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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

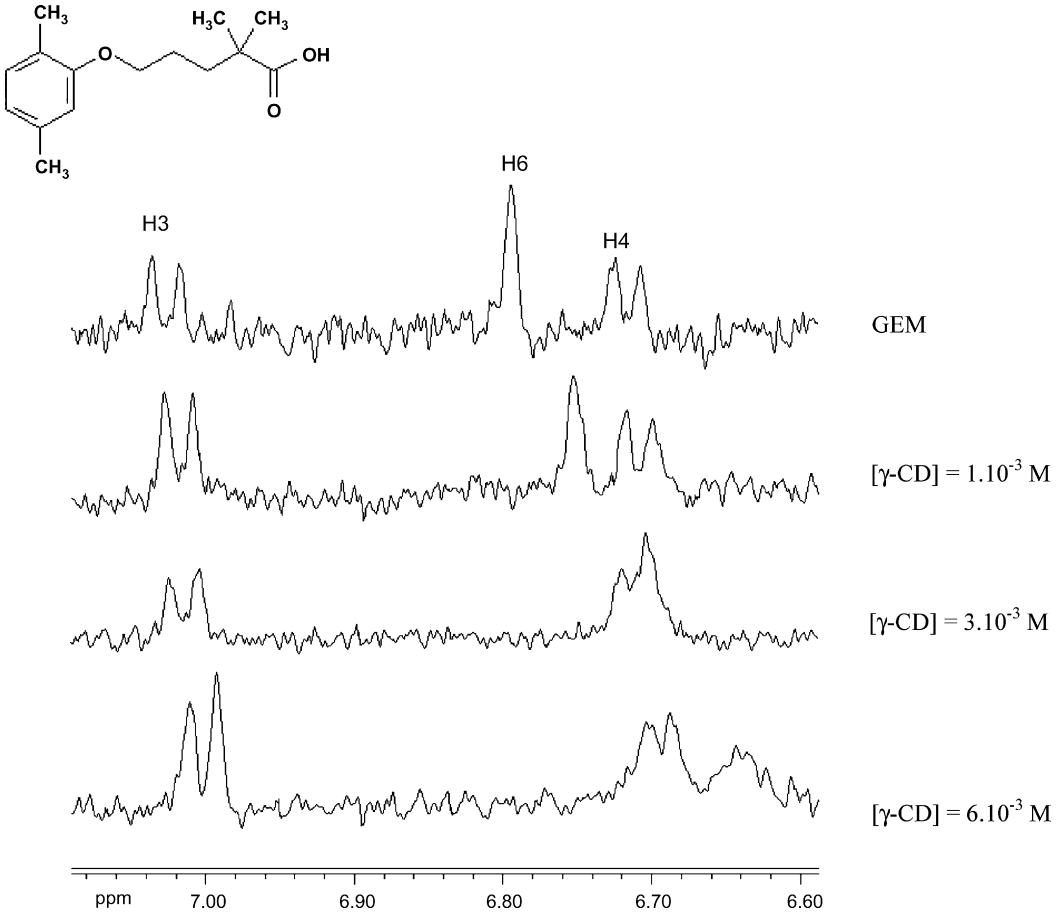

Fig. 1. Aromatic region of the ^{1}H NMR spectra of gemfibrozil in the presence of increasing concentrations of γ-CD in D_2O at pH 2.8.

from 15 to 45 °C, as shown in Fig. 2. The tight non-linear fittings to Eq. (1) are consistent with the assumed 1:1 stoichiometry.

The binding parameters obtained for the supramolecular interaction between gemfibrozil and γ-CDs are collected in Table 1. The higher binding constants of the complex with HP-γ-CD can be related with the cavity volume and rims polarity of the substituted oligomer. The values of the stability constants obtained with γ-CD are higher than those of the γ-CD complexes formed with benzene, toluene and ethylbenzene [19], probably due to a dependence of the stability constant on chain length in benzene derivatives. The complexation constants obtained with γ-CD are lower than those of the complexes with β-CD [4,8–10], probably due to its greater volume and polarity [11].

With respect to the *a* values, defined by the ratio $\phi_c\varepsilon_c/\phi_g\varepsilon_g$, it has been found that they increase at high temperatures (Table 1). It was previously observed that the band at the longest wavelength in the absorption spectrum of gemfibrozil does not change in the presence of increasing concentrations of γ- and HP-γ-CD. In addition, the fluorescence of gemfibrozil decreases markedly with temperature in an almost lineal way [3]. In consequence, from the dependence of the ratio ϕ_c/ϕ_g on the temperature, it is possible to infer a protective effect of the host molecule for the excited state of the fluorophore, taking into account that the values of the molar absorptivity of the

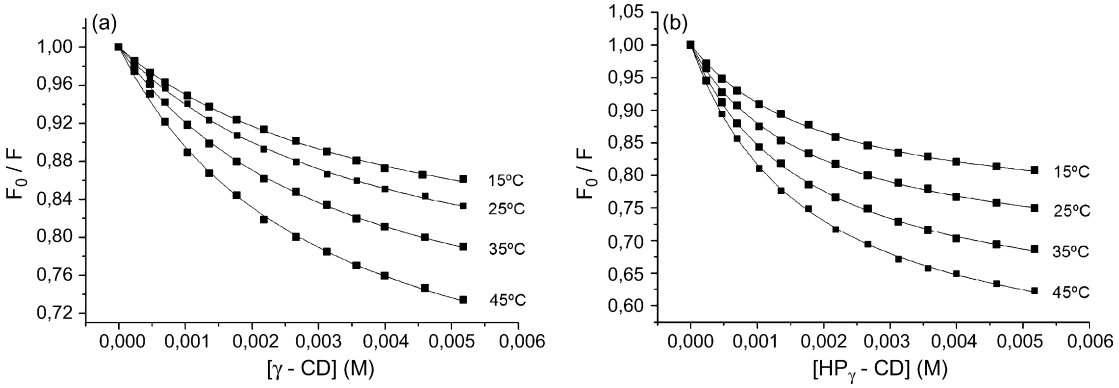

Fig. 2. Plots of the fluorescence data of gemfibrozil in the presence of γ-CD (a) and HP-γ-CD (b) at different temperatures.

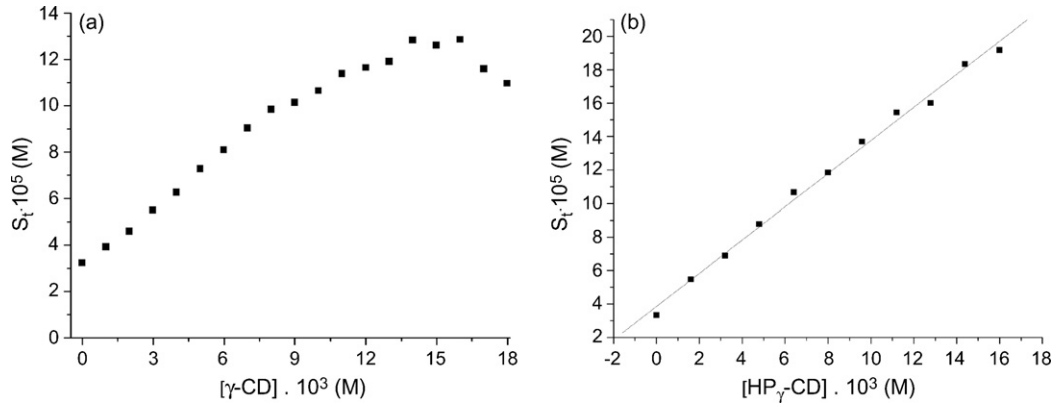

Fig. 3. Solubility isotherms of the complexes of gemfibrozil with γ-CD (a) and HP-γ-CD (b) at 25 °C.

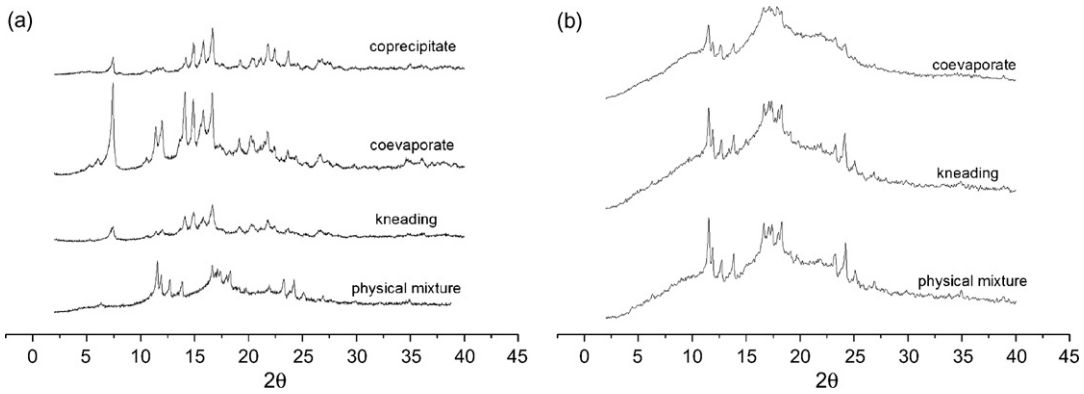

Fig. 4. X-ray diffraction patterns of the physical mixtures and kneaded and coevaporated systems of gemfibrozil with γ-CD (a) and HP-γ-CD (b) and the coprecipitated with γ-CD (a).

complex (ε_c) and the free guest (ε_g) are nearly the same at the excitation wavelength. The quantum yields of the complexes (ϕ_c) were calculated taking as reference the quantum yield of gemfibrozil determined experimentally ($\phi_g = 0.22 \pm 0.02$). The values of ϕ_c obtained for the complexes with γ- and HP-γ-CD at 25 °C were 0.30 and 0.32, respectively.

The enthalpy and entropy of the binding (Table 1) have been calculated from the temperature dependence of the association constant by using the van't Hoff equation. Good correlation coefficients ($r > 0.996$) were obtained in both cases. The positive entropies and negative enthalpies show that the inclusion complexation is a both entropy and enthalpy driven process, with near to a 30% of complex stabilisation coming from the entropy in both types of complexes. The increase of entropy is probably associated to hydrophobic interactions involving the release of water molecules from the CD cavity and the loss of structured water around the guest. The small differences obtained in the values of enthalpy and entropy for the complexes may indicate a very slight influence of the CD rims in the interaction of gemfibrozil with both CDs.

The slightly higher enthalpy value of HP-γ-CD can be explained in terms of the difference in volume and flexibility between both CDs [5]. The binding forces associated to the interaction of the non-polar aromatic moiety of gemfibrozil with the CD cavity should be quite similar in both cases. Therefore, it seems that the presence of a carboxylic group at the end of the aliphatic chain, which is capable of forming H-bonds with the CD rims, might contribute to the enthalpy of each system in a slightly different way, due to steric implications.

Table 1
Stability constants of the complexes of gemfibrozil with γ- and HP-γ-CD at different temperatures and pH 2.8 (mean values of 4 assays). Enthalpy (ΔH°) and entropy (ΔS°) of complexation

T (°C)	γ-CD		HP-γ-CD	
	$K_{11} \times 10^{-2}$ (M^{-1})	a	$K_{11} \times 10^{-2}$ (M^{-1})	a
15	2.0 ± 0.1	1.30 ± 0.02	3.7 ± 0.1	1.32 ± 0.01
25	1.8 ± 0.2	1.39 ± 0.02	3.2 ± 0.1	1.49 ± 0.04
35	1.6 ± 0.2	1.55 ± 0.05	2.8 ± 0.2	1.80 ± 0.04
45	1.4 ± 0.1	1.85 ± 0.03	2.5 ± 0.1	2.02 ± 0.01
ΔH° (kJ mol^{-1})	−9.3 ± 0.2		−9.8 ± 0.2	
ΔS° (J mol^{-1} K^{-1})	12 ± 1		15 ± 1	

3.2. Phase solubility diagrams

The diagrams of the isotherms representing the solubility of gemfibrozil in the presence of increasing concentrations of γ- and HP-γ-CD are depicted in Fig. 3. There are 2.3 and 3.1-fold enhancements in the solubility of gemfibrozil in the presence of 6×10^{-3} M γ- and HP-γ-CD, respectively. The intrinsic solubility of gemfibrozil was $(3.2 \pm 0.3) \times 10^{-5}$ M (mean of 10 assays).

An inclusion complex with limited solubility is formed with γ-CD and the isotherm can be classified as Bs type [17]. This type of diagram usually allows the determination of the stoichiometry of the complex from the plateau region. However, the turbidity and viscosity of the solutions containing concentrations of γ-CD higher than 9×10^{-3} M made difficult to assign the initial and final

points of the plateau [13]. The stability constant of the complex was determined from the regression analysis of the initial linear portion of the diagram, being $250 \pm 10\,M^{-1}$. In relation with the fluorimetric determination, the higher value arising from the solubility method can be related to a problem of self-aggregation of the γ-CD molecules in solution, which was manifested by the turbidity detected [12,13]. The aggregates formed are able to solubilize drugs by a micellar type-mechanism [7]. When there are non-inclusion mechanisms implicated in the solubility enhancement together with complexation, it is more accurate to use the parameter called complexation efficiency to quantify the solubilizing effect of CDs [7]. The complexation efficiency of γ-CD with gemfibrozil is 3.4×10^{-3}.

The solubility of gemfibrozil in the presence of HP-γ-CD increases in a linear way, so the diagram can be classified as A_L type. The stability constant arising from the solubility profile ($300 \pm 10\,M^{-1}$) agrees with that obtained by fluorescence spectroscopy ($320 \pm 10\,M^{-1}$ at 25 °C), indicating that the solubility technique is accurate to determine the stability constant of this complex, because derivative CDs have more difficulties to aggregate than natural CDs.

3.3. X-ray diffraction and thermal analysis

The PM exhibited the main reflections of gemfibrozil superposed with the amorphous profile of each CD (Fig. 4). However, the reflections of gemfibrozil are not present in the kneaded, coevaporated and coprecipitated systems with γ-CD; these systems showed the channel crystalline structure of the oligomer. This structure presents a characteristic reflection at $2\theta = 7.5°$, which differentiates the channel from the cage form [15]. There are other peaks such as those at $2\theta = 14.2°$, 14.9°, 15.8° and 16.7° that can also be assigned to the channel structure of γ-CD. It seems probable that the drug is included within the empty channels of the crystalline structure of γ-CD, as occurs with other guest molecules [5,20]. In accordance with the X-ray diffraction experiments, the melting of the drug is hardly detected in the DTA curves of the kneaded, coevaporated and coprecipitated systems with γ-CD; this fact also evidences the formation of the complex in the solid state.

On the contrary, the diffraction patterns of the coevaporated and kneaded systems with HP-γ-CD present the main reflections of the drug (Fig. 4), although the intensity is much lower in the coevaporated, indicating perhaps the partial formation of an amorphous complex.

Despite the interaction in solution was more favourable with HP-γ-CD, the presence of hydroxypropyl groups in the rims of HP-γ-CD probably makes difficult the complexation in the solid state. The interior cavities of the columnar crystalline structure of γ-CD seem to play an important role in the formation of the complex in the solid state, as complexation was achieved by all the preparation methods.

3.4. FTIR spectroscopy

The FTIR spectra of the systems gemfibrozil-γ-CD and those of the pure components are shown in Fig. 5. When the systems are compared with the physical mixture, it can be observed that the carbonyl stretching band at 1708 cm^{-1} broadens and shifts towards higher wavenumbers, indicating changes in the intermolecular H-bonds of the drug upon complexation [21]. Similar modifications have been found in the combination signal of the carboxyl group (C–O–H) at 1403 cm^{-1}, which point out changes in the interactions of this group when the complex is formed. In addition, the bands at 1271 and 1214 cm^{-1}, corresponding to antisymmetric vibrations of the aryl-ether group [22] broaden and shift upon complexation. Finally, the decreased intensity of the band associated to the out of plane bending of the aromatic C–H bonds at 803 cm^{-1} (data not shown) evidence the inclusion of the benzene ring.

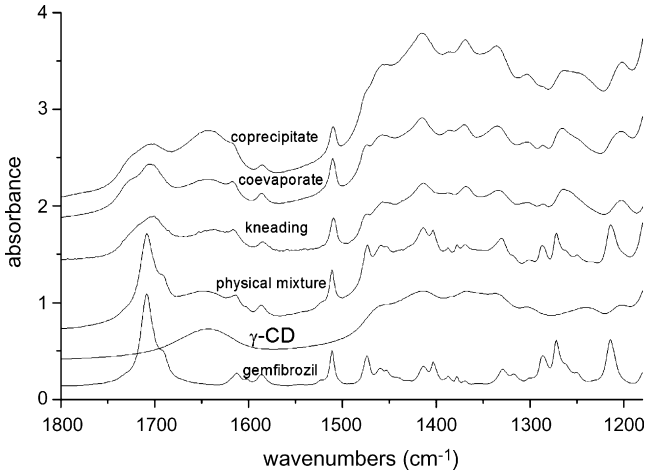

Fig. 5. FTIR spectra of gemfibrozil, γ-CD, the physical mixture and the kneaded, coevaporated and coprecipitated systems.

In relation with the HP-γ-CD systems, no changes were detected in the FTIR spectra.

4. Conclusions

The performance of fluorescence spectroscopy and phase solubility techniques in the study of complexation of gemfibrozil with γ-CD and HP-γ-CD has been analysed by comparing the apparent stability constants obtained. The solubility technique is less accurate for the determination of the constant with γ-CD due to aggregation phenomena that are less patent with the derivative CD. It has been also found that the crystallisation of γ-CD in the channel structure may have an important role in the formation of the complexes in the solid state.

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