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Spectroscopic characterisation of the inclusion complexes between the antifungal drugs naftifine and terbinafine and cyclodextrins

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The complexation of naftifine (NF) and terbinafine (TB) with cyclodextrins (CDs) has been investigated by UV/visible and ^{1}H NMR spectroscopy, ROESY techniques and also ESI-MS. Both drugs form 1:1 inclusion complexes with all the CDs tested except with α-CD, as deduced from the Benesi–Hildebrand plots and confirmed by ESI-MS and NMR spectroscopy (Job plot method). The K_{11} values for NF decrease in the order β-CD > methylated β-CD > 2-hydroxypropyl-β-CD > γ-CD. The determination of the enthalpy and entropy provides information about the main driving forces in the process. The stability constants of the complexes NF–β-CD, TB–β-CD and TB–γ-CD determined by ^{1}H NMR spectroscopy are in agreement with the values obtained by UV. For TB–β-CD, the value is higher, due to the fact that the length of the TB aliphatic chain allows a deeper inclusion of the naphthalene group inside the corresponding β-CD molecule, according to the 2D ROESY experiments.

Keywords: terbinafine; naftifine; cyclodextrins; inclusion complex characterisation

1. Introduction

Cyclodextrins (CDs) are torus-shaped cyclic oligosaccharides made up of α-D-glucopyranose residues linked by glycosidic bonds, the most common are those formed by six, seven or eight glucose units (α-, β- and γ-CD, respectively; Figure 1). This class of carbohydrates exhibits a doughnut shape, in which the cavity has a hydrophobic character compared with water, whereas the rims, bearing the OH groups, are hydrophilic. This structure makes CDs capable of forming inclusion compounds with a variety of guest molecules both in solution and in the solid state (*1*). The first condition required for a molecule to form an inclusion complex with CD is to fit in the cavity, either completely or partially. In addition, a favourable energetic balance is required, which depends on the nature of the guest, the inner diameter of the CD and its substitution degree (*2*). The formation of these inclusion compounds has been widely used to improve the aqueous solubility of poorly soluble drugs, together with their bioavailability, dissolution rate, permeability and stability (*3*). Natural CDs can be modified by substitution of the hydroxyl groups by methyl or hydroxypropyl groups in order to improve properties such as the solubility or to avoid undesired effects. 2-Hydroxypropyl-β-cyclodextrin (HPβ-CD) is a hydroxyalkyl β-CD derivative that has been studied most thoroughly. It is highly hydrophilic and generally forms complexes with many poorly water-soluble substances. In addition, both β- and HPβ-CD can be safely used in oral and parenteral formulations. The preparation of the methylated β-CD (Mβ-CD) is relatively simple and inexpensive. Both CD derivatives (Mβ- and HPβ-CD) are found to be permeable into the skin, particularly when they are applied under the occlusive dressing conditions and/or by using vehicles containing absorption-promoting agents (*4*, *5*).

In this study, naftifine (NF) and terbinafine (TB) have been selected as guests to characterise their respective complexes with CDs. Naftifine, (*E*)-*N*-methyl-*N*-(1-naphthylmethyl)-3-phenyl-2-propen-1-amine ($pK_{aNF} = 8.0 \pm 0.2$), and terbinafine, (*E*)-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalene methanamine ($pK_{aTB} = 6.7 \pm 0.3$) hydrochlorides, are two antifungal agents of the chemical group of allylamines (Figure 2). They present a high selectivity for a single fungal enzyme and an apparent lack of interferences with other enzymes of fungal and mammalian origin (*6*). Their mechanism of action is based on the blocking of the biosynthesis of ergosterol, which is an essential component of fungal cell membranes (*7*). TB is indicated for both oral and topical treatment of mycoses and NF only for topical treatment. These drugs present gastrointestinal adverse effects, which are expected to be reduced or avoided by microencapsulation in CDs.

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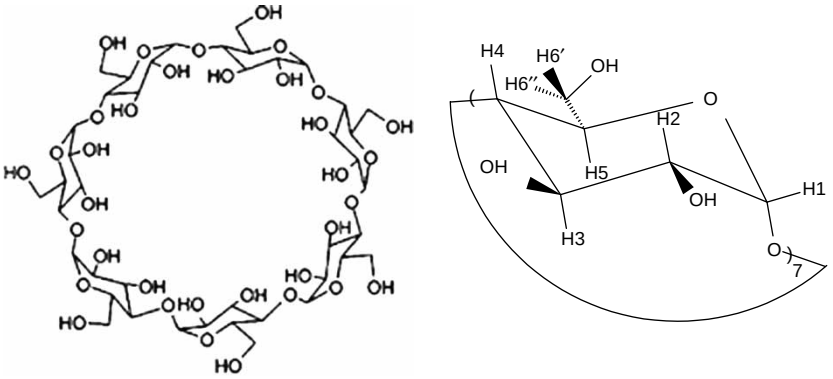

Figure 1. β-CD molecule.

The study of complexation with CDs is usually focused on two main objectives: the accurate determination of the stability constants and the structural elucidation of the complex.

There is a wide variety of techniques that can be used to determine the stability constants (*1*). The accuracy of the constant is improved by the use of different methods in its calculation, although disagreements between techniques are not uncommon.

In relation to the study of the mode of inclusion or topology of the complex, ^{1}H NMR has become the most important method for structural elucidation of inclusion complexes with CDs, particularly in solution. For structural studies, the nuclear Overhauser effect (NOE) is one of the most important tools for the study of the topology of the complexes (*8*), because the dipolar interactions that are detected as NOE enhancements reflect the spatial proximity between protons. For medium-sized systems, such as those studied here, the appropriate technique is rotating-frame Overhauser effect spectroscopy (ROESY) (*9*).

The aim of this paper is to study the inclusion process of NF and TB with natural and derivative CDs by different techniques. This characterisation includes the determination of the stability constants, stoichiometries and the corresponding thermodynamic parameters together with the structural elucidation of the complexes.

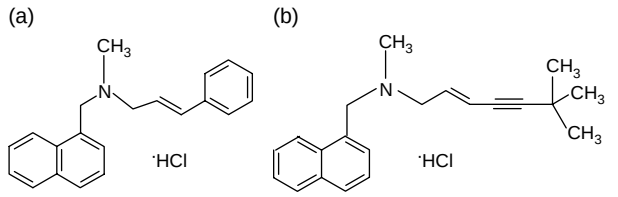

Figure 2. Chemical structures of (a) NF and (b) TB hydrochlorides.

2. Experimental

2.1 Materials

NF hydrochloride (molecular weight 323.5) was kindly supplied by Schering (Milan, Italy) and TB hydrochloride (molecular weight 327.9) by Novartis (Basel, Switzerland). α- and β-CD were from Wacker Chemie GmbH (Munich, Germany), having a water content of 9.8 and 11.2%, respectively, as determined by thermal analysis. γ-CD was from Roquette (Lestrem, France), with 11.0% of water. Mβ- and HPβ-CD were from Sigma (St Louis, MO, USA). Mβ- and HPβ-CD had average substitution degrees DS ≈ 12 and 4 and a water content of 5.2 and 6.5%, respectively. D_2O (deuterium content 99.9%) was obtained from Aldrich Chemical (Barcelona, Spain).

2.2 Spectrophotometric method

The interactions of NF with α-, β-, Mβ-, HPβ- and γ-CD in aqueous solutions (pH 4) were studied at different temperatures: 290, 293, 298, 303 and 310 K. The temperature was controlled by recirculating water from a thermostat bath Lauda Ecoline RE 104. The spectra of NF and NF + CD were recorded with a diode array spectrophotometer HP 8452. The NF concentration was kept constant at 5.0×10^{-5} M while that of CD varied between 4.8×10^{-4} and 3.8×10^{-3} M by the addition of a concentrated solution of NF + CD to the cell. This procedure permits to minimise the errors due to the manipulation of the cuvette or to slight changes in its position in relation to the source. The measurements were made at 254 nm. The Benesi–Hildebrand equation was used to calculate the stability constant (K_{11}) (*10*):

$$\frac{1}{\Delta A} = \frac{1}{S_t K_{11} \Delta\varepsilon [L]} + \frac{1}{S_t \Delta\varepsilon},$$

where ΔA is the difference between the absorbance of the drug in the presence and absence of CD, $\Delta\varepsilon$ is the

difference between the molar absorption coefficient of NF and the complex, S_t and $[L]$ are the initial concentrations of NF and CD, respectively. K_{11} can be calculated from the slope of the straight line obtained by plotting $1/\Delta A$ against $1/[L]$.

The thermodynamic parameters of the inclusion process were determined from the temperature dependence of the association constant using the van't Hoff equation.

2.3 ^{1}H NMR measurements

All the NMR measurements were performed at 25°C in D_2O as the solvent. For collecting the 1D spectra, we used a Bruker Avance 400 Ultrashield spectrometer, by averaging 600 scans. The solvent signal (HDO, at 4.792 ppm) was used as the reference. For NF, the concentration was kept constant at 2.7 mM and that of β-CD varied from 0.5 to 10 mM by adding NF to vials with different amounts of β-CD. In the case of TB, the Job plot method was used to ascertain the stoichiometry of the complexes with both β- and γ-CD (*10*), by mixing a 4 mM solution of the drug with either 4 mM β- or γ-CD in different amounts.

For the 2D ROESY experiments, a Bruker Avance AV-500 spectrometer (11.7 T) was used by applying the pulse sequence defined in the literature (*11*). Different spin-lock mixing times were tested until obtaining the best signal-to-noise ratio with 600 ms. The Fourier transform of the free-induction decays (1024 × 1024 matrices, obtained by averaging at least 64 scans), 2D phase tuning and signal integration were performed with MestRe-C software (version 4.7; Mestrelab Research, 2004). For determining the stability constants of the complexes with TB and β- or γ-CD, we used a multivariable nonlinear analysis with selected protons of the host and the guest. The main advantage of the multivariable analysis arises from the increase in the number of fitted data. The protons chosen for the analysis were those that underwent more pronounced shifts, assuming that the stability constant is the same for each proton. The equation used for the determination of the stability constants is based on the fast exchange between the host and the guest in the NMR time scale. If such is the case, the measured chemical shifts are the average of the chemical shifts of the species present (*10*, *12*). $\Delta\delta$ (the difference between the chemical shift of the host or the guest alone and the measured chemical shift at each concentration) can be related to the concentration of TB and CD at each point:

$$\Delta\delta=\Delta\delta_0\frac{1}{2}\left\{\left(1+\frac{w}{x}+\frac{1}{K_{11}x}\right)-\sqrt{\left(1+\frac{w}{x}+\frac{1}{K_{11}x}\right)^2-4\frac{w}{x}}\right\},$$

where $\Delta\delta_0$ is the difference in the limit when all the drugs are complexed, w is the TB or CD concentration and x is that of CD or TB, depending on whether the stability constant is obtained from the host or the guest. All the calculations were performed with ORIGIN software (version 7.0; Microcal Software, Inc., Northampton, MA, USA).

2.4 Electrospray ionisation mass spectrometry

The ESI-MS spectra of NF, TB and their complexes with CDs (α-, β-, Mβ-, HPβ- and γ-CD) were obtained using a Waters micromass ZQ mass spectrometer equipped with an electrospray interface. CD–TB and CD–NF mixtures (1:1, 0.1 mg/ml) were prepared in methanol/water (50:50, v/v) and were introduced into the ion source of the mass spectrometer with a flow rate of 20 μl/min using a syringe pump. The ionisation voltage was 3.51 and 3.33 kV for NF and TB, respectively. The temperature of the inlet capillary was 200°C and the detection was performed in the positive ion mode.

3. Results and discussion

3.1 Spectrophotometric characterisation of NF–CDs complexes

The addition of increasing amounts of every CD, except for α-CD, caused a decrease in the absorbance intensity at 254 nm and also the appearance of an isosbestic point; both facts indicate complex formation (*10*) (Figure 3).

These changes in the spectral properties are probably due to the influence of the high electron density inside the CD cavity on the electrons of the guest (*13*). Plots of $1/\Delta A$ as a function of 1/[CD] for NF confirm the formation of a 1:1 inclusion complex. As an example, Figure 4 shows the Benesi–Hildebrand plot of NF in the presence of β-CD at 25°C. The stability constants determined for the complexes at the different temperatures are compiled in Table 1.

The K_{11} values decrease in the order β-CD > Mβ-CD > HPβ-CD > γ-CD. In the case of α-CD, the absence of changes in the UV spectra suggests that its cavity is too narrow to include NF. With respect to β-CD and its derivatives, the complexation between NF and the natural CD is stronger, probably due to interactions between the amino group and the hydroxyls of the β-CD rims, which are not present in the corresponding derivatives of β-CD. In addition, taking into account that the drug is charged in aqueous solution ($pK_a = 8.0 \pm 0.2$), the substitution of some of the hydroxyls of the CD by the hydroxypropyl and the methyl groups probably reduces the affinity of the charged drug for the CD. Although HPβ-CD bears more OH groups than Mβ-CD, the lower constant obtained for HPβ-CD in relation to Mβ-CD could

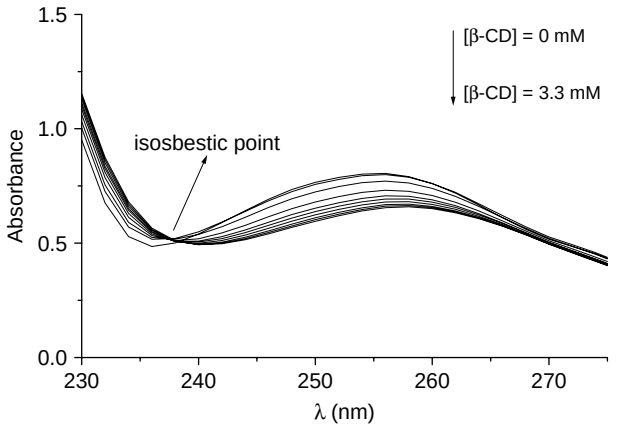

Figure 3. Absorption spectra of 5.0×10^{-5} M NF as a function of [β-CD] at 25°C.

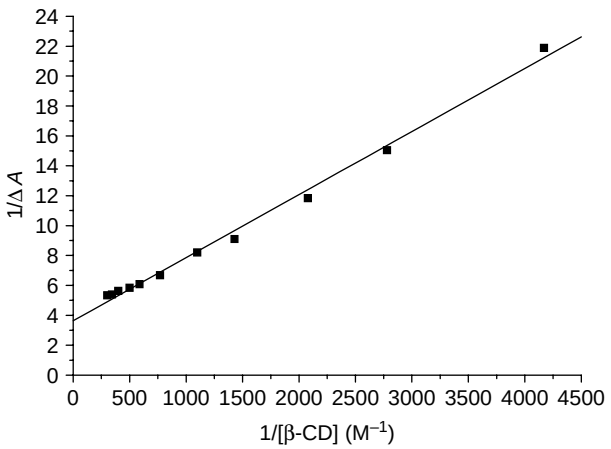

Figure 4. Benesi–Hildebrand plot of $1/\Delta A$ vs. 1/[β-CD] for NF at 25°C.

be explained by steric hindrance of the hydroxypropyl group, which could hamper the guest molecule entering the CD cavity (*14*).

The corresponding enthalpy (ΔH) and entropy (ΔS) values were calculated from the van't Hoff relationship from the temperature dependence of the equilibrium constants. The results are shown in Table 1. The enthalpies are always negative and close to each other, meaning that the attractive forces involved in the complexation of NF with the different CDs, such as van der Waals and hydrogen bonding between the drug and the CD cavity, are similar (*15*). In relation to the entropy, it is positive for the complex with β-CD whereas it is negative with the other macrocycles; this can be interpreted in terms of the specific interactions between the CD and the guest. The OH groups of β-CD are initially solvated by structured water, but the formation of hydrogen bonds between the amino group of NF and the hydroxyl groups of the CD would break this structure and release the water molecules in the bulk. However, for the β-CD derivatives, the negative values of entropy are related to an increase in order in the system upon complexation. This could be associated with the solvation of the amino group by water molecules in the complex and also with a pronounced restriction of the rotational and vibrational degrees of freedom in the complex due to the presence of the hydroxyl and methyl groups.

The interactions of neutral NF with CDs have been studied by the solubility method at pH 12 and 298 K in a previous work (*16*), resulting in $K_{11} = 512$, 829, 1123 and 184 M^{-1} for the complexation with β-, HPβ-, Mβ- and γ-CD, respectively. The stability constants resulted higher at pH 12, except for β-CD, showing that the interaction is stronger for the neutral form of NF, according to the explanation of Szejtli (*13*). Since the drug is uncharged at pH 12, it can penetrate deeper into the CD cavity.

It was intended to study the interactions between TB and CDs but no changes in the TB spectra were observed.

3.2 ^{1}H NMR spectroscopy

As a preliminary step, we have investigated the possible concentration dependence of the chemical shifts of both NF and TB (from 0.5 to 3.5 mM). This is important if aggregation processes in solution are expected, but this has not been the case. The signal assignation of NF and TB is shown in Figures 5 and 6, respectively. These antifungal drugs have similar chemical structures and in both cases the signal of proton d overlaps that of HDO in the ^{1}H NMR spectra.

3.2.1 *NF–β-CD interactions*

The ^{1}H NMR spectrum for NF is quite similar to that of TB (Figures 5 and 6). The difference is the presence of the benzene ring in the case of NF (signals between 7.4 and 7.3 ppm) instead of the *tert*-butyl group of TB. Upon

Table 1. Stability constants[a] ($K_{11}\cdot10^{-2}/M^{-1}$) and thermodynamic parameters of NF complexes with CDs in water.

	290 K	293 K	298 K	303 K	310 K	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})
β-CD	10.8 ± 0.4	10.2 ± 0.3	9.0 ± 0.3	8.2 ± 0.3	6.9 ± 0.6	−17.0 ± 0.8	0.6 ± 3
Mβ-CD	4.1 ± 0.1	3.8 ± 0.1	3.3 ± 0.4	2.9 ± 0.4	2.5 ± 0.4	−19.2 ± 0.5	−16.3 ± 1.7
HPβ-CD	3.0 ± 0.2	2.8 ± 0.1	2.5 ± 0.4	2.2 ± 0.1	1.9 ± 0.1	−18.7 ± 0.5	−16.9 ± 0.2
γ-CD	2.3 ± 0.1	–	2.1 ± 0.2	1.7 ± 0.2	–	−14.0 ± 0.1	−3.0 ± 0.4

[a]Standard errors are indicated.

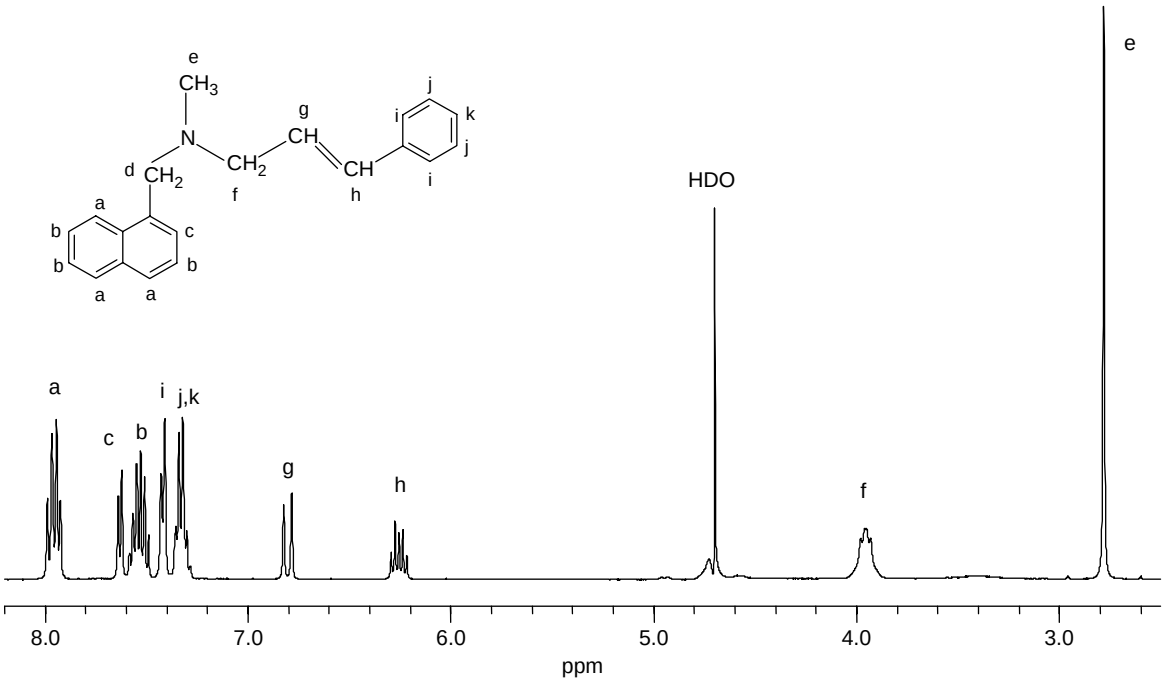

Figure 5. ^{1}H NMR spectrum of 2.7 mM NF in D_2O.

addition of a solution of NF to variable amounts of β-CD, the protons that shift are a, g, h, i of NF, the inner protons of β-CD (H3 and H5) and H6. All of them move upfield, with the maximum changes corresponding to those of NF g, h and i, which shift −0.592, −0.963 and −0.515 ppm, respectively, while in the case of CD H5 moves −0.065 ppm. Figure 7 displays the spectra for all the measured molar ratios in the regions, where the changes are more remarkable.

The changes in the chemical shifts with respect to those of the pure substances in water, $\Delta\delta$, have been plotted vs. the molar ratio in Figure 8. These plots indicate

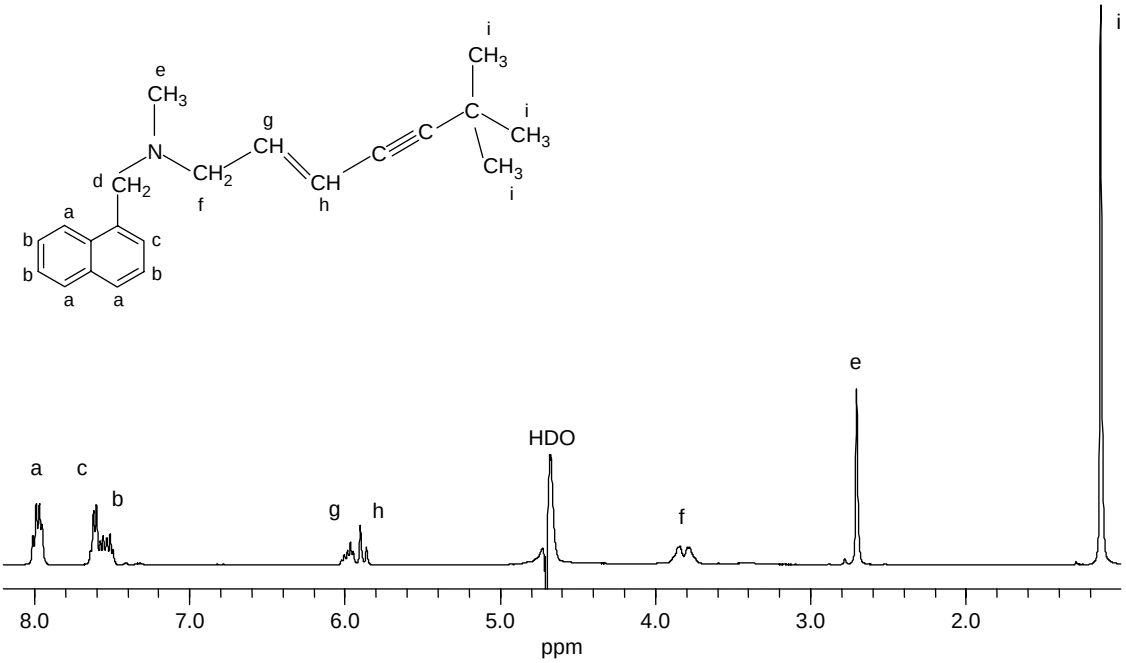

Figure 6. ^{1}H NMR spectrum of 0.5 mM TB in D_2O.

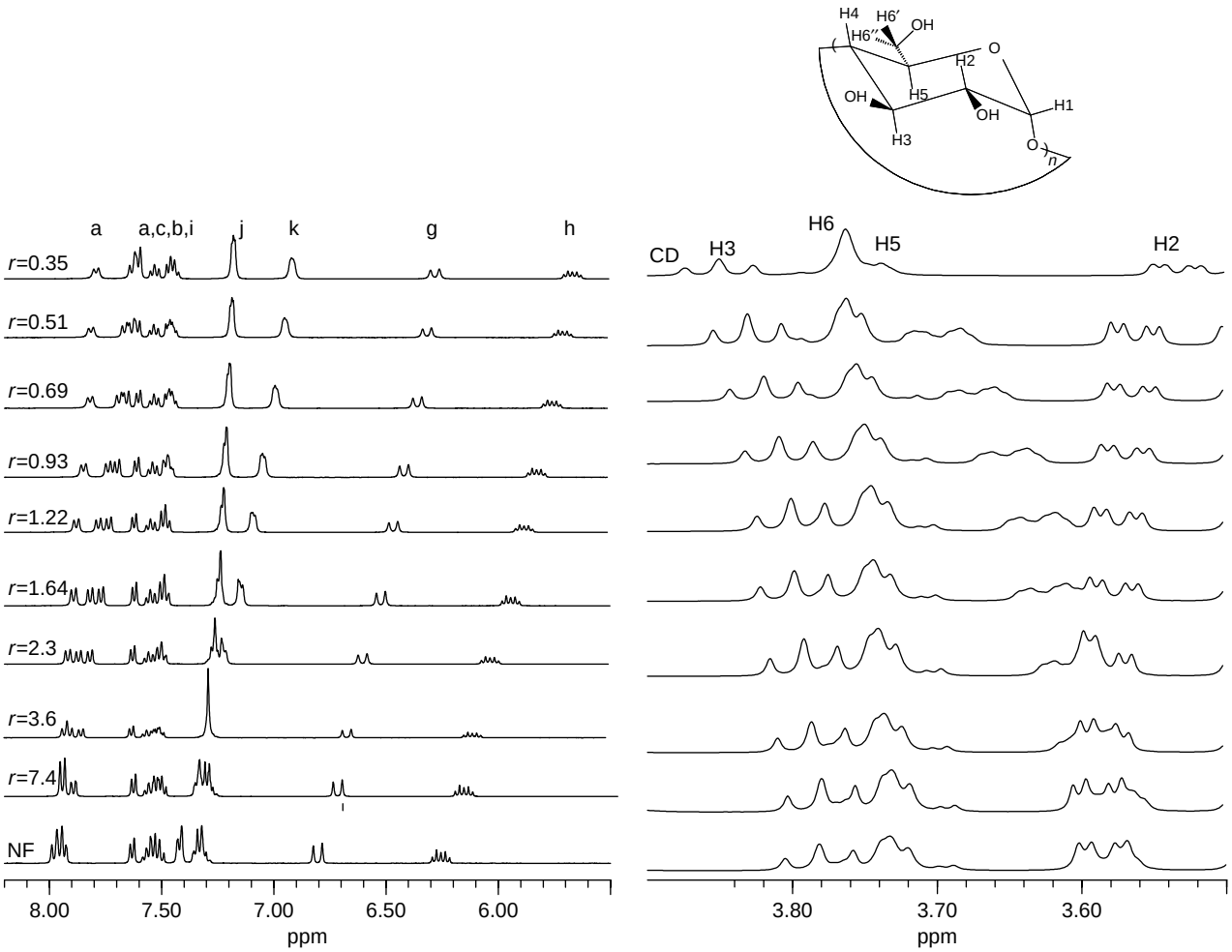

Figure 7. Selected regions of the ^{1}H NMR spectra in D_2O at different [NF]/[β-CD] ratios.

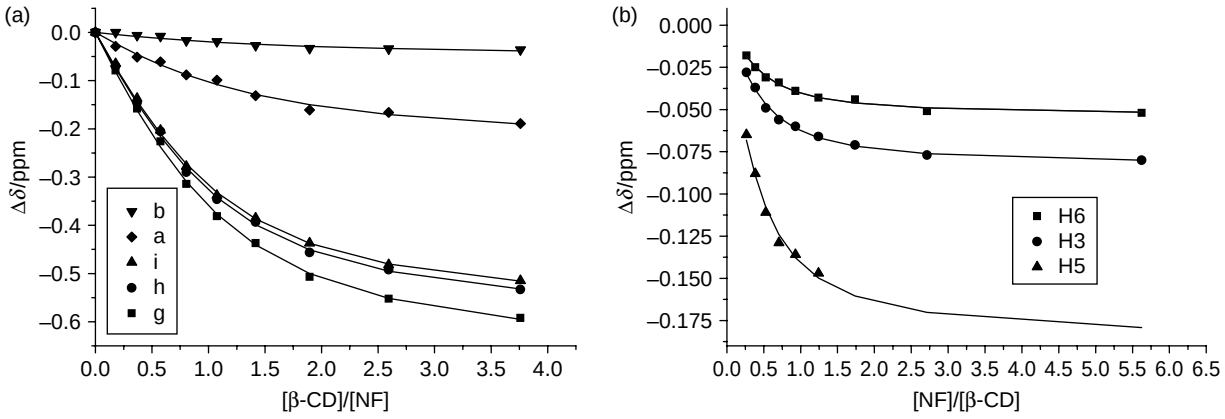

Figure 8. Increments in the chemical shifts vs. the molar ratio for selected protons of (a) NF and (b) β-CD. Solid lines are obtained by a nonlinear fit assuming a 1:1 stoichiometry.

Table 2. Binding constants[a] ($K_{11}\cdot10^{-2}/M^{-1}$) corresponding to the NF–β-CD system, calculated with NF and β-CD protons.

NF proton	K_{11}	β-CD proton	K_{11}
a	5.1 ± 1.1	H3	7.0 ± 1.0
g	8.9 ± 0.6	H5	5.5 ± 1.2
h	9.6 ± 0.8	H6	7.1 ± 1.6
i	9.7 ± 0.7		

[a]Standard errors are indicated.

a 1:1 stoichiometry, as deduced from the extrapolations from high and low molar ratios at $R = 1$.

Provided a fast exchange in the scale of NMR, and a 1:1 stoichiometry, the observed chemical shift δ is the sum of the chemical shift due to the complex ($\delta_{NF:CD}$) and that due to the host/guest (δ_i, where i = CD or NF), each one averaged with its molar fraction (X_i, $X_{NF:CD}$), that is (*17*)

$$\delta = \delta_i X_i + \delta_{NF:CD} X_{NF:CD}.$$

The binding constant for each proton can be estimated by a nonlinear fit of the increments of the chemical shifts with respect to that of the pure host or guest compound, $\Delta\delta$, vs. the molar ratio (Figure 8). The values deduced for the stability constants are compiled in Table 2. The average K_{11} values obtained with the protons of NF and β-CD are $(8.3 \pm 0.8) \times 10^2$ and $(6.5 \pm 1.3) \times 10^2$ M^{-1}, respectively. These values are within the experimental uncertainty and agree with the stability constant calculated by UV spectrophotometry at 25°C, despite the different range of concentrations used.

3.2.2 *TB–β-CD and TB–γ-CD interactions*

The presence of β- or γ-CD induces important shifts in the signals of TB, as well as changes in those of the CDs, especially in the inner H3 and H5. These protons move upfield in the presence of TB (Figure 9). Those that shift to a larger extent are H3 and H5 (−0.070 and −0.092 ppm, respectively). It can also be observed that H1, H2 and H4 change −0.018, +0.043 and −0.002 ppm, respectively. The displacement of H2 may suggest a possible contact of TB with the external surface of the CD (*18*). The shifts of the TB protons are, in general, greater than those of the CD (Figure 9). The most remarkable changes occur in g, h (vinylic) and e, in an extent of −0.413, −0.263 and +0.153 ppm, respectively. The methyl protons (i) and those of the naphthalene group (a) shift less (−0.016 and −0.099 ppm, respectively).

In the case of γ-CD, the protons of the macrocycle, which exhibit the most remarkable changes, are H3 (−0.065 ppm) and H5 (−0.069 ppm). Unlike β-CD, H6 moves less (−0.019 ppm) and the external protons do not shift. The protons of TB, which undergo larger shifts, are those of the vinyl group and of the *tert*-butyl group, which move upfield (g, −0.49 ppm; h, −0.353; i, −0.086). On the other hand, *e* proton moves downfield (+0.158 ppm) as it occurred with β-CD. The interaction with γ-CD is weaker than that with β-CD, as deduced from the lower shifts with the former.

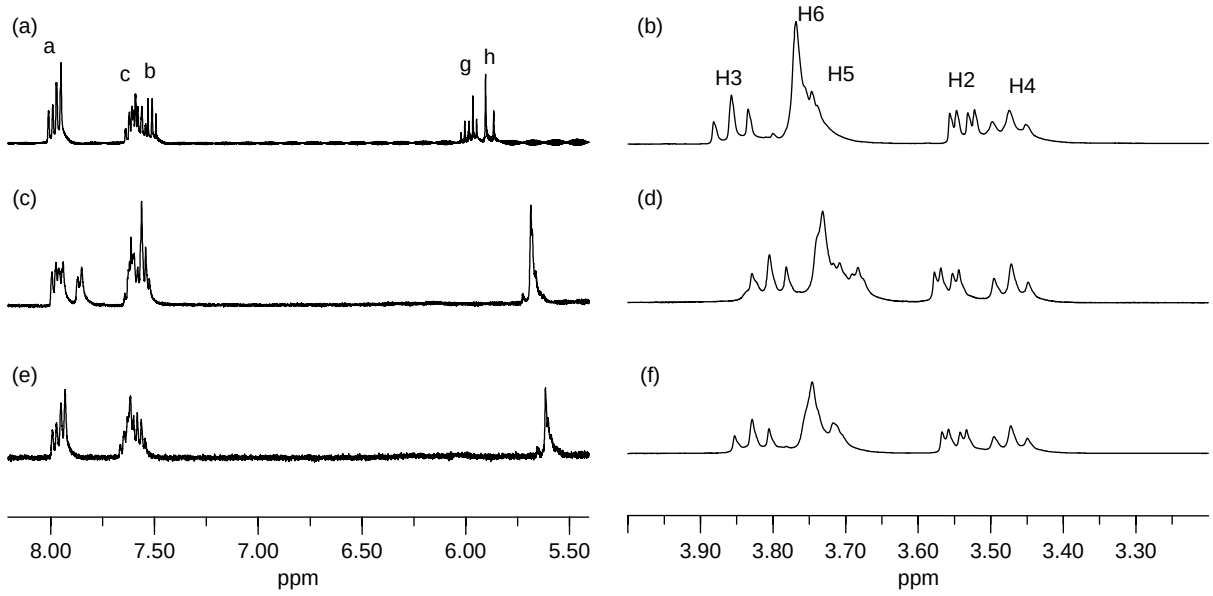

Figure 9. Selected regions of the ^{1}H NMR spectra of (a) TB 4 mM; (b) β-CD 4 mM; (c) and (d) [TB]/[β-CD] = 0.5; (e) and (f) [TB]/[β-CD] = 0.3.

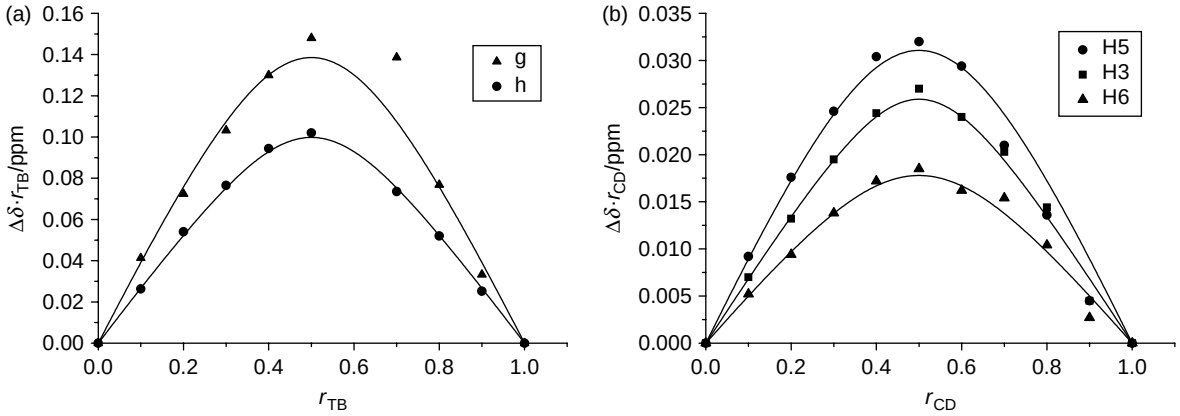

Figure 10. Job plots for selected protons of (a) TB and (b) β-CD.

With both CDs, it seems that the interaction takes place between the internal protons of the CD and the aliphatic part of TB, although the inclusion of the naphthalene nucleus cannot be discarded. Its size fits both CDs (*19*), and the changes in some of their protons are significant enough to conclude that inclusion has taken place. This could suggest as most probable a 2:1 stoichiometry. In order to elucidate whether this is the case, an NMR titration according to the Job plot method (*10*) has been performed. The results for TB–β-CD are shown in Figure 10, where r_{TB} and r_{CD} are the mole fractions of the host and the guest, respectively, and $\Delta\delta$ is the change in the chemical shift of the CD and TB protons. In both cases, the maxima appear at a mole fraction of ca. 0.5, which unquestionably indicates a 1:1 stoichiometry for the complex.

The stability constants have also been deduced for both complexes. For TB–β-CD, protons g, h, H3, H5 and H6 yield a binding constant of $(1.4 \pm 0.3) \times 10^3 \mathrm{l\,mol^{-1}}$. In the case of TB–γ-CD, the protons chosen were i, H3 and H5 (Figure 11). The multivariable analysis produces $K_{11} = (2.2 \pm 1.1) \times 10^2 \mathrm{l\,mol^{-1}}$. This constant is lower than that of the complex with β-CD and reflects the fact that the cavity size of γ-CD is wider for TB, whereas that of β-CD provides a tighter fitting.

3.3 2D structure of the complexes

2D ROESY experiments provide valuable information about the actual topology of the inclusion complexes. Figure 12 shows an expanded region of the ROESY spectrum of the system NF–β-CD. Although the interpretation is complicated due to signal overlapping, the spectrum reveals intense correlations between signals of the aromatic protons of the guest and those of the inner part of the β-CD (H3 and H5), which confirms the intracavity binding. The most intense are the NOEs between i, j and k protons (phenyl group) and H3 and H5, although those of the naphthalene nucleus also correlate strongly with H3 and scarcely with H5, indicating that the naphthalene enters the cavity by its wider side. In view of the 1:1 stoichiometry evidenced by the ^{1}H NMR data, the only explanation is that there must be a mixture of 1:1 complexes, in which either the benzene part or the naphthalene moiety of NF is included. There would be no

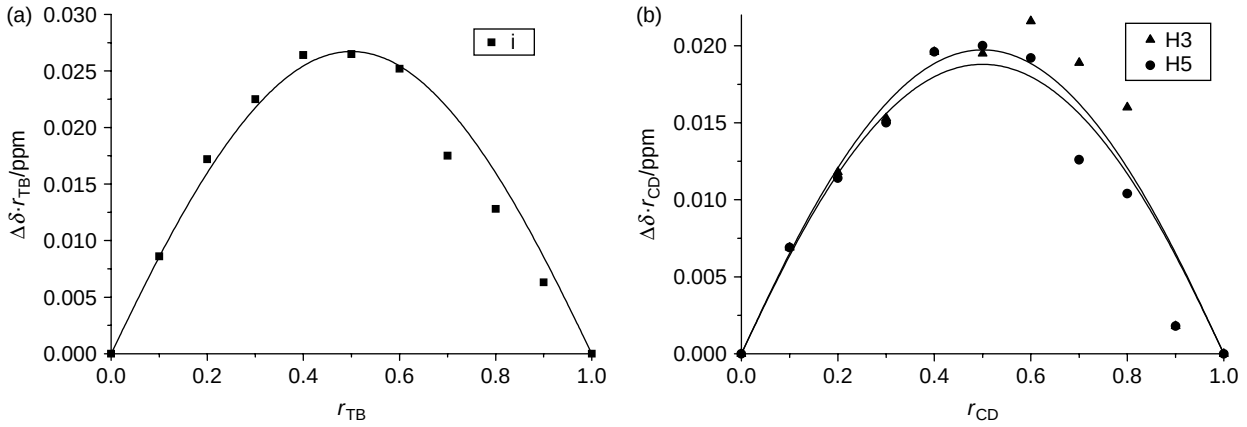

Figure 11. Job plots for selected protons of (a) TB and (b) γ-CD.

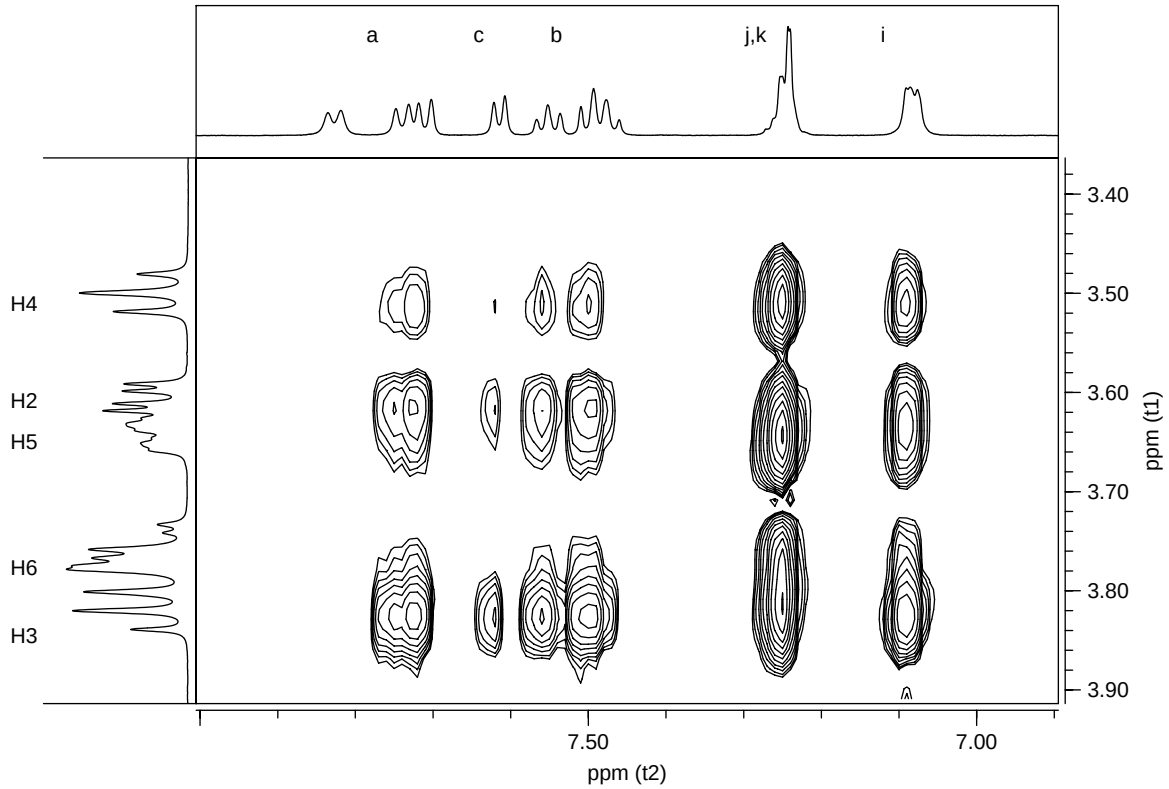

Figure 12. Zoomed view of the ROESY spectrum of the system NF–β-CD.

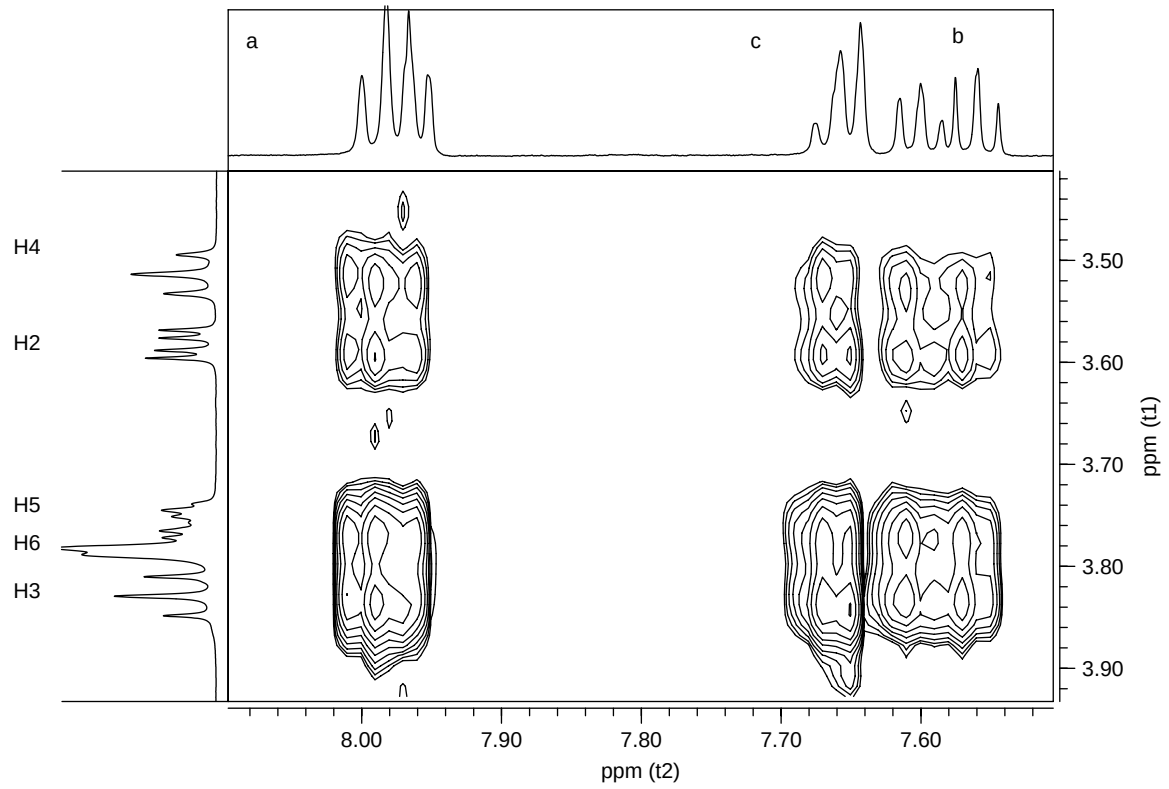

Figure 13. Partial view of the ROESY spectrum of the TB–β-CD system (aromatic region).

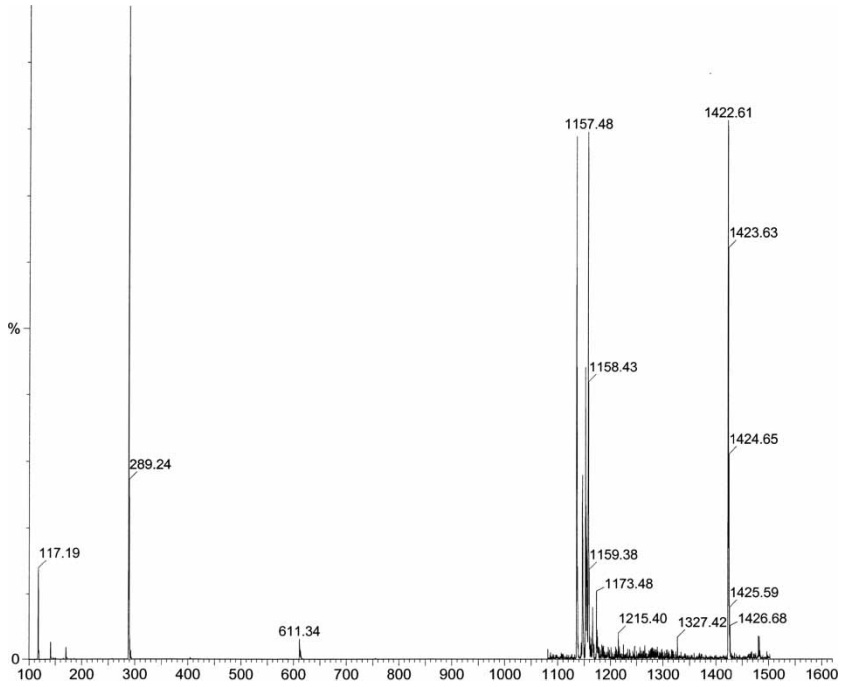

Figure 14. ESI-MS spectrum of β-CD with NF.

possibility to share the molecule between two CDs, at least within the interval of concentration used.

In the case of TB and β-CD, the spectrum (Figure 13) shows a clear correlation between the naphthalene group and the inner protons H3 and H5 of the β-CD. Also, the *tert*-butyl signal of the TB (i protons) correlates strongly with H5 (figure not shown), whereas H2 and H4 give weaker cross-peaks with both the naphthalene and *tert*-butyl groups. The fact that the most intense NOE signals are paired as naphthalene–H3 and H5 and i–H5 suggests, as in the case of NF, that the naphthalene group enters the cavity by its wider rim and also the *tert*-butyl group (this one more deeply buried than the naphthalene). The 1:1 stoichiometry, confirmed by the Job plot, must imply that both topologies must be present in solution.

In the case of TB with the wider γ-CD, the qualitative interpretation is the same as that of the precedent cases: intense cross-peaks appear between the inner protons of γ-CD and i ones (relative NOE intensities = 100), being weaker than those between the protons of the naphthalene group and H3 and H5 (NOE volume = 14). This suggests that the affinity of TB for this macrocycle is mainly due to the inclusion of the aliphatic part of the guest.

3.4 Electrospray ionisation mass spectrometry

Full-scan positive-ion mass spectra of TB and NF showed the protonated molecular species $[M + H]^+$ at *m/z* 292.2 and 288.2, respectively. The most abundant ion in the product ion spectra was at *m/z* 140.7 for TB and 116.9 for NF, as a result of the dissociation of the drugs.

Ions due to the inclusion of Na^+ by α-, β-, Mβ-, HPβ- and γ-CD are observed at *m/z* 995, 1157, 1353, 1563 and 1319, respectively. All the CD–NF and CD–TB complexes, except those with HPβ-CD, can be detected by ESI-MS in the positive ion mode. As an example, the spectrum obtained for NF–β-CD is shown in Figure 14. The complexes of α-, β-, Mβ-, HPβ- and γ-CD with NF give ions at *m/z* 1260, 1422, 1618 and 1584 corresponding to the respective drug–CD complexes, which demonstrate the presence of 1:1 stoichiometry in all the cases. Complexes of the same CDs with TB give ions at *m/z* 1264, 1426, 1622 and 1588, respectively. Therefore, ESI-MS can provide a direct proof of the formation of 1:1 non-covalent complexes in the gas phase. Thus, the stoichiometries determined by ESI are in agreement with those obtained by ^{1}H NMR and UV spectroscopy.

The signals for TB–α-CD and NF–α-CD are weaker than those of α-CD alone, probably due to a weak host–guest interaction. As expected, the interactions of the antifungal drugs with β-CD are stronger, in agreement with the fact that the cavity of α-CD is too narrow for lodging aromatic molecules, while β-CD fits adequately (*19*). The interaction of NF with γ-CD is weak, but it seems to be stronger for TB, in accordance with a better fit in this wider macrocycle.

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