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# THE INTERACTION OF FUROSEMIDE WITH HEPTAKIS (2,6-DI-O-METHYL)-ß-CYCLODEXTRIN IN SOLUTION AND IN SOLID STATE

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إن عقار الفيورسيمايد يعتبر من مدرات البول القوية والذى له قابلية ضعيفة جدا للذوبان. ويعتبر غير ثابت فى الوسط الحامضى مما يؤدى إلى قلة التوافر الحيوى للعقار عند تعاطيه عن طريق الفم. فى هذا البحث تم دراسة تأثير الداى ميثيل-بيتا-سيكلودكسترين على ذوبان العقار فى الماء ودرجة ثباته فى الوسط الحامضى. ولقد تم تحديد منحنى الإذابة ووجد أنه يتبع نوع Ap والذى يعنى أن عقار الفيورسيمايد يكون متراكبين منغمسين أحدهما بنسبة ١:١ والآخر بنسبة ٢:١ مع الداى ميثيل-بيتا-سيكلودكسترين. ولقد وجد أن ثابت التكوين للمتراكبين ١:١ ، ٢:١ يساوى ٣٧٧٥ مول$^{-1}$ و٦٥ مول$^{-1}$ على التوالى. وأيضا تم دراسة هذه المتراكبات فى المحلول عن طريق الإنحراف الطيفى ووجد أن ثابت التكوين يساوى ١٠٠ مول$^{-1}$ بإستخدام معادلة سكوت وأيضا وجد أنه يساوى ١٥٧ مول$^{-1}$ بإستخدام منحنى بنيز-هيلدبراند. أيضا تم دراسة تلك المتراكبات المنغمسة فى الحالة الصلبة بواسطة التحليل السعرى التفاضلى ، والأشعة تحت الحمراء وبواسطة أشعة إكس ومعدل الانطلاق. هذه الدراسة أكدت وجود المتراكبات المنغمسة للعقار. ولقد وجد أن معدل ذوبان العقار من تلك المتراكبات قد زادت بدرجة كبيرة جدا خصوصا مع المركب المحضر بواسطة الطحن الآلى. وقد يعزى هذا إلى تكوين الشكل اللابلورى الذى يؤدى إلى زيادة بلل العقار وأيضا تكوين المتراكب المنغمس. من جهة أخرى ، فقد وجد أن عقار الفيورسيمايد يتحلل فى الوسط الحامضى ولكن تحلله فى الوسط القاعدى ضعيف جدا. ومن دراسة ثبات هذا المركب وجد أن وجود الداى ميثيل-بيتا-سيكلودكسترين له تأثير غير ملحوظ على درجة تحلل العقار ، حيث أن باستخدام معادلة أرهنيس وجد أن فترة نصف عمر العقار تساوى ٥٨ دقيقة للعقار و٦٠ دقيقة للمتراكب عند الأس الهيدروجينى ١,٤ وعند درجة الحرارة ٣٧°م.

*Furosemide (FSD) is a potent diuretic, has a low aqueous solubility and an instability in acidic medium which hinder its bioavailability when administered orally. In the present study, the effect of dimethyl-ß-cyclodextrin (DM-ß-CD) on the aqueous solubility, stability of FSD was studied. The phase solubility diagram with DM-ß-CD was classified as Ap-type, which mean that, the FSD formed 1:1 and 1:2 inclusion complexes with DM-ß-CD. The stability constants ($K_{1:1}$ and $K_{1:2}$) were calculated to be 3557 $M^{-1}$ and 65 $M^{-1}$, respectively. The complex formation of FSD and DM-ß-CD in solution was also calculated using spectral shift method. The value of stability constant, $K_c$, in this case, was found to be 100.0 $M^{-1}$ using Scott's equation and was 157 $M^{-1}$ using Benesi-Hildebrand plot. Characterization of the products was carried out by differential scanning calorimetry, IR spectrophotometry, X-ray analysis and dissolution study, which confirmed the existance of an inclusion complexation. FSD solubility was dramatically enhanced by inclusion, especially in the ground system with DM-ß-CD. This might be attributed to the amorphous state, the increased wettability of the drug and the inclusion complex formation. FSD was found to be rapidly hydrolysed in the acidic pH region, but in the basic pH region, the drug hydrolysis is extremely low. The presence of DM-ß-CD had nonsignificant effect on the hydrolysis of the drug. Using the Arrhenius parameters obtained from the studies, FSD would have a half-life of 58 and 60 min. for drug and drug-DM-ß-CD complex, respectively under conditions of pH 1.4 and temperature of 37°.*

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## INTRODUCTION

Furosemide (FSD) is an anthranilic acid derivatives used to treat edematous state of hepatic, cardiac and renal origin.[1,2] Because FSD has limited solubility in water, which respresents a problem in its bioavailability, attempts were done to overcome this problem on the basis of the complexation of the drug with a variety of cyclodextrin derivatives. ß-Cyclodextrin is a cyclic oligosaccharide of seven glucose residues with a cavity formed through an α-1,4-linkage cyclization. ß-cyclodextrin as well as its alkylated and hydroxyalkylated derivatives are used extensively as pharmaceutical excipients due to their remarkable molecular complexation property with many drugs.[3,4]

Cyclodextrins and their derivatives play an important role in the formulation development due to their effect on solubility, dissolution rate,[5-8] chemical stability,[9-11] absorption of a drug.[12-18] Currently, some of their derivatives, especially 2-hydroxypropyl-ß-cyclodextrin, are being investigated for toxicity for use in parenteral dosage form.[19] The goal of this study was to determine if the aqueous solubility and stability of FSD can be increased by complexation with DM-ß-CD. Phase solubility diagram of the complex was constructed and evaluated. The complexes were prepared by co-grinding and coprecipitating methods. Each complex formed was characterized by UV spectroscopy, differential scanning calorimetry, powder-X-ray diffractometry and IR spectroscopy. The dissolution rate of each complex made by either co-grinding and coprecipitating methods was a comparatively evaluated. The hydrolysis of the drug was also investigated and evaluated at a different pHs and in the absence and presence of DM-ß-CD.

## EXPERIMENTAL

### Materials

Furosemide (FSD) (APS Cleckheaton, UK) was used without any preliminary treatment and Di-O-methyl-ß-cyclodextrin (DM-ß-CD) was purchased from Toshin Chemical Company (Tokyo-Japan). All other chemicals and solvents were of analytical reagent grade.

### Apparatus

The IR spectra were obtained with a Hitachi 295 infrared spectrophotometer (Hitachi, Tokyo, Japan) using KBr disk method. The disks made under a pressure equal to 400 kg $cm^{-2}$. Powder X-ray diffraction patterns (XRD) were taken on a Rigakudenki 2027 diffractometer (Rigakudenki Co. Ltd.) with Ni-filtered Cu-K radiation, a voltage of 30 KV, and a scanning speed of 2° $min^{-1}$. Differential scanning calorimetric (DSC) patterns were carried out by a Perkin-Elmer Model DSC-1B (Perkin-Elmer, USA-Norwalk, CT), operated at the conditions of sample weights 4.5 mg, using the sample pan for liquid sample, at a scanning speed of 8 K $min^{-1}$ under $N_2$ stream, and a temperature range of about 320 to 500 K. Ultraviolet (UV) spectra were taken on a Hitachi U-557 UV-spectrophotometer (Tokyo, Japan).

### Preparation of solid complexes

Solid complexes of FSD with DM-ß-CD were prepared by the co-grinding method, using a vibrational mill of Heiko Seisakusho-Model TJ-220 (Rigakudenki Co., Ltd., Tokyo, Japan), and by coprecipitating method, by dissolving equimolar amount of FSD and DM-ß-CD into methanol and evaporating the solvent.

### Solubility studies

The stability constant for inclusion complex formation between FSD and DM-ß-CD was determined using the phase-solubility method.[20] Excess amount of the drug (0.04 mM) was added to water containing various concentrations (2.5-60 mM) of DM-ß-CD. The suspensions were shaken at 25° for 72 h. After equilibrium was attained, the suspensions were filtered, and the filtrates were properly diluted with water and analyzed spectrophotometrically at 276 nm for total FSD content.

### Spectral shift method

The UV absorption changes of FSD in the presence of DM-ß-CD (varied from 0 to 10

mM) in water at 25° were recorded. The change in absorbance of the drug by the addition of various concentration of DM-ß-CD was measured at 276 nm to evaluate the stability constant of the complex.

**Dissolution studies**

The dissolution rates of FSD, its physical mixture, ground mixture and coprecipitate with DM-ß-CD, were measured according to the dispersed amount method.[21] The equivalent amount of 50 mg FSD was put into 900 ml of phosphate buffer (pH 5.8) and stirred at 50 rpm at 37° (USP XXIII). At appropriate time intervals, 5 ml, was withdrawn, then diluted with the dissolution medium, and analyzed spectrophotometrically at 276 nm for FSD concentration. An equal volume of dissolution medium preadjusted at the same temperature was immediately replaced after each sample withdrawn.

**Stability studies**

The stability of FSD in 0.1 N HCl was studied in absence and presence of DM-ß-CD at 60°, 70° and 80°. Solutions were prepared by dissolving 16.55 mg of the FSD in 100 ml of the tested solution containing $1x10^{-5}$ M of DM-ß-CD. The solutions were placed at a constant temperature and the samples were taken at the appropriate intervals. The remaining FSD was determined spectrophotometrically at 276 nm. The stability rate constant for the overall hydrolysis of FSD were determined from the slopes of the linear semilogarithmic plots of remaining concentration of FSD vs. time.

## RESULTS AND DISCUSSION

**Solubility studies**

Figure 1 shows the phase-solubility digarsm of FSD with DM-ß-CD which is Ap-type indicating formation of 1:1 and 1:2 drug/DM-ß-CD complexes.[20] The stability constant for 1:1 and 1:2 complexes were calculated after constructing a plot by using equation 1:

$$\frac{([S_t]-[S_0])}{[L_t]} = K_{1:1}[S_0] + K_{1:1}K_{1:2}[S_0][L_t] \quad \ldots (1)$$

where $[S_t]$ is the total drug concentration at total CD concentration $[L_t]$, $[S_0]$ is the solubility of FSD in the absence of CD and $K_{1:1}$, and $K_{1:2}$ represent the stability constants for the 1:1 and 1:2 complexes respectively. A plot of $([S_t]-[S_0])$ / $[L_t]$ vs $[L_t]$ results in a linear plot with an intercept of $K_{1:1}[S_0]$ and a slope of $K_{1:1}K_{1:2}[S_0]$. The stability constants, $K_{1:1}$ and $K_{1:2}$, for 1:1 and 1:2 inclusion complexes were calculated to be 3557 $M^{-1}$ and 65 $M^{-1}$, respectively.

The high stability constant of the 1:1 complex is probably not to be taken into consideration, due to the very low water solubility of furosemide and to the difficulty in measuring its exact value. On the other hand, the 65 $M^{-1}$ stability constant of the 1:2 complex corresponds better to the data obtained in the physicochemical, sepctral shift and dissolution studies.

**Determination of stability constant spectrophotometrically**

Figure 2 shows the effect of different molar concentration of DM-ß-CD on the absorption spectrum of FSD. the stability constant, $K_c$, was also determined according to conventional Scott's equation 2:[22]

$$\frac{a.b}{d} = \frac{1}{K_c.\varepsilon_c} + \frac{b}{\varepsilon_c} \quad \ldots (2)$$

where $a$ is the total concentration of FSD, $b$ is the total concentration of DM-ß-CD, $\varepsilon_c$ is the difference of the molar absorpativities for free and complexed FSD, and $d$ is the change in absorbance of the drug by addition of DM-ß-CD, $K_c$ was calculated and was found to be 100 $M^{-1}$.

On the other hand, the spectral data were also, analyzed by the double reciprocal plot as showin in Figure 3. The plot of $1/\Delta A$ vs $1/[DM\text{-}\beta\text{-}CD]$ is linear, indicating the presence of 1:1 complex.

The apparent 1:1 stability constant was estimated by the Benesi-Hildebrand equation[23]

$$\frac{1}{\Delta A} = \frac{1}{[D]\ K_c\ \Delta\varepsilon}\ \frac{1}{[DM\text{-}\beta\text{-}CD]} + \frac{1}{[D]\ \Delta\varepsilon}$$

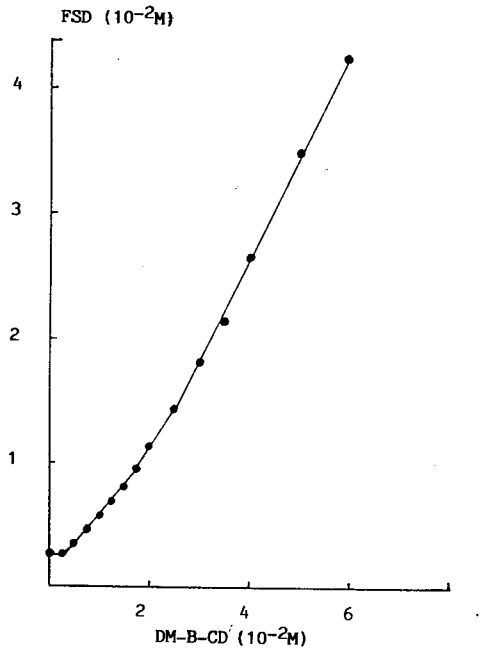


**Fig. 1:** Phase-solubility diagram of furosemide-DM-ß-CD system at 25°.

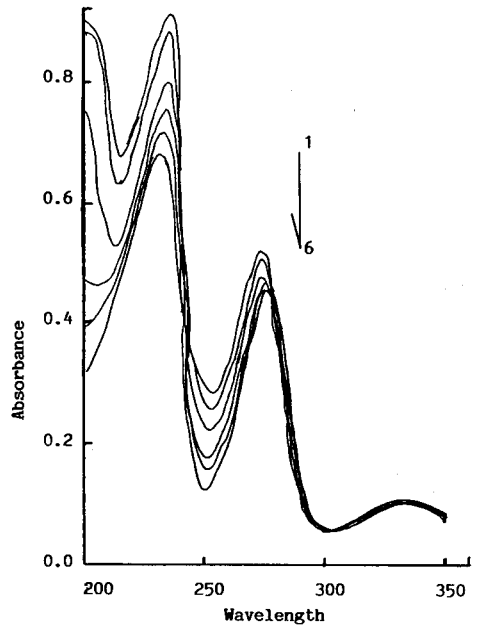


**Fig. 2:** Effect of DM-ß-CD concentrations on UV absorption spectra of furosemide in water. The concetnration of drug was $1.0x10^{-4}$ M: (1) drug alone; (2) drug in presence of $1x10^{-3}$ M DM-ß-CD; (3) drug in presence of $2x10^{-3}$ M DM-ß-CD; (4) drug in presence of $5x10^{-3}$ M DM-ß-CD; (5) drug in presence of $8x10^{-3}$ M DM-ß-CD; (6) drug in presence of $1.0x10^{-2}$ M DM-ß-CD.

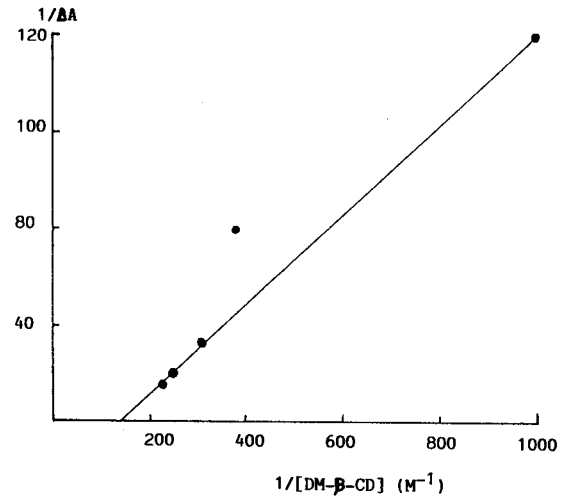


**Fig. 3:** Benesi-Hildebrand plot for the furosemide-DM-ß-CD system (ΔA displays the change of absorbance at $\lambda_{max}$= 276 nm and [DM-ß-CD] the concentraton of DM-ß-CD.

where ΔA is the difference of absorbance at 276 nm, [DM-ß-CD] is the DM-ß-CD concentration, [D] is the total drug concentration (constant) and $\Delta\varepsilon$ is the difference of molar absorpativities between complexed and free drug. The stability constant; $K_c$ was obtained from the intercept/slop ratio and this value was 157 $M^{-1}$, that is approximately of the same value calculated by the Scott's equation (100 $M^{-1}$).

## XRD

The XRD patterns of the FSD-DM-ß-CD systems are respresented in Figure 4. The diffractograms of FSD and DM-ß-CD exhibit a series of intense peaks, which are indicative of their crystallinity. The spectrum relevant to the physical mixture is constituted practically by the superposition of the spectra of the single components. The spectrum of coprecipitated sample showed with respect to the components the disappearance of some important spectral peaks for both FSD and DM-ß-CD indicating the presence of new solid crystalline phases, corresponding to inclusion complexes. It is important also to remark that the peak intensities

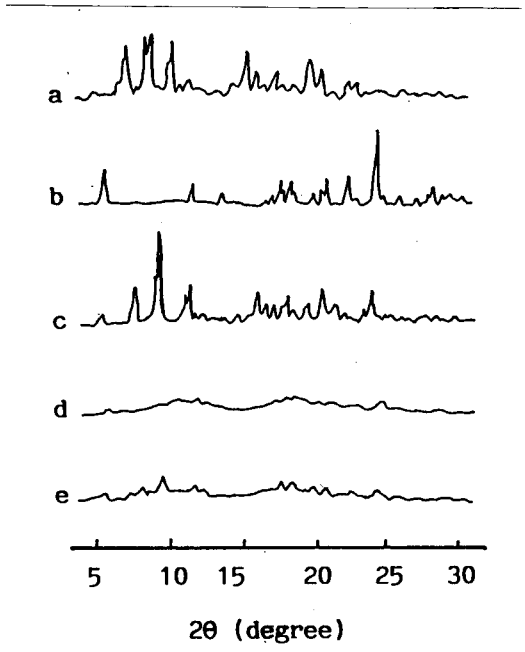


**Fig. 4:** X-ray diffraction patterns for the following products: (a) DM-ß-CD alone; (b) FSD alone; (c) physical mixture; (d) ground mixture; and (e) coprecipitate.

diminished with respect to the spectra of the starting materials, indicating a lower degree of crystallinity for these complexes. This fact may be attributed to the very rapid precipitation of the complexes during their preparation and insufficient for a regular crystal growth or the preparation method in which evaporation can reduce the crystallization possibility. The co-ground showed a halo pattern, typical of amorphous samples. In this case, the formation of an amorphous inclusion complex is supposable.

**IR spectra**

Figures 5 and 6 show the IR spectra of the samples under study. IR spectrum of furosemide (Fig. 5b) is characterized by the absorption of the $NH_2$ group, located in the bands at 3410, 3358, 3280 and 3196 $cm^{-1}$. The first two bands (forked band) are assigned to asymmetric and symmetric stretching vibrations of the free $NH_2$ groups in the molecule of the pure drug. The rest of the bands at 3280 and 3196 $cm^{-1}$ are caused by the amino-groups bonded by intermolecular hydrogen bonds. On the other hand, Fig. 5b is characterized by peaks appearing between 1700 and 1500 $cm^{-1}$, which can not be confused with DM-ß-CD peaks (Fig. 5a) around 1200-1000 $cm^{-1}$. In the physical mixture, the spectrum is the superposition of these of the pure components with attenuation of the FSD peaks (Fig. 5c). For the complexes the FSD peaks mostly shifted to lower wave number (1680, 1595 and 1565 $cm^{-1}$) in the case of the carbonyl stretching vibrations and to higher wave number (3400, 3350 and 3280 $cm^{-1}$) in the case of amino stretching vibrations. On the other hand, the observed peak at 3125 $cm^{-1}$ was disappeared in the case of ground mixture and coprecipitate (Figs. 5d and e). The C=O and $NH_2$ absorption wave numbers of the drug are summarized in Fig. 6.

The IR spectrum of DM-ß-CD (Fig. 5a) is characterized by intense bands at 3500-3300 $cm^{-1}$, associated with the absorption of hydrogen-bonded-OH groups of the DM-ß-CD.

These results are in favour of the existence of an inclusion complex which may be formed by hydrogen bonds among the above mentioned groups of the host-guest molecules.

**Differential scanning calorimetry**

This technique was used to study the prepared systems. The obtained calorimetric curves are shown in Fig. 7. Furosemide itself melted at 497 K (Fig. 7b). DSC curves of the physical mixure is just the superposition of DSC thermograms of the pure components.

The ground sample of FSD and DM-ß-CD (Fig. 7d) shows the disappearance of the fusion peak of the drug, indicating the existence of an interaction between the two components.[24] The endothermic peak observed around 488 K in this thermogram, probably, indicates the fusion of a new solid complex. In this thermogram is also evident an exothermic peak at around 400 K, probably due to amorphous nature of the sample that became crystalline after heating.[24]

DSC thermogram of coprecipitate of FSD and DM-ß-CD does not show a similar trend to that observed for ground sample. This thermogram showes the disappearance of the fusion peak of the drug and new endothermic peak was observed at 413 K showing that an interaction is present between two components (Fig. 7e).

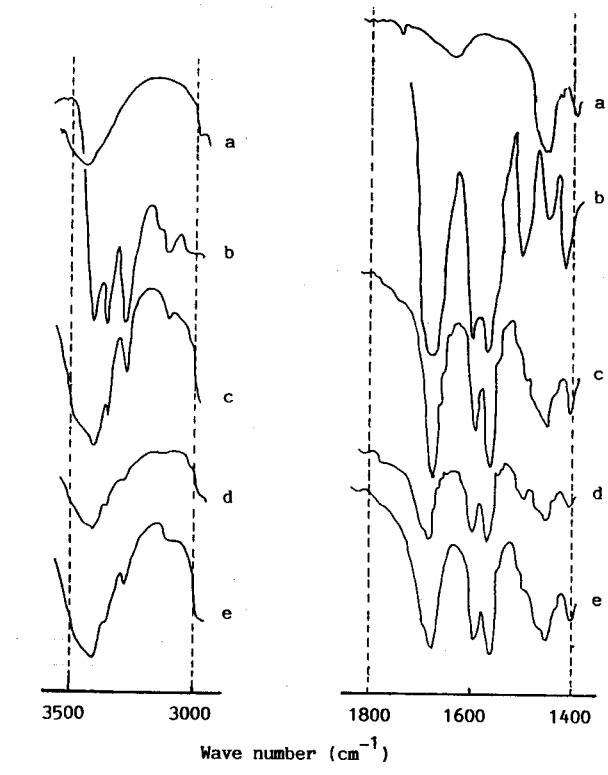


**Fig. 5:** IR spectra of (a) DM-ß-CD alone; (b) FSD alone; (c) physical mixture; (d) ground mixture and (e) coprecipitate.

| Wavenumber (cm$^{-1}$) | 3500 1 2 3 4 3000 | 1800 5 1600 6 7 1400 |
|---|---|---|
| (a) Furosemide (FSD) | 10 58 80 25 | 70 90 60 |
| (b) FSD-DM-B-CD physical mixture | 3400 50 80 25 | 80 90 60 |
| (c) FSD-DM-B-CD ground mixture | 3400 50 80 | 80 95 65 |
| (d) FSD-DM-B-CD coprecipitation | 3400 50 80 | 80 90 60 |

**Fig. 6:** Comparison of amino stretching and carbonyl stretching vibrations of furosemide in various states

(1) N-H stretch, sulfonamide
(2) N-H stretch, secondary amine, intramolecular bonded
(3) N-H stretch, sulfonamide
(4) N-H stretch, secondary amine, intramolecular bonded
(5) C=O intramolecular bonded
(6) N-H bending
(7) N-H bending

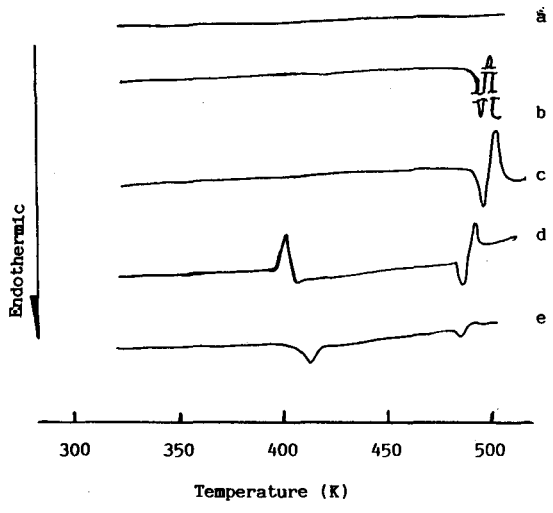


**Fig. 7:** DSC patterns of (a) DM-ß-CD alone, (b) FSD alone; (c) physical mixture; (d) ground mixture and (e) coprecipitate.

**Dissolution rate studies**

The dissolution profiles of FSD and FSD-DM-ß-CD systems are shown in Fig. 8. It is evident that the co-ground system exhibits a very higher dissolution rate than the coprecipitate, the physical mixture, and the pure drug. The dissolution profiles are evaluated by the dissolution efficiency parameter[25] at 60 min ($DE_{60}$) and the dissolved percentage at 60 min ($DP_{60}$) (Table 1). The enhancement of the dissolution rate for the physical mixture may be attributed to the wetting effect of the DM-ß-CD. This improvement has been attributed to the formation of an inclusion complex in the solid state and to the reduction of the crystallinity of the products, as confirmed by DSC and XRD studies.

**Stability studies**

The hydrolysis of furosemide in 0.1 N HCl (pH 1.4) followed first-order kinetics at all temperatures investigated. Introduction of DM-ß-CD did not affect the hydrolysis rate and kinetic order. First-order plots at all temperatures studied typically exhibited linear relationship, some typical plots at 70° are shown in Fig. 9. The pH values of solutions were maintained constant throughout the course of kinetic studied at all temperatures in the absence and in the presence of DM-ß-CD. The influence of DM-ß-CD was evaluated by comparing K values determined in the presence of DM-ß-CD with that in its absence, in 0.1 N HCl solution, at each temperature. The stability of drug was dependent on the temperature. On the other hand, the effect of DM-ß-CD on the stability of the drug was found to be nonsignificant. The Arrhenius temperature-dependencies for the hydrolysis of FSD in the absence and pesence of DM-ß-CD, were evaluated over 60 to 80° (Fig. 10). Activation parameters for these reactions, employing the transition-state theory of temperature-dependence of reaction rate[26,27] and Gibbs-Helmholtz equation have also been determined.

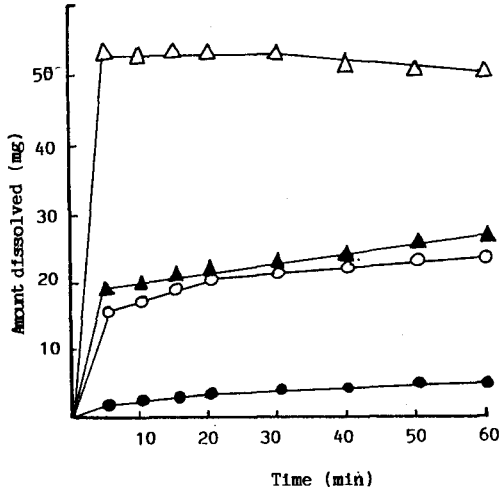


**Fig. 8:** Dissolution rate profiles of (•) furosemide alone; (○) physical mixture of FSD-DM-ß-CD; (▲) coprecipitation of FSD-DM-ß-CD; (Δ) ground mixture of FSD-DM-ß-CD.

Values of the standard free energy (ΔF°), enthalpy (ΔH°) and entropy (ΔS°) for the hydrolytic reactions were computed and it were found in Table 2. The activation energy (Ea) for the hydrolysis of furosemide (44.7 Kcal $mol^{-1}$) agreed well with a value at pH 1.2 reported in the literature.[28] When these parameters were determined for the hydrolysis of the drug-DM-ß-CD complex from K data, no significant difference from those values obtained in the absence of DM-ß-CD was found (Table 2). This might indicate that the mechanism of hydrolysis of FSD was probably unchanged in the presence of DM-ß-CD, as suggested in the literature.[29,30]

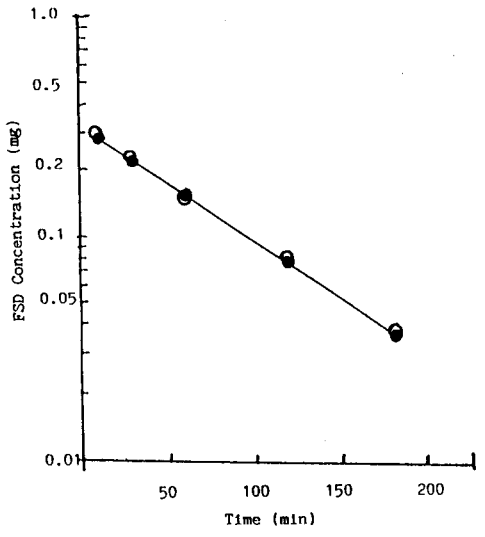


**Fig. 9:** Apparent first-order plot for the hydrolysis of furosemide in 0.1 N HCl at 70° (•) FSD alone; (○) FSD in presence of DM-ß-CD.

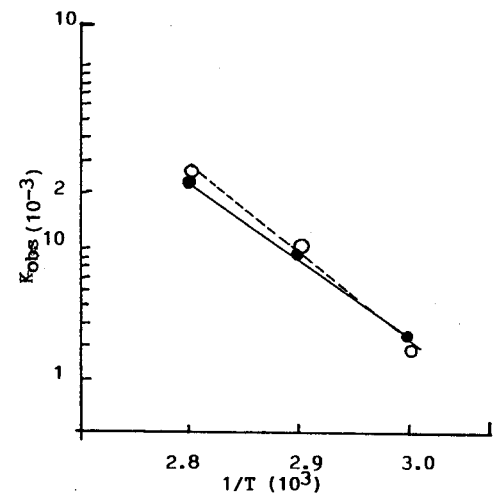


**Fig. 10:** Arrhenius plot for the hydrolysis of furosemide in 0.1 N HCl at 60, 70 and 80° (•) FSD alone; (○) FSD in presence of DM-ß-CD.

**Table 1:** Dissolution percentage ($DP_{60}$) and dissolution efficiency ($DE_{60}$) over the first 60 min corresponding to the binary systems FSD-DM-β-CD.

| Binary systems | Elaboration method | DP60 | DE60 |
|---|---|---|---|
| Furosemide | - | 9.5 | 0.077 |
| Fursosemide-DM-β-CD | Physical mixture | 46.62 | 0.397 |
| | Coprecipitate | 51.44 | 0.435 |
| | Ground mixture | 99.60 | 0.989 |

**Table 2:** Kinetic and thermodynamic parameters of the hydrolysis of FSD in absence and presence of DM-β-CD in 0.1 N HCl at different temperatures.

| System | Temperature (°C) | K ($10^{-3}$ $min^{-1}$) | ΔF° (cal/mole) | ΔH° (cal/mole) | ΔS° (cal/mole deg) |
|---|---|---|---|---|---|
| Furosemide | 60 | 3.10 | 3822.6 | -204.55 | -12.10 |
| | 70 | 9.88 | 3147.4 | | -9.77 |
| | 80 | 12.80 | 3057.5 | | -9.27 |
| Furosemide-DM-β-CD | 60 | 2.73 | 3906.8 | -257.40 | -12.51 |
| | 70 | 10.43 | 3110.5 | | -9.81 |
| | 80 | 12.65 | 3065.7 | | -9.41 |

## Conclusion

The results obtained demonstrate that DM-ß-CD used in this study is able to interact with FSD in solution and solid states. Aqueous solubility of the drug significantly increases when complexed with DM-ß-CD, all drug-DM-ß-CD systems have a high dissolution rate, in particular, 100% of the FSD-DM-ß-CD ground mixture dissolves within 5 min. In general, the results show that DM-ß-CD is very useful additive for increasing the aqueous solubility of furosemide.

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