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A fluorimetric study of pindolol and its complexes with cyclodextrins

Carmen Gazpio, Miguel Sánchez *, Arantza Zornoza, Carmen Martín, Cristina Martínez-Ohárriz, Itziar Vélaz

Departamento de Química y Edafología (Sección de Química-Física), Facultad de Ciencias, Universidad de Navarra, 31080 Pamplona, Spain

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Abstract

Spectrofluorimetric characteristics of pindolol have been investigated with the aim of using this technique for analytical determinations. Other monosubstituted indole derivatives, 4-methoxy and 5-methoxyindole, have been also studied for comparative purposes. Corrected excitation and emission wavelengths in different solvents are reported and the effect of solvent on the Stokes shifts of these compounds has been analysed using the Lippert equation. In addition, the Stokes shift of pindolol has been determined in dioxan–water solvent mixtures and the presence of specific solvent effects is discussed. The fluorescence of pindolol is pH dependent, the quantum yields determined in water are lower than those in other solvents. With respect to the sensitivity, it has been found that the detection limits in aqueous solutions are improved in the presence of β and methyl-β-cyclodextrin. Finally, a fluorimetric analysis of the interaction between pindolol and different cyclodextrins has been carried out in order to determine the apparent stability constants of the complexes and the thermodynamic parameters associated to complexation.

Keywords: Pindolol; Cyclodextrin; Fluorescence quantum yield

1. Introduction

Pindolol [1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol] (Fig. 1) is a noncardioselective beta-blocker commonly used in the treatment of cardiovascular disorders. This drug enhances the clinical effects of antidepressant drugs [1].

α-, β- and γ-Cyclodextrins (CD) are cyclic oligosaccharides having six, seven or eight glucopyranose units, respectively. They are toruslike macrorings which have a nonpolar cavity and two hydrophilic rims. Cyclodextrins can form inclusion complexes which usually lead to an improvement in some of the characteristics of the guest molecule such as solubility, dissolution rate, bioavailability and stability [2].

Recent investigations have been carried out on the complexation of pindolol with CDs with the

* Corresponding author. Fax: +34-48-425649.
E-mail address: misango@unav.es (M. Sánchez).

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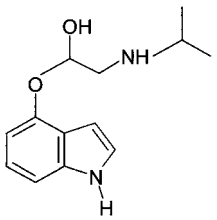

Fig. 1. Molecular structure of pindolol.

aim of improving the in vitro transcorneal permeability of the drug in eye drop formulations [3].

This work presents a study of the spectrofluorimetric characteristics of pindolol in different solvents, with the aim of using this technique for analytical determinations. 4-Methoxy and 5-methoxyindole have been also analysed for comparative purposes. In addition, a fluorimetric analysis of the interaction between pindolol and different CDs has been carried out in order to determine the apparent stability constants of the complexes and the thermodynamic parameters associated to complexation, as well as the fluorimetric detection limits of the complexes formed.

2. Experimental

2.1. Reagents

Pindolol, 4-, 5-methoxyindole, propranolol hydrochloride and 1-methoxynaphtalene were obtained from Sigma–Aldrich. β-CD was purchased from Roquette, M-β-CD (DS: ~12–13 methyl groups/CD ring) from Cyclolab and α-CD as well as γ-CD from Wacker. Buffer of pH 12.1 was prepared with NaOH and KCl solutions. Aqueous solutions were made with distilled water.

2.2. Apparatus

pH measurements were carried out with a micropH 2002 of CRISON equipped with glass and Ag/AgCl reference electrode. Absorption spectra were recorded using a single beam HP8452A Diode-Array UV–Visible spectrophotometer. Fluorescence measurements were performed using a LS50 Perkin–Elmer spectrofluorimeter equipped with a Xenon lamp, using 1.00 cm quartz cells and slit widths of 4 nm. The cell housing was thermostatised with ±0.4 °C precision.

3. Results and discussion

3.1. Spectrofluorimetric study

3.1.1. Solvent effects

The Stokes shifts of pindolol and other indole derivatives in different solvents have been analysed by the application of Lippert equation, Δv was plotted against the solvent parameter Δf where $\Delta\nu$ (cm^{-1}) is the difference in wavenumber between the absorption and emission maxima and

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}.$$

Pindolol, 4-methoxy and 5-methoxyindole exhibit solvent dependent fluorescence characteristics. The position of the absorption and emission bands for the 4-substituted indoles are similar whereas 5-methoxyindole presents longer wavelengths. In these compounds, the emission wavelengths present a bathochromic shift as solvent polarity increases (Table 1). Lippert plots are shown in Fig. 2, the positive slopes may indicate an increase of the dipolar moments upon excitation and there are marked deviations from linearity in methanol and water which are associated to specific solvent effects. These deviations have been explained on the basis of the fluorescence of the indole parent chromophore. The fluorescence red shift in alcohols could be due to a charge transfer type of interaction between excited indole and solvent molecule, which may give rise to a stabilisation of the emitting state. The larger red shift observed in water might be associated to an excited state proton transfer [4].

The fluorescence of pindolol has been studied in dioxan–water mixtures (Fig. 3) as these mixtures have been frequently used as models to study the behaviour of molecular probes in membranes [5]. It has been found that dielectric enrichment has a slight influence on Stokes shift and specific solvent effects appear for water molar fractions above 0.8.

Table 1
Absorption and emission wavelengths of pindolol, 4-methoxyindole and 5-methoxyindole in different solvents

Solvent	Pindolol		4-Methoxyindole		5-Methoxyindole	
	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)
Water	286.0	319.0	286.0	316.2	–	–
Acetonitrile	287.5	303.8	286.0	304.1	306.0	327.6
Methanol	285.3	312.2	286.5	313.0	–	–
Dichloromethane	287.0	305.0	286.0	305.0	306.0	327.9
Cyclohexane	287.0	302.5	287.0	301.8	–	–
p-Dioxan	287.0	304.0	286.5	304.5	308.0	326.2
Hexane	287.0	302.2	286.0	300.9	305.5	321.8

3.1.2. Fluorescence quantum yields

The fluorescence quantum yields at 25 °C were determined by comparison of the corrected emission spectra with the spectrum of a fluorescence standard (quinine bisulphate in 0.1 N sulphuric acid) using optically diluted solutions [6].

Fluorescence quantum yields are summarised in Table 2. It can be observed that 5-methoxyindole is more fluorescent than pindolol and 4-methoxyindole, this fact could be attributed to a higher electronic delocalization through the π-system. A lower quantum yield in water has been observed in all these compounds, the value obtained for 5-methoxyindole is coincident with data previously reported [7]. The fluorescence of these indole derivatives is quenched in dichloromethane; it could be due to the donation of an electron from the fluorophore to the solvent.

Similar quantum yields are obtained for pindolol and 4-methoxyindole, so it seems that the chain present in the drug does not have a significant effect on the emission process. This fact has been confirmed by comparing the fluorescence quantum yields of another pair of compounds, 1-methox-

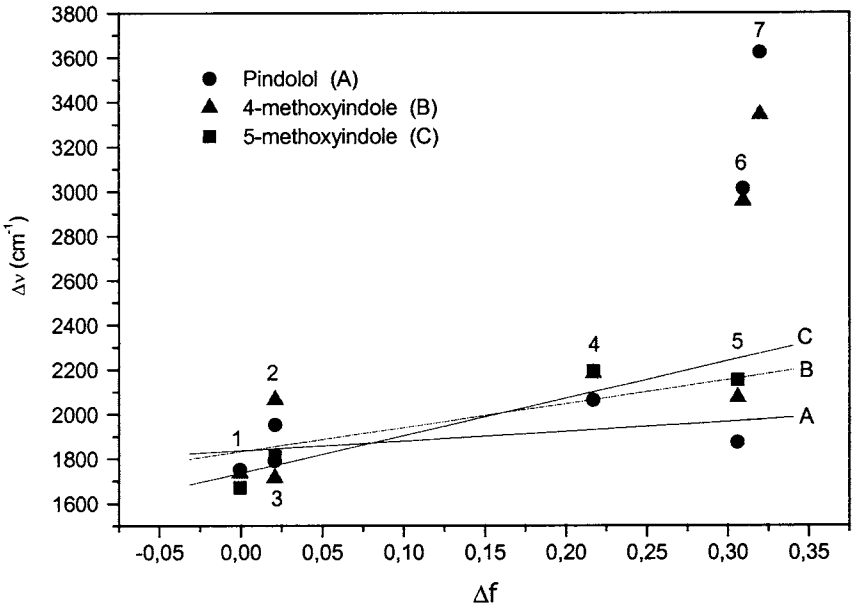

Fig. 2. Plot of $\Delta\nu$ (cm^{-1}) vs. Δf. (1) *n*-hexane; (2) cyclohexane; (3) *p*-dioxan; (4) dichloromethane; (5) acetonitrile; (6) methanol; (7) water.

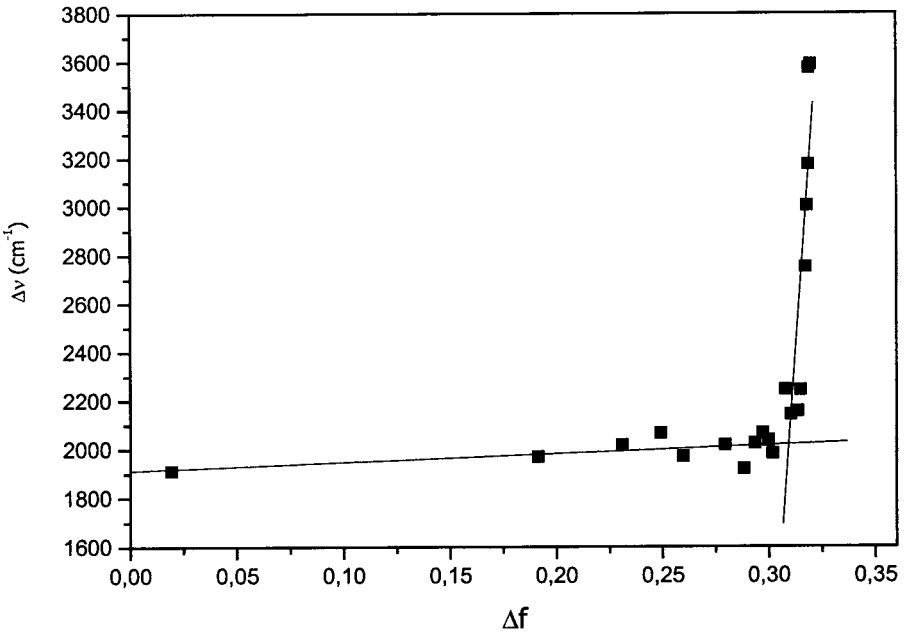

Fig. 3. Plot of Δv (cm^{-1}) vs. Δf for pindolol in dioxan–water mixtures.

Table 2
Fluorescence quantum yields (ϕ) of pindolol, 4-methoxyindole and 5-methoxyindole in different solvents at 25 °C

Solvent	ϕ Pindolol	ϕ 4-Methoxyindole	ϕ 5-Methoxyindole
Water	0.013±0.003	0.010±0.001	0.27±0.02
Acetonitrilie	0.17±0.02	0.16±0.01	0.58±0.01
Methanol	0.16±0.02	0.23±0.08	0.61±0.01
Ethanol	0.23±0.01	–	0.36±0.02
Dichlorometane	0.027±0.003	0.027±0.01	–

ynaphtalene and propranolol hydrochloride, a beta-blocker which has a similar chain bound to a naphthalene ring. It has been found that both values are comparable as well.

Finally, an increase of the quantum yields of pindolol and 4-methoxyindole in water (pH 6.5) has been found in presence of β-CD, the values obtained were 0.032±0.004 and 0.025±0.002, respectively.

3.1.3. Sensitivity and detection limits

Sensitivity and detection limits of pindolol in different solvents have been calculated by measuring the fluorescence of pindolol at different concentrations within its linear dynamic range (0.3–3 μg ml^{-1}). The detection limits have been determined according to the equation $C_L = Ks_{bl}/S$, where s_{bl} is the standard deviation of the measurements of solvent fluorescence intensity, S is the sensitivity calculated from the slope of the plot fluorescence intensity versus concentration and K is a numeric factor ($K = 3$) chosen according to a 90% confidence level [8].

The detection limits of pindolol are compiled in Table 3. The sensitivity is lower in water and it is dependent on pH, pindolol is less fluorescent in alkaline solutions. The detection limits in aqueous solutions can be improved by complexation with CDs; this can be applied for analytical determinations of the drug.

3.2. Complexation with cyclodextrins

The interactions of pindolol with β-CD and M-β-CD were studied at pH 12.1 by fluorescence measurements. For determining the stability constants of the complexes, the concentration of pindolol was kept constant at 1.21×10^{-5} M

Table 3
Detection limits of pindolol in different media

Solvent	λ_{exc} (nm)	λ_{em} (nm)	Detection limit (ng ml^{-1})
Ethanol	266	303	1.3
Acetonitrile	264	305	4.1
Water (pH 6.5)	264	319	36.7
Water–β-CD (pH 6.5)	264	319	23.9
Aqueous solution (pH 12.1)	264	319	38.7
Aqueous solution β-CD (pH 12.1)	264	319	18.6

while the concentrations of the β-CDs varied from 0 to 2.6×10^{-3} M. The excitation and emission wavelengths were 264 and 319 nm, respectively.

The calculation of complex stability constants has been made following a fluorimetric method [9]. Assuming a 1:1 stoichiometry, the following equation was used:

$$1 - \frac{F_o}{F} = \frac{(a-1)[L]_t K_s}{1 + a[L]_t K_s}, \quad (1)$$

where F_o and F are the intensities in absence and in presence of the different CDs (ligand), respectively. $[L]_t$ is the CD concentration in the equilibrium and $a = \varepsilon_c \phi_c / \varepsilon_x \phi_x$, being ε and ϕ the molar absorptivities and fluorescence quantum yields of the complex and the substrate (pindolol), respectively.

In order to obtain linear plots, this equation becomes:

$$\frac{[L]_t}{[1 - (F_o/F)]} = \frac{1}{(a-1)K_s} + \frac{a}{(a-1)}[L]_t. \quad (2)$$

The stability constants can be calculated from the intercept and the slope of the straight lines obtained by plotting the first member of Eq. (2) against $[L]_t$.

The thermodynamic parameters of the inclusion process were determined from the temperature

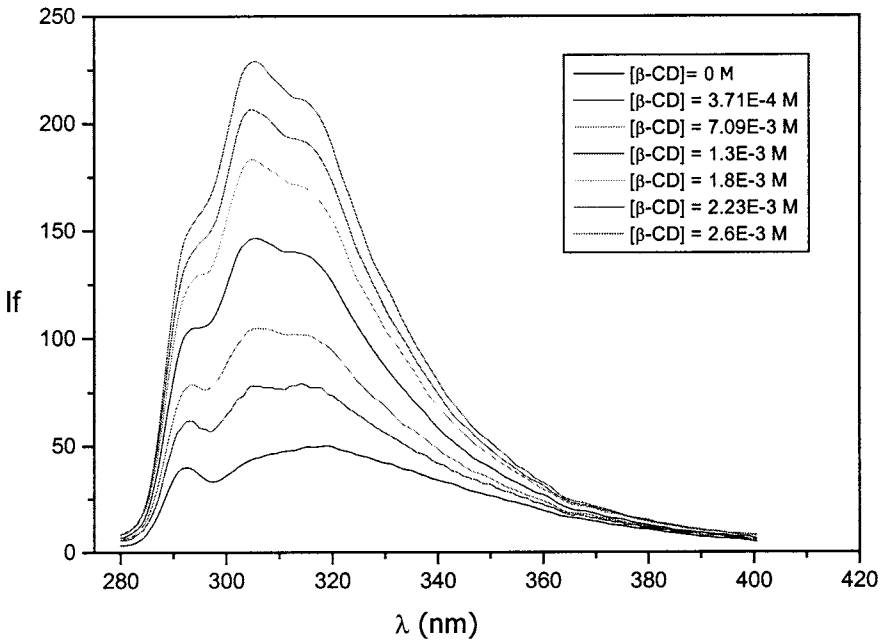

Fig. 4. Emission enhancement of pindolol in pH 12.1 aqueous solution in the presence of increasing concentrations of β-CD at 25 °C ([pindolol] = 1.21×10^{-5} M).

Table 4
Stability constants and thermodynamic parameters of complexation

	K (M^{-1})				ΔH° (kJ mol^{-1})	ΔS° (J K^{-1} mol^{-1})
	17 °C	20 °C	25 °C	30 °C		
β-CD	118	109	97	84	−19.1±0.7	−26.4±2.5
M-β-CD	196	188	183	173	−6.6±0.5	21.0±1.7

dependence of the association constant by using the Van't Hoff equation.

The interactions with CDs involve an increase of fluorescence in pindolol, a fourfold enhancement has been observed in the presence of β-CD, this increase is even higher with M-β-CD (tenfold) and HP-β-CD (sixfold). This fact is less marked with γ-CD and negligible with α-CD; this could be attributed to a less tight fitting of the drug within the cavity. In Fig. 4 it is shown, as an example, the effect of β-CD on the emission spectrum of pindolol.

The fluorescence enhancement with β-CD and M-β-CD allows to calculate the stability constants of the respective complexes at different temperatures (Table 4). Previously, we studied the complexation of this drug with different CDs in pH 5.5 aqueous solution [10]. The drug is a weak base (pK_a 9.46) [11] and it has been found that the apparent stability constants are higher in alkaline solutions, this fact evidences the importance of ionisation in the formation of these complexes. In pH 5.5 aqueous solution, pindolol is in an ionised state so it has lost part of its hydrophobicity, therefore hydrophobic interactions may play an important role in complexation.

In addition to the increased fluorescence, at pH 12.1 the emission wavelength of pindolol presents a slight hypsochromic shift in presence of increasing concentrations of β-CD. This is consistent with the blue shift that takes place in nonpolar solvents, as the formation of this type of complexes involves the inclusion of the drug in the relatively nonpolar cavity of the CD.

The thermodynamics of the complexation process is usually complicated [12,13], the higher negative ΔH value obtained with β-CD could be related to stronger hydrogen bonding with the hydroxyl groups and the positive entropy obtained with M-β-CD could be associated to hydrophobic interactions with the methyl substituents of the CD.

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