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HPLC and solubility study of the interaction between pindolol and cyclodextrins

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Abstract

The complexation with β-cyclodextrin (β-CD) has been investigated using reversed-phase liquid chromatography. The compounds tested have been pindolol and, for comparison purposes, indole and 4-methoxyindole. The retention behaviour has been analysed on a Kromasil 100 C18 column and the mobile phase used was methanol–pH 6 phosphate buffer (15/85 v/v) in which β-CD was incorporated as a mobile phase additive. The decrease in the retention times with increasing concentrations of β-CD enables the determination of the apparent stability constants of the complexes. In addition, the low solubility of pindolol, a weak base, in pH 12 aqueous solution has been improved by complexation with different cyclodextrins. The solubility enhancements with 1.4×10^{-2} M β-, hydroxypropyl-β, and γ-CD have been 1.9, 1.8 and 1.4-fold, respectively, with 2.4×10^{-2} M methyl-β-CD it was 2.8-fold whilst no effect was observed with α-CD. The stability constants of the complexes at pH 12 have been determined from the solubility isotherms.

Keywords: Pindolol; Cyclodextrin; HPLC; Complex formation; Solubility isotherm

1. Introduction

Cyclodextrins (CDs) are torus shaped cyclic oligosaccharides made up of α-(1,4) linked glucose units, the most common cyclodextrins are α, β and γ-cyclodextrin, which contain six, seven and eight glucose units, respectively. The non-polar cavity of CDs can form inclusion compounds with a variety of guest molecules, the binding is governed by the molecular polarity and ability to closely fit within the cavity [1]. The formation of these inclusion compounds has been widely used to improve the solubility, bioavailability and stability of pharmaceuticals [2].

The role of CDs in a HPLC system is based on their different extent of complexation depending on the structural features of the guests, this fact leads to differences in the retention behaviour which can improve the selectivity of the chromatographic method. Cyclodextrins have been employed in separating closely related compounds such as structural isomers [3,4] and, due to their chiral nature, they have also been used in enantioseparations, as they offer versatile chiral recognition features [5,6].

There are two approaches for applying CDs in reversed-phase HPLC, either CDs can be in the stationary phase chemically bonded to silica gel, or they can be used as mobile phase additives. As a result of host–guest interactions, the retention time of the guest will change, it will be shorter when complexation occurs in the mobile phase and longer when it takes place in the stationary phase. These changes in the retention behaviour are closely related to the stability constants of the complexes formed. The determination of apparent stability constants of different inclusion compounds has been previously reported [7–10].

The aims of this work were to study the HPLC retention behaviour of pindolol and other indole derivatives in the presence of β-cyclodextrin and to estimate the stability constants

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of the respective inclusion compounds from the influence of the cyclodextrin concentration on their capacity factors. The suitability of the proposed HPLC method for the calculation of the stability constants has been evaluated by comparing the values determined by HPLC with literature data obtained using other methods such as fluorescence spectroscopy and spectrophotometry [11,12,13].

The compounds selected for this investigation were pindolol, 4-methoxyindole, and indole. Pindolol [1-(1H-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol] (PIN) is a non-selective β-adrenergic antagonist used for the clinical treatment of angina pectoris and hypertension [14], the other compounds were studied for comparison purposes. It has been previously reported the use of different cyclodextrins in eye drop formulations of pindolol and mepindolol sulfate, which improved the permeation rates of the drugs through isolated porcine cornea [13].

Finally, taking into consideration the low aqueous solubility of pindolol, specially in alkaline media, as it is a weak base with $pK_a = 9.4$ [15], an attempt has been made to increase it by means of complexation with different cyclodextrins. The assays have been carried out in pH 12 aqueous solution because it is well known that complex formation is usually favoured when the guest is in a non-ionised state.

2. Experimental

2.1. Reagents and chemicals

Pindolol, 4-methoxyindole and indole were purchased from Sigma–Aldrich, β-cyclodextrin (β-CD) was purchased from Roquette, methyl-β-cyclodextrin (Mβ-CD) (DS: 12–13 methyl groups/CD ring) from Cyclolab and α-cyclodextrin (α-CD) as well as γ-cyclodextrin (γ-CD) from Wacker.

HPLC-grade methanol (MeOH) and triethylamine (TEA) were obtained from Scharlau. Potassium dihydrogen phosphate and disodium hydrogen phosphate (anhydrous) were from Panreac. Sodium nitrate was acquired from Aldrich. All water used was deionised at 18 MΩ using a Wasserlab ultrapure water system. The buffer solution of pH 12 contained 0.012 M NaOH and 0.05 M KCl.

2.2. Apparatus and chromatographic conditions

The chromatographic experiments were performed using a Waters 600E Controller pump, a Waters 717 plus autosampler and a Waters (Mildford, USA) 996 PDA detector (detection: 264 nm for pindolol and 4-methoxyindole, 270 nm for indole) and the Millenium 32 as software. The column employed was a Kromasil 100 C18, 5 μm, 15 cm × 0.46 cm (Scharlau, Barcelona, Spain). The mobile phase used for these studies was methanol–pH 6 phosphate buffer (15/85 v/v), the phosphate buffer contained 3.71×10^{-2} M KH_2PO_4 and 4.29×10^{-3} M Na_2HPO_4. An amount of TEA was added to obtain a 10 mM concentration in the mobile phase; 7.62 g/L of β-CD were then dissolved in the mobile phase. The final apparent pH measured was 7.0. It was freshly prepared, filtered through a 0.20 μm pore size Albet nylon membrane filter and degassed using helium sparging prior to use and continuously while in operation. The mobile phase was pumped at a flow rate of 0.8 mL/min. All the chromatographic experiments were carried out at room temperature, which was fixed at 18 ± 1 °C using an air conditioning system. The dead time (t_o) was obtained using sodium nitrate. The concentration of the solutes indole, 4-methoxyindole and pindolol in the solutions injected was 8.05×10^{-5} M and the volume of injection was 100 μL in all the experiments.

2.3. Determination of the apparent stability constants by HPLC

The retention behaviour of the solutes in RP-HPLC is governed by their partition coefficients between the mobile and stationary phases. In the presence of cyclodextrins there is an additional contribution which is the complexation process.

The capacity factors for each indole derivative were monitored in the presence of increasing concentrations of β-CD. The apparent formation constants, K_{11}, of the complexes were determined in triplicate using the following expression [16]:

$$\frac{1}{k'} = \frac{1}{k'_s} + \frac{K_{11}\,[\beta\text{-CD}]}{k'_s}$$

where k' is the capacity factor at each cyclodextrin concentration, [β-CD], and k'_s is the capacity factor of the solute in absence of cyclodextrin. For a complex with a 1:1 stoichiometry, a plot of $1/k$ versus [β-CD] yields a straight line and K_{11} is obtained from the slope to intercept ratio.

2.4. Solubility studies

As the drug is a weak base, the solubility assays were carried out in pH 12 phosphate buffer solutions at 25 °C. The cyclodextrins used were α-, β-, Mβ-, HPβ- and γ-CD. Excess amounts of PIN were added to 25 mL glass tubes containing different concentrations of CD. The tubes were placed in a water bath at a constant temperature and shacked until equilibrium was reached (24 h), then, the solutions were filtered (0.8 μm pore) and the concentration of PIN was spectrophotometrically determined at 264 nm using a HP8452A diode-array spectrophotometer. The presence of CDs did not interfere the spectrophotometric assay of pindolol.

When a linear relationship between the solubility of PIN and the concentration of CD is obtained, the diagram is classified as A_L according to Higuchi and Connors [17] and the experimental data fit the following equation:

$$S_t = S_0 + \frac{K_{1:1} S_0\,[\text{CD}]}{1 + K_{1:1} S_0}$$

where S_0 is the molar solubility of PIN and S_t is the molar solubility of PIN in presence of cyclodextrin. The apparent

stability constant of the complex formed, $K_{1:1}$, can be obtained from the slope of the straight line.

3. Results and discussion

3.1. Chromatographic conditions

An investigation has been carried out in order to choose the most suitable conditions for the analysis. The effect of methanol concentration and flow rate are discussed.

Firstly, it has to be taken into consideration that complexation with cyclodextrins is usually studied in water, in absence of organic solvents, because the presence of organic solvents could interfere the host–guest interaction, as it is discussed later.

The chromatographic experiments were carried out using a Kromasil C18 stationary phase. The use of that non-polar stationary phase did not allow the determination of the apparent formation constants using water alone as mobile phase, as it would involve very long retention times with the associated experimental error, therefore, the use of an amount of an organic modifier in the mobile phase was required. This fact led us to determine the formation constants of the complexes in the presence of methanol. Methanol was the organic component of the mobile phase, whereas a pH 6 phosphate buffer was the aqueous component of the eluent. There were two reasons to use phosphate buffer; first, under such conditions the mobile phase presents a negligible absorbance at the range of wavelengths where the measurements are taken (260–280 nm) [18] and it is possible to adjust the pH at 7.0 by adding TEA.

As an example, the capacity factors of pindolol at different MeOH concentrations are shown in Fig. 1, with the abscise axis representing the volume fraction of MeOH in the mobile phase. As it was expected, it is observed that an increase in MeOH concentration led to a decrease in the retention time for pindolol, owing to the weaker adsorption of solute molecules on the stationary phase as a result of solvation phenomena [7].

The effect of the mobile phase flow rate on peak shape was examined for a mobile phase consisting of methanol–phosphate buffer (25:75) (data not shown). A mobile phase flow rate of 0.8 mL/min was selected as a compromise between peak shape and time of analysis.

3.2. Chromatographic determination of the apparent association constants

When cyclodextrins are added to the mobile phase, solute retention is governed by its partition between the mobile and stationary phases and the solute complexation with cyclodextrins. With the solute retention time and the void time, the capacity factors were calculated for each solute in the presence of increasing concentrations of β-CD. As expected, the retention times decrease as the concentration of β-CD in the mobile phase increases, due to the formation of the analyte–cyclodextrin complex, which enhances the guest solubility in the mobile phase and reduces its residency time in the column; an assay is shown as an example in Fig. 2. The variation coefficient in the retention times by replication has always been lower than 4%.

The formation constants between the above mentioned indole derivatives and β-CD have been calculated. The linear relationship between $1/k'$ and β-CD concentration (Fig. 3), with correlation coefficients higher than 0.99, indicates that the behaviour of these compounds is well described by the model assuming 1:1 stoichiometry between the guest and β-CD [19].

The apparent stability constants obtained are shown in Table 1; the results are in good agreement with the litera-

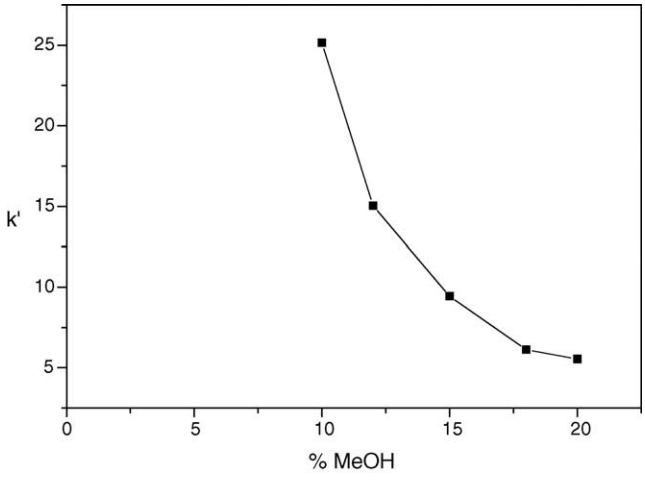

Fig. 1. Capacity factor of pindolol plotted vs. methanol volume fraction in the mobile phase. Chromatographic conditions – column: Kromasil 100 C18, 5 μm, 15 cm × 0.46 cm ID, mobile phase: methanol–pH 6 phosphate buffer, flow rate: 0.8 mL/min.

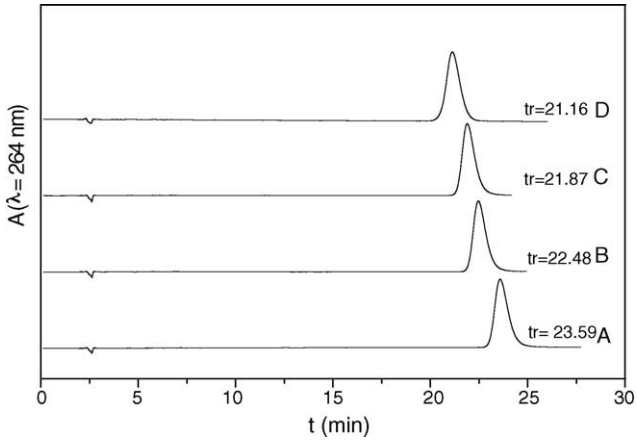

Fig. 2. Decrease in the retention time of pindolol in the presence of increasing concentrations of β-CD in the mobile phase (A = 0, B = 1.2, C = 2.4 and D = 3.6 mM) at 18 °C. Chromatographic conditions – column: Kromasil 100 C18, 5 μm, 15 cm × 0.46 cm ID; mobile phase: methanol–pH 6 phosphate buffer (15/85 v/v) containing 10 mM TEA.

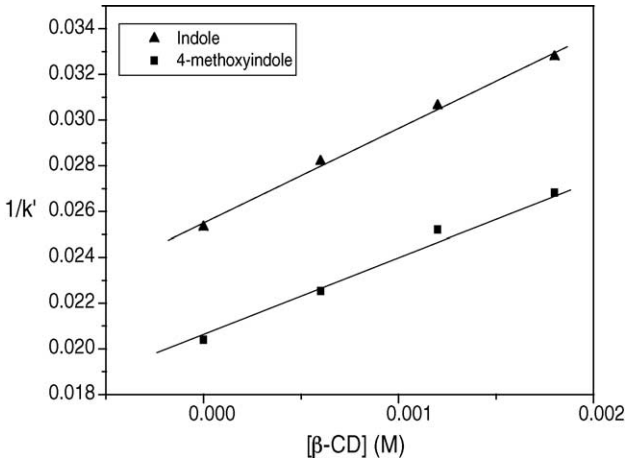

Fig. 3. Plot of $1/k'$ vs. β-CD concentration for indole and 4-methoxyindole. Chromatographic conditions as in Fig. 2.

ture data. These values are slightly lower than those determined by fluorimetric and spectrophotometric measurements which were carried out in an aqueous medium with no organic solvent. For example, Örstan and Ross [11] reported the determination of the apparent stability constant for the indole-β-CD complex at 25 °C using fluorimetric and spectrophotometric measurements, the values obtained were 184 and 196 M^{-1}, respectively. In relation to the complexation of 4-methoxyindole and pindolol with β-CD, our previous investigations using fluorescence spectroscopy led to apparent stability constants of 164 M^{-1} for the 4-methoxyindole-β-CD complex and 73 M^{-1} for the complex with pindolol, both calculated in water at 20 °C [12].

An explanation for the differences detected could be the amount of methanol present in the mobile phase, as well as small differences in the temperature of operation. It is well known that the addition of methanol may have a negative effect on complex formation with β-CD. For these systems studied, it seems that the addition of methanol led to a decrease in the apparent binding constants. There are several factors which may contribute to this decrease, firstly, the amount of methanol present results in a less polar mobile phase in which the non-polar solutes become more soluble, as a consequence, the solute affinity for the hydrophobic cavity of β-CD diminishes and part of the driving force for inclusion is removed. Secondly, a phenomenon of competence between the solute and the alcohol for binding β-CD may take place, even though methanol binds weakly to β-CD, being its association constant 0.32 M^{-1} [20].

As can be observed in Table 1, the apparent stability constants of the β-CD complexes with indole and 4-methoxyindole are similar, but they are clearly higher than that of pindolol The inclusion complex formation is related to the hydrophobic character of the guest molecule; so at pH 7, the protonation of the amine group of pindolol, with pK_a 9.4, probably has a negative influence in the inclusion within the β-CD non-polar cavity, resulting in a lower apparent stability constant. Besides the hydrophobic nature, the values obtained point out that the substitution of the indole ring may play an important role in the complexation, being the indole and 4-methoxyindole complexes more stable than the complex formed with pindolol. Probably, the presence of a bulky substituent in the indole ring may induce steric hindrance during the inclusion process.

3.3. Solubility studies

The formation of inclusion complexes between PIN and the CDs studied involves solubility enhancements in all the cases except for that of α-CD. The increase of the solubility of PIN in the presence of 1.4×10^{-2} M β-, HPβ- and γ-CD was 1.9, 1.8 and 1.4-fold, respectively; in the presence of 2.4×10^{-2} M Mβ-CD it was 2.8-fold. The solubility enhancements obtained with cyclodextrins have been widely employed for improving the bioavailability of drugs [2,21].

The solubility diagrams have been constructed by plotting the solubility of PIN as a function of the CD concentration. As it is shown in Fig. 4, the diagrams obtained present a linear

Table 1
Apparent formation constants, K_{11}, for solute/β-CD inclusion complexes in water at 18 °C determined by HPLC

Compound	Correlation coefficient, r	Slope, m	Intercept, b ($\times 10^3$)	Formation constant, K_{11} (M^{-1})	Bibliography data, K_{11} (M^{-1})
Indole	0.997	4.3 ± 0.2	25.4 ± 0.2	170 ± 12	184 (25 °C) [11]
4-Methoxyindole	0.994	3.3 ± 0.2	20.6 ± 0.3	163 ± 3	164 (20 °C) [12]
Pindolol	0.998	5.8 ± 0.1	113.0 ± 0.4	51 ± 2	73 (20 °C) [12]

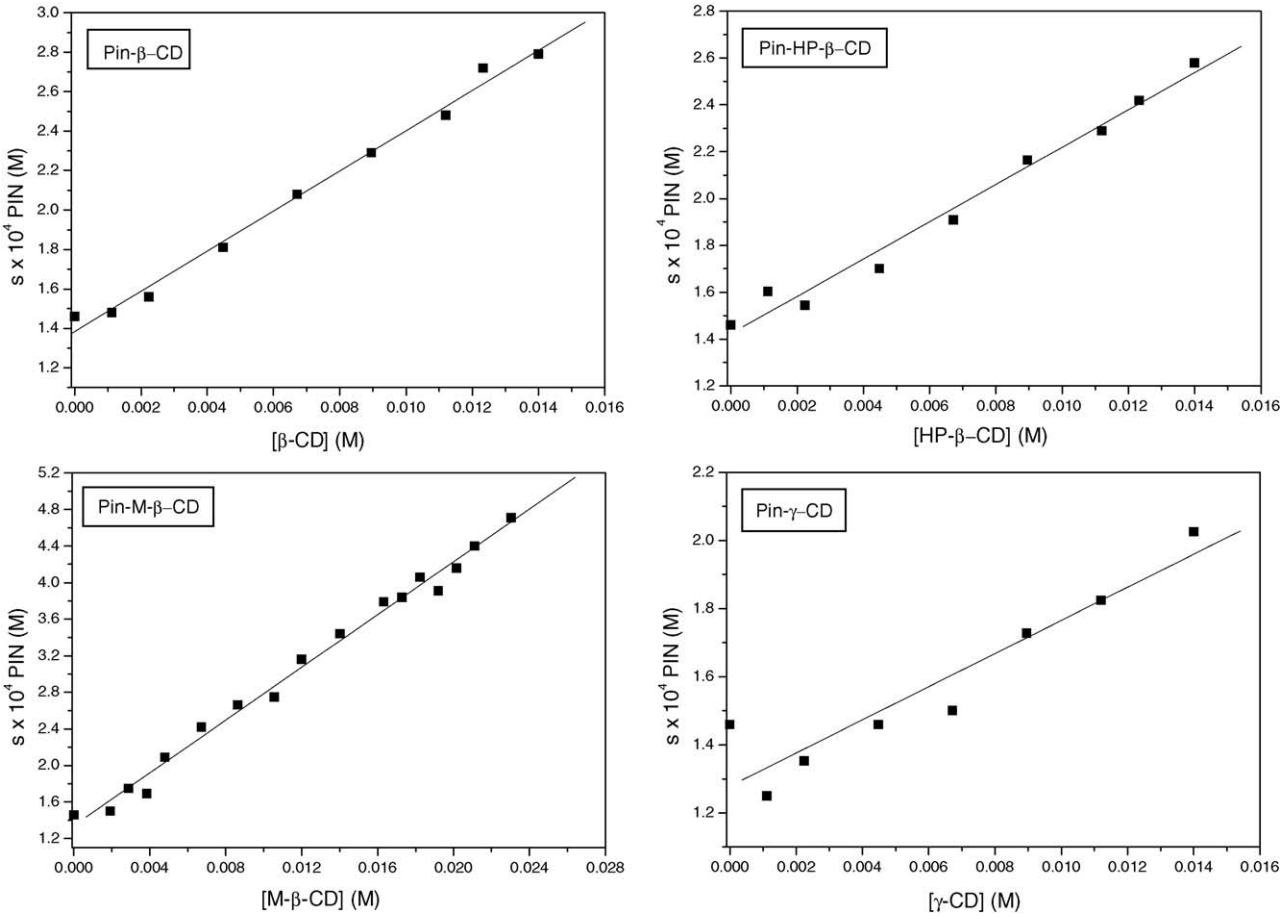

Fig. 4. Solubility diagrams of pindolol with β-, Mβ-, HPβ- and γ-CD in pH 12 aqueous solution at 25 °C.

increase of solubility with the concentration of CD so they can be classified as A_L type [17]. The apparent stability constants ($K_{1:1}$) determined from the slope and the intercept of these plots are summarised in Table 2. The differences in the results obtained with α-, β- and γ-CD reveal the importance of the cavity size to get an adequate fitting between the host and guest molecules. On one hand, it seems that the cavity of α-CD is too small and complexation does not take place and, on the other hand, the large cavity of γ-CD gives rise to a loose fitting of the drug, therefore, the cavity of β-CD appears to reach the best fitting for PIN.

The values of K_{11} obtained with β- and Mβ-CD are in reasonable agreement with the constants reported previously (97 and 183 M^{-1}, respectively) using a fluorimetric method [22]. It is not unusual to find discrepancies in the stability constants obtained using different techniques [23]. In this case, it has to be taken into account that the phase solubility assays are carried out in saturated solutions whilst the fluorimetric method employs diluted solutions. In saturation conditions, the formation of aggregates or micelles might take place [24], as cyclodextrins are known to self-associate [25] and, under such conditions, non-ideality phenomena might arise as well.

Finally, the comparison of the values for the constants of the complex PIN-βCD obtained by HPLC (pH 7) and by solubility studies (pH 12) evidences the negative effect of the ionisation state of the drug on the complexation, due to the hydrophobic nature of the interaction.

Table 2
Stability constants of the complexes of PIN with different cyclodextrins in pH 12 aqueous solution at 25 °C determined by phase solubility techniques

	β-CD	HP-β-CD	M-β-CD	γ-CD
m ($\times 10^3$)	10.2 ± 0.4	8.0 ± 0.4	14.4 ± 0.4	4.9 ± 0.7
b ($\times 10^5$)	13.8 ± 0.3	14.2 ± 0.3	13.4 ± 0.5	12.9 ± 0.6
R	0.99	0.99	0.99	0.94
K_{11} (M^{-1})	74 ± 5	57 ± 3	109 ± 12	38 ± 7

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