1}	0.0262
FSM/β-CD with 0.5% SLS	0.0343	373 M^{-1}	0.0355

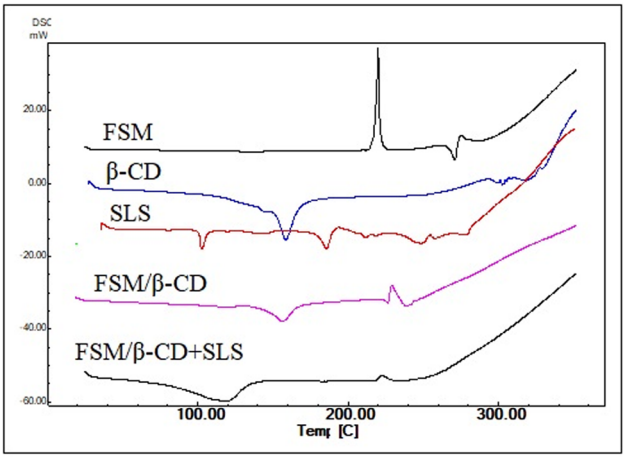

Figure 3: Comparative DSC spectra of complexes.

>1.5, respectively [24]. The experimental procedure was approved by the Animal Ethics Committee of Pharmacy Faculty, Jadara University, Jordan.

3 Results and discussion

3.1 Phase solubility studies of FSM in binary and in ternary systems

The phase solubility diagrams of binary (FSM/β-CD) and ternary (FSM/β-CD with 0.5% SLS) systems showed a linear A.L. type solubility curves of FSM against increasing concentration of β-CD and β-CD with 0.5% SLS (Figure 2). According to Higuchi Connors [22], A.L. type curve with slope values less than 1 was suggested for the formation of 1:1 stoichiometric complex. The data of apparent stability constants (K_s) and complexation efficiencies of the prepared complexes were presented in Table 1. The apparent stability constants (K_s) of 280 M^{-1} and 326 M^{-1} were obtained for FSM/β-CD and FSM/β-CD with 0.5% SLS systems, respectively. CE was considered as more reliable data for complexation. CE of FSM/β-CD and FSM/β-CD with 0.5% SLS systems was obtained as 0.0266 and 0.0301, respectively. It was revealed from the data that the addition of 0.5% SLS to the β-CD did not change the phase solubility curve, but enhanced the interaction of FSM with β-CD by increasing the stability constant.

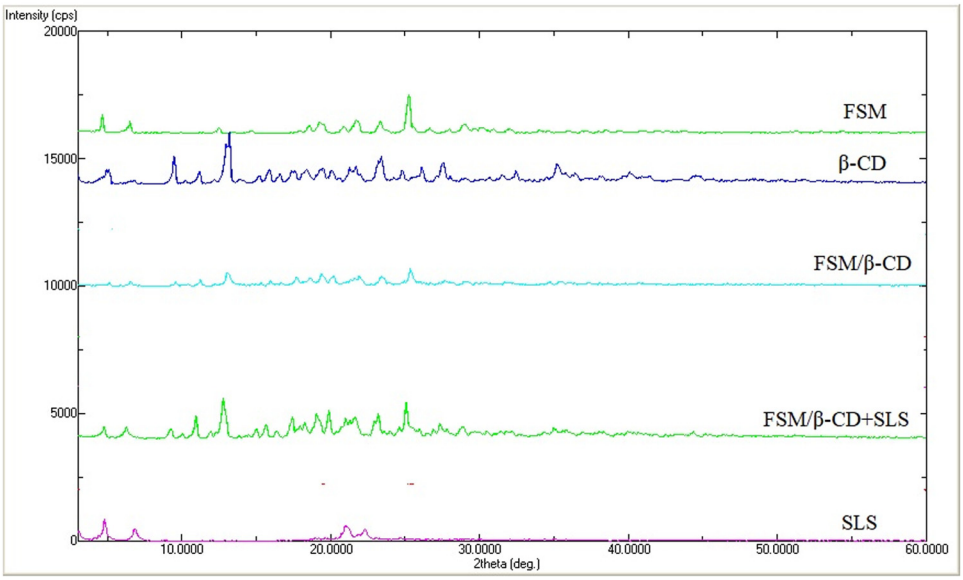

Figure 4: Comparative PXRD pattern of complexes.

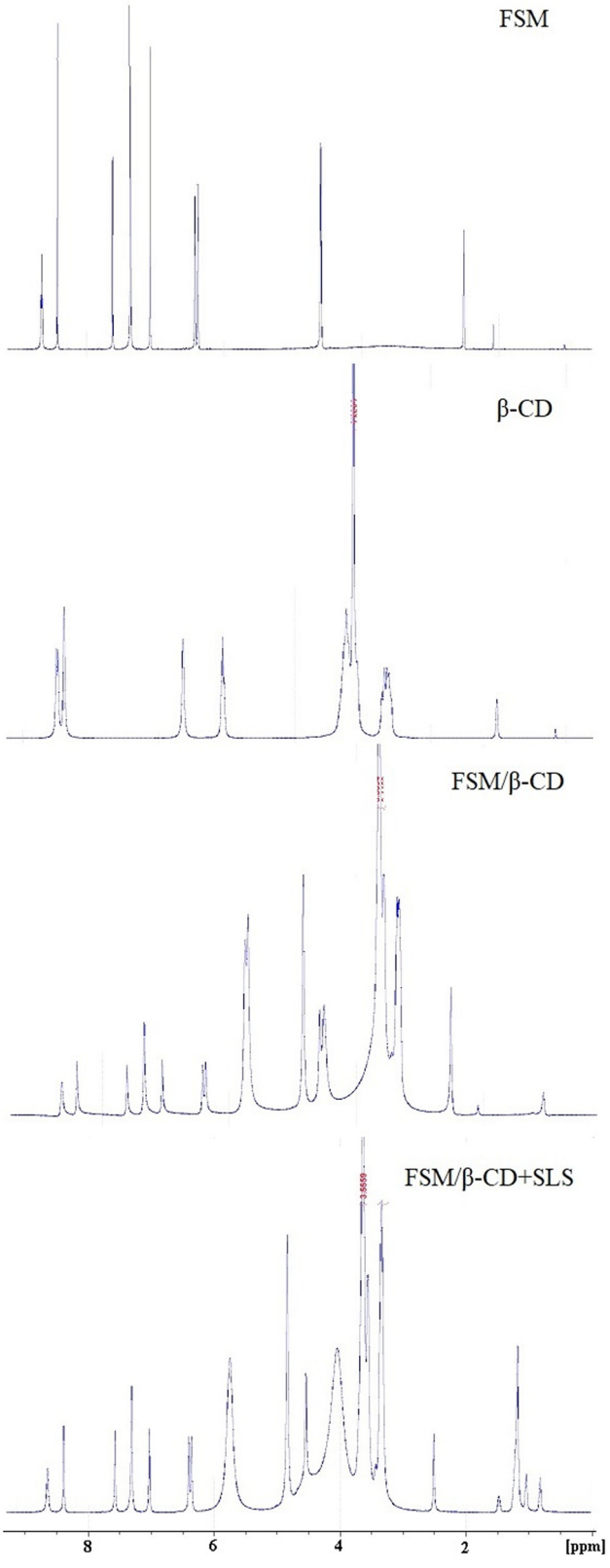

Figure 5: Comparative ^{1}H NMR spectra of complexes.

3.2 DSC studies

The scanned DSC curves FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS are shown in Figure 3. A sharp endothermic peak of FSM at 216°C was shown, which evidenced drug purity. An endothermic peak of β-CD was observed around 143°C due to the loss of water. The DSC curve of FSM/β-CD was evidenced with a diminished endothermic peak of FSM. However, FSM/β-CD with SLS showed complete disappearance of the endothermic peak of FSM, suggesting complex formation.

3.3 PXRD studies

The PXRD is a valuable technique to identify the crystallinity/amorphocity of compounds. The PXRD patterns of FSM, β-CD, SLS, FSM/β-CD, and FSM/β-CD with SLS are shown in Figure 4. The FSM pure drug showed many sharp peaks confirming its crystalline nature. The FSM/β-CD and FSM/β-CD with SLS exhibited broad and diffused peaks evidenced its amorphocity of FSM after complexation.

3.4 ^{1}H NMR spectral analysis

The ^{1}H NMR spectra of FSM showed a characteristic peak in the range of 7.0–8.4 ppm. The hydrogen atoms (H_3 and H_5) are present in the inner portion of β-CD molecules, which are responsible for interaction with drug molecules. The ^{1}H NMR spectra of FSM/β-CD and FSM/β-CD with SLS complexes evidenced with the change in chemical shift of H_3 and H_5 atoms β-CD cavity, which confirms the inclusion of FSM inside the β-CD cavity (Figure 5).

3.5 *In vitro* drug release studies

Dissolution of FSM alone was found to be very slow (22.6% in 1 h), which was due to insolubility of FSM in water. A release of 59.3% and 77.2% was noted after 1 h from complex FSM/β-CD and FSM/β-CD with SLS, respectively. The maximum release of FSM from FSM/β-CD with SLS was probably due to the presence of SLS surfactant (Figure 6). This study demonstrates that when FSM is complexed with β-CD with SLS, there is a significant elevation of the rate of dissolution of the drug.

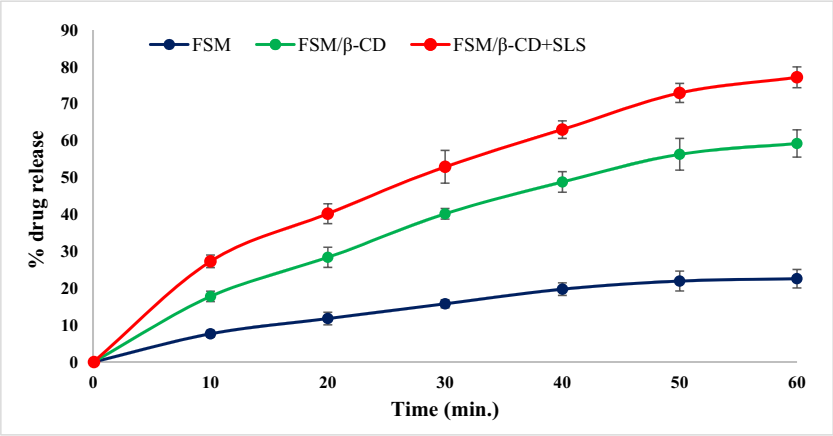

Figure 6: Comparative release profile of complexes.

Table 2: Diuretic activity parameters of complexes in rats

Group	Urine volume (mL)	pH	Conductivity (ms)	TDS (g/L)	Diuretic activity (V_t/V_r)
Control	8.23 ± 0.79	7.02	8.47	3.11	—
FSM	29.75 ± 4.57*	6.67	5.18	2.20	—
FSM/β-CD	37.42 ± 6.43**	6.81	7.66	4.55	1.25
FSM/β-CD + SLS	46.83 ± 3.88***	6.72	9.75	5.84	1.57

Values represent the mean ± SEM (n = 6). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. *p < 0.05, **p < 0.01, ***p < 0.001.

3.6 Diuretic activity

The urine output and its measured parameters are presented in Table 2. It was observed that the measured urine volume was found significantly higher in FSM/β-CD-treated (**p < 0.01) and FSM/β-CD + SLS-treated (**p < 0.001) groups in comparison to the FSM-treated group. The pH and conductivity of the complex treated group also increased in comparison to the control group. The diuretic activity of the FSM/β-CD complex was found moderate with a value of 1.25. However, a good diuretic activity was shown by FSM/β-CD + SLS with a value of 1.57. This enhancement in the diuretic activity was probably because of the presence of SLS. In conclusion, the bioavailability of FSM was increased when complexed with β-CD and SLS.

4 Conclusion

The inclusion complexes of FSM in binary (FSM/β-CD) and ternary (FSM/β-CD with SLS) were successfully developed. Phase solubility studies confirmed the inclusion complexation of FSM with β-CD in the absence or presence of SLS. The release of the FSM by β-CD complexation in the presence of SLS showed a synergistic action in the solubility of FSM due to the surfactant properties of SLS. In conclusion, the SLS blend with β-CD offers a unique formulation approach to enhance the solubility and diuretic action of FSM.

Conflict of interest: The author reports no conflict of interest associated with this manuscript.

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