

## STUDY OF THE INTERACTION AND HYDROLYSIS OF FUROSEMIDE WITH CYCLODEXTRINS IN SOLUTION AND IN THE SOLID STATES

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إن عقار الفيروسيמיד والذي يستخدم كمدر قوي للبول ، عند تعاطيه عن طريق الفم يعطى توافراً حيويًا ضعيفاً وهذا قد يعزى إلى عدم قابلية ذوبانه في الماء أو تحلله قبل أن يتم امتصاصه. إن عملية تكوين مترابكات من العقار وكل من ألفا-سيكلودكسترين وبيتا-سيكلودكسترين في المحلول قد تم دراسته بطريقة الإذابة وطريقة الإنحراف الطيفي. وقد وجد أن ثابت التكوين للمترابكات تساوي ٦٢٥ مول<sup>-١</sup> و٤٨٥ مول<sup>-١</sup> مع ألفا-سيكلودكسترين و١٠١٥ مول<sup>-١</sup> و١٣٧٧ مول<sup>-١</sup> مع البيتا-سيكلودكسترين بهاتين الطريقتين على التوالي.

ولقد تم دراسة المترابكات المنغمسة للعقار مع مركبات السيكلودكسترين في الحالة الصلبة بواسطة التحليل السعري التفاضلي والأشعة تحت الحمراء وبواسطة أشعة إكس ومعدل الإنطلاق والذي تبين أن هناك تفاعلات بين العقار والحوامل والتي أدت إلى زيادة تكوين الشكل اللابلوري للعقار وخصوصاً في حالة الطحن الآلي.

وكما هو متوقع فإن معدل ذوبان العقار من المترابكات مع السيكلودكسترين قد وجد أنه زاد بدرجة كبيرة بالنسبة للعقار نفسه وقد يعزى هذا إلى تكوين الشكل اللابلوري وزيادة درجة الإذابة بطريقة الطحن الآلي.

من ناحية أخرى ، فقد تم دراسة حركية تحلل العقار لمعرفة تأثير الأس الهيدروجيني ودرجة الحرارة في وجود أو غياب مركبات السيكلودكسترين على ميكانيقية تحلل العقار ، ولقد إتضح أن العقار يتم تحلله بسرعة في الوسط الحمضي ولا يتأثر معدل التحلل هذا بوجود مركبات السيكلودكسترين ، كما أن العقار يعتبر ثابتاً في الوسط المتعادل والقلوي.

*Furosemide, a potent diuretic, has been found to give a low bioavailability profile when administered orally which potentially could be due to its insolubility in water, and hydrolysis of the drug prior to absorption.*

*Complex formation of furosemide and both  $\alpha$ -cyclodextrin and  $\beta$ -cyclodextrin in solution was studied by phase solubility and spectral shift methods. The values of the apparent stability constant,  $K_c$ , by these techniques, were 625  $M^{-1}$  and 485  $M^{-1}$  with  $\alpha$ -cyclodextrin and were 1015  $M^{-1}$  and 1377  $M^{-1}$  with  $\beta$ -cyclodextrin, respectively. Binary systems of furosemide with  $\alpha$ -CyD and with  $\beta$ -CyD were prepared using the physical mixture, co-grinding and coprecipitation methods.*

*The interaction in the solid state was investigated by differential scanning calorimetry (DSC), infrared spectroscopy (IR), X-ray powder diffractometry (XPD) and a dissolution-rate method.*

*The main conclusion arising from these studies has been a high interaction between drug and carrier, linked to a clear increase in the amorphous nature of the drug in these systems, specially in the co-ground system. As expected, the dissolution rate of CyDs complexes was much faster than that of the pure drug. This might be attributed to the amorphous state, the increased wettability of the drug and the inclusion complex formation at the liquid and solid state. On the other hand, an in-vitro kinetic study was performed to elucidate the effect of pH and temperature in absence and presence of  $\alpha$ - and  $\beta$ -CyDs on the kinetics and hydrolysis mechanism of furosemide as a function of pH and temperature.*

## INTRODUCTION

Furosemide is a potent orally effective diuretic that is widely prescribed to treat oedema associated with congestive heart failure, cirrhosis of the liver, and renal diseases.<sup>1,2</sup> It is practically insoluble in water. Its limited aqueous solubility creates variation in its dissolution rate and consequently, in its bioavailability.<sup>3</sup> If it could be dissolved in water to a greater extent, its effect would be more intensive. For this purpose, CyDs appear to be very suitable. Attempts have been made to increase the solubility and dissolution rate of it.

Cyclodextrins (CyD) are natural cyclic oligosaccharides which form inclusion complexes with a wide range of inorganic and organic guests as long as they have a size which suitably fits within the CyD cavity. Their use as solubilizing and stabilizing agent in pharmaceutical development has recently been reported.<sup>4-7</sup> Penetration-enhancing activity has been demonstrated for CyDs with several drugs.<sup>8-10</sup> Recent literatures<sup>11-13</sup> has reported the increased stability, solubility, dissolution rate and bioavailability of a variety of chemically distinct pharmaceutical compounds as a result of complex formation with cyclodextrins and with a chemically modified derivatives of  $\beta$ -CyD. For instance, the hydrolysis of cephalothin complex with hydroxypropyl  $\beta$ -CyD, was slower than that of the uncomplexed cephalosporin by 4-8 fold at pH 6.6.<sup>13</sup>

The present paper is orientated to the investigation of the inclusion made between furosemide and  $\alpha$ - and  $\beta$ -CyDs, both in solution and in the solid state, to evaluate the influence of the complexation in the dissolution performance of the drug. In addition, this study was evaluated the effect of CyDs on the pH-catalysed hydrolysis of FSD in different buffer systems at different pH over the temperature range 60 to 80°C. Temperature-dependence data for this hydrolysis reaction in the absence and presence of CyDs, and the equilibrium reaction of complex formation, will be determined for the kinetic data obtained.

## MATERIALS

Furosemide (FSD) or 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid (APS Cleckheaton, UK) was used without any preliminary treatment.  $\alpha$ -Cyclodextrin ( $\alpha$ -CyD) was purchased from Nacalai Tesque Inc. (Kyoto, Japan).  $\beta$ -Cyclodextrin ( $\beta$ -CyD) was purchased from Toshin Chemical Company (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade.

## METHODS

### Phase solubility studies

The solubility measurements were carried out according to the method described by Higuchi and Connors.<sup>14</sup> Furosemide, in an amounts that exceeded its aqueous solubility (100 mg) were accurately weighed in well stoppered tubes to which were added 10 ml of water containing various concentrations of  $\alpha$ -CyD ( $0.25-5.0 \times 10^{-2}$  M) or  $\beta$ -CyD ( $0.25-12.0 \times 10^{-3}$  M). These tubes were sealed and shaken at 25°C. After equilibrium was attained (about one week), the solutions were properly diluted with water and analyzed spectrophotometrically at 276 nm for total FSD content. The apparent 1:1 stability constants  $K_c$ , was calculated from the initial linear part of phase solubility diagrams using the following equation.<sup>14</sup>

$$K_c = \text{slope} / \text{intercept} (1 - \text{slope}) \dots \dots \dots (1)$$

### Spectroscopic studies

Complex formation between FSD and  $\alpha$ -CyD or  $\beta$ -CyD was also studied by the spectral shift method.<sup>15</sup> The concentration of the drug in these studies was  $1 \times 10^{-4}$  M, where as the CyD concentration were increased from  $1 \times 10^{-3}$  to  $1 \times 10^{-2}$  M and UV spectra of FSD were recorded on a Hitachi U-557 UV-spectrophotometer. The change in absorbance of the substrate (FSD) by the addition of various concentrations of the ligand ( $\alpha$ -CyD or  $\beta$ -CyD) was measured at 276 nm to evaluate the stability constant of complexes.

### Preparation of solid complexes

The preparation of solid complexes FSD- $\alpha$ -CyD and FSD- $\beta$ -CyD was performed by different techniques, which are described below in details. The molar ratio was 1:1.

### Coprecipitation

The preparation of the inclusion complexes of furosemide with CyDs in 1:1 molar ratio were carried out by addition of 0.6615 g of FSD to 200 ml of 0.002 M solution of  $\alpha$ -CyD or  $\beta$ -CyD. The system was kept under magnetic agitation to 60°C until evaporation of the liquid was completed, the system was protected from the light, due to FSD photolability. The formed precipitate was separated and dried at 40°C in an oven.

### Co-grinding

The physical mixture of furosemide and CyDs in 1:1 molar ratio was introduced in a tungsten carbide vibrational mill (Heiko Seisakusho TJ-200). Total weight of the sample was about 2.0 g. The grinding process was continued for 10 min.

### Infrared absorption spectroscopy (IR)

IR measurements were made with a Hitachi 295 infrared spectrophotometer using KBr disc method.

### Differential-scanning calorimetry (DSC)

The Perkin-Elmer DSC-1B was operated at a scanning speed 8 K/min under  $N_2$  stream from 320 to 480 K. The measurements were done using the sample pan for the liquid sample. Sample weight was about 4.5 mg.

### X-ray powder diffraction (XPD)

A Rigakudenki 2027 (Japan) powder diffractometer was used. Measuring conditions were: target Cu, filter Ni, voltage 30 KV, current 5 mA, time-constant 0.5 s, scanning speed 2°/min<sup>-1</sup>.

### Dissolution rate studies

The dissolution rate were performed according to the USP XXIII paddle method, the dissolution medium was USP XXIII, pH 5.8 phosphate buffer, 900 ml. The stirring speed 50

rpm and the temperature 37±0.5°C. The samples, 5 ml, were withdrawn at various time intervals using a syringe, an equal volume of dissolution medium preadjusted at the same temperature was immediately replaced after each sample withdrawn. The samples were analyzed spectrophotometrically at 276 nm against a similarly treated blank.

### Kinetic measurements

Furosemide was dissolved in methanol in volumetric flask previously rapped with foil to produce a 1x10<sup>-3</sup> M stock solution of the drug. In a typical kinetic run, one milliliter of the stock solution was added to 100 ml volumetric flask contain buffer solution to produce approximately 1x10<sup>-5</sup> M solution of the drug. The reaction flasks were maintained at the reaction temperature (60-80°C±0.5°C) in water bath. Kinetics reactions was carried out in absence and in presence of CyDs. Samples were withdrawn at certain times intervals without any replacement during the course of the reactions. Kinetic runs were conducted throughout the pH regions.

## RESULTS AND DISCUSSION

### Phase solubility studies

Figures 1 and 2 show the phase solubility diagrams of FSD with  $\alpha$ -CyD and  $\beta$ -CyD in water. The solubility curves can be classified as Bs type,<sup>14</sup> where the initial rising portions are followed by plateau regions and then the total FSD concentration decreased with the precipitation of microcrystalline complexes.

The apparent 1:1 stability constants ( $K_c$ ) were calculated by Eq. (1), from the initial line portion of the solubility diagram, assuming the 1:1 inclusion complex formation and were found to be 625 M<sup>-1</sup> with  $\alpha$ -CyD and 1015 M<sup>-1</sup> with  $\beta$ -CyD.

### Spectral shift method

The effect of different molar concentrations of  $\alpha$ -CyD and  $\beta$ -CyD on the absorption spectrum of furosemide is given in Figures 3 and 4. It appears as a bathochromic effect in the absorption maxima of the drug, with a diminution of the absorbance are attributed,

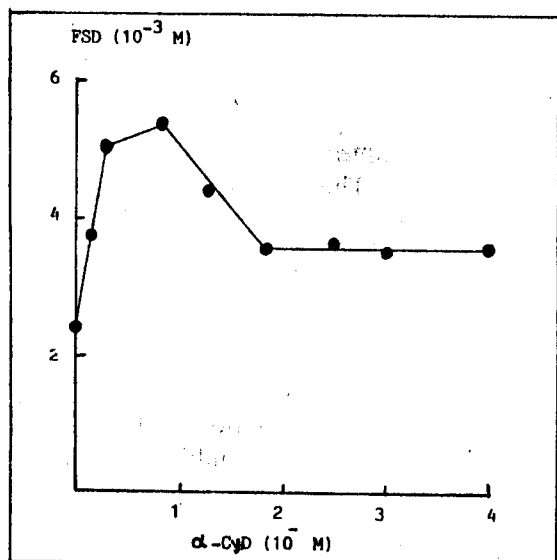


Fig. 1: Phase-solubility diagram of furosemide- $\alpha$ -CyD system at 25°C.

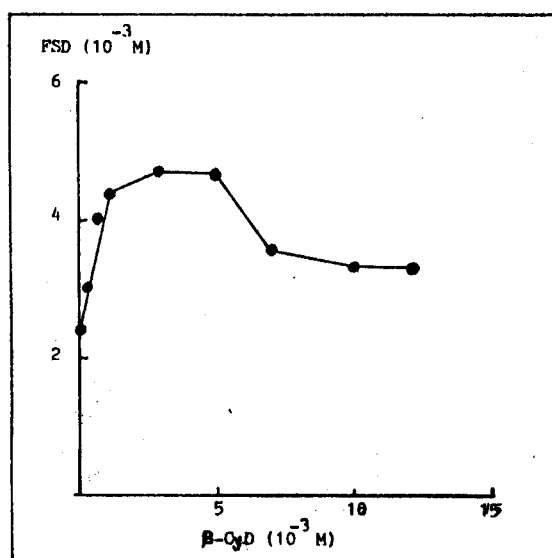


Fig. 2: Phase-solubility diagram of furosemide- $\beta$ -CyD system at 25°C.

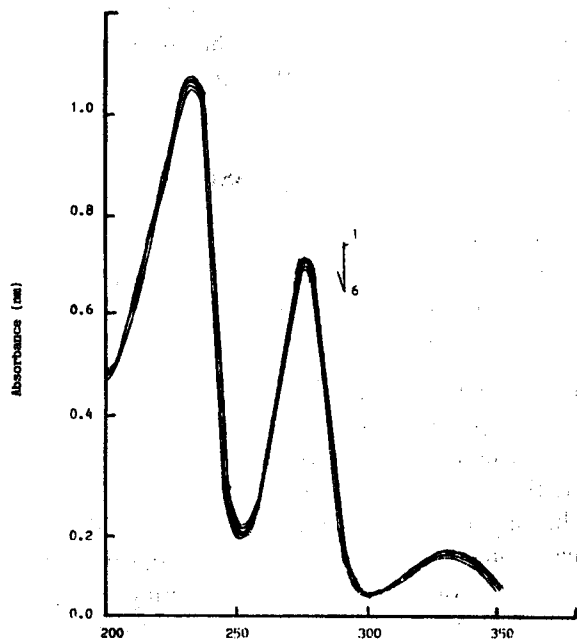


Fig. 3: Effect of  $\alpha$ -CyD concentrations on UV absorption spectra of furosemide in water. The concentration of drug was  $1.0 \times 10^{-4}$  M: (1) drug alone; (2) drug in presence of  $1 \times 10^{-3}$  M  $\alpha$ -CyD; (3) drug in presence of  $2 \times 10^{-3}$  M  $\alpha$ -CyD; (4) drug in presence of  $5 \times 10^{-3}$  M  $\alpha$ -CyD; (5) drug in presence of  $8 \times 10^{-3}$  M  $\alpha$ -CyD; (6) drug in presence of  $1.0 \times 10^{-2}$  M  $\alpha$ -CyD.

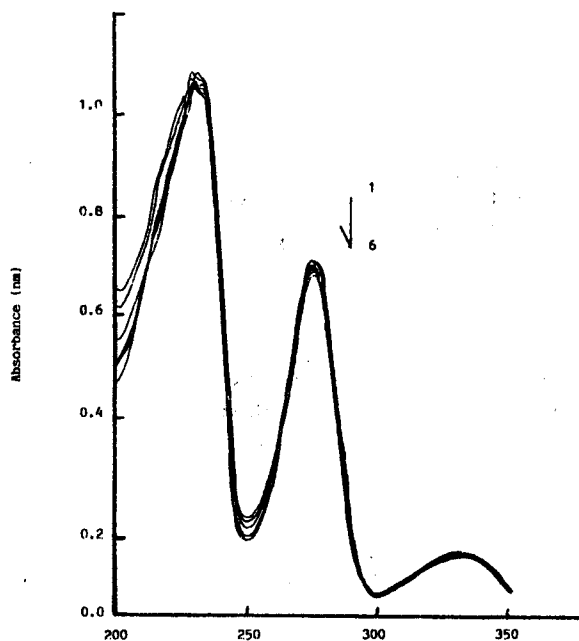


Fig. 4: Effect of  $\beta$ -CyD concentration on UV absorption spectra of furosemide in water. The concentration of drug and drug with  $\beta$ -CyD are the same as in Fig. 3.

primarily, to the formation of a inclusion complex in the liquid state. The changes of the peak intensity may be the result of the moving of the guest molecules from water to the CyD cavity. This is reasonable in the light of fact that there are no proton donating groups inside cavity of the CyD molecule.<sup>16</sup>

The spectral data were analyzed by the double reciprocal plot as shown in Figure 5. The plot of  $1/\Delta A$  vs  $1/[CD]$  is linear, indicating the presence of 1:1 complex.

The apparent 1:1 stability constants were determined by the Benesi-Hildebrand equation:<sup>17</sup>

$$\frac{1}{\Delta A} = \frac{1}{[D] K_c \Delta \epsilon} + \frac{1}{[CyD] \Delta \epsilon} \dots (2)$$

where  $\Delta A$  is the difference of absorbance at 276 nm,  $[CyD]$  is the CyD concentration,  $[D]$  is the total drug concentration (constant) and  $\Delta \epsilon$  is the difference of molar absorptivities between the complex and free drug. The stability constants;  $K_c$ , were obtained from the intercept/slope ratio and this value was 485 and 1377  $M^{-1}$  with  $\alpha$ -CyD and  $\beta$ -CyD, respectively, that is approximately of the same order of value calculated by solubility studies.

### IR spectra

Figures 6 and 7 show IR spectra of the samples under study. IR spectrum of furosemide (Fig. -b) is characterized by the absorption of the C=O group, located in the band at 1670  $cm^{-1}$ . This band is assigned to asymmetric stretching vibration of the C=O group in the molecule of pure drug. The large shift of this vibration to higher wave numbers proves the hydrogen-bonded state of the carbonyl group. Finally, wave numbers observed at 1590 and 1560  $cm^{-1}$  may be assigned to the N-H and N-H bending<sup>18</sup> respectively, and which is typical for condensed cyclic hydrogen-containing systems. The vibrations of N-H and N-H bending groups are shifted to 1598 and 1570  $cm^{-1}$ , respectively, and reduced in their intensities. Thus, as spectral changes always concern C=O, N-H and N-H bending groups of the furosemide, it should be suggested that the host-guest interactions are dominated by hydrogen bonds among the above mentioned groups.

### DSC data

Figures 8 and 9 show the DSC thermograms of powders prepared from furosemide with  $\alpha$ -CyD and  $\beta$ -CyD by co-grinding, and coevaporation, compared with those of their physical mixtures.

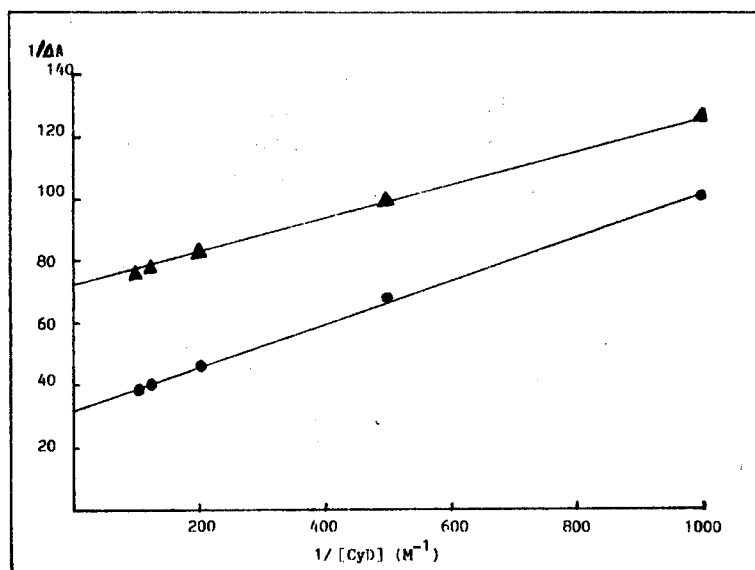
There is no different between physical mixture and processed powders with 1:1 drug/ $\beta$ -CyD molar ratio, the physical mixture of drug/ $\alpha$ -CyD in molar ratio 1:1 shows a drug melting peak lower than that of the drug powder. This may be due to a partial complexation during heating processes. In conclusion, all complexes formed, by co-grinding and coevaporation methods, were characterized by differential scanning calorimetry to ensure that the inclusion complexes were formed. As shown in Figures 8 and 9, the sharp transition at 495 K is the melting point of furosemide. This transition was disappeared or shifted to lower melting point for the inclusion complexes formed by co-grinding and coprecipitation methods. This indicated that complete complexation was formed by the above mentioned methods for complexation.

### Powder X-ray diffractograms

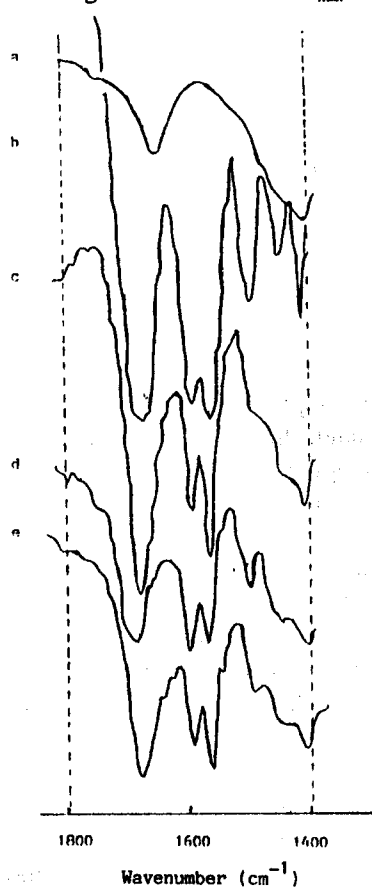
Figures 10 and 11 show the changes in the powder X-ray diffraction patterns of furosemide- $\alpha$ -CyD and  $\beta$ -CyD powders. The co-grinding FSD-CyD complexes showed halo-patterns. On the other hand, the FSD, CyDs physical mixture and coprecipitation complexes showed many diffraction peaks, with just a certain crystallinity reduction in the case of physical mixtures and coprecipitations. It is remarkable that the grinding effects extended not only to the destruction of crystal lattice of FSD and CyDs, but also to the inclusion formation between drug and CyDs which was predicted from the results of DSC and IR.

### Dissolution studies

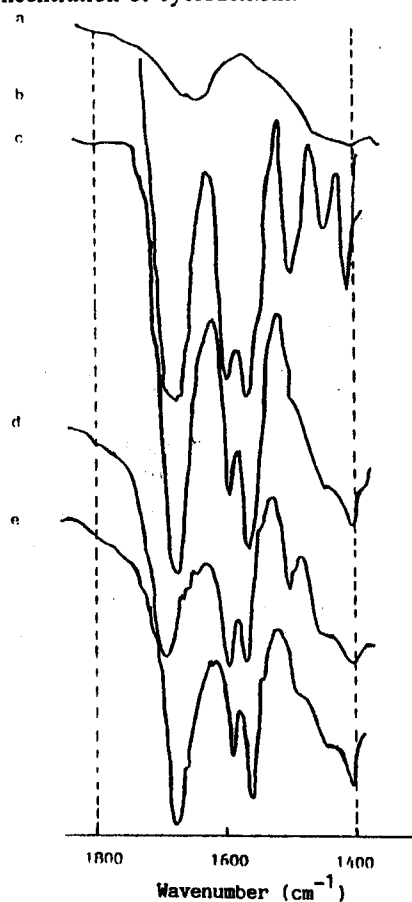
Figure 12 shows the dissolution profiles of powders with molar ratio 1:1, composed by FSD- $\alpha$ -CyD and FSD- $\beta$ -CyD, respectively, compared with the profiles of furosemide alone and its physical mixture.



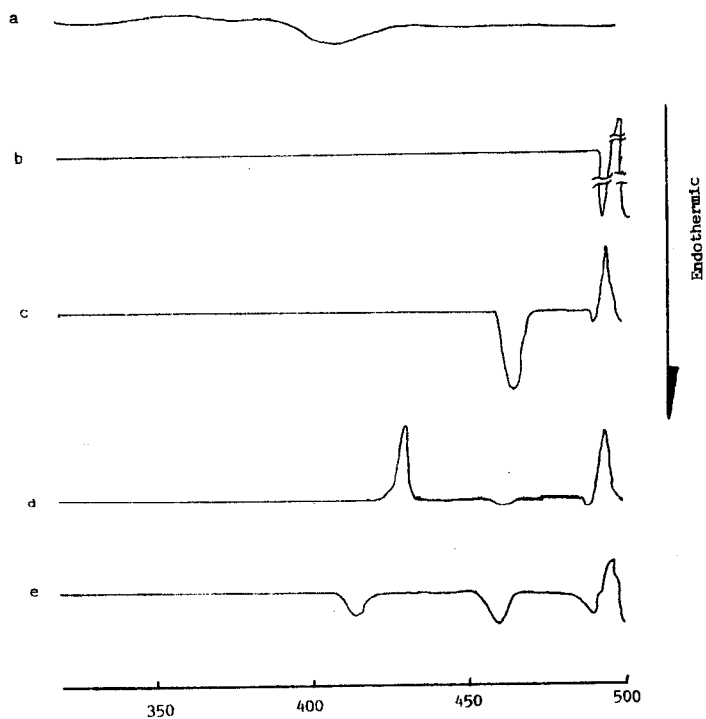
**Fig. 5:** Benesi-Hildebrand plot for (●) the furosemide- $\alpha$ -CyD system and (▲) the drug- $\beta$ -CyD system ( $\Delta A$  displays the change of absorbance at  $\lambda_{max} = 276$  nm and  $[CyD]$  the concentration of cyclodextrin).



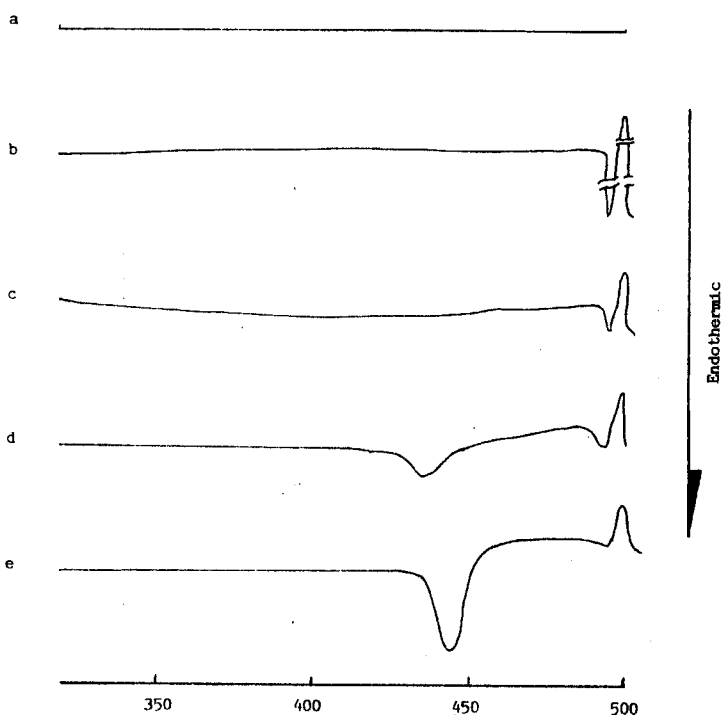
**Fig. 6:** Infrared spectra of furosemide- $\alpha$ -CyD systems. a)  $\alpha$ -CyD alone; b) furosemide alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



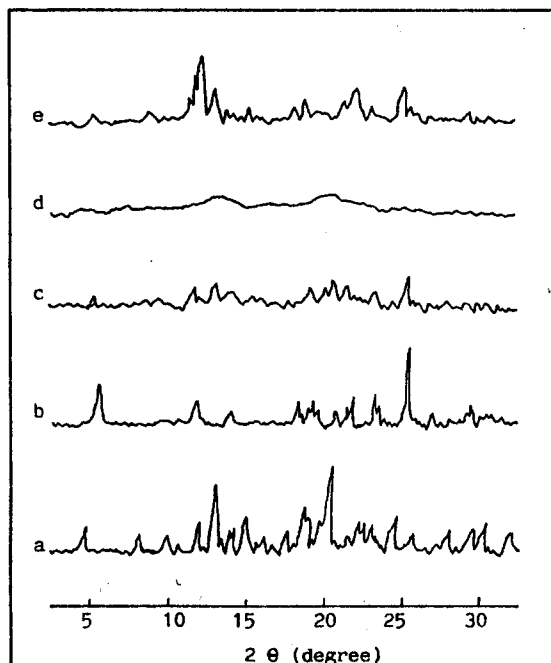
**Fig. 7:** Infrared spectra of furosemide- $\beta$ -CyD systems. a)  $\beta$ -CyD alone; b) furosemide alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



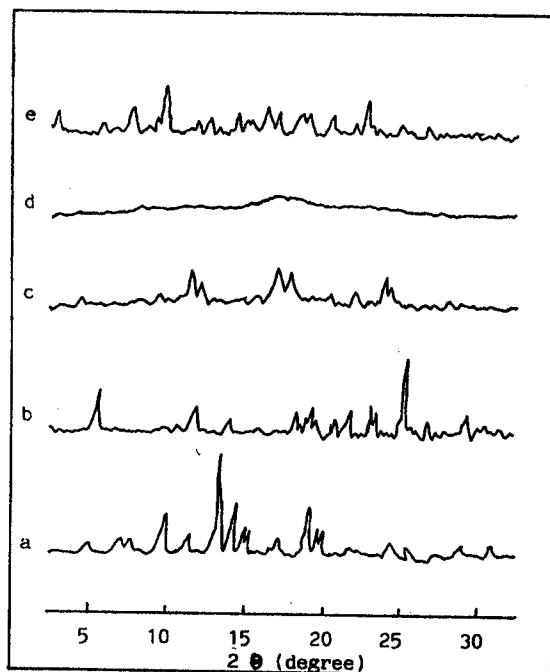
**Fig. 8:** Differential scanning calorimetry patterns of furosemide- $\alpha$ -CyD systems. a)  $\alpha$ -CyD alone; b) drug alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



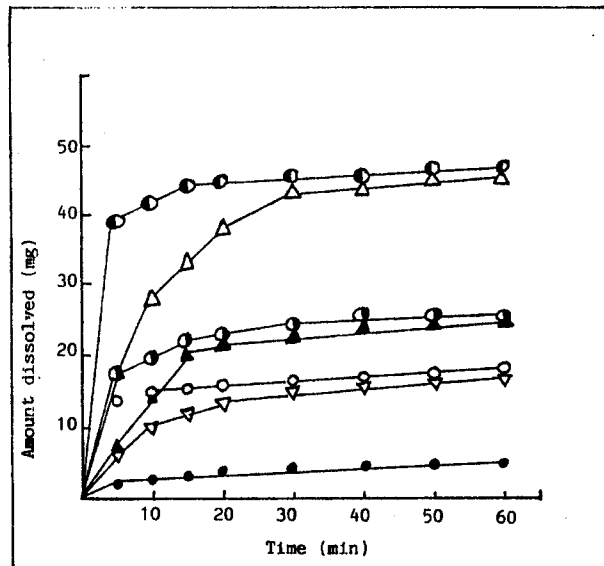
**Fig. 9:** Differential scanning calorimetry patterns of furosemide- $\beta$ -CyD systems. a)  $\beta$ -CyD alone; b) furosemide alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



**Fig. 10:** Powder X-ray diffraction patterns of furosemide- $\alpha$ -CyD systems. a)  $\alpha$ -CyD alone; b) furosemide alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



**Fig. 11:** Powder X-ray diffraction patterns of furosemide- $\beta$ -CyD systems. a)  $\beta$ -CyD alone; b) furosemide alone; c) physical mixture (1:1 molar ratio); d) complex prepared by grinding; e) complex prepared by coprecipitation.



**Fig. 12:** In vitro dissolution rate profiles of furosemide-CyDs mixtures: ●, furosemide alone; ▽, coprecipitation of FSD- $\beta$ -CyD; ○, coprecipitation of FSD- $\alpha$ -CyD; ▲, physical mixture of FSD- $\beta$ -CyD; ○ physical mixture of FSD- $\alpha$ -CyD; Δ, ground mixture of FSD- $\beta$ -CyD; ○, ground mixture of FSD- $\alpha$ -CyD.



It is evident that the dissolution rate of furosemide was significantly improved through the complex formation. This enhancement in the dissolution rate, especially from ground mixtures with CyD than from physical mixtures and coprecipitates, may be attributed not only to the dispersion of drug in CyDs after co-grinding, but also to the nearly amorphous state of such systems. This assumption has been confirmed by XPD and DSC. The increase in dissolution rate recorded for the physical mixtures may be explained on the basis of the solubility of the drug in aqueous CyDs solutions. Since the CyDs dissolve more rapidly in the dissolution medium than the pure drug, it can be assumed that, in early stages of the dissolution process, the CyDs molecule will operate locally on the hydrodynamic layer surrounding the particles of the drug. This action resulting in an *in situ* inclusion process, which produces a rapid increase of the amount of dissolved drug. This situation has been similarly described by other authors.<sup>19</sup> The marked increase in the dissolution rate of physical mixtures could be also due, although to a lesser extent, to the hydrophilic effect of CyDs, which can reduce the interfacial tension between water-insoluble drugs and the dissolution medium, thus leading to a higher dissolution rate.<sup>20</sup>

#### Kinetic hydrolysis

Figure 13 shows a typical first-order kinetic plot for the hydrolysis of furosemide in 0.01 N HCl at 70°C, in presence or absence of CyDs. Table 1 presents the rate constants derived from these plots.

In the pH region above 6.6, the hydrolysis reaction is extremely slow. Furosemide hydrolysis is apparently negligible in the basic pH region, in presence and in absence of CyDs.

#### Rate-pH profile

The log K - pH profile for the hydrolysis of furosemide was constructed from the logarithm of the apparent first rate constants and pH values at 70°C in presence and absence of CyDs. In the acidic pH region, the log K - pH profile indicates specific hydrogen ion catalysis on the

neutral species. In this region, the log K - pH profile may be described by:

$$K_{\text{obs}} = K_{\text{H}^+} [\text{H}^+] \dots\dots\dots (3)$$

or

$$\log K_{\text{obs}} = \log K_{\text{H}^+} - \text{pH} \dots\dots\dots (4)$$

Due to the slow hydrolysis rate in the neutral and basic pH regions, there are too few data points to construct a complete log K - pH profile, in presence and absence of CyDs.

#### Stability parameters

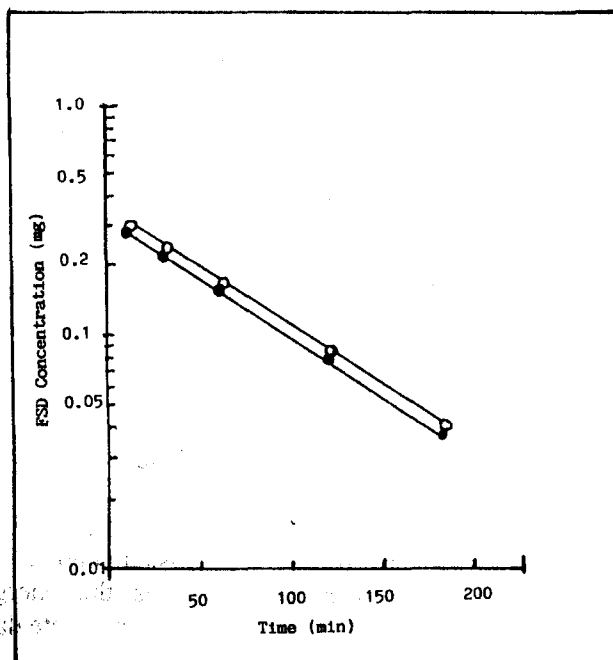
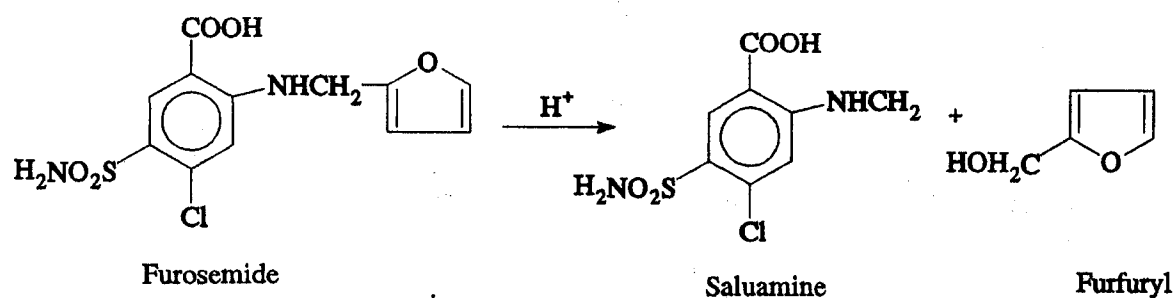
Hydrolysis kinetics as a function of buffer concentration, temperature and CyDs were studied to give a complete profile of furosemide stability. Buffer catalysis was ascertained by varying the original buffer concentration, in presence and absence of CyDs, in a gradient manner while keeping the buffer pH constant. No significant buffer catalysis and CyDs effects was observed. The Arrhenius parameters were obtained from a plot of the logarithm stability constant, K, versus the reciprocal of the absolute temperature, T, in accordance with this equation.

$$\log K = \log A - (E_a/2.303) (1/RT) \dots (5)$$

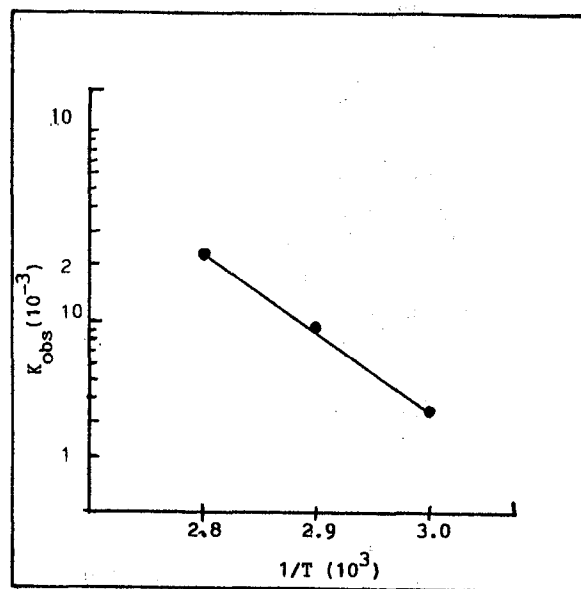
where R is the gas constant (1.987 cal deg<sup>-1</sup> mol<sup>-1</sup>) and log A is a constant known as the frequency factor, and E<sub>a</sub> is the energy of activation of the hydrolysis. To generate data for this plot, reactions were carried out at different temperatures in buffered solutions with or without CyDs at constant pH. The Arrhenius plots of these systems, show that there is no significant effect upon the addition of the CyDs, where the slope (E<sub>a</sub>) of these system was the same (45 kcal/mol) and an intercept (log A) of 16.0. These values will allow stability predictions in the acidic pH region where the degradation is hydrogen-ion catalyzed (Fig. 14).

From the observed spectra changes and kinetic behavior of furosemide, the hydrolysis of furosemide may be expected to proceed as depicted in the following scheme 1.

Scheme 1



**Fig. 13:** Apparent first-order plot for the hydrolysis of FSD in 0.1 N HCl at 70°C. ●, FSD alone; ○, FSD in presence of CyDs.



**Fig. 14:** Arrhenius plot for the hydrolysis of FSD in 0.1 N HCl.

**Table 1:** Apparent first-order rate constant of FSD in absence and presence of CyDs at 70°C.

pH	$K_{obs} (\text{min}^{-1}) \times 10^{-4}$		
	FSD	FSD- $\alpha$ -CyD	FSD- $\beta$ -CyD
1.4	114.2	103.00	106.00
3.0	21.8	16.53	15.6
4.4	1.01	4.05	1.01
6.6	1.01	2.02	1.01

## Conclusions

The results obtained demonstrate that, the grinding and coprecipitation methods are simple and suitable methods for obtaining inclusion complex of FSD with  $\alpha$ -CyD and  $\beta$ -CyD. The dry co-grinding method, used for preparing FSD-CyDs binary systems, has yielded products, characterized by their rapid dissolution rate, while the dissolution rates of inclusion complexes prepared by coprecipitation method were low. This difference may be attributed to the difference of the degree of crystallinity, that is, the ground mixture was in an amorphous state while the coprecipitate was in a crystalline state. Kinetic study showed that, there was no significant effects on the hydrolysis of the furosemide.

Finally, it is important to remark the utility of the co-grinding process to produce systems presenting a very rapid amorphization of the drug, which significantly improve the solubility. This increase of the solubility could improve the bioavailability of orally administered furosemide.

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