Drug Development and Industrial Pharmacy, 32:1123–1134, 2006

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600920663

informa
healthcare

Cyclodextrin Inclusion Complexes of the Central Analgesic Drug Nefopam

H. Brun, M. Paul and N. Razzouq
Laboratoire de Pharmacotechnie, Service de Pharmacie, Hôpital Henri Mondor, Créteil, France

M. Binhas
Service d' Anesthésie-Réanimation, Hôpital Henri Mondor, APHP, Créteil, France

S. Gibaud
Laboratoire de Pharmacie Clinique et Biotechniques, EA 3452, Faculté de Pharmacie, Université Nancy-1, Nancy, France

A. Astier
Laboratoire de Pharmacotechnie, Service de Pharmacie, Hôpital Henri Mondor, Créteil, France; and Laboratoire de Pharmacie Clinique et Biotechniques, EA 3452, Faculté de Pharmacie, Université Nancy-1, Nancy, France

Address correspondence to A. Astier, Pharmacy Department, Henri Mondor Hospital, 51, Av. du Maréchal de Lattre de Tassigny, 94010 Créteil cedex, France; Fax: + (33) 1-49-81-27-64; E-mail: alain.astier@hmn.ap-hop-paris.fr

ABSTRACT Inclusion complexes of nefopam base (NEF) with various β-cyclodextrins (βCDs) were investigated. All tested βCDs increased the apparent solubility of NEF according to a Higuchi A_L type plot (except βCD: A_N type plot), which indicates the formation of 1:1 stoichiometry inclusion complexes. ^{1}H-NMR and ^{13}C-NMR experiments showed that complexation by CDs allowed an easy separation of the *R* and *S* enantiomers. Based on spectral data obtained from the two-dimensional rotating frame nuclear Overhauser effect spectroscopy (2D-ROESY), a reasonable geometry for the complexes could be proposed implicating the insertion of the benzoxazocine ring into the wide end of the torus cavity.

KEYWORDS Nefopam, Cyclodextrins, Solubility diagram, MNR, ROESY

INTRODUCTION

Nefopam (NEF), 5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine, a non-narcotic drug with central analgesic effects can be used as adjuvant to morphine administration after surgery (McLintock et al., 1988: Mimoz et al., 2001). NEF has a distinct profile from that of opioides, for it induces neither tolerance nor physical dependence or respiratory depression even in the postoperative period (Heel et al., 1980). Moreover, NEF has a 50% morphine-sparing effect when administered intravenously (Mimoz et al., 2001).

Analgesia used in the postoperative period must have a rapid action to relieve patients' pain; therefore, the intravenous administration of NEF is the most adapted way to handle this pain (short T_{max}). But adverse effects (nausea, vomiting, sedation, dry mouth, tachycardia, and profuse sweating) are observed during the rapid infusion (Mimoz et al., 2001). Thus, continuous NEF administration could decrease the frequency of adverse effects.

The sublingual route could be considered a good alternative to parenteral administration. Indeed, the sublingual mucosa is relatively permeable, giving a rapid absorption and acceptable bioavailability for many drugs, and is a convenient, accessible, and generally, a well-accepted administration route (Harris & Robinson, 1992). Thus, the sublingual route seems well adapted to obtain an easy and rapid analgesia, especially in ambulatory practice (Lin et al., 2005). Moreover, drugs are delivered directly to the systemic circulation avoiding the hepatic first-pass metabolism (De Vries et al., 1991).

The commercialized drug (Acupan®, Biocodex Laboratories, Compiègne, France) is the hydrochloride salt of NEF (solubility in water = 20 g/L), which is not adapted for buccal absorption because the drug is fully protonated. Indeed, the passive diffusion of molecules through the buccal mucosa is more important for the non-ionized and liposoluble forms (Harris & Robinson, 1992). Therefore, the unprotonated base form of NEF appears to be more convenient. However, NEF base is poorly water soluble (1×10^{-2} –0.15 M), hence saliva. Thus, to improve its aqueous solubility and absorption across the buccal epithelium, complexation with cyclodextrins (CDs) could be a good approach (Mannila et al., 2005).

CDs are able to enhance the permeation of poorly soluble drugs through the biological membranes (Duchêne & Wouessidjewe, 1990; Loftsson & Brewster, 1996; Veiga et al., 1998). Sure enough, CDs increase the apparent aqueous solubility of lipophilic drugs without changing their molecular structure and their intrinsic abilities to permeate the lipophilic biological membranes. CDs act like true carriers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane (Masson et al., 1999). The relatively lipophilic membrane (buccal mucosa) has low affinity for the hydrophilic CD molecules that remained in the aqueous phase (saliva). Thus, CDs can act as penetration enhancers by increasing drug availability at the surface of the biological barriers and by influencing the membrane permeability because CDs are able to include some of the biological membrane components (phospholipids and cholesterol), thus altering their permeability (Masson et al., 1999).

The complex formation of NEF with hydroxypropyle-βCD and sulfated-βCD was only reported from a viewpoint of enantiomeric separation of mixtures of several racemic drugs by capillary electrophoresis (Heuermann & Blaschke, 1994; Ding & Fritz, 1999; Wang & Khaledi, 1999). No detailed study has ever been dedicated to study the complexation of NEF by CDs. Thus, the main purpose of the present study was the characterization of the complexes between NEF and different CDs in order to evaluate the possibility of using NEF by the sublingual route.

MATERIALS AND METHODS

Materials

The base form of racemic nefopam (5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-benzo[f][1,4] oxazocine; MW: 253.3) was kindly supplied by Biocodex Laboratories (Compiègne, France). β-Cyclodextrin (βCD), methyl-β-cyclodextrin (1.6-2.0 methyl unit per anhydroglucose unit; MeβCD), hydroxypropyl-β-cyclodextrin (degree of substitution = 4.4; HPβCD), and hydroxyethyl-β-cyclodextrin (HEβCD) were purchased from Aldrich (Saint Quentin, France). Sulfobuthylether-β-cyclodextrin (SBEβCD) was kindly donated by Cydex (Lexena, KS, USA). All other reagents were of analytical grade either from Merck Eurolab (Fontenay-sous-Bois, France) or Acros organics (Noisy-le-Grand, France) and were used as received. Demineralized water was used throughout the study.

Phase-Solubility Studies

Solubility studies were performed according to the Higuchi and Connors method (1965). An excess of NEF (0.015 and 0.15 M) was added to 5 mL of NaOH 0.1 M^{-1} solution containing various concentrations of CD (0 to 0.01 M^{-1} for βCD or 0 to 0.1 M^{-1} for βCD derivatives). To test the influence of pH on NEF complexation, HPβCD solutions at pH 7.5, 8, and 9 (Tris[hydroxymethyl]aminomethane buffer; Tris) were prepared. The mixtures were stirred in screw-capped amber vials during 2 h on a rock-and-roller agitator at 25°C. Preliminary experiments on time dependence showed that equilibrium was reached after this stirring period. Then, the solutions were centrifuged at 9000×*g* for 5 min, after filtration (0.45 μm Millipore membrane filter) and adequate dilution, NEF concentrations, in the supernatant, were determined spectrophotometrically at 262 nm (1 cm quartz cuve; Carry 50 spectrophotometer; Varian, les Ulis, France). The apparent solubility of the substrate ($[NEF]_{tot}$) was determined as a function of the added ligand concentration ($[CD]_{tot}$) (Frömming & Szejtli, 1994). Since the phase-solubility diagrams were of A_L-type for all βCD derivatives and assuming a 1:1 complex, the apparent 1:1 stability (or formation) constant K_s (or $K_{1:1}$) was calculated for each CD using the slope from the linear regression analysis of the phase-solubility isotherm using the following equation:

$$Ks = \frac{slope}{[NEF]_s \,.\, (1 - slope)} \qquad (1)$$

The inherent solubility of NEF ($[NEF]_s$) was determined in pure water under identical conditions.

Determinations were performed in triplicate and the constants were expressed as the mean ± SD.

Determination of the Partition Coefficient

The partition coefficient *P* of NEF, alone or in the presence of various CDs, was determined using octanol as the lipophilic phase and buffer solutions at pH 5 (acetate 0.36 M), pH 9 (Tris 0.1 M), and pH 13 (NaOH 0.1 M) as the hydrophilic phase. Each phase was presaturated by the other, by mixing equal volumes, vigorous shaking, and funnel separation.

Equimolar amounts of NEF and HPβCD (0.1 M) were solubilized in the buffer solutions, respectively, by stirring during 2 h. After centrifugation at 9000×*g* for 5 min, the supernatant containing the complexed NEF was sampled. Equal volumes (5 mL) of buffered solutions of free (0.1 M) or complexed NEF and octanol were mixed and shaken for 30 min with a horizontal agitator. The two phases were separated by centrifugation at 9000×*g* for 15 min and assayed spectrophotometrically as described. The partition coefficient was expressed as the logarithm of the ratio of the NEF concentration in the organic phase to that in the aqueous phase (log *P*).

Determination of Stoichiometry by Continuous Variation Method (Job's Plot)

The well-known continuous variation method (Job's plot; Job, 1928) is based on the absorbance difference, ΔA ($\Delta = A - A_0$) of NEF in absence (A_0) and in presence (A) of complexant (Dotsikas et al., 2000). The inclusion complexes of NEF were prepared by mixing, during 2 h, various amounts of NEF with aqueous solutions of HPβCD, resulting in certain molar ratios to keep the total concentration constant ($[NEF]_t + [HP\beta CD]_t = M$). The absorbances were measured at 259 nm. Subsequently, $\Delta A \times [NEF]_t$ for HPβCD was plotted against *r*, ($r = [NEF]_t / ([NEF]_t + [HP\beta CD]_t)$). The quantities $\Delta A \times [NEF]_t$ were proportional to the concentration of the complexes and the stoichiometry of the complex, which is 1:1 when $A \times [NEF]$ rise to a maximum at $r = 0.5$.

^{1}H- and ^{13}C-NMR Studies of NEF/MeβCD Complex

All experiments were performed on a Bruker Avance DRX NMR spectrometer operating at 9.4 Tesla (proton frequency: 400.133 MHz; carbon frequency: 100.62 MHz) and at a sample temperature of 300 K. The two-dimensional rotating frame nuclear Overhauser effect spectroscopy (2D-ROESY) experiments were recorded with the following parameters: mixing times 300 msec with a radiofrequency field of 8 kHz, acquisition map 2 K×256, number of scans 16; final 2D map after FT: 1 K×1 K.

The lyophilized NEF/MeβCD complex was dissolved in D_2O. For solubility reasons, spectra of free NEF were obtained in d_6-DMSO. Chemical shifts of protons were given in parts per million (ppm) relative to the solvent signal (monodeuterated water, HOD at 4.84 ppm). Since the commercial MeβCD, which was used in all experiments, contained 1.6–2.0 methyl units per anhydroglucose unit and its ^{1}H-MNR spectrum was very similar to that of heptakis (2,6-di-O-methyl)-β-cyclodextrin, chemical shift assessments and calculations were performed considering that two hydroxyl hydrogen atoms per unit were substituted, one from the primary hydroxyl group (O-6) and one from the secondary hydroxyl groups (O-2 or O-3). The hydrogen and carbon atoms from NEF and the corresponding numbering *n* are represented as H*n* or C*n*. The chemical shifts δ are expressed in ppm. Considering that the dynamic exchange between the free and the complexed forms of each enantiomer of NEF is short on the NMR time scale, the observed chemical shift signal δ_R or δ_S represents the mole fraction weighted average shift of the *R* or *S* enantiomer of the free NEF ($\delta_{R \text{ or } S \text{ (free)}}$ at χ molar fraction) and complexed NEF ($\delta_{R \text{ or } S \text{ (complexed)}}$ at $1 - \chi$ molar fraction):

$$\delta = \left[\chi \delta_{R \text{ or } S \text{ (free)}}\right] + \left[(1-\chi)\delta_{R \text{ or } S \text{ (complexed)}}\right] \quad (2)$$

Since pure enantiomers were not available, the corresponding δ of each atom was unknown, and thus, the averaged chemical shifts of the corresponding split signals, due to the enantiomeric recognition by complexation, were not attributed to the respective *R* or *S* enantiomer of the pair and were individually computed as δ_L for the lower value and δ_H for the higher value.

The complexation-induced shift for each enantiomeric pair was calculated from the following equation:

$$\Delta\delta_{H \text{ or } L} = \delta_{H \text{ or } L\ (complexed)} - \delta_{(free)} \quad (3)$$

and its average value as following:

$$\Delta\delta_{(mean)} = \tfrac{1}{2}(\Delta\delta_H + \Delta\delta_L) \quad (4)$$

The intensity of the split effect was estimated according to the equation:

$$\Delta\delta_{R/S} \mid \delta_H - \delta_L \mid \quad (5)$$

IR Study of the NEF/HPβCD Complex

Infrared spectra of mixtures prepared with molar ratios *r* (CD/NEF) from 0.2 to 5 were obtained using a Perkin Elmer FT/IR 2000 spectrometer. Increasing concentrations of HPβCD were mixed with constant concentrations of NEF for 2 h. Then 1 mL of ethanol was added to solubilize the excess of NEF. The mixtures were dried at 40°C under vacuum. After dilution of dry residues to $1/10^e$ with KCl powder, the samples were analyzed by diffuse reflectance from 4000 to 450 cm^{-1} (40 scans; Kubelka-Nunk transformation). Spectra of physical mixtures at identical ratio of NEF and HPβCD were also recorded as non-inclusion controls.

RESULTS AND DISCUSSION

Phase-Solubility Studies

The phase-solubility diagrams for the complex formation between NEF and CDs in different media (Fig. 1) were of A_N type for βCD and A_L type for HPβCD, HEβCD, MeβCD, and SBEβCD, according to the classification of Higuchi and Connors (1965). The inherent solubility of NEF, determined in 0.1 M NaOH solution (pH 13), was 3.67 ± 0.25 $g.L^{-1}$ (0.0145 ± 0.0010 M; $n = 12$).

In the case of βCD, the A_N type of solubility curve could be associated with an alteration in the effective nature of the solvent in the presence of large amounts of CDs, thus leading to a change in the complex formation constant. An alternative explanation for the A_N type curve is self-association of βCD molecules at higher concentrations (Higuchi & Connors, 1965; Loftsson & Brewster, 1996; Loftsson et al., 2002).

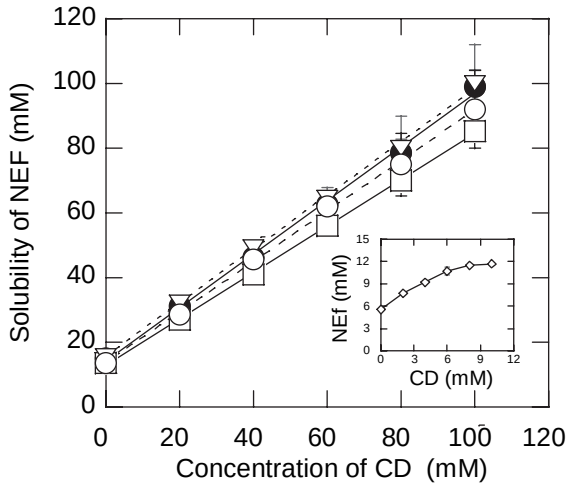


FIGURE 1 **Phase-Solubility Diagrams for NEF in the Presence of HPβCD (-●-), MeβCD (-Δ-), HEβCD (-□-), SBEβCD (-○-) at pH13 (NaOH 0.1 M; 25°C). Inset: Diagram in the Presence of βCD. Each Point Represents the Mean ± SD of 3 Determinations.**

Similar results were obtained for miconazole/βCD complex (Tenjarla et al., 1998) and for dexamethasone/βCD complex (Vianna et al., 1998).

For all CD derivatives, the solubility of NEF increased linearly as a function of the CD concentration (type A_L). This linear host/guest correlation with slope lower than 1 suggested that the complexes could be of the first order with respect to CDs (1:1 stoichiometry). The apparent 1:1 stability constants (*Ks*) were higher for HPβCD and MeβCD (346.2 ± 8.0 M^{-1} and 302.0 ± 5.6 M^{-1}, respectively) than for HEβCD and SBEβCD (197.9 ± 2.1 M^{-1} and 276.4 ± 12.7 M^{-1}, respectively), indicating more stable complexes with dimethyl and hydroxypropyl derivatives.However, it has been demonstrated that the slope of a phase-solubility diagram, in a drug/cyclodextrin system, could be linear despite that enhanced solubility occurred through both inclusion and non-inclusion processes, such as complex aggregates or micellar formation (Loftsson et al., 2002). Thus, the A_L-type isotherm does not necessarily demonstrate the formation of an inclusion complex and other experiments are required to ascertain the process. Nevertheless, the complexation efficiency of these CDs, as defined by the product $S_0 \times K_{1:1}$ (Loftsson et al., 1999, 2002), was about 0.50. The solubility enhancement factor, as defined by the ratio NEF solubility in 1×10^{-1} M solution of CD to its inherent solubility S_0, ranged from 5.9 to 6.9. This limited factor is due to the relatively high water solubility of NEF base and, thus, coherent with the well-admitted principle claiming that the higher the aqueous solubility of the

pure drug, the lower the relative solubility enhancement by CD complexation (Loftsson & Brewster, 1996).

The similar binding constant values suggest that the solubilization process was identical among the different CD derivatives by the formation of inclusion complexes, regardless of the substitution of the external hydrogen atoms of the toroidal shape. Moreover, despite the basic properties of NEF, the charged sulfonated SBβCD exhibited no better complexing efficiency than neutral derivatives.

The influence of pH on the apparent stability constant of NEF/HPβCD complex is presented in Fig 2. The decrease of pH led to a decrease in the apparent stability constants, indicating that apparent stability constants of NEF inclusion complexes were higher in the less ionized form (pKa of NEF = 9.3). Ionized molecules, with lower hydrophobicity, should produce weaker interactions with the hydrophobic cavities of CDs than the unionized ones. However, although CDs demonstrated lower affinity to the ionized form of NEF, both forms of NEF can be complexed. Similar results were observed for the complexation of tolbutamide (Veiga et al., 1996) and nicardipine (Fernandes et al., 2002).

Determination of the Partition Coefficient

The lipophilicity values determined at pH 5, 9, and 13 for NEF, alone or in the presence of an equimolar amount of HPβCD, are reported in Table 1. first mention At every pH value examined, the apparent lipophilicity of NEF in the presence of HPβCD was lower than this of NEF alone, confirming the capability of CD to enhance the apparent hydrophilicity of NEF via the formation of a complex. Interestingly, this effect was evident not only at pH 13, where NEF is non-ionized but also at pH 5, where nefopam is fully ionized. This suggests that the ionized NEF can form a complex with HPβCD, which further increases the hydrophilicity of the drug. However, HPβCD presents a greater attraction for the lipophilic form since the difference of the log *P* in the presence and absence of CDs was greater at pH 13 (log $P = 1.01$) than at pH 5 (log $P = 0.39$) and pH 9 (log $P = 0.35$). Similar results were observed with papaverine (Ventura et al., 1998), nimesulide (Miro et al., 2000), and dexamethasone (Vianna et al., 1998).

TABLE 1 **Logarithm of the Partition Coefficient *P* of Nefopam (NEF) Alone and in the Presence of Hydroxypropyl-β-CD (HPβCD) at pH 5, 9, and 13**

	pH 5	pH 9	pH 13
NEF/HPβCD	−0.65 ± 0.01	0.53 ± 0.03	0.26 ± 0.06
NEF	−0.26 ± 0.01	0.88 ± 0.05	1.27 ± 0.14

Each point represents the mean ± SD ($n = 3$).

According to the general rule that the ionized form of a molecule is less lipophilic than the respective neutral form, the apparent lipophilicity of NEF alone decreased as the pH of the buffer decreased. In the presence of CDs, the log *P* of NEF decreased as the pH decreased, except at pH 9. This could be explained by weaker interactions between the NEF and the CDs, which promotes an easier dissociation of the complex and the subsequent migration of the basic form to the organic phase.

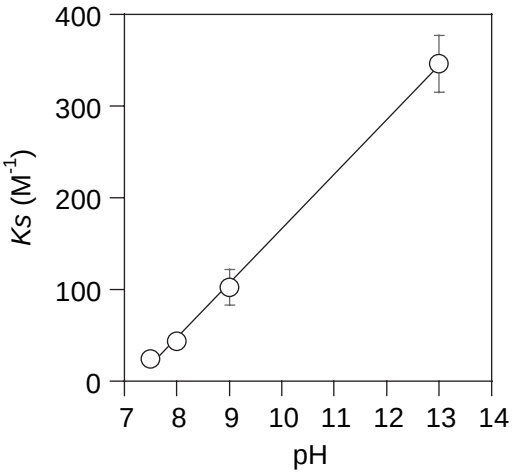


FIGURE 2 **pH Influence on the Apparent Stability Constant *Ks* of NEF/HPβCD Complex. Each Point Represents the Mean ± SD of 3 Determinations.**

Determination of the Complex Stoichiometry by Continuous Variation Method (Job's Plot)

The postulated 1:1 stoichiometry of the complexes was ascertained by the continuous variation method using UV-spectrophotometry. Since the complexation of NEF by increasing concentrations of CDs induced a proportional hypochromic effect at 259 nm, the calculated quantity $\Delta A[NEF]$ was used as the concentration-dependant physical parameter to construct the corresponding Job's plot.

As presented in Fig. 3, the plot shows a maximum value at $r = 0.5$ and a highly symmetrical shape, which

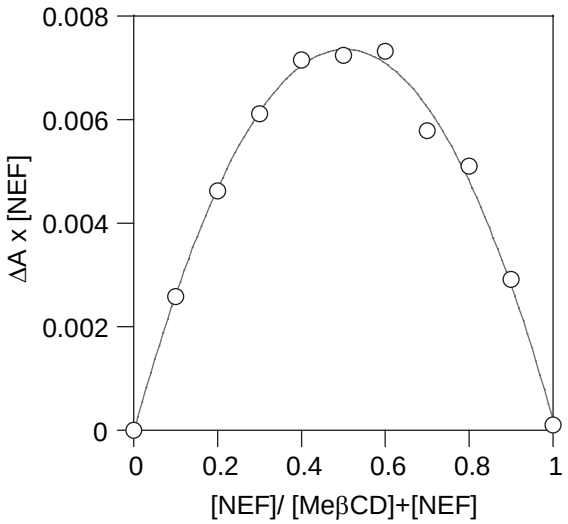


FIGURE 3 **Continuous Variation Plot (Job's Plot) of NEF/ HPβCD Inclusion Complex. The Total Concentration of NEF and CD was 1.0×10^{-2} M in NaOH 0.1 M. $\Delta A = A - A_0$: The Difference of Absorbance at 259 nm With (A) and Without (A_0) Cyclodextrin.**

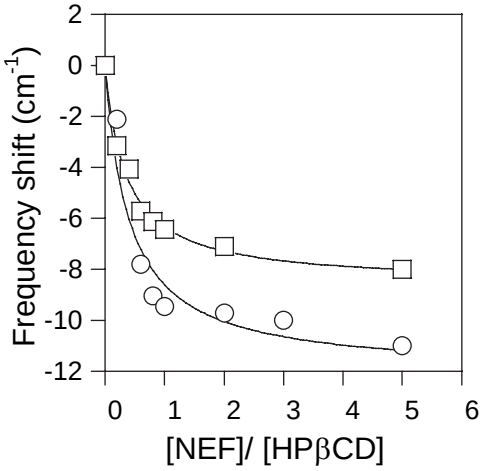


FIGURE 4 **Vibration Frequency Shifts of Two Characteristic Absorption Bands of NEF as a Function of the Molar Ratio NEF / HPβCD. Bands at 865.3 cm^{-1} (-○-) and 954.9 cm^{-1} (-□-). FT-IR Spectra of the Lyophilized Complexes Were Recorded in KCl by Diffuse Reflectance.**

demonstrates the existence of a NEF/HPβCD complex with a 1:1 stoichiometry and confirming therefore the assumption made from the phase-solubility diagrams.

IR Studies

FT-IR spectra of the solid inclusion complexes, obtained by freeze-drying of an equimolar solution of NEF and HPβCD, revealed the most significant changes in the NEF spectrum in the range 450–1000 cm^{-1}. Titration of NEF by increasing concentrations of HPβCD induced proportional bathochromic shifts of the absorption bands at 865.5 cm^{-1} and 954.9 cm^{-1} (tentatively attributed to antisymmetric stretching vibrations of the oxazocine ring), whereas physical mixtures at identical ratio showed no significant modification (Fig. 4). first mention Moreover, the easy data fitting to a hyperbolic model gave additional evidence for a 1:1 stoichiometry complex. This effect could also suggest an inclusion scheme involving the benzoxazocine ring of NEF.

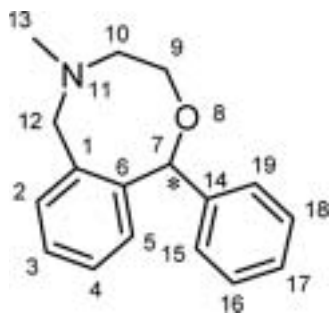
NMR Studies

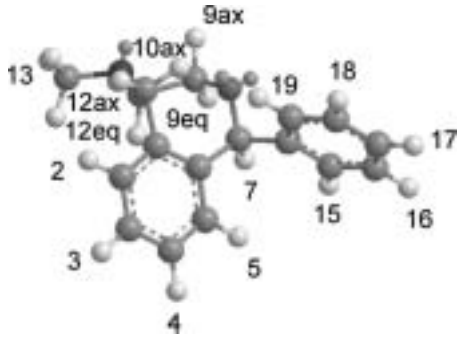
NMR spectroscopy is one of the most useful methods to obtain information about the geometry of inclusion complexes (Djedaïni et al., 1990; Ganza-Gonzalez et al., 1994; Roselli et al., 1999; Kim et al., 2004). Thus, several ^{1}H and ^{13}C NMR experiments were performed using an equimolar solution of racemic NEF and MeβCD, HPβCD, or HEβCD. For practical reasons, only the complex with MeβCD was studied extensively. However, very similar qualitative results were obtained with other CDs. Tables 2 and 3 show ^{1}H and ^{13}C chemical shifts of NEF in the presence and the absence of MeβCD. For an easy visualization of the data, the 2D and 3D structures of NEF and its *R* and *S* enantiomers with the proton numbering use were inserted into Table 2. The corresponding spectra are represented in Fig. 5.

The ^{1}H NMR spectrum was assigned by comparing the dipolar correlation figures from the ROESY map and considering that the conformation most favorable energetically for the oxazocine ring is a twisted chair with the bulky methyl group corresponding to N-CH_3 in equatorial position (MM2 force field method; Chem3D Ultra 8.0 software, Cambridge Soft, Cambridge, MA, USA).

In the presence of MeβCD, all distinguishable proton signals were split into two peaks of equal intensity (proton H7 attached to the asymmetric carbon atom, protons H2, H12, and H13 from N-CH_3 group). As demonstrated by ^{13}C NMR experiments, all carbon peaks were also split except the C13. Since NEF is a racemic mixture and MeβCD is a chiral selector, these findings can be explained by the formation of two separate complexes with different geometry and magnetic environment, which leads to nonequivalent complexation-induced chemical shifts of (*R*)-NEF and (*S*)-NEF

TABLE 2 ^{1}H Chemical Shifts of Racemic Nefopam (NEF) in the Presence (Complex) and the Absence (Free) of Methyl-β-CD (MeβCD)





	R-NEF			*S*-NEF			
Position and number of protons		$\delta_{(complex)}$		$\Delta\delta$			
	$\delta_{(free)}$	δ_L	δ_H	$\Delta\delta_L$	$\Delta\delta_H$	$\Delta\delta_{(mean)}$	$\Delta\delta_{R/S}$
H13 (3H)	*s* 2.32	*d* 2.40	*d* 2.42	+0.10	+0.12	+0.11	0.02
$H10_{eq}$	*dd* 2.50	*dd* 2.74	*dd* 2.76	+0.24	+0.26	+0.22	0.02
$H10_{ax}$	*dd* 2.70	*dd* 2.80	*dd* 2.86	+0.10	+0.16	+0.13	0.06
$H9_{eq}$	*d* 3.80	*d* 3.88	*d* 3.92	+0.08	+0.12	+0.10	0.04
$H9_{ax}$	*d* 4.00	*d* 4.05	*d* 4.28	+0.05	+0.28	+0.17	0.04
$H12_{ax}$	*d* 3.70	*d* 3.77	*d* 3.87	+0.07	+0.17	+0.12	0.13
$H12_{eq}$	*d* 4.70	*d* 4.47	*d* 4.67	–0.23	–0.03	–0.13	0.20
H7	*s* 5.86	*s* 5.84	*s* 5.90	–0.02	+0.04	+0.02	0.06
H5	*d* 7.00	*d* 6.90	*d* 6.96	–0.10	–0.04	–0.12	0.06
H2–4 (3H)	*m* 7.16	*m* 7.16 (n.r.)		n.d.		n.d.	n.d.
H15–19 (5H)	*m* 7.33	*m* 7.38 (n.r.)		+0.05		+0.05	n.d.

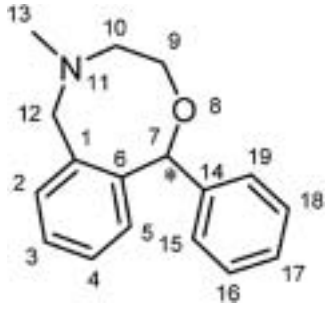
Chemical shifts (δ) are expressed in ppm; $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$; δ_H and δ_L = higher and lower δ values for each *R/S* diastereomer pair. $\Delta\delta_{R/S} = |\delta_H - \delta_L|$; *s* = singlet; *d* = doublet; *dd* = double doublet; *m* = multiple. n.r. = not resolved. n.d. = not determined.

with MeβCD. The formation of these diastereomeric pairs of complexes strongly confirms the postulated inclusion process since the chiral discrimination properties of CDs could be observed only if the guest molecule is inserted into the host cavity (Chankvetdaze et al., 1995; Fanali, 2000; Lai et al., 2003; Dodziuk et al., 2004). Furthermore, in earlier works, the separation of NEF enantiomers was obtained by capillary electrophoresis using HPβCD or sulfated β-CD as chiral selector (Heuermann & Blaschke, 1993; Ding & Fritz, 1999; Wang & Khaledi, 1999).

Since only two peaks were obtained for each nucleus, this result indicates that the dynamic complexation process was fast on the NMR time scale and that only averaged signals of the involved species were observed, as described in all cases pertaining to enantioseparation by CDs (Dodziuk et al., 2004). Since most observations of splitting due to CD-induced chiral recognition pertain to ^{1}H, there are only few studies describing this effect in ^{13}C spectra, especially with drugs (Chankvetadze et al., 1995, 2000; Lai et al., 2003). With NEF, both the ^{1}H and ^{13}C signals were clearly duplicated. The complexation-induced shifts ranged from –0.23 to +0.28 ppm for ^{1}H and from –1.6 to +0.5 ppm for ^{13}C (Tables 2 and 3). Comparable values were observed for ^{13}C signals during the chiral recognition of (±) α-pinene with α-CD, but lower values were observed for ^{1}H signals (Dodziuk et al., 2000).

The absolute difference between the chemical shifts of the respective *R* and *S* enantiomers $\Delta\delta_{R/S}$ (Eq. [5]) was more pronounced for the protons H7, H10', and H12,12′ and the carbon atoms C10, C12, and C7, corresponding to the oxazocine cycle, than for those located on the phenyl side. These findings could suggest the occurrence of increased geometrical constraints and decreased flexibility of the molecule, with limited rotation of the phenyl ring around the C7-C14 bond and strong involvement of the oxazocine ring in the inclusion process. Indeed, for a particular enantiomer, the amplitude of the signal separation of each atom, reflected by $\Delta\delta_{R/S}$, should depend on the extent of its chemical environment modification induced by

TABLE 3 ^{13}C Chemical Shifts of Nefopam (NEF) in the Presence (Complex) and the Absence (Free) of Methyl-β-CD (MeβCD)



Position of carbon	$\delta_{(free)}$	$\delta_{(complex)}$		$\Delta\delta$		$\Delta\delta_{(mean)}$	$\Delta\delta_{R/S}$
		δ_L	δ_H	$\Delta\delta_L$	$\Delta\delta_H$		
C13	44.2	44.0		–0.2		0.2	
C12	54.3	53.0	54.0	–1.3	–0.3	–0.8	1.0
C10	58.5	57.0	58.0	–1.5	–0.5	–1.0	1.0
C9	68.4	67.0	67.5	–1.4	–0.9	–1.1	0.5
C7	83.2	82.0	83.0	–1.2	–0.2	–0.7	1.0
C3–5,15–18	n.r. (127.7 to 129.1)	n.r. (127.0 to 129.1)		n.d		n.d.	n.d.
C19	133.7	133.5	133.9	–0.2	–0.2	0.0	0.4
C1	134.4	134.4	134.6	0.0	+0.4	+0.2	0.4
C2	134.7	134.7	134.9	0.0	+0.2	+0.1	0.2
C14	142.6	141.0	141.2	–1.6	–1.4	–1.5	0.2
C-6	144.1	143.0	143.2	–1.1	–0.9	–1.0	0.2

Chemical shifts (δ) are expressed in ppm. $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$. δ_H and δ_L = higher and lower values of δ for each *R/S* diastereomer pair. $\Delta\delta_{R/S} = |\delta_H - \delta_L|$. n.r.= not resolved; n.d.= not determined.

the complexation. Moreover, the signal split also depends on the respective binding constants and on the kinetics of host/guest interaction. However, since the binding of the racemic with a chiral selector is a prerequisite for efficient enantioseparation, no proportional relation can necessarily exist between the stereoselectivity and the average binding strength, estimated by the determination of the pseudo-first-order constant *Ks* by solubility diagrams. Thus, the enantioseparation efficiency seems more likely related to the difference between the binding strength of each enantiomer (Chankvetdaze et al., 1995).

Beside the peak splitting due to the enantiomeric separation in the presence of MeβCD, NEF proton signals also displayed significant shifts. Irrespectively of the individual shift of each enantiomer,the sign and the intensity of $\Delta\delta_{(mean)}$ (Eq. [4]) of one enantiomeric pair reflect the modification of the chemical environment by complexation. Therefore, this parameter was used to study the inclusion process. Downfield shifts were observed for all protons except $H12_{eq}$ and H5. The most affected protons were $H10_{ax}$, $H10_{eq}$, $H12_{ax}$, $H12_{eq}$, and H13 ($N\text{-}CH_3$). The downfield displacement of a guest proton is a sign of weaker interactions with hydrogen atoms of the CDs, corresponding to a deshielding effect due to the Van der Walls forces (Djedaïni et al, 1990), the variation in the local polarity when these protons are inserted into the cavity (Djedaïni et al., 1990; Ganza-Gonzales et al., 1994; Dollo et al., 1996; Ventura et al., 1998; Faucci et al., 2000; Fernandes et al., 2003) or steric perturbations induced by the inclusion process. Interestingly, the protons H2 to H4 from the aromatic moiety of the benzoxazocine ring were not affected and the protons from the phenyl group (H15 to H19) and the proton H7, connected to the asymmetric carbon C7, were only slightly displaced. The upfield shifts of protons $H12_{eq}$ and H5 could suggest an association with some oxygen atoms of the CDs, rich in π electrons (Ganza-Gonzales et al., 1994).

Upfield shifts were observed in all carbon nuclei by ^{13}C NMR experiments. As for protons, the aromatic carbons were only slightly affected and the carbons corresponding to the heteroatomic ring were strongly

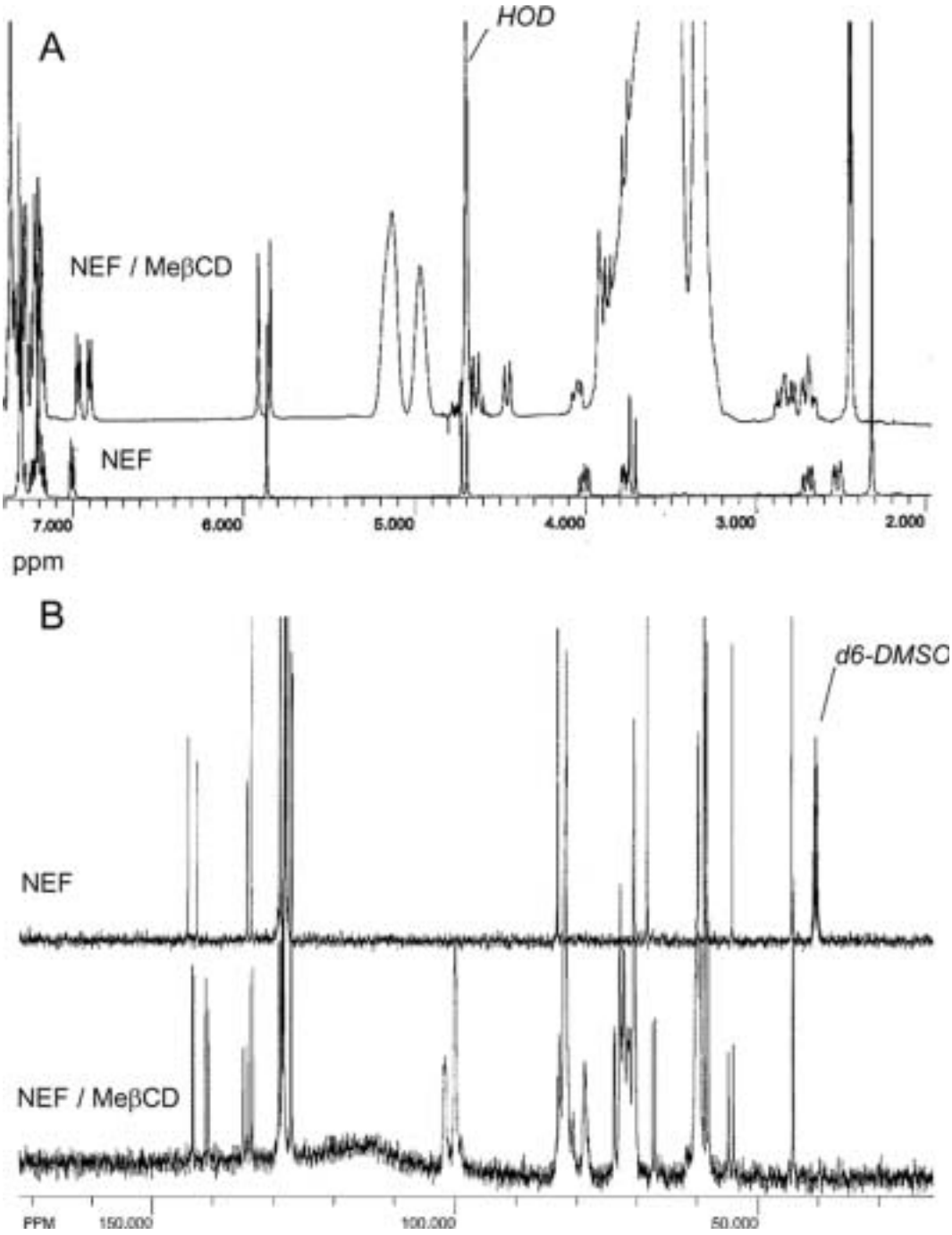


FIGURE 5 ^{1}H-NMR (A) and ^{13}C-NMR (B) Spectra of 1:1 NEF/MeβCD Complex. Spectra Were Recorded in d_6-DMSO (NEF) and D_2O (Complexes) at 300 K. Structures of NEF Enantiomers With the Corresponding Numbering Are Presented in Table 2.

shifted. These findings suggest that the oxazocine ring is more involved in the insertion into the CD cavity than the aromatic rings. These results are also in accordance with the requirement that, in order to obtain an efficient chiral recognition by CDs, the chiral center of the guest molecule must be located close of the entrance of the cavity (Gosnat et al., 1995). However, since the nefopam spectra were recorded in different solvents for its free and complexed forms for solubility reasons, a solvent effect cannot be ruled out for the differences in δ values.

Despite the fact that the peak assignments obtained with the randomly methylated βCD, used throughout experiments, were in good agreement with those previously reported by Correia et al. (2002) for heptakis (2,6-di-O-methyl)-β-cyclodextrin (DMβCD), only a

tentative assignment for the chemical shifts of H2 to H5 protons were made. Indeed, MeβCD consists of a mixture of several closely related derivatives producing broadening of NMR peaks. Furthermore, the spectral region ranging from 3.50 to 4.00 ppm corresponds to the H3′ and H5′ protons of MeβCD (H3-CD or H5-CD) and to the $H9_{eq}$, $H9_{ax}$, and $H12_{ax}$ protons from NEF, leading to some overlapped peaks. However, the peak assessment of the proton H1′ (H1-CD) and those from the 2- or 3-OCH_3 and 6-OCH_3 groups of the CD could be considered as unambiguous. Although the protons from the 2- or 3-OCH_3 group (wide end of the cavity) were strongly shifted downfield ($\delta = +0.18$ ppm), those from the 6-OCH_3 group (narrow end) were only slightly affected (+0.03 ppm). Moreover, the H1-CD located at the exterior of the torus was not significantly affected ($\delta < 0.01$ ppm). These findings strongly suggest an interaction between the guest molecule and the CD, implicating the wide end rather than the narrow end of the cavity.

Considering the difficulty to ascertain the chemical shifts of the H3′ and H5′ protons of MeβCD (H3-CD or H5-CD) by 1D experiments, a two-dimensional ROESY study was conducted to evaluate the inclusion geometry of the NEF/MeβCD complexes. Figure 6 first mention shows partial contour plots of ROESY spectra for the NEF/MeβCD system. The $H12_{eq}$ proton of one of the enantiomer pair ($\delta_L = 4.47$ ppm), the H7 proton (both enantiomers) and the H13 protons of one enantiomer ($\delta_H = 2.42$ ppm) gave cross interactions with a CD resonance at $\delta = 3.86$–3.90 ppm. This region corresponds to the internal H3-CD proton located near the wide side of the MeβCD cavity (reported δ for DMβCD = 3.94 ppm; Correia et al., 2002). Furthermore, additional interferences were observed between the 3.77–3.81 ppm spectral region of the CD (attributed to the H5-CD proton located near the narrow rim; reported value = 3.87 ppm for DMβCD) and the $H12_{eq}$ proton of the other enantiomer (δ_H=4.67 ppm), the H7 proton of one enantiomer (δ_L=5.84 ppm), and the H13 protons of the other enantiomer ($\delta_L = 2.40$ ppm). The H2-H4 aromatic protons (benzoxazocine ring) gave evidence of spatial proximity with the 2- or 3-OCH_3 protons of the CD but not

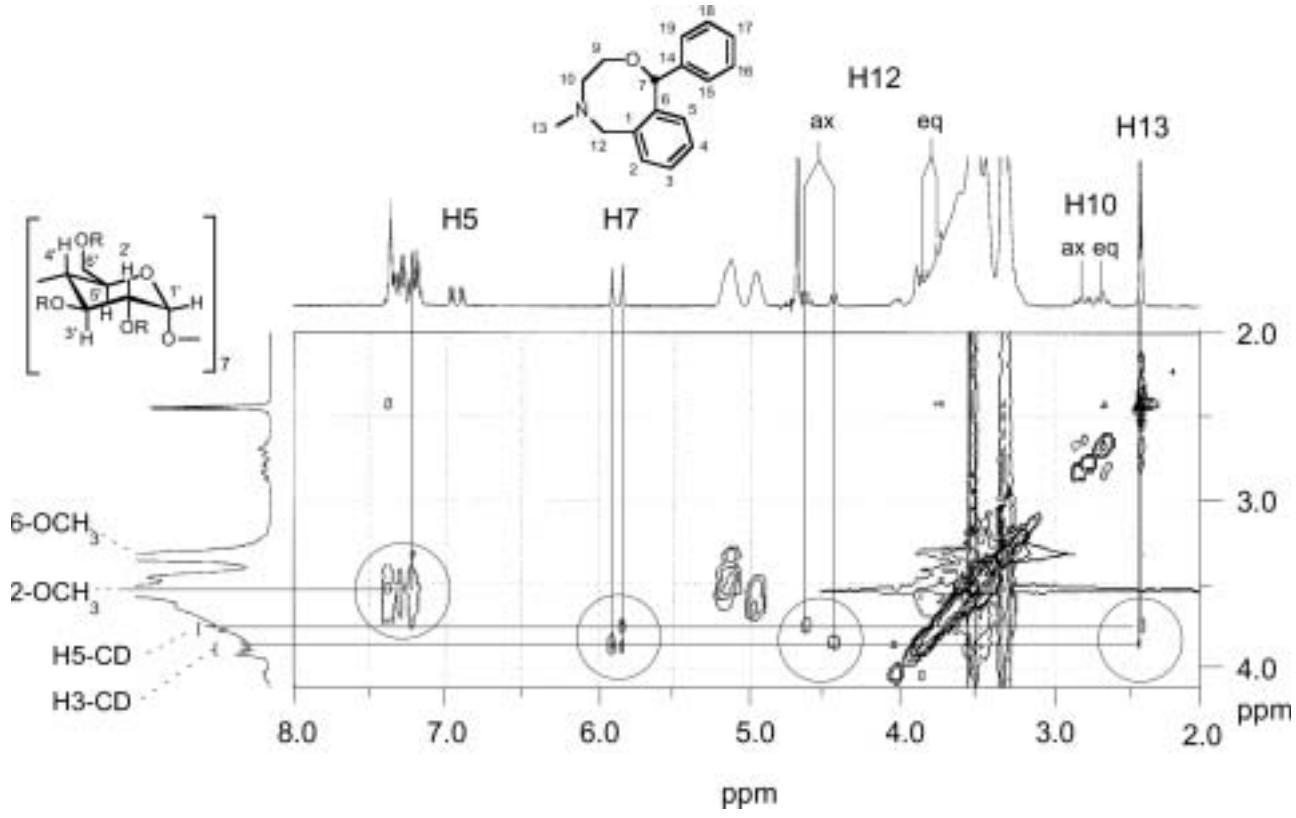


FIGURE 6 **Partial Contour Plot of ROESY Spectrum of NEF/MeβCD in D_2O at 300 K. The Circles and Lines Indicate the Areas of Interaction Between Several Protons of NEF and MeβCD. The Chemicals Shifts of H3-CD and H5-CD Were Tentatively Assigned Considering MeβCD as Heptakis (2,6-di-O-methyl)-β-Cyclodextrin.**

significantly with the 6-OCH_3 protons located at the narrow rim or with the H3-CD and H5-CD regions. Moreover, the H5 proton gave no cross peak with any other proton.

These findings demonstrate that the geometry of the drug moiety inserted into the MeβCD cavity strongly differs between both NEF enantiomers, as indicated by the peak splits and the corresponding marked complexation-induced shift observed for most of the drug protons. These 2D-ROESY results confirm also that NEF was included from the wide side by its benzoxazocine moiety, as suggested by 1D experiments. However, a complex cross-peak area was observed between H15, 19 protons from the phenyl group, and most of CD protons, including mainly the 2- and 3-OCH_3 protons but also partly the 6-OCH_3 protons, which could reflect a possible insertion of this aromatic ring into both wide and narrow sides of the cavity. This hypothesis, which implies the formation of several alternative complexes, is not supported by the results issued from phase-solubility diagrams, 1D ^1H and ^{13}C experiments.

CONCLUSION

This study concludes that the complexation of NEF by various βCD derivatives results in a significant increase of its water solubility by the formation of true inclusion complexes. All studied CDs produced a concentration-dependent linear increase of NEF water solubility by forming 1:1 stoichiometry inclusion complexes (except for βCD). The formation of complexes was pH dependent. Both non-ionized and ionized forms of NEF can interact with the CDs, but the complexes were more stable with the non-ionized form of NEF. ^1H-NMR, ^{13}C-NMR, and 2D ROESY studies provided strong evidence that the interaction was a true inclusion phenomenon.

The easy enantiomeric selection by the complexation implies sufficient differences between the geometry of the diastereomer pairs. There is no significant difference in binding affinities and basic complexation mechanisms among the different CDs. Based on the spectral data, a reasonable geometry of the complexes could be proposed, implicating the insertion of the benzoxazocine ring into the wide end of the torus cavity of the CDs. In addition, our results show that the enantioselective complexation of Nefopam did not lead to differences in its overall solubility. However, the release of each enatiomers from the corresponding complex could be different and, thus, influencing their bioavailability.

The understanding of the interaction mechanism and the demonstration of the similarities between the complexing capacities of several βCDs were prerequisites to evaluate extensively the possible enhancement of the sublingual bioavailability of NEF. In particular, ex vivo experiments, using a model of isolated pig buccal epithelium, are showing preliminary results that suggest an improved absorption of NEF from their CD complexes.

ACKNOWLEDGEMENTS

We gratefully acknowledge Biocodex Laboratories and Cydex Laboratories for the generous supplies of nefopam and SEBβCD, respectively.

We thank Pr. P. Mutzenhardt and Pr. D. Canet, "Service Commun de RMN, Université Henri Poincaré Nancy 1, Faculté des Sciences, BP 239, 54506 Vandœuvre-lès-Nancy, France," for their help in the NMR measurements and the very fruitful discussions.

REFERENCES

Chankvetadze, B., Endresz, D., Bergenthal, D., & Blaschke, G. (1995). Enantioseparation of mianserine analogues using capillary electrophoresis with neutral and charged cyclodextrins buffer modifiers. ^{13}C study of the chiral recognition mechanism. *J. Chromatrogr. A., 717,* 245–253.

Chankvetadze, B., Burjanadze, N., Pintore, G., Bergenthal, D., Bergander, G., Mülenbrock, C., Breitkreuz, J., & Blaschke, G. (2000). Separation of brompheniramine enantiomers by capillary electrophoresis and study of chiral recognition mechanisms of cyclodextrins using NMR-spectroscopy, UV spectrometry, electrospray ionization mass spectrometry and X-ray crystallography. *J. Chromatogr. A., 878,* 471–484.

Correia, I., Bezzenine, N., Ronzani, N., Platzer, N., Beloeil, J. C., & Doan, B. T. (2002). Study of inclusion complexes of acridine with β- and (2,6-di-o-methyl)-β-cyclodextrin by use of solubility diagrams and NMR spectroscopy. *J. Phys. Org. Chem., 15,* 647–659.

De Vries, M. E., Boddé, H. E., Coos Verhoef, J., & Junginger, H. E. (1991). Developments in buccal drug delivery. *Crit. Rev. Ther. Drug Carrier Syst., 8,* 271–303.

Ding, W., & Fritz, J. S. (1999). Carbamate chiral surfactants for capillary electrophoresis. *J. Chromatogr. A, 831,* 311–320.

Djedaïni, F., Lin, S. Z., Perly, B., & Wouessidjewe, D. (1990). High-field nuclear magnetic resonance techniques for the investigation of a β-cyclodextrin: indometacin inclusion complex. *J. Pharm. Sci., 79,* 643–646.

Dollo, G., Le Corre, P., Chevanne, F., & Le Verge, R. (1996). Inclusion complexation of amide-typed local anaesthetics with β-cyclodextrin and its derivatives. I. Physicochemical characterization. *Int. J. Pharm., 131,* 219–228.

Dotsikas, Y., Kontopanou, E., Allagiannis, C., & Loukas, Y. L. (2000). Interaction of 6-p-toluidinylnaphtalene-2-sulphonate with β-cyclodextrin. *J. Pharm. Biomed. Anal., 23,* 997–1003.

Dodziuk, H., Kozminski, W., Lukin, O., & Sybilska, D. (2000). NMR manifextations and molecular dynamics modelling of chiral recognition of α-pinene by α-cyclodextrins. *J. Mol. Struct., 523,* 205–212.

Dodziuk, H., Kozminski, W., & Ejchart, A. (2004). NMR studies of chiral recognition by cyclodextrins. *Chirality.,* 90–105.

Duchêne, D., & Wouessidjewe, D. (1990). Pharmaceutical uses of cyclodextrines and derivatives. *Drug. Dev. Ind. Pharm., 17*, 2487–2499.

Fanali, S. (2000). Identification of chiral drug isomers by capillary electrophoresis. *J. Chromatogr. A., 875,* 89–122.

Faucci, M. T., Melani, F., & Mura, P. (2000). 1H-NMR and molecular modelling techniques for the investigation of the inclusion complex of econazole with α-cyclodextrin in the presence of malic acid. *J. Pharm. Biomed. Anal., 23,* 25–31.

Fernandes, C. M., Vieira, M. T., & Veiga, F. J. B. (2001). Physicochemical characterization and in vitro dissolution behavior of nicardipine-cyclodextrins inclusion compounds. *Eur. J. Pharm. Sci., 15,* 79–88.

Fernandes, C. M., Carvalho, R. A., Pereira da Costa, S., & Veiga, F. J. B. (2003). Multimodal molecular encapsulation of nicardipine hydrochloride by β-cyclodextrin, hydroxypropyl-β-cyclodextrin and triacetyl-β-cyclodextrin. Structural studies by ^{1}H NMR and ROESY experiments. *Eur. J. Pharm. Sci., 18,* 285–296.

Frömming, K. H., & Szejtli, J. (1994). *Cyclodextrin in Pharmacy*. Dordrecht: Kluwer Academic.

Ganza-Gonzales, A., Vila-Jato, J. L., Anguinao-Igea, S., Otero-Espinar, F. J., & Blanco-Mendez, J. (1994). A proton nuclear magnetic resonance study of the inclusion complex with β-cyclodextrin. *Int. J. Pharm., 106,* 179–185.

Gosnat, M., Djedaïni-Pilard, F., & Perly, B. (1995). Etude par RMN de la reconnaissance chirale par des cyclodextrines modifiées. *J. Chim. Phys., 92,* 1777–1781.

Harris, D., & Robinson, J. R. (1992). Drug delivery via the mucous membranes of the oral cavity. *J. Pharm. Sci., 81,* 1–10.

Heel, R. C., Brogden, R. N., Pakes, G. E., Speight, T. M., & Avery, G. S. (1980). Nefopam: a review of its pharmacological properties and therapeutic efficacity. *Drugs, 19,* 249–267.

Heuermann, H., & Blaschke, G. (1994). Simultaneous enantioselective determination and quantification of dimethindene and its metabolite N-demethyl-dimethindene in human urine using cyclodextrins as chiral additives in capillary electrophoresis. *J. Pharm. Biomed. Anal., 12,* 753–760.

Higuchi, T., & Connors, K. A. (1965). Phase-solubility techniques. *Adv. Anal. Chem. Instr., 4,* 117–212.

Job, P. (1928). Recherches sur la formation de complexes minéraux en solution et sur leur stabilité. *Ann. Chem., 9,* 113–114.

Kim, J. H., Lee, S. K., Ki, M. H., Choi, W. K., Ahn, S. K., Shin, H. J., & Hong, C. I. (2004). Development of parenteral formulation for a novel angiogenesis inhibitor, CKD-732 through complexation with hydroxypropyl-[beta]-cyclodextrin. *Int. J. Pharm., 272,* 79–89.

Lai, S., Locci, E., Piras, A., Porcedda, S., Lai, A., & Marongiu, B. (2003). Imazil-cyclomaltoheptaose (β-cyclodextrin) inclusion complex: preparation by supercritical carbon dioxide and ^{13}C CPMAS and 1H NMR characterization. *Carbohydr. Res., 338,* 2227–2232.

Lin, Y. H., Hwang, J. L., Huang, L. W., & Chen, H. J. (2005). Use of sublingual buprenorphine for pain relief in office hysteroscopy. *J. Minim. Invasive Gynecol., 12,* 347–350.

Loftsson, T., & Brewster, M. E. (1996). Pharmaceutical applications of cyclodextrins. l. Drug solubilisation and stabilization. *J. Pharm. Sci., 85,* 1017–1025.

Loftsson, T., Magnusdottir, A., Masson, M., & Sigurjonsdottir, J. F. (2002). Self-association and cyclodextrin solubilization of drugs. *J. Pharm. Sci., 91,* 2307–2316.

Loftsson, T., Masson, M., & Sigurjonsdottir, J. F. (1999). Methods to enhance the complexation efficiency of cyclodextrins. *S.T.P. Pharma. Sci., 9,* 237–242.

Mannila, J., Jarvinen, T., Jarvinen, K., Tarvainen, M., & Jarho, P. (2005). Effects of RM-beta-CD on sublingual bioavailability of delta(9)-tetrahydrocannabinol in rabbits. *Eur. J. Pharm. Sci., 26,* 71–77.

Masson, M., Loftsson, T., Masson, G., & Stefanson, E. (1999). Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. *J. Control. Release., 59,* 107–118.

McLintock, T. T. C., Kenny, G. N. C., Howie, J. C., McArdle, C. S., Lawrie, S., & Aitken, H. (1988). Assessment of the analgesic efficacity of nefopam hydrochloride after upper abdominal surgery: a study using patient controlled analgesia. *Br. J. Surg., 75 (8),* 779–781.

Mimoz, O., Incagnoli, P., Josse, C., Gillon, M. C., Kuhlman, L., Soilleux, H., & Fletcher, D. (2001). Analgesy efficacy and safety of nefopam vs. propacetamol following hepatic resection. *Anaesthesia, 56,* 520–525.

Miro, A., Quaglia, F., Calignano, A., Barbato, F., Cappello, B., & La Rotonda, M. I. (2000). Physicochemical and pharmacological properties of nimesulide/β-cyclodextrin formulations. *S.T.P. Pharma. Sci., 10,* 157–164.

Pose-Vilarnovo, B., Perdomo-Lopez, I., Echezarreta-Lopez, M., Schroth-Pardo, P., Estrada, E., & Torres-Labandeira, J. J. (2001). Improvement of water solubility of sulfamethizole through its complexation with β- and hydroxypropyl-β-cyclodextrin; Characterization of the interaction in solution and in solid state. *Eur. J. Pharm. Sci., 13,* 325–331.

Roselli, C., Perly, B., Duchêne, D., & Wouessidjewe, D. (1999). β-Cyclodextrin and its derivatives as solubilizing fillers for tablet formulations: ^{1}H-RMN contribution as the ultimate tool for the study of drug/cyclodextrin interaction. *S.T.P. Pharma. Sci., 9,* 267–271.

Tenjarla, S., Puranajoti, P., Kasina, R., & Mandal, T. (1998). Preparation, characterization, and evaluation of miconazole-cyclodextrin complexes for improved oral and topical delivery. *J. Pharm. Sci., 87,* 425–429.

Veiga, F., Teixeira-Dias, J. J. C., Kedzierewicz, F., Sousa, A., & Maincent, P. (1996). Inclusion complexation of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. *Int. J. Pharm., 129,* 63–71.

Ventura, C. A., Puglisi, G., Zappala, M., & Mazzone, G. (1998). A physico-chemical study on the interaction between papaverine and modified β-cyclodextrins. *Int. J. Pharm., 160,* 163–172.

Vianna, R. F. L., Bentley, M. V. L. B., Ribeiro, G., Carvalho, F. S., Neto, A. F., de Oliveira, D. C. R., & Collett, J. H. (1998). Formation of cyclodextrin inclusion complexes with corticosteroids: their characterization and stability. *Int. J. Pharm., 167,* 205–213.

Wang, F., & Khaledi, M. G. (1999). Non-aqueous capillary electrophoresis chiral separations with sulphated β-cyclodextrin. *J. Chromatogr. B., 731,* 187–197.