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The Terfenadine/β-Cyclodextrin Inclusion Complex

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Abstract. Terfenadine (TFN) is a very hydrophobic antiallergic drug. It exists in three polymorphic and two solvated forms and is practically insoluble in water. These properties make a pharmaceutical formulation with acceptable biopharmaceutical characteristics difficult to prepare. Inclusion complexation with β-cyclodextrin (βCD) may eliminate such problems. The properties of the TFN/βCD system have been studied in liquid, gaseous and solid phases by ^{1}H and ^{13}C NMR spectroscopy, powder X-ray diffractometry, differential scanning calorimetry and fast atom bombardment mass spectrometry. The solubility phase diagram was also recorded. In solution and in the gaseous phase the 1 : 1 complex prevails, whereas a 1 : 2 TFN/βCD complex has been isolated by precipitation from homogeneous solution.

Key words: Terfenadine, β-cyclodextrin, inclusion complex.

1. Introduction

Terfenadine ((±)-α-[4-(1,1-dimethylethyl)phenyl]-4-(hydroxy-diphenylmethyl)-1-piperidinebutanol: TFN) is a selective histamine H_1 receptor antagonist currently used against hay fever, rush and most other allergic diseases (Figure 1). Since it lacks CNS depressant activity, TFN has been shown to be a clinically effective antihistamine which has a less adverse sedative effect in comparison to the classical histamine H_1-receptor antagonists [1].

The drug is practically insoluble in water (10 μg/mL), very hydrophobic, highly adhesive and it tends to adsorb on surfaces. It exists in three polymorphic and two solvated forms [2]. TFN chemically belongs to the group of amine compounds bearing a diphenylmethyl functionality.

Successful CD complexation of some members of this class (fendiline, adiphenine [3] and cinnirazine [4]) has been reported. Recently, the stability constants and enthalpy changes associated with the complex formation of other amine type drugs

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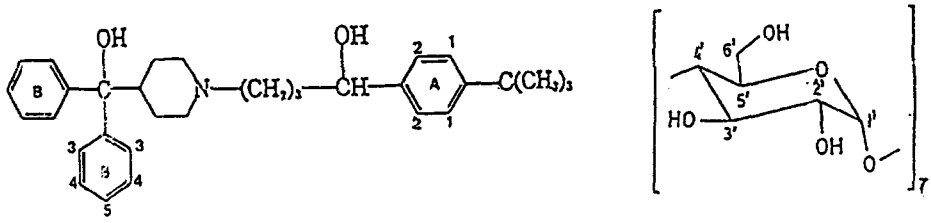

Fig. 1.

(including TFN hydrochloride) with β-, 2-hydroxy-propyl-β- and γ-CD have been systematically studied by solution microcalorimetry [5–6].

In another paper [7], molecular modelling has been used to generate the three dimensional structure of the complexes of 13 amine compounds with α-, β- and γ-CD.

Here the TFN/βCD system in different states (liquid, gaseous and solid) has been studied by the solubility method, ^{1}H and ^{13}C NMR spectroscopy, powder X-ray diffractometry, differential scanning calorimetry (DSC) and fast atom bombardment mass spectrometry (FAB-MS). Moreover, the structure of the supramolecular assembly in aqueous solution has been elucidated by measuring the intermolecular nuclear Overhauser effects (nOe) in the rotating frame. Some of these findings have already been presented as a preliminary communication [8].

2. Experimental

2.1 Materials

The following materials were used: β-cyclodextrin (Roquette Co., Lestrem, France) and Terfenadine (Sibefat SpA, Milano, Italy). D_2O, CD_3COOD and CD_3COCD_3 were purchased from Merck Chemical Co., Milano, Italy. All other chemicals and reagents were of analytical grade.

2.2 Preparation of the TFN/βCD Complex by Freeze Drying

16.6 g (12.7 mmole) of βCD (drying loss 13.2%) and 2.5 g (5.3 mmole) of TFN were vigorously stirred in 100 mL of water. Initially the very hydrophobic TFN floats on the surface of the system but after 10–12 h of vigorous stirring a pastelike, homogeneous, creamy suspension was obtained. Microscopic observation showed only a very finely dispersed amorphous suspension, without any trace of crystalline structure. As the filtration was very difficult, the solid complex was isolated by freeze drying the suspension. Yield: 18 g (14% TFN content).

2.3 PREPARATION OF THE TFN/βCD COMPLEX BY PRECIPITATION

16.6 g (12.7 mmole) of βCD (drying loss 13.2%) and 2.5 g (5.3 mmole) of TFN were stirred in 1000 mL of distilled water for 11 days at room temperature. In the originally clean, homogeneous solution a microcrystalline product slowly formed and was isolated by filtration. After drying, the yield was 12 g (19.8% TFN content).

2.4 MEASUREMENTS

The solubility isotherms were recorded according to Ref. [9]. Five series of aqueous TFN and βCD suspensions were equilibrated at 25°C for different periods (1/2–4 days). The quantitative determination of TFN was carried out by HPLC, using a Hewlett-Packard 1050 system provided with a UV-Vis detector. A commercially produced ultrasphere ODS analytical column (Beckmann-Astec, C18, 5 μm, 250 × 4.6 mm i.d.) was employed. The mobile phase 4 : 6 (v/v) 0.1 M ammonium acetate buffer (pH = 6.1): CH_3CN was filtered and degassed prior to use.

The ^1H NMR spectra were recorded at 400.13, 300.13 and 200.13 MHz on Bruker AMX 400, CXP 300 and ACF 200 spectrometers respectively, in D_2O/CD_3COOD at pD = 2.8. The assignment of the protons of TFN was accomplished by a two dimensional homonuclear correlation double quantum filtered experiment [10, 11] to reduce the intensity of the t-butyl group resonance. The ^{13}C NMR spectra were recorded at 50.3 MHz on a Bruker ACF spectrometer in D_2O/CD_3COOD or CD_3COCD_3. The carbon assignments were achieved through a two dimensional heteronuclear correlaton experiment in the ^1H decoupled version. All chemical shifts were determined relative to external sodium 3-trimethyl-silyl-propionate (TSP) at 0 ppm (accurate to = 0.001 ppm). The nuclear Overhauser effects in the rotating frame (ROESY) were measured at 303 K, applying a 4 KHz spin-lock field during two different mixing periods (τ_m 180, 250 ms) and with selective presaturation of the solvent signal.

FAB mass spectra were obtained with a Finnigan Mat TSQ 700 spectrometer utilizing thioglycerol as the ionization matrix. An accelerating voltage of 8 kV was used for the experiment. Powder X-ray diffraction patterns were recorded on a Philips PW 1130 diffractometer using Cu K_α radiation over the interval 3–30°/2Θ (time constant: 8 s, speed: 1°/min).

The DSC analysis was carried out on a DuPont 1090 Thermal Analysis System using a scanning rate of 5°C/min under an argon atmosphere.

3. Results and Discussion

3.1 THE TFN/βCD SYSTEM IN SOLUTION

Figure 2 shows different equilibrium phase solubility diagrams obtained for the TFN/βCD system in water. The solubility behaviour seems to be rather complicated and depends strongly on the stirring time. Isotherms I, II and III were taken after an

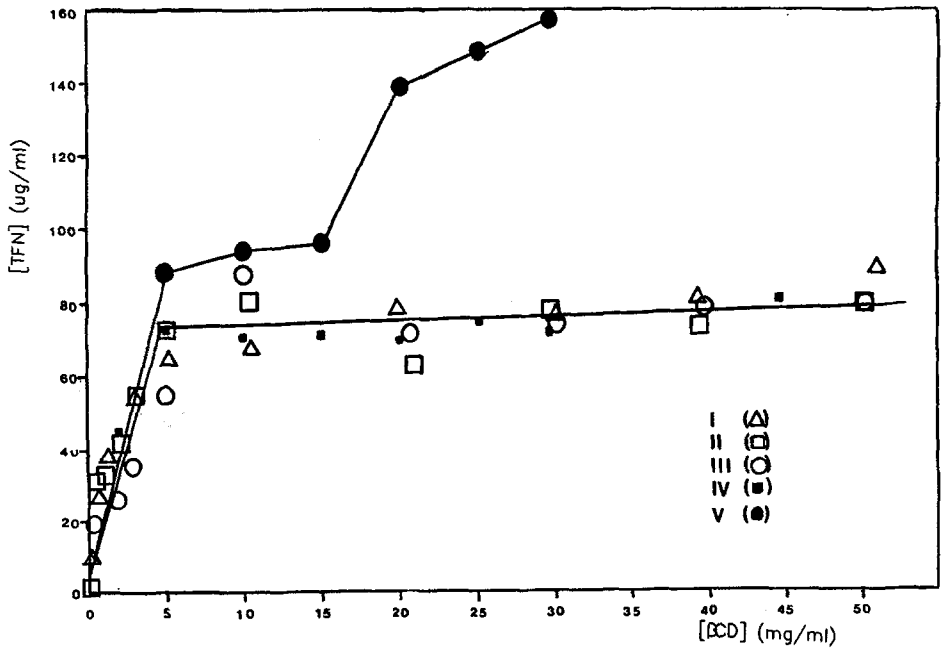

Fig. 2. Solubility of TFN as a function of βCD concentration in water at 25°C: (I), (II) and (III) after stirring for half a day; (IV) after stirring for a day; (V) after stirring for four days.

equilibration time of half a day and isotherm IV after 1 day. All these four isotherms show an initial linear section up to 5–10 mg/mL βCD concentration and a plateau corresponding to about 80 μg/mL of TFN. This indicates that a soluble complex (1 : 1) is formed by a fast process and the solubility limit of this complex is at about 80 μg/mL of TFN. Interestingly, typical Bs type solubility curves [8] could not be obtained either with a large molar excess of βCD, or with a dramatic reduction of the TFN input. Isotherm V was taken after equilibration for 4 days and it is seen to be quite different from the previous ones. It shows two intervals of a sharp solubility increase: one, as with isotherms I, II, III and IV, up to about 5 mg/mL βCD concentration but reaching about 95 μg/mL TFN solubility and another, starting at about the solubility limit of the βCD itself, between 15 and 20 mg/mL βCD. Above 20 mg/mL βCD concentration, solubility isotherm V shows a second, but less steep, linear section. This behaviour reflects the formation of another complex having a different stoichiometry (1 : 2). The solubility limit of the latter complex could not apparently be attained even at 30 mg/mL βCD concentration. The explanation of this behaviour is not known. TFN exists in several different polymorphic forms which have different crystal structures and solubilities. It is conceivable that, upon complexation with βCD, these different forms interconvert through precipitation and redissolution processes; a complicated quasi equilibrium may therefore rise, which depends on various factors such as the βCD concentration, stirring time,

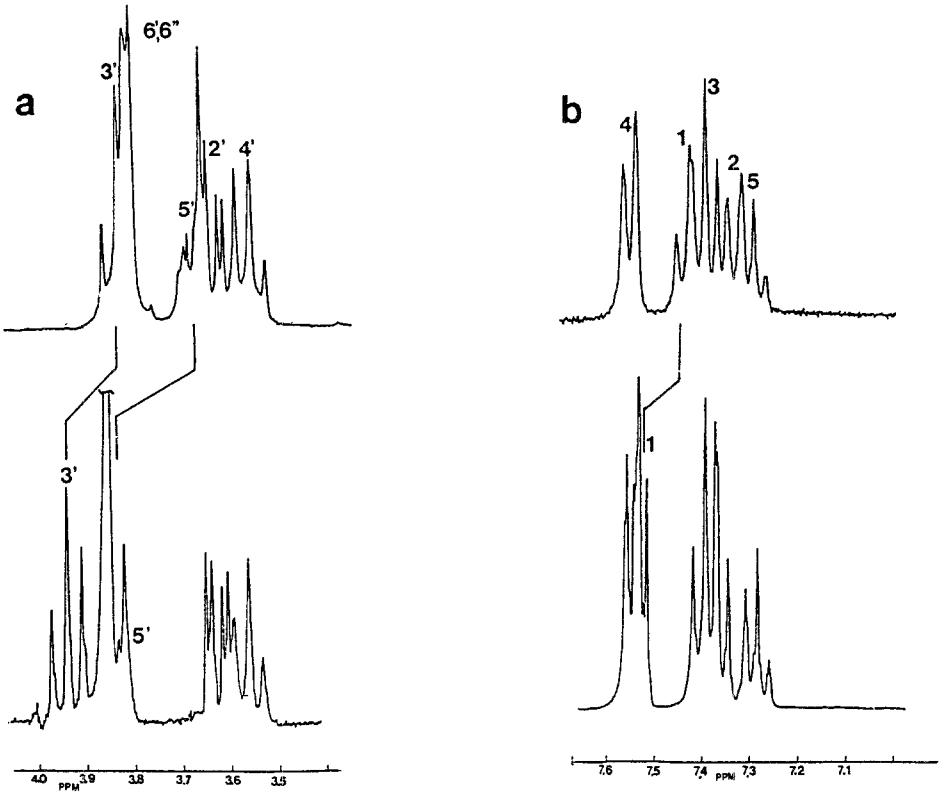

Fig. 3. Partial 300 MHz ^{1}H-NMR spectra (4 mM) of (a) the aliphatic region of the 1 : 1 TFN/βCD mixture (upper trace) in comparison with pure βCD (lower trace); (b) the aromatic region of the 1 : 1 TFN/βCD mixture (upper trace) in comparison with pure TFN (lower trace).

temperature, pH etc. The phase solubility technique by no means provides direct proof of inclusion [12] and therefore the system was investigated by ^{1}H and ^{13}C NMR.

Figure 3 shows the effect of βCD on the ^{1}H NMR spectrum of TFN and *vice versa* in an acidic aqueous solution (pD 2.8). The spectrum consists of only one set of resonances, showing that the whole system is in rapid exchange on the NMR time scale. The presence of TFN resulted in dramatic upfield shifts of the resonances of the protons H-3′ and H-5′ which are located on the inner surface of the β-CD cavity, clearly proving the reality of the inclusion [13–15]. Also the aromatic protons H-1 belonging to ring A of TFN (see Figure 1) show a noticeably larger upfield shift than the other aromatic protons upon addition of βCD. This fact indicates that at least the *para-t*-butyl substituted aromatic ring is inserted into the βCD cavity, driven primarily by hydrophobic interactions.

The results of the ^{13}C NMR measurements are shown in Tables I and II. Slight shifts of βCD carbons were detectable whereas the C-1 and C-2 signals of the *para-*

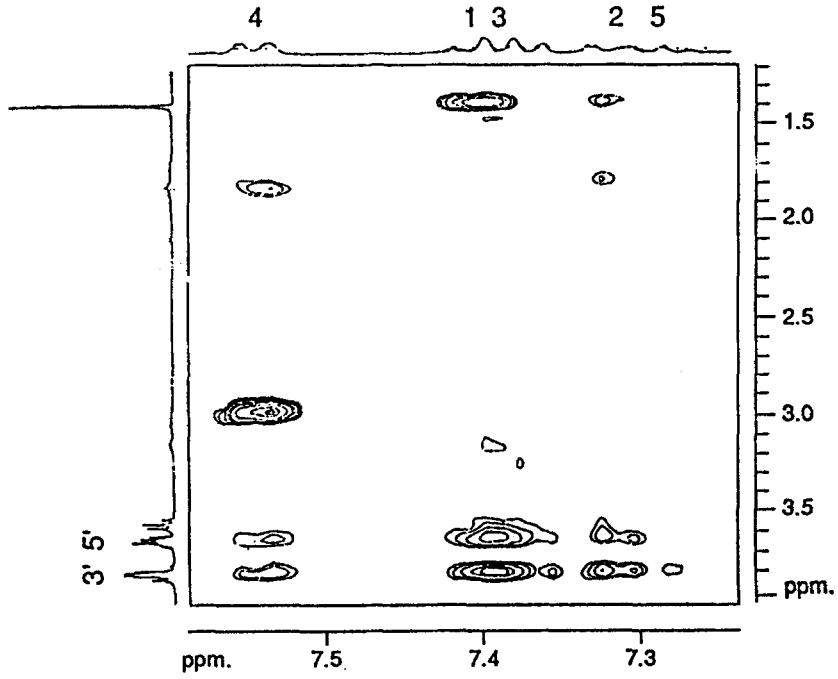

Fig. 4. Expanded region of the ROESY spectrum carried out with $\tau_m = 250$ ms (the spectrum with $\tau_m = 180$ ms does not show any qualitative difference).

TABLE I

^{13}C Chemical shifts (ppm) of β-CD in the absence and in the presence of Terfenadine (TFN).

Carbon	free δ_0	with TFN δ	$\delta - \delta_0$
1′	104.556	104.799	0.243
2′	74.764	74.699	−0.065
3′	75.803	75.962	0.159
4′	83.776	83.930	0.154
5′	74.492	74.560	0.068
6′	62.913	62.674	−0.239

t-butyl substituted ring showed remarkable highfield shifts. According to studies on the effect of solvent polarity on ^{13}C shifts [16, 17], it was observed that these

TABLE II

^{13}C Chemical shifts (ppm) of the aromatic carbons of Terfenadine in the absence and in the presence of β-CD.

Carbon	free δ_0	with β-CD δ	$\delta - \delta_0$
1	128.421	127.792	−0.629
2	128.729	128.360	−0.369
3	128.161	128.360	0.199
4	131.381	131.257	−0.124
5	129.867	129.826	−0.041

signals in organic solvents, such as CD_3COCD_3, appear at higher field than those in water. The results may again reasonably support the statement that the *para-t*-butyl ring is located in the hydrophobic cavity. In order to gain some further and more reliable information regarding the βCD/TFN molecular assembly in solution, two-dimensional nOe measurements were performed on the 1 : 1 mixture ($c = 4$ mM). As the nOes in the laboratory frame are almost zero at room temperature, they were measured under spin-locked conditions [18–20]. The result is displayed in Figure 4; a set of cross peaks connects both the H-5′ and H-3′ resonances of βCD to all the aromatic signals of TFN, indicating that not only the *para-t*-butyl phenyl ring, but also the other two aromatic rings are alternatively included in the cavity. However, the larger volume of the cross-peak area in correspondence with the nOe concerning the *para-t*-butyl phenyl ring confirms that the complex involving inclusion of this ring is the most stable one. TFN hydrochloride forms both 1 : 1 and 1 : 2 complexes in aqueous solution; the association constants for the two equilibria:

$$H + G \overset{K_{11}}{\rightleftharpoons} HG_1$$

$$HG_1 + H \overset{K_{12}}{\rightleftharpoons} HG_2$$

were found by microcalorimetry to be ≈20 000 M^{-1} and ≈600 M^{-1}, respectively [5]. These values were confirmed by NMR titration using nonlinear regression [21]. When K_{11} is large and $\gg K_{12}$, G is negligible and $[HG_2]$ can be calculated by solving the equation:

$$[HG_2] = (K_{12}[G]_t[H]_t - K_{12}[G]^2)/(1 + K_{12}[H]_t - K_{12}[G]_t)$$

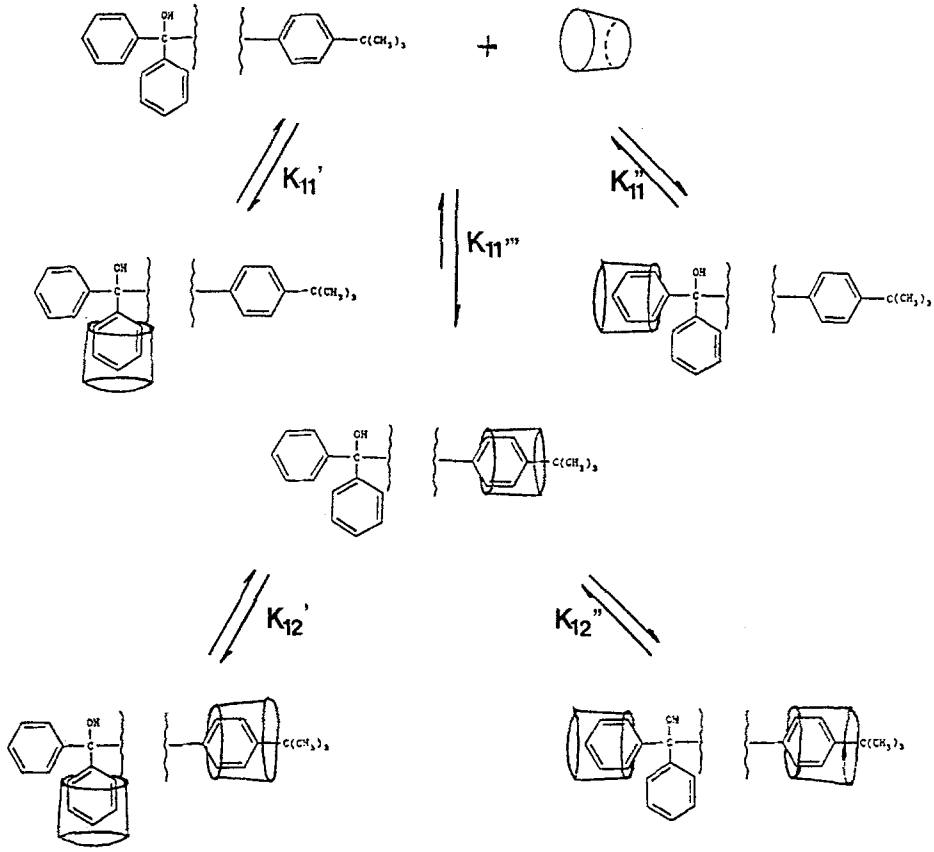

Fig. 5. Supposed representation of the possible inclusion equilibria for the TFN/βCD system. $K_{11} = K_{11'} + K_{11''} + K_{11'''}$; $K_{12} = K_{12'} + K_{12''}$.

where K_{11} and K_{12} are the association constants for the complexes; $[G]_t$ and $[H]_t$ are the total concentrations of TFN and βCD, respectively. If $[H]_t = [G]_t$, $[HG_2]$ is zero, therefore, in the conditions described above, three isomeric 1 : 1 complexes must be present in solution, each one involving inclusion of a different end of the molecule. Figure 5 shows all the possible inclusion equilibria for the 1 : 1 TFN/βCD system. $K_{11'}$ and $K_{11''}$ are not necessarily equivalent since TFN presents a chiral center [22]. On the basis of our findings, the equilibria for the 1 : 2 stoichiometry can also be presumed assuming that, at higher βCD concentrations, the most stable 1 : 1 complex may take up a second βCD molecule at one of the other two aromatic terminals.

3.2 THE TFN/βCD SYSTEM IN THE GASEOUS PHASE

Recently, some authors [23–25] have reported the use of FAB-MS to detect the formation of supramolecular adducts in the gaseous phase. Figure 6 shows the

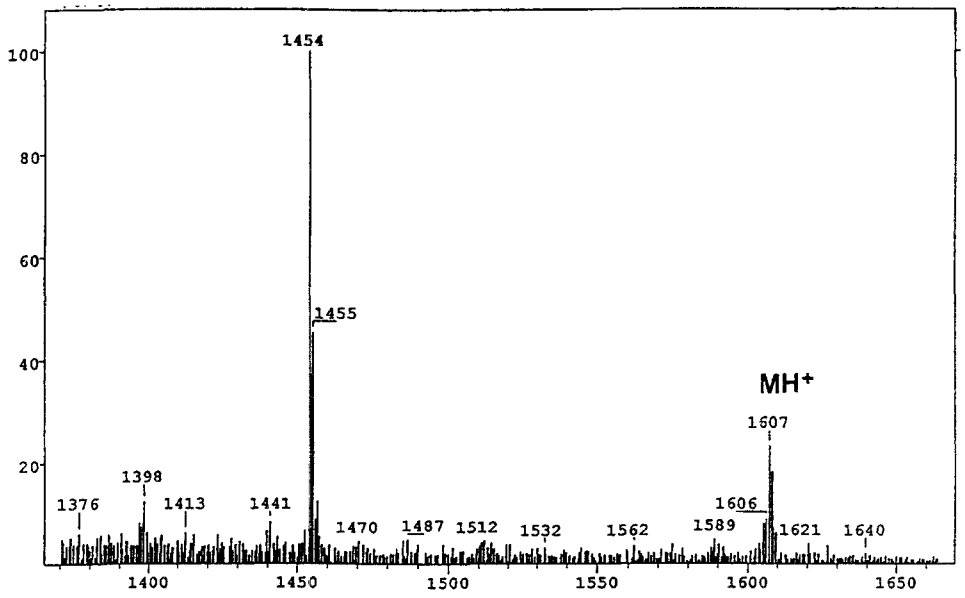

Fig. 6. FAB mass spectrum of the 1 : 1 TFN/βCD mixture.

FAB-MS spectrum of the TFN-βCD mixture in a 1 : 1 molar ratio. The spectrum reveals the presence of a distinct peak at *m/z* 1607 corresponding to the protonated molecular ion of the 1 : 1 adduct (the peak at *m/z* 1454 has not been assigned). The same analysis carried out on the 1 : 2 mixture gave the same result, indicating that only the 1 : 1 adduct is stable in the gaseous phase.

3.3 THE TFN/βCD SYSTEM IN THE SOLID STATE

DSC curves of TFN, a TFN-βCD physical mixture (17% active ingredient) and complexes prepared by precipitation and by freeze drying are shown in Figure 7. The uncomplexed TFN content can be calculated from the enthalpy change under melting at 151°C. The quantitative evaluation of the DSC curve of the TFN/βCD complex prepared by precpitation revealed that only about 10% TFN (related to the active ingredient content) remained uncomplexed. This suggests that the product contains at least 17% TFN in the complexed form, which corresponds exactly to the theoretical composition of the 1 : 2 TFN/βCD complex.

The melting of TFN could not be observed on the DSC curve of the complex prepared by freeze drying, confirming that no crystalline TFN was present, probably because a real inclusion complex has been obtained by this method. X-ray diffractograms of TFN itself and of the complexes prepared in the two different ways are shown in Figure 8. The diffractograms (b) and (c) do not show any sharp peak with respect to (a), indicating the amorphous character of the complexes.

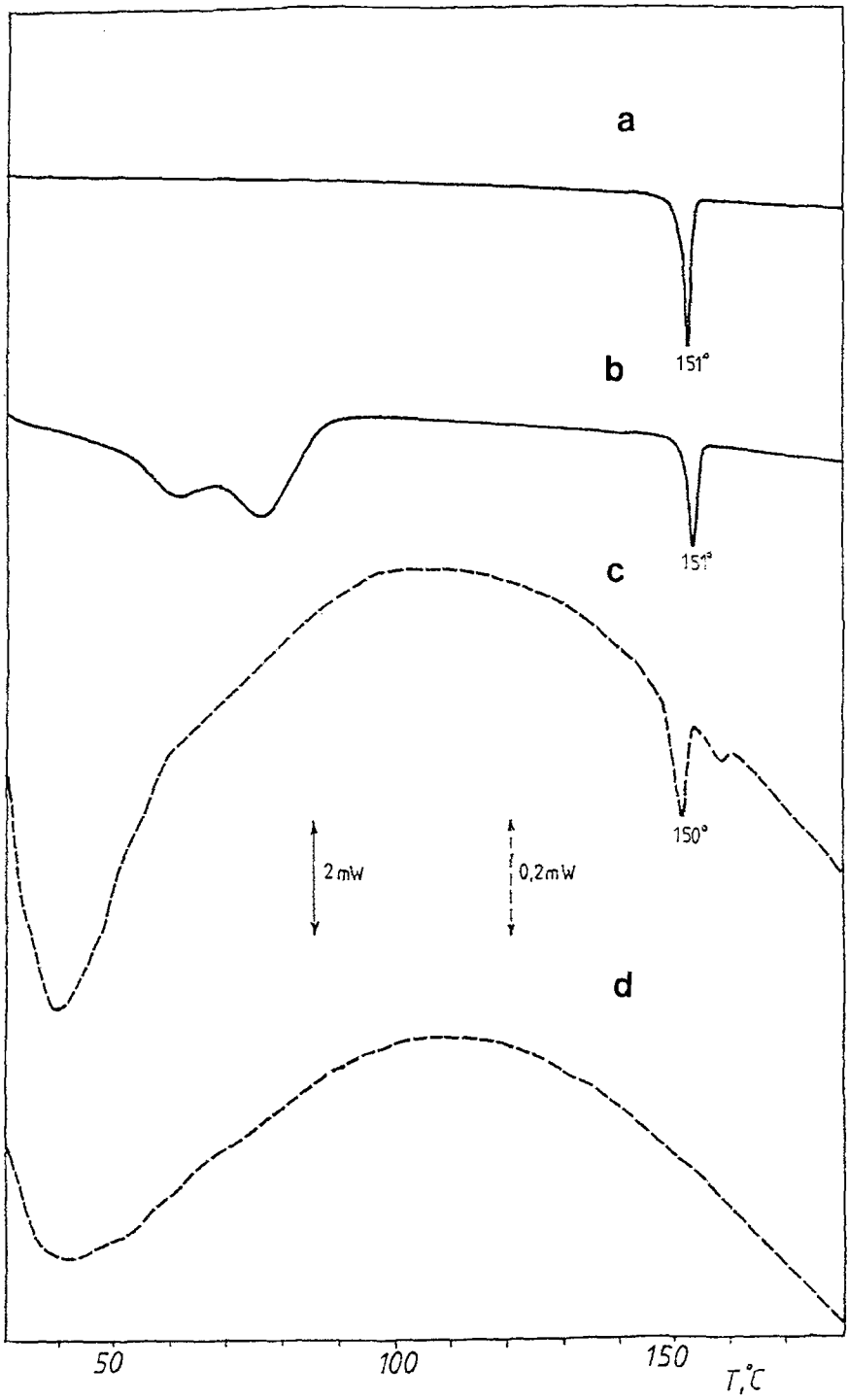

Fig. 7. DSC curves of: (a) TFN (mass: 0.503 mg, ΔH: 119 mJ/mg); (b) physical mixture with 17.2% TFN (mass: 5.146 mg, ΔH: 17.9 mJ/mg); (c) TFN/βCD complex obtained by precipitation (mass: 5.347 mg, ΔH: 2.24 mJ/mg); (d) TFN/βCD complex obtained by freeze drying (mass: 5.190 mg, ΔH: 0.11 mJ/mg).

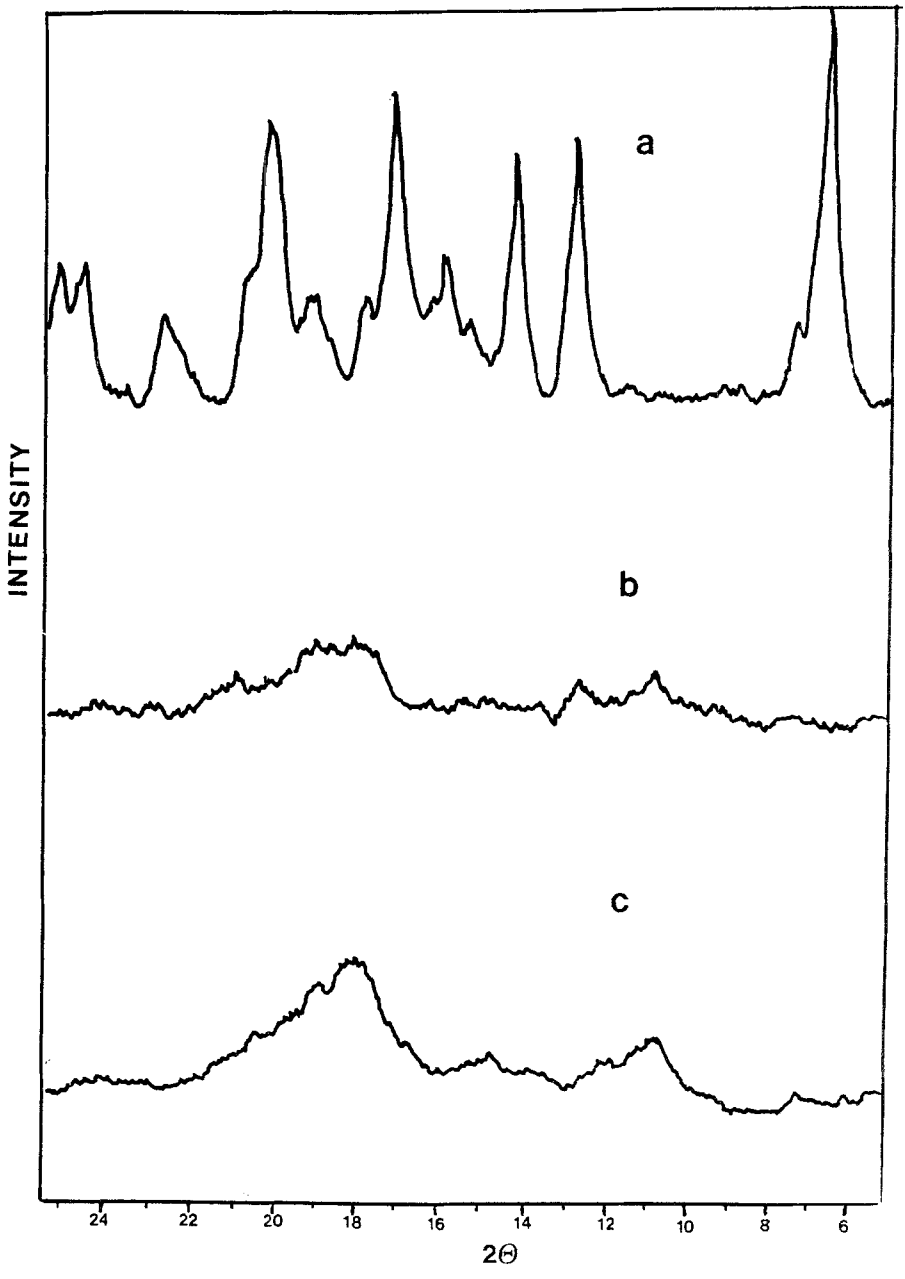

Fig. 8. X-ray diffractograms of: (a) TFN itself; (b) TFN/βCD complex obtained by precipitation; (c) TFN/βCD complex obtained by freeze drying.

4. Conclusions

The formation of the TFN/βCD complex has been demonstrated in the liquid, solid and gaseous phases by ^{1}H and ^{13}C NMR spectroscopy, powder X-ray diffractometry, DSC and FAB mass spectrometry. In solution and gaseous phases the 1 : 1 complex prevails, whereas a 1 : 2 TFN/βCD complex has been isolated by precipitation from homogeneous solution.

The association constant for the second βCD molecule is not very high, therefore in aqueous solution the 1 : 2 complex would exist only at rather high βCD concentration. On the basis of the intermolecular nOes, the geometry of the supramolecular system in solution has been hypothesized.

The preparation of the 1 : 2 TFN/βCD complex may eliminate the problems associated with the existence of various polymorphic modifications of TFN, as cyclodextrin complexation is able to 'freeze' the amorphous form of the molecule.

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