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Short communication

Analysis of the complexation of gemfibrozil with γ- and hydroxypropyl-γ-cyclodextrins

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ABSTRACT

The interactions of gemfibrozil with γ- and HP-γ-cyclodextrin (CD) have been studied in aqueous solution by fluorescence and NMR spectroscopy and by solubility measurements and in the solid state by X-ray diffraction, thermal analysis and FTIR spectroscopy. The influence of the technique employed in the analysis of complexation is discussed. The fluorescence of gemfibrozil increased in the presence of γ- and hydroxypropyl-γ-cyclodextrin (HP-γ-CD), especially with the later, because the inclusion of the aromatic ring in the cavity, evidenced by 1H NMR, has a protective effect on the excited state of the drug. The fluorescence enhancement allowed the determination of the binding constants at pH 2.8. Complexation was a both entropy and enthalpy driven process. The solubility diagrams obtained with γ-CD and HP-γ-CD were B_s and A_L type, respectively. The apparent stability constants calculated from the solubility data at 25 °C were compared with those obtained from the fluorescence assays. It was found that drug solubilization with γ-CD involves other contributions together with the inclusion phenomena. Solid complexes of gemfibrozil with γ-CD (and not with HP-γ-CD) have been obtained by kneading, coevaporation and coprecipitation methods. The solid complexes crystallised in the channel structure, in a process involving the carboxyl and aryl-ether groups.

1. Introduction

Gemfibrozil, 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a benzene derivative of valeric acid with lipophilic character and poor water solubility. It is a hypolipidemic agent which is effective in reducing serum cholesterol and triglyceride levels [1].

Gemfibrozil exhibits native fluorescence. This property has been used for the determination of the drug in plasma by HPLC with fluorescence detection [2] and to develop other spectrofluorimetric methods [3,4].

It is well known the ability of native cyclodextrins (CD) to form inclusion complexes, whose stability constants can be determined by different methods [5,6]. In general, the spectroscopic methods employ lower concentrations of the components than the solubility isotherms. The comparison of the stability constants obtained by both spectroscopic and solubility methods allows the separation of the total solubilizing effect of CDs from their ability to form true inclusion complexes with a guest [7].

It has been proved, using fluorescence, NMR and solubility methods, that the non-polar moiety of gemfibrozil can be housed into the hydrophobic cavity of β-CDs [4,8–10]. The volume and polarity of the γ-CD cavity are higher than those corresponding to β-CD [5,11] and these properties condition the value of the binding constants. Complexation with γ-CD may have advantages in comparison with β-CD, because the magnitude of the association constant can condition the release of the drug from a particular formulation [5]. The use of γ-CD to increase drug solubility presents additional benefits in relation to other natural CDs (α- and β-CD) because it is more soluble and exhibits the lowest toxicity. However, one of the drawbacks of using natural CDs is their tendency to form molecular aggregates by intermolecular hydrogen bonding in aqueous solution. This phenomenon causes turbidity and even precipitation of the solutes at high concentrations [12]. One way to avoid the opalescence of the solutions is the use of chemically modified CDs such as hydroxypropyl-γ-cyclodextrin (HP-γ-CD) [13]. HP-γ-CD is more soluble than γ-CD and its tendency to aggregate is much lower because the formation of hydrogen bonds between the hydroxyls is hindered.

There are also differences in the behaviour of natural and derivative CDs in the solid state. The precipitation of γ-CD results in crystals that exhibit a cage structure, but it has been reported the preparation of columnar crystals by rapid crystallisation [14] and also the formation of an amorphous solid upon desiccation [15]. On the contrary, in the case of HP-γ-CD, the etherification of the

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hydroxyl groups of γ-CD usually yields a mixture of different isomers that precipitate as amorphous solids [16].

The aim of the present work was to compare the performance of fluorescence spectroscopy and phase solubility techniques in the determination of the stoichiometry and the stability constants of the complexes of gemfibrozil with γ- and HP-γ-CD and also to study the supramolecular interaction by NMR spectroscopy. In addition, it was intended to evidence solid-state complexation in order to identify amorphous or crystalline structures.

2. Materials and methods

2.1. Materials

Gemfibrozil was purchased from Sigma. γ- and the randomly substituted HP-γ-CD were from Wacker Chemie GmbH. All other reagents and solvents were from Panreac.

2.2. Steady-state fluorescence

The measurements of fluorescence were performed using a LS50 PerkinElmer spectrofluorimeter.

Regarding that gemfibrozil is a weak acid (pK_a 4.7), the binding constants between the uncharged form of the acid and γ-CDs were calculated at pH 2.8.

The excitation wavelength used was 276 nm, the emission of fluorescence was obtained at 303 nm and both the excitation and emission slit widths were 2.5 nm. In each titration, the fluorophore concentration was held constant at 8.0×10^{-6} M and the CD concentration increased from 0 to 6×10^{-3} M.

In order to elucidate the stoichiometry of a fluorescent complex, the following equation [17], which assumes a 1:1 stoichiometry, can be used:

$$\frac{F_o}{F} = \frac{1 + K_{11}[\mathrm{CD}]}{1 + aK_{11}[\mathrm{CD}]} \quad (1)$$

where F_o represents the fluorescence intensity of free gemfibrozil, F is the intensity in the presence of CD, K_{11} is the binding constant and a parameter is defined by:

$$a = \frac{\phi_c \varepsilon_c}{\phi_g \varepsilon_g}, \quad (2)$$

ε and ϕ being the molar absorptivities and fluorescence quantum yields of the complex (c) and the free guest (g), respectively.

The fluorescence quantum yield of the complex was determined from the a values, after determining the quantum yield of the drug in aqueous solution. The fluorescence quantum yield of the drug at pH 2.8 was measured using optically diluted solutions, by comparison of the corrected emission spectra of gemfibrozil with that of quinine bisulphate in 0.1N sulphuric acid [18], the excitation wavelength employed was 260 nm.

Finally, it was possible to obtain the enthalpy (ΔH°) and entropy (ΔS°) of complexation from the temperature dependence of the binding constants by considering the van't Hoff equation.

2.3. Nuclear magnetic resonance spectroscopy

The ^{1}H NMR spectra of a set of solutions containing the same concentration of gemfibrozil (3.2×10^{-5} M) and different concentrations of CD ranging from 0 to 6×10^{-3} M at pH 2.8 have been obtained. A Brucker Avance 400 Ultra Shield TM 400 MHz ^{1}H NMR spectrometer has been employed. The measurements have been carried out at 295 K in D_2O containing HCl. The chemical shifts have been determined taking as reference the signal of the OH present in the solvent.

2.4. Phase solubility studies

The solubility experiments have been carried out in pH 2.8 aqueous solution by adding amounts of 20 mg of gemfibrozil to test tubes containing 20 mL of different concentrations of CD, ranging from 0 to 1.6×10^{-2} M, which were shaken in a bath at 25 °C until equilibrium was reached (14 h approximately). Samples were taken by filtration and measured at 216 nm using a HP8452A diode-array spectrophotometer. From the phase solubility diagrams obtained by plotting the solubility of gemfibrozil versus CD concentration, it is possible to estimate the apparent stability constant as well as the stoichiometry of the complexes [17]. The equation that describes linear diagrams, named A_L by Connors [17], is:

$$S_t = S_o + \frac{K_{1:1} S_o [CD]}{1 + K_{1:1} S_o} \quad (3)$$

where S_o is the solubility of pure gemfibrozil and S_t is the solubility in the presence of a CD concentration and $K_{1:1}$ is the apparent stability constant of the complex.

2.5. Solid state complexation

2.5.1. Preparation of solid systems

Different methods such as coevaporation (E), coprecipitation (CP) and kneading (KN) have been employed to prepare solid systems to assess complexation in the solid state by comparison with the corresponding physical mixtures (PM). All the systems contained 0.250 mmol of each component (1:1 drug–CD molar ratios). The coevaporated (E) systems were prepared by dissolving both gemfibrozil and CD in a methanol/water 70/30 solution at 55 °C, being the solvent subsequently eliminated by rotary evaporation at 85 °C under vacuum. The coprecipitated systems (CP) were the solid residues obtained from the solubility experiments which gave rise to B_s type isotherms at the end of the plateau region of the isotherm. The kneaded products (KP) were prepared by careful mix of drug and CD with a minimum volume of a 3:5 methanol:water solution. The paste obtained was kneaded for a period of time until it became more dense, then it was dried at 70 °C.

2.5.2. Characterisation of solid systems

The solid systems prepared were characterised using X-ray powder diffraction (Bruker axs D8 Advance diffractometer, Cu Kα, 40 kV, 30 mA), differential thermal analysis (DTA/TGA 851 Mettler Toledo) and FTIR spectroscopy (Nicolet FTIR spectrometer with KBr discs) by comparison with physical mixtures prepared in the same molar ratio.

3. Results and discussion

3.1. Steady-state fluorescence and NMR spectroscopy

The fluorescence of gemfibrozil in aqueous solution is enhanced in the presence of increasing concentrations of γ- and HP-γ-CDs, especially with the later. The supramolecular interaction between the guest and both CDs involves the inclusion of the phenoxy group of gemfibrozil inside the host central void, as has been evidenced by ^{1}H NMR spectroscopy. The aromatic region of the ^{1}H NMR spectra of gemfibrozil (3.2×10^{-5} M) in the presence of increasing concentrations of γ-CD are shown in Fig. 1. The signals of H6, H4 and H3 aromatic protons shift towards higher fields when increasing the concentration of CD, indicating the inclusion of the aromatic moiety in the cavity of γ-CD.

The fluorescence values of F_o/F versus CD concentration [CD] have been fitted to Eq. (1) at different temperatures ranging