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Inclusion complexation of furosemide in cyclodextrins

Part 1: Effect of cyclodextrins on the physicochemical characteristics of furosemide

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The interaction of furosemide with five cyclodextrins (CyDs), namely, α-, β-, γ-, dimethyl-β-CyD (DM-β-CyD) and trimethyl-β-CyD (TM-β-CyD) was investigated. Differential UV spectrophotometry revealed marked effect of CyDs on the extinction coefficient of the drug at different wavelengths. The continuous variation method was used to elucidate the stoichiometry of the molecular interaction. The data revealed formation of 1 : 1 complexes between furosemide and the investigated CyDs. The investigated CyDs were found to increase the solubility of the drug in water to a marked extent. Complexes of furosemide with CyDs were prepared. The chemical stability of the drug and its complexes was also investigated. The results revealed first-order degradation kinetics. The degradation rate constant as well as the half-life time were determined and revealed that inclusion complexation of furosemide in CyDs leads to a marked protection of the drug against chemical degradation. The dissolution behaviour of furosemide and its complexes with CyDs or their physical mixtures was examined. The solubility of furosemide in CyD-complexes is improved.

1. Introduction

Furosemide is practically insoluble in water, and improvement of its dissolution properties is essential because the *in vitro* dissolution behaviour of furosemide tablets is closely related to bioavailability [1–3]. On the other hand, Rowbotham et al. [4] reported that aqueous furosemide solutions undergo hydrolysis and photochemical degradation. The pharmaceutical application of CyDs at present mainly concern the preparation of inclusion compounds in order to achieve some definite peculiar advantages at the molecular level, improvement of dissolution rate, bioavailability and stability. In this context, Loftsson and Brewster [5] reviewed the use of cyclodextrins for solubilization, stabilization and formulation of drugs through the formation of inclusion complexes while Irie and Uekama [6] summarized findings on the safety profile of cyclodextrins.

The present communication deals with the inclusion complexation of furosemide in five CyDs, namely, α-CyD, β-CyD, γ-CyD, DM-β-CyD and TM-β-CyD, and the effect of these CyDs on the solubility, dissolution rate as well as the stability of the drug.

2. Investigations, results and discussion

The interaction of furosemide with CyDs was monitored spectrophotometrically. The UV absorption spectrum of furosemide solution (0.01 mmol/l) was investigated in the presence of different CyD concentrations (5–20 mmol/l). A distinct change in the extinction coefficient of the drug was noticed in the presence of the CyDs as a function of their concentration. The absorbance-value decreases with increasing β-CyD and DM-β-CyD concentrations and increases with increasing α-, γ- and TM-β-CyD concentrations. These spectral changes may give an indication of a sort of molecular interaction taking place between furosemide and these CyDs. These interactions were further investigated through other spectral measurements using the continuous variation method [7]. The absorbance of solutions containing different mole fractions of the drug and each of the investigated CyDs was measured at 276 nm. The total concentration of furosemide and CyD in each solution was fixed at a concentration of 0.036 mmol/l. The absorbance-values of all the investigated solutions were found to differ from the summation of the absorbance-values of their corresponding components. This gives evidence for complex formation between furosemide and these CyDs. The measured absorbance-values were subtracted from the calculated ones and the resulting absorbance differences were plotted against the mole fraction of each system. An illustration of such plots is shown in Fig. 1. An abrupt change in slope was found to take place at 0.5 mole fraction in all the systems. This indicates that furosemide forms equimolar complexes with the investigated CyDs.

Fig. 2 shows the solubility diagrams obtained for furosemide with α-, β- and γ-CyDs as well as with methylated derivatives of β-CyD. The solubility of furosemide increases linearly as a function of CyD concentration. This indicates that furosemide forms inclusion complexes with these CyDs in equimolar ratios. The solubilizing power increases in the following order: β- > α- > γ-CyD and β- > DM-β- > TM-β-CyD.

The apparent stability constants (K_c) of the formed complexes were calculated from the solubility diagrams according to the equation:

$$K_c = \text{slope}/S_0(1 - \text{slope}) \qquad (1)$$

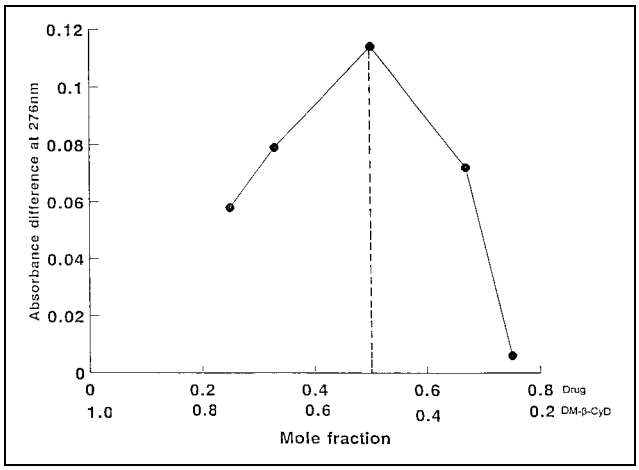

Fig. 1: Elucidation of the stoichiometric ratio of furosemide-DM-β-cyclodextrin complex by a spectrophotometric method

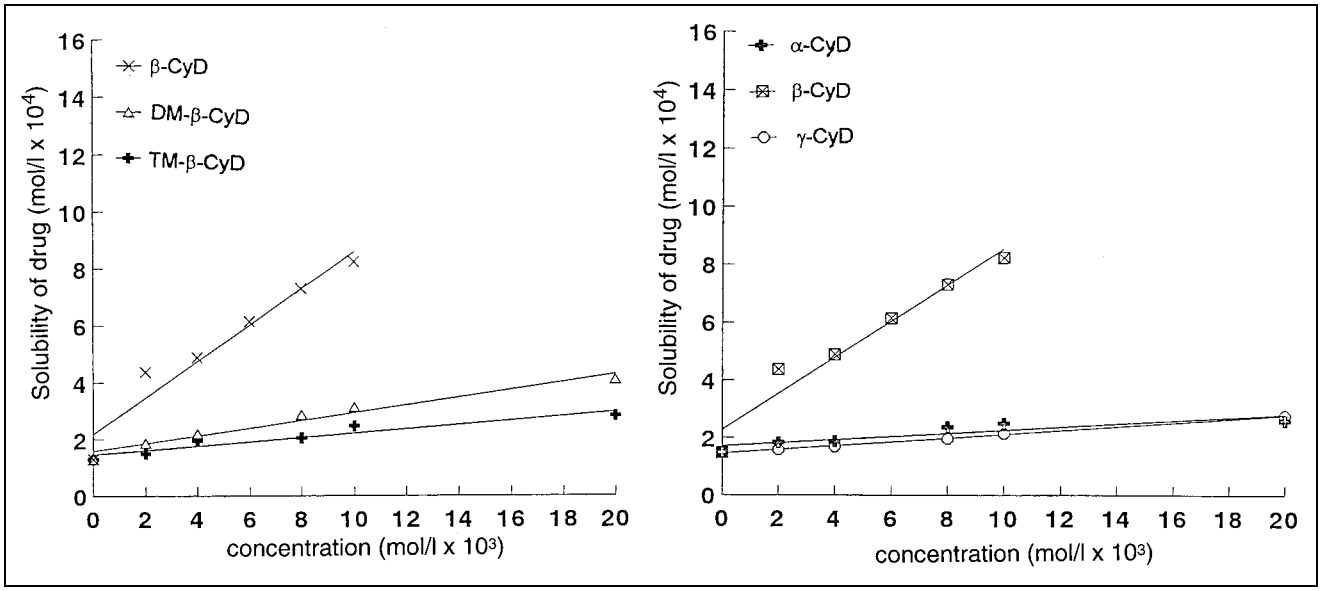

Fig. 2: Effect of cyclodextrins on the solubility of furosemide in water at 25 °C

where S_0 = solubility of furosemide in water (1.49×10^{-4} mol/l). The K_c values increase in the order: DM-β- > β- > α- > TM-β- > γ-CyD (117.3, 88.5, 74.7, 72.5 and 44.3 mol^{-1}, respectively).

The solid complexes of furosemide with each of the investigated CyDs were prepared by the kneading method [8] and the microcrystalline complexes were examined by IR spectroscopy.

IR spectrophotometry confirmed these results. Furosemide is characterized in particular by peaks between 1100 and 3500 cm^{-1}, corresponding to its different functional groups. For CyDs, the spectrum shows only the vibration of free OH between 3500 and 3300 cm^{-1} and those of bound OH at 2900 cm^{-1}. On the other hand, in the spectra of inclusion complexes, a change in the peaks of furosemide appears, as if they were bound. This spectrum appears to confirm a blocking of furosemide molecule in the CyD cavity, characteristic of an inclusion. Moreover, no new peak appears, which indicates that no chemical bonds were created in the compound formed.

The UV absorption spectrum of a furosemide solution (0.01 mmol/l) after photolysis for different time intervals shows a gradual decrease in the absorbance at 276 nm during photolysis pointing out the involvement of only the intact drug for the absorbance at this wave-length. Thus, a stability study was conducted by measuring the absorbance at this wavelength.

The investigation of the chemical stability of furosemide and furosemide-CyD complexes reveals a distinctly higher stability of the prepared complexes compared to the drug per se. The graphical illustration of the logarithm of the retained amount of drug as a function of time reveals a linear relationship, pointing to first-order degradation kinetics (Fig. 3). The degradation rate constant as well as the half-life time of the drug (Table) clearly indicate that inclusion complexation of furosemide with the investigated CyDs leads to a decrease in its degradation rate constant by 23, 50, 63, 70 and 83% for DM-β-, γ-, β-, α- and TM-β-CyD, respectively. This result runs parallel with

Table: Degradation rate constant (K) and t_{50} for furosemide and furosemide-CyD complexes

System	K (h^{-1})	t_{50} (h)
Furosemide	0.040	17.3
Furosemide-α-CyD complex	0.012	57.8
Furosemide-β-CyD complex	0.015	46.2
Furosemide-γ-CyD complex	0.020	34.7
Furosemide-DM-β-CyD complex	0.031	22.3
Furosemide-TM-β-CyD complex	0.007	99.0

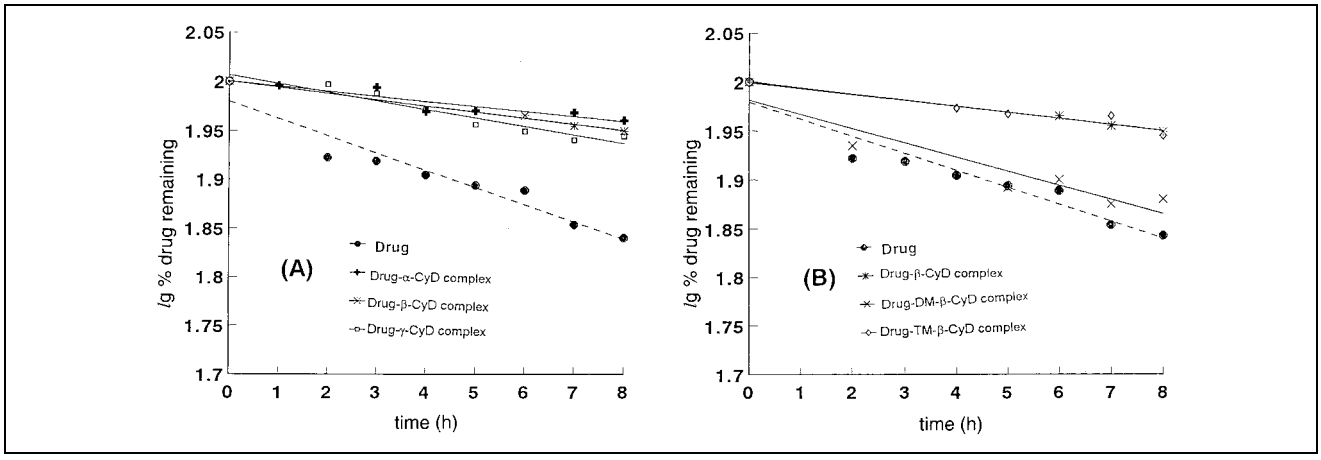

Fig. 3: Hydrolysis of furosemide and furosemide-cyclodextrin complexes in solutions

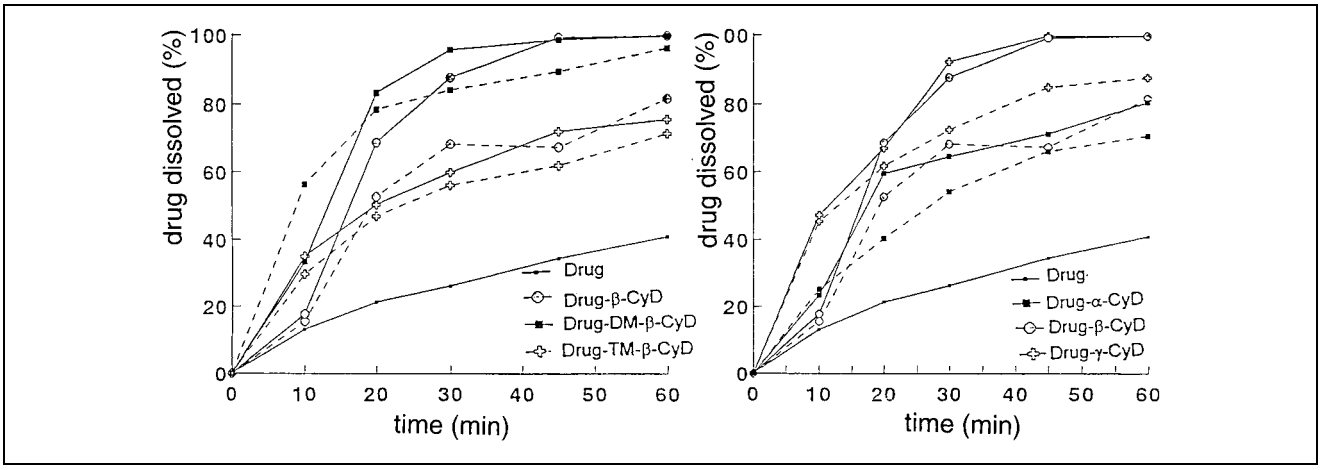

Fig. 4: Effect of cyclodextrins on the dissolution profile of furosemide
——— complexes, – – – – physical mixtures

other previously reported results [9–13] regarding improvement of the stability of drugs by complexation with CyDs.

Dissolution curves for furosemide, its physical mixtures with CyDs as well as the prepared complexes are shown in Fig. 4. It is evident that the dissolution rate of furosemide is significantly improved by complex formation. The dissolution efficiency of furosemide and the prepared complexes was found to be 25, 55, 70, 76, 76 and 54% for furosemide, furosemide-α-CyD, -β-CyD, -γ-CyD, -DM-β-CyD and -TM-β-CyD complexes, respectively. There is no good correlation between the dissolution efficiency of the prepared complexes and the solubilizing power of CyD. This may be due to the fact that the apparent dissolution rate of the complex is known to be dependent upon various factors such as solubility, diffusion coefficient and the dissociation of the complex in the dissolution medium [14].

3. Experimental

3.1. Materials

Furosemide (Hoechst), α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, heptakis (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CyD), all supplied from Sigma Chemical Co., St. Louis, USA.

3.2. Methods

3.2.1. Solubility studies

Solubility measurements were carried out according to Higuchi and Lach [15]. Excess amounts of furosemide were added to aqueous solutions containing various concentrations of CyD and shaken at 25 ± 0.5 °C. After equilibrium was attained (approximately 24 h), an aliquot was filtered, diluted with water and analyzed spectrophotometrically at 276 nm.

3.2.2. Preparation of solid complexes

The complexes of furosemide with each of CyDs under investigation were prepared by the kneading method [8], whereby furosemide was added to CyD in an equimolar concentration (molar ratio), then kneaded thoroughly with the least amount of water to obtain a paste which was then dried under vacuum at 40 °C. Physical mixtures of furosemide and each of CyDs were prepared by just mixing furosemide and each of CyD in equimolar concentrations.

3.2.3. IR-Spectroscopy

The IR spectra were measured in KBr discs using a Shimadzu 435μ-04 IR spectrophotometer.

3.2.4. Chemical stability study

The chemical stability study was conducted on a 0.01 mmol/l furosemide solution or its CyD complex in buffer phosphate (pH 5.8). All the prepared solutions were filtered and filled into 10 ml colourless glass ampoules which were then sealed. The ampoules were exposed to direct sunlight. The furosemide content of the different solutions was determined before and after exposure to light for different time intervals.

3.2.5. Dissolution studies

The dissolution rate of 40 mg of furosemide or its equivalent weight of furosemide-CyD complexes or physical mixtures, all of the same particle size contained in gelatin capsules was determined using the USP rotating basket method at 37 °C at 50 r.p.m. Phosphate buffer of pH 5.8 (900 ml) was used as the dissolution medium. At different time intervals for a period of 60 min, a 5 ml aliquot portion of the dissolution medium was withdrawn, filtered through a Millipore filter (0.45 μm pore size) and replaced by an equal volume of fresh dissolution medium. The drug was determined spectrophotometrically at 276 nm.

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