

RESEARCH PAPER

Improvement of Dissolution Properties of Furosemide by Complexation with β -Cyclodextrin

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ABSTRACT

The purpose of this study was to increase the solubility of furosemide (FR) with inclusion compound of β -cyclodextrin (β -CD). The interaction between FR and β -CD in solution was studied by the solubility method. The phase solubility studies reveal a B_s -type diagram with an inclusion complex of 1:1 molar ratio and a stability constant of 823.5 M^{-1} . The solid complexes of FR with β -CD were prepared by using freeze-drying, kneading, and co-precipitation methods. In addition, the physical mixture was prepared for comparison. Inclusion complexation was confirmed by the results from the studies of x-ray diffraction, differential scanning calorimetry, and infrared spectroscopy. The rates of release of the active material from the resulting complexes were determined from dissolution studies using the flow-through cell method.

The dissolution rate of FR was significantly enhanced by inclusion of the β -CD in the formulations. The rate of release of the active material was found to be dependent on the preparation method of the complexes, and the drug prepared by the kneading method was shown to have the fastest dissolution profile compared to the other methods used in this study.

INTRODUCTION

Furosemide is a potent diuretic which is often administered orally. In the rat, Chungi et al. (2) have shown a greater absorptive capacity for FR from the stomach than from the intestine, despite the much smaller absorptive area. The oral bioavailability of FR was found to be very poor likely due to the insufficient aqueous solubility in pH 1.2, and the solubility of FR in pH 1.2 may be the rate-determining step in the absorption process (1,2).

Inclusion complexation of lipophilic drug molecules with cyclodextrins has been extensively applied to optimize the biopharmaceutical parameters such as solubility, stability, and bioavailability (3,4). β -Cyclodextrin (CD) is one of the natural cyclodextrins and numerous works concerning its effect on improving the physico-chemical characteristics of many lipophilic drugs have been published (5-7).

The work presented in this paper was undertaken to improve both the solubility of FR and its oral bioavailability characteristics by inclusion complex formation with β -CD.

MATERIALS AND METHODS

Materials

β -CD was purchased by Sigma Company (St. Louis, MO) and FR was supplied by Deva A. Ş. (Istanbul, Turkey). All other compounds and solvents used in this study were of analytical reagent grade.

Phase Solubility Studies

Solubility measurements were performed by the method of Higuchi and solutions containing various concentrations of β -CD ranging from 1×10^{-4} M to 2.4×10^{-4} M were shaken in sealed flasks in a thermostated water bath at a constant temperature of 25°C. After an equilibrium was attained (approximately 3 days) aliquots were withdrawn and filtered through 0.45- μ m filters. A portion of the filtrate was then diluted with water and analyzed spectrophotometrically. The solubility constant and the ratios of FR/ β -CD in the complexes were calculated from the phase solubility diagram.

Preparation of Solid Complexes

The solid complexes of FR with β -CD were prepared by using the following four different methods.

Kneading Method

Aqueous ethanol solution (50%) was added to the mixture of FR/ β -CD (1:1) until a creamy homogenous product was obtained. This mixture was transferred to a mortar and kneaded for 15 min. Then it was dried in an oven under a vacuum at 50°C until a constant weight was obtained to complete the process.

Freeze-Drying Method

The mixture of FR/ β -CD (1:1) was dissolved in the minimum volume of 28% aqueous ammonium solution and freeze-dried using a Hotesic freeze-dryer.

Coprecipitation Method

The mixture of FR/ β -CD (1:1) was dissolved in 50% ethanol, the solvent was allowed to evaporate, and then it was further dried under a vacuum at 50°C for 24 hr.

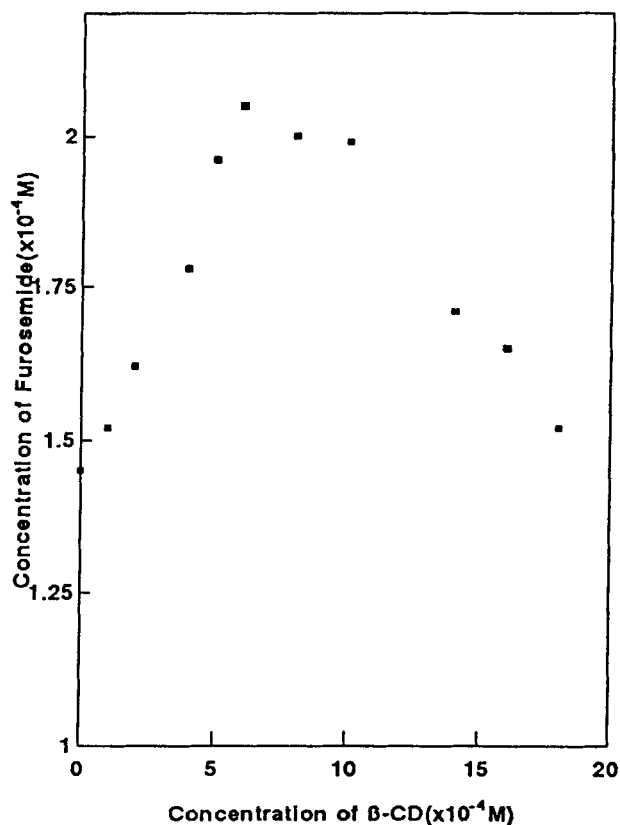


Figure 1. Phase solubility diagram of FR/ β -CD system in water at 25°C.

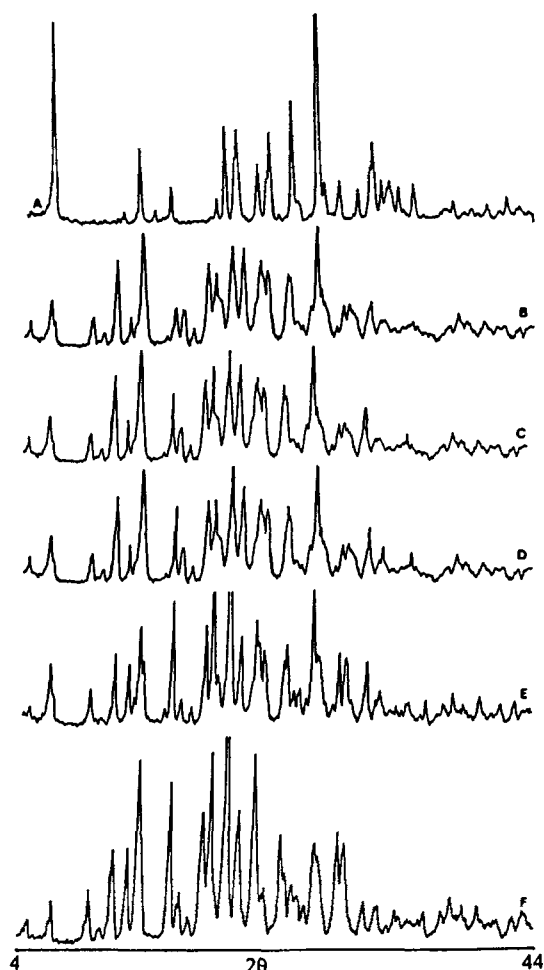


Figure 2. X-ray diffraction pattern of FR/ β -CD systems. (a) FR; (b) kneaded product; (c) freeze-dried product; (d) coprecipitated product; (e) physical mixture; (f) β -CD.

Physical Mixture

The physical mixture was prepared by a simple dry mixing of equimolar quantities of FR and β -CD in a mortar for 10 min.

The solid samples obtained by the use of four different methods were sieve analyzed and the particle sizes between 0.125 and 0.250 mm were used for the dissolution tests.

Characterization of Complexes

X-ray powder diffraction patterns were obtained by the use of a Jeol JDX-8P diffractometer with $\text{CuK}(\alpha)$ radiation under 40 kV at a current of 20 mA. The IR spectra were obtained using a Pye Unicam SP 1000 IR

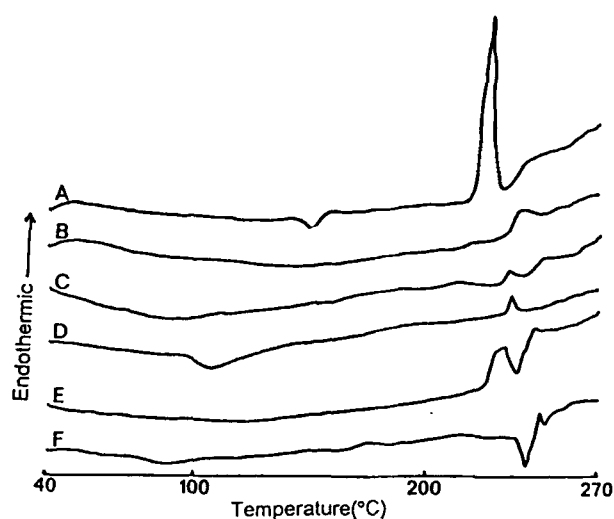


Figure 3. DSC thermograms of FR/ β -CD systems. (a) FR; (b) kneaded product; (c) freeze-dried product; (d) coprecipitated product; (e) physical mixture; (f) β -CD.

spectrophotometer with potassium bromide pellets. Differential scanning calorimetry (DSC) with a Shimadzu DT-40 was used for each sample at a constant scanning speed of $10^\circ\text{C}/\text{min}$ between 40°C and 300°C under nitrogen as purging gas.

Dissolution Rate Studies

Flow-through cell dissolution apparatus at 9 ml/min flow rate was employed for dissolution rate studies. The sink conditions were satisfied in all the dissolution tests. Simulated gastric fluid was used without enzymes and 0.02% polysorbate 20 added to bring the surface tension to about $45\text{--}50\text{ mNm}^{-1}$ for mimicking the in vivo value. Forty milligrams of powdered FR sample or an equivalent amount of complexes was tested for 8 hr. Samples were taken at predetermined intervals of 30 min and 1 hr for the first 3 hr, and then at 5 hr. If no statistically significant differences existed between the results obtained; the mean of three dissolution test results were taken and their fits to different kinetic models were evaluated.

Solubility Studies

Solubilities of active material and active material/ β -CD complex were studied at pH 1.2. To this aim, an excess amount of active material (an amount of active material, more than could be dissolved) was added to a closed flask with pH 1.2, and then mixed with a mag-

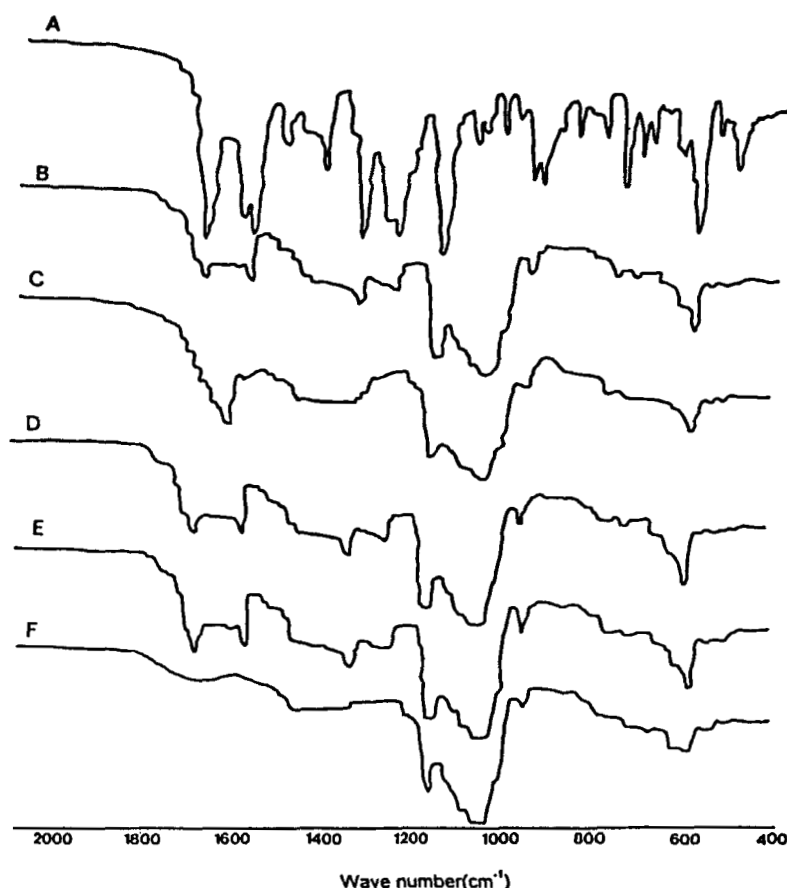


Figure 4. Infrared spectra of (a) FR; (b) kneaded product; (c) freeze-dried product; (d) coprecipitated product; (e) physical mixture; (f) β -CD.

netic mixer at 37°C and left at rest. Thereafter, the liquid phase was filtered through 0.45- μ m filters and the amount of active material in this solution was determined. Solubility of active material was calculated by the point measured during formation of the equilibrium status.

Stability Studies

The stability of FR and FR/ β -CD (1:1) complex in room temperature was studied for 9 months. Samples taken monthly were tested for the dissolution rate; in addition, presence of any structural change was investigated by DSC measurements.

RESULTS AND DISCUSSION

Phase Solubility Diagram

The phase solubility diagram obtained for FR and β -CD is shown in Fig. 1. The solubility curve can be

classified as type B_s. The stoichiometric ratio of complex determined from the descending part of the diagram was found to be 1:1 (FR/ β -CD).

The 1:1 stability constant (k) of the soluble complex was calculated according to the following equation:

$$k = \frac{S_t - S_0}{S_0(L_t - S_t + S_0)} \quad (1)$$

where S_t is total concentration of dissolved FR, S_0 is the equilibrium solubility of FR in the presence of β -CD, and L_t is the total concentration of β -CD (8).

The value of the stability constant k is found to be 823.5 M⁻¹.

Characterization of Inclusion Formation

Evidence for an inclusion formation between FR and β -CD was provided by the analysis of the results obtained from x-ray diffractometry, differential scanning calorimetry, and infrared spectroscopy.

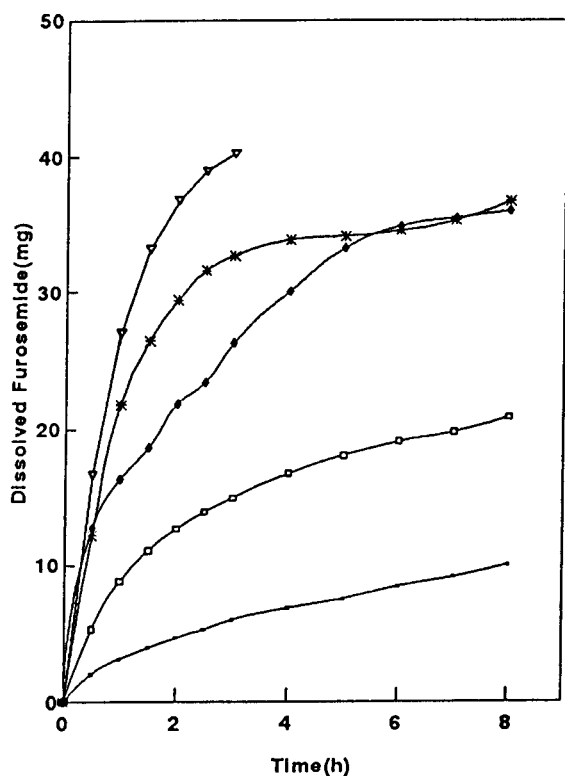


Figure 5. Dissolution profiles of FR from FR/ β -CD systems prepared by different methods (molar ratio 1:1). (■) FR; (★) freeze-drying method; (▽) kneading method; (□) coprecipitation method; (◆) physical mixture.

Figure 2 shows the x-ray diffraction patterns of FR, its inclusion complexes with β -CD, and β -CD itself. The diffraction pattern of the kneaded samples was found to be considerably different than that of the other samples investigated in this study. Some of the peaks appeared either with lower intensity or completely disappeared from the pattern of the complexes as a result of formation of inclusion compounds.

DSC thermograms of FR, its physical mixture, and the inclusion complexes of β -CD are shown in Fig. 3. The β -CD displayed no peaks in the temperature range between 40°C and 300°C, while the FR exhibited its characteristic endothermic peak associated with the melting point of the drug at 226°C. The peak of the active material for kneaded products in the DSC thermogram disappeared and the physical mixture had a less intense peak. The absence of the peak in DSC curves may be considered as a strong indication for the formation of inclusion of the drug into the β -CD cavity.

The IR spectra of the complexes together with the pure drug are shown in Fig. 4 for comparison. As can

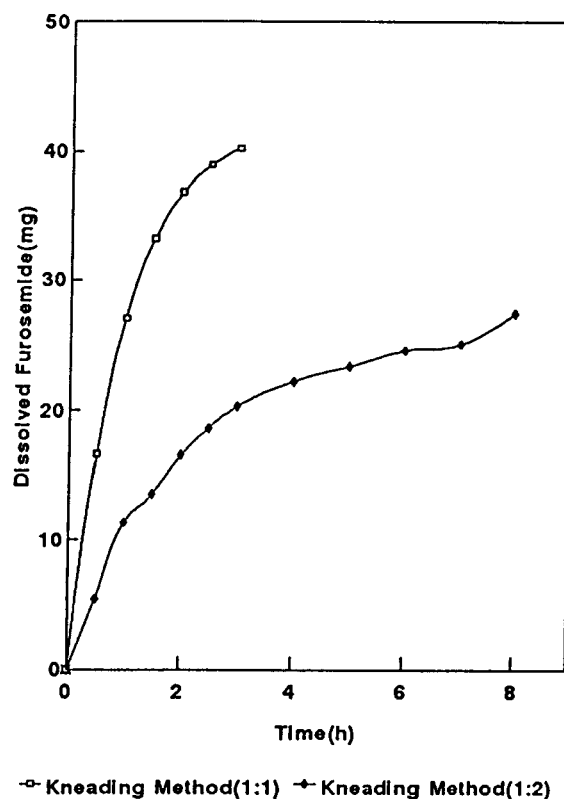


Figure 6. The effect of FR/ β -CD ratio on the release of FR from formulations prepared by kneading method. (□) FR/ β -CD (1:1); (◆) FR/ β -CD (1:2).

be clearly seen from the figure, the characteristic bands of FR at 1671 cm^{-1} ($-\text{COOH}$), 1596 cm^{-1} ($-\text{NH}_2$), 1322 cm^{-1} ($-\text{SO}_2$), and 582 cm^{-1} ($-\text{Cl}$) were modified significantly in the solid complexes as a result of complex formation. The observed decreases in the intensities for the bands, especially for the carbonyl band, may be due to its restriction within the β -CD cavity.

Dissolution Rate Studies

The dissolution profiles of both FR and the inclusion compounds are shown in Fig. 5. The figure illustrates that the release of active material was strongly affected by the method of formulation. The kneaded product exhibited the best dissolution properties and was followed by the freeze-dried product, physical mixture, and coprecipitated products, respectively. All the active material of kneaded product dissolved within 180 min, whereas the values measured for the freeze-dried, coprecipitated, physical mixture, and pure drug were 82, 37, 66, and 15%, respectively. The all-inclusion

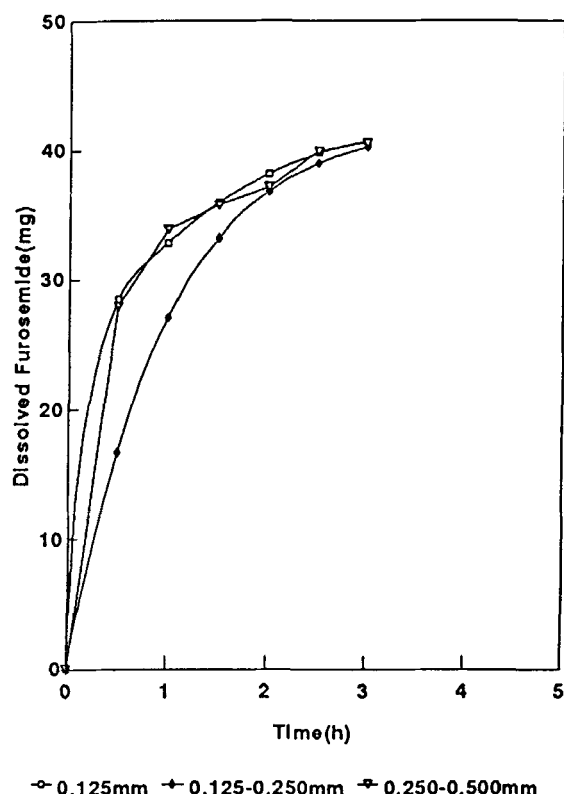


Figure 7. The effect of particle size on the release rate of FR from formulations prepared by kneading method. (□) 0.125 mm; (◆) 0.125–0.250 mm; (▽) 0.250–0.500 mm.

compounds exhibited better dissolution properties than the pure drug alone. The solubility of active material was also enhanced from 30.2 $\mu\text{g/ml}$ to 92 $\mu\text{g/ml}$ in pH 1.2 by the complexation with β -CD (1:1). The improve-

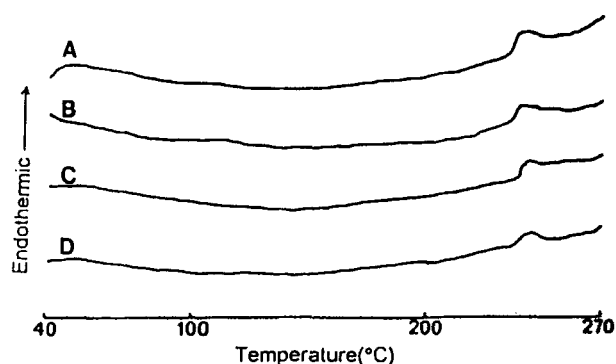


Figure 8. DSC thermograms of FR/ β -CD complex. (a) not stored; (b) 3 months storage; (c) 6 months storage; (d) 9 months storage.

ment in dissolution rate of the drug/ β -CD systems may be attributed to the degree of crystallinity of the active material, together with the increase in both the wettability and the solubility of the drug.

The effect of FR/ β -CD ratio on the release rate of drug was also investigated in kneaded product and the highest release was obtained with a 1:1 ratio (Fig. 6). This profile also confirms that the molar ratio of drug/ β -CD complex was 1:1.

The release rates of active material from different sizes of powder prepared by kneading method are given in Fig. 7. No differences were found between the release rates of formulations with the particle size of powder ranging from 0.125 mm to 0.500 mm.

The room temperature stability of FR/ β -CD complexes prepared by kneading method was checked for 9 months after production and no significant changes in their stabilities were found from the dissolution rate profiles and DSC thermograms of the sample (Fig. 8).

The inclusion compound of FR/ β -CD (1:1) prepared by kneading method was used in the tablet formulations. In vivo studies, the testing of these formulations on six healthy subjects, have been carried out. The bio-availability of FR was increased by 1.8 times when it was compared with the classical tablet. The results of this study indicate that FR is completely available in the stomach from its complex with β -CD and the β -CD may be considered as an effective additive to solid FR formulations for a rapid and uniform release of the drug. The details of this subject will be presented in the near future.

CONCLUSION

As a result of this study it may be concluded that the solubility and the dissolution rate of FR was significantly enhanced by the complex formation. The release of the active material was strongly affected by the method of preparation of inclusion compounds and the inclusion compound prepared by kneading method showed the fastest dissolution profile. The particle size of granules had no detectable effect on the rate of release of the active material.

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