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Journal of Chromatography A 817 (1998) 91–104

JOURNAL OF CHROMATOGRAPHY A

Chiral separations by capillary zone electrophoresis with the use of cyanoethylated-β-cyclodextrin as chiral selector

Zeineb Aturki[a], Claudia Desiderio[a], Luisa Mannina[b], Salvatore Fanali[a,*]

[a]*Istituto di Cromatografia del Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy*

[b]*Università del Molise, Facoltà di Scienze M.M.F.F.N.N., 86170 Isernia, Italy*

Abstract

The uncharged β-cyclodextrin derivative, cyanoethylated-β-cyclodextrin, was successfully used, as chiral selector, in capillary zone electrophoresis in a polyacrylamide coated capillary. Several basic and acidic analytes belonging to different classes of compounds of pharmaceutical interest were analyzed and their enantiomers resolved. The chiral resolution was strongly influenced by the concentration of the cyclodextrin as well as by the pH of the background electrolyte and the capillary temperature. Compared with the results previously obtained for the separation of naproxen enantiomers employing trimethylated-β-CD, the use of cyanoethylated-β-CD caused an inversion of migration order [*R*-(−)-naproxen faster than the *S*-(+)-isomer]. ^{1}H NMR spectra of *R*,*S*-naproxen without cyclodextrin and *R*,*S*- and *S*-naproxen in the presence of the chiral selector revealed a strong interaction between the methyl group and H proton of the aromatic moiety of naproxen and the cyanoethylated-β-cyclodextrin.

Keywords: Enantiomer separation; Buffer composition; Naproxen; Drugs

1. Introduction

Chiral analysis is one of the most studied topics in analytical chemistry especially in the pharmaceutical field where a large number of compounds, possessing an asymmetric center, are used as a pair of enantiomers. Very often the two optical isomers exhibit different pharmacological activity and the tendency now is to produce drugs containing only the single active molecule. Thus separation methods capable of rapidly performing chiral analysis with good precision, high sensitivity, high resolution and at low costs are increasingly required for e.g., chiral purity control of drugs, monitoring the synthesis, pharmacokinetic studies etc.

Gas chromatography (GC) [1] and high-performance liquid chromatography (HPLC) [2–4] have been widely employed in this field. Recently capillary electrophoresis (CE) has become popular because of its high efficiency, low cost, wide range of separation mechanisms, simplicity and flexibility, very low consumption of buffer and samples etc. [5–13].

The separation of chiral compounds can be achieved in CE by simply adding to the background electrolyte (BGE) an appropriate chiral selector (direct separation method), e.g., native and/or derivatized cyclodextrins [5–13], proteins [14–17], modified crown-ethers [18,19], chiral surfactants

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00426-9

[5,20,21], metal–chiral amino acid complexes [22,23], antibiotics [24–29] etc. Among them, cyclodextrins (CDs) and their derivatives are the most widely employed chiral additives because of their high resolution capability towards racemic compounds belonging to different classes, low absorption in the UV region and good solubility in aqueous buffers.

The resolution mechanism, when CDs are used as chiral selectors, is usually based on inclusion complexation where the analyte fits into the CD cavity and the chiral recognition is achieved because of the secondary bonds with the substituent groups on the rim of the chiral agent. Thus the dimensions of the cavity of the CD and the shape and structure of analyte are of paramount importance in the chiral recognition process [30]. β-CD, which has cavity dimensions very similar to that of a wide number of analytes, seems to be the most appropriate chiral inclusion complexing agent. However, its solubility in both aqueous and organic solvents is relatively low (e.g., 1.85% w/v in water) and thus modified β-CDs are usually selected in order to increase the solubility and/or to improve the enantiomeric resolution (different secondary interactions and/or different separation mechanism).

In this study we used cyanoethylated-β-cyclodextrin (β-CD-CN) for the enantiomeric resolution of several compounds of pharmaceutical interest belonging to different classes, namely anesthetic, β-adrenergic blocker, non-steroidal anti-inflammatory drugs (NSAIDs), β-adrenergic agonist, antihistaminic, β-adrenergic agonist and anticoagulant. The effect of CD concentration, pH of the BGE on chiral resolution and migration separation factor of the studied racemic compounds were investigated. For basic compounds the influence of organic modifier as well as capillary temperature was also studied. Furthermore, the interaction between naproxen and β-CD-CN was investigated by ^{1}H NMR.

2. Experimental

2.1. Instrumentation

A Biofocus 3000 automated capillary electrophoresis apparatus (Bio-Rad, Hercules, CA, USA), equipped with a multiwavelength UV–visible detector and a thermostating liquid system was used for the electrophoretic experiments. The UV signals were recorded at 206 nm. Analyses were carried out in a polyacrylamide-coated capillary 35 cm (effective length, 30.5 cm) ×50 μm I.D. housed in a Bio-Rad user assembler cartridge. Fused-silica capillaries were from Composite Metal Services (Worcestershire, UK) and polyacrylamide coating was prepared using the modified method described by Hjerten [31]. Both capillary and carousel temperature were set at 25°C or otherwise stated. Samples were injected by pressure at 5 p.s.i. for 2 s (1 p.s.i.= 6894.76 Pa). The applied voltage was constant at + or −20 kV.

^{1}H NMR spectra were performed in a Bruker AMX600 spectrometer (Karlsruhe, Germany) operating at 600.13 MHz. All one- (1D) and two-dimensional (2D) used sequences were performed with suppression of the water resonance. Literature pulse sequences [32] were used for 2D experiments: ^{1}H-^{1}H COSYPR (2D correlated spectroscopy experiment with presaturation), data matrix size 512×512; time domain (td) 512 in F1 and 1024 in F2; relaxation delay (rd)=2 s; number of scans (n_s)=4; dummy scans (n_{ds})=8; ^{1}H-^{1}H TOCSYPR (2D total correlation spectroscopy experiment with presaturation): data matrix size 512×512; td 512 in F1 and 1024 in F2; rd=2 s; n_s=24; n_{ds}=8; mixing time=80 ms: ^{1}H-^{1}H ROESYPRTP (2D rotating frame Overhauser enhancement spectroscopy experiment phase sensitive using time-proportional phase incrementation with presaturation): data matrix size 512×512; td 512 in F1 and 1024 in F2; rd=2 s; n_s=32; n_{ds}=8; cw pulse for spinlock=50 ms.

2.2. Chemicals

Acetic acid, phosphoric acid (85%, w/w), boric acid and sodium hydroxide were purchased from Carlo Erba (Milan, Italy). Racemic ibuprofen, indoprofen, ketoprofen, flurbiprofen, bupivacaine, chlorpheniramine, clenbuterol, ephedrine, epinephrine, ketamine, isoproterenol, metoprolol, norepinephrine, norphenylephrine, oxprenolol, propranolol were from Sigma (St. Louis, MO, USA); racemic suprofen, carprofen, naproxen, cicloprofen, (−)- and (+)-naproxen were kindly provided by Dr. Cecilia Bartoluc-

ci, Istituto di Strutturistica Chimica, C.N.R. (Montelibretti, Roma, Italy). Etilefrin was kindly provided by Professor G. Blaschke, Institute of Pharmaceutical Chemistry, University of Munster (Munster, Germany). Warfarin, pindolol, promethazine were from Aldrich (Steinheim, Germany). Cyanoethylated-β-cyclodextrin (β-CD-CN) was kindly provided by Cyclolab (Budapest, Hungary).

Stock solutions of buffer contained 50 m*M* phosphoric acid, 50 m*M* acetic acid and 50 m*M* boric acid in water were titrated with concentrated sodium hydroxide (150 m*M* Britton Robinson buffer, B.R.B.). 75 m*M* B.R.B. used for the experiments was daily prepared by diluting the stock solution with water and adding the appropriate amount of cyclodextrin. The capillary was rinsed between runs with water (70 s) and then with the BGE with the chiral selector for 100 s prior the electrophoretic run.

Standard solutions of samples (10^{-3} *M*) were prepared in methanol and diluted at the desired concentration with 7.5 m*M* B.R.B. without chiral selector.

For ^{1}H NMR spectra a 75 m*M* B.R.B. was prepared in $^{2}H_2O$ by adding equimolar concentrations of undeuterated boric acid, phosphoric acid and [$^{2}H_4$]acetic acid and titrated with NaO^{2}H to pH 5.0. The aqueous solvent was obtained diluting this buffer with $^{2}H_2O$ in the ratio 1:10. In order to obtain the proper concentration stock solutions of *S*- and *R,S*-naproxen 10^{-3} *M* were prepared in C$^{2}H_3O^{2}$H and diluted in the aqueous solvent.

3. Results and discussion

Several basic and acidic compounds of pharmaceutical interest including anticoagulants, anesthetics, antihistaminics, β-adrenergic blockers, α- and β-adrenergic agonists and NSAIDs were analyzed by CE for the chiral resolution using an uncharged derivatized β-cyclodextrin (β-CD-CN) in the BGE.

Fig. 1a, b and c show the chemical structures of the studied racemic compounds while the physicochemical properties of the CD employed are summarized in Table 1. β-CD-CN differs from its parent compound in the modification at the hydroxyl groups on the rim at position 2, 3 and 6 with cyanoethyl and possesses a degree of substitution (DS) 3.8 with solubility, in water, >25 g/100 ml (in Table 1 only the substitution at position 1 is represented).

It has been shown that the most important parameters affecting the chiral resolution of racemic compounds using CDs, include the CD type and concentration, BGE type, concentration and pH, organic modifier, capillary temperature and electroosmotic flow (EOF) [13]. In order to suppress/minimize the EOF a polyacrylamide coated capillary was used thus maximizing the mobility of the CD-complexed analyte and free analyte [33] with the aim of improving the resolution. The capillary temperature was 25°C.

Basic compounds were analyzed in a BGE at pH 2.5 without the chiral selector. As expected all the studied compounds moved as cations showing a single peak in a relatively short time (2.5–8.3 min). β-CD-CN was added to the BGE at different concentrations (10–150 m*M*) and the electrophoresis run after injecting, separately, the basic analytes in order to study the effect of CD concentration on resolution (R) and migration separation factor (α). The two parameters were calculated using the following equations:

$$\alpha = \frac{t_2}{t_1} \quad (1)$$

$$R = 2\frac{t_2 - t_1}{w_2 - w_1} \quad (2)$$

where t_2 and t_1 are the migration times of the two enantiomers ($t_2 > t_1$) while w_2 and w_1 are the width of the separated peaks at the baseline. α is a parameter not influenced by the peak shape that can give useful information concerning the degree of enantiorecognition [34].

Table 2 gives the calculated resolution and migration separation factor when the electrophoretic runs were performed at pH 2.5 and the BGE contained increasing concentrations of β-CD-CN in the range 10–150 m*M*.

Enantiomeric resolution was achieved for all the studied basic analytes except for ketamine, metoprolol, oxprenolol and promethazine while propranolol and ephedrine exhibited poor resolution.

The increase of CD concentration in the BGE caused a general increase of migration time due to the complexation with the chiral selector as well as

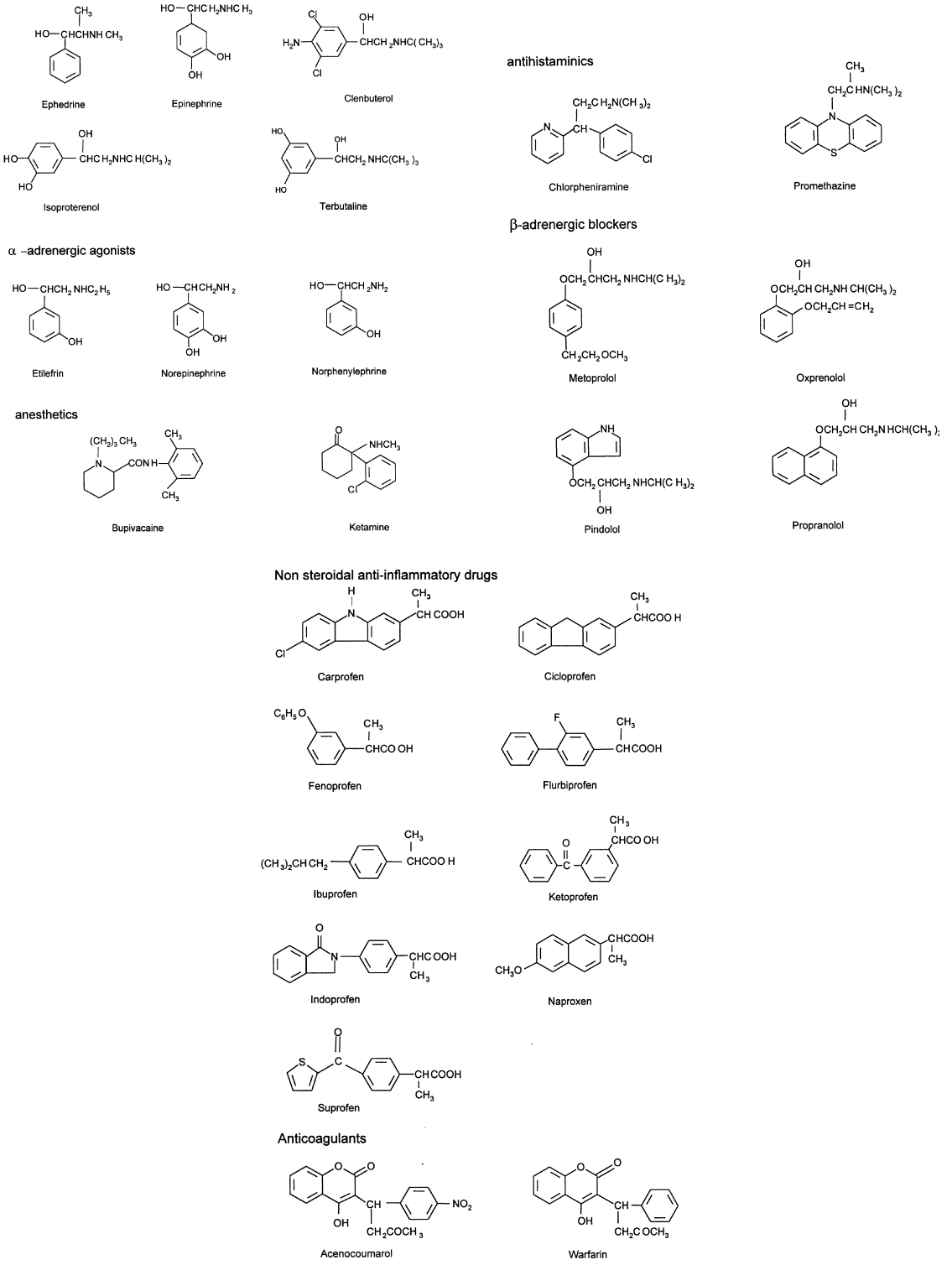
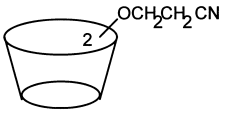


Fig. 1. Chemical structure of the studied basic and acidic racemic compounds.

Table 1
Physicochemical properties of cyanoethylated-β-cyclodextrin (β-CD-CN)



	Cyanoethylated-β-cyclodextrin
Formula	$C_{54}H_{78}O_{35}N_4$
Molecular weight	1343.3
Degree of substitution (DS)	3.8 cyanoethylgroups/CD ring
	primary/secondary substitution, 1:3
	O_2/O_3 substitution, 4:1
Solubility (g/100 ml)	water >25
	methanol >5
	DMSO >25

to the increase of the viscosity. The resolution increased by increasing the chiral selector concentration for bupivacaine, epinephrine, isoproterenol, norepinephrine, norphenylephrine while for other analytes such as clenbuterol, etilefrin, terbutaline a maximum of resolution was achieved at different concentrations of CD (30, 100 and 20 m*M*, respectively).

The migration separation factor was also influenced by the concentration of the chiral agent; α increased on raising the CD concentration for bupivacaine, clenbuterol, ephedrine, etilefrin, isoproterenol, pindolol. A maximum value of α was recorded for the following compounds: chlorpheniramine (at 30 m*M* of CD), norepinephrine (at 70 m*M* of CD) and epinephrine, norphenyephrine, terbutaline (at 100 m*M* of CD).

The migration separation order was verified for epinephrine and isoproterenol injecting, separately, two mixtures containing (−)- and (+)-isomers (2:1) and the electrophoresis run in the BGE at pH 2.5 containing 40 or 100 m*M* of β-CD-CN. In all cases the migration order was (−)-epinephrine and (−)-isoproterenol faster than their antipodes. Thus, from the above results the (+)-enantiomers of the two analytes formed more stable complexes than those of their chiral isomers. For example Fig. 2 shows the electrophoretic separation of racemic clenbuterol, etilefrin, epinephrine, isoproterenol, norphenyephrine and terbutaline enantiomers under the optimum experimental conditions.

3.1. Chiral separation of acidic compounds

Based on our experience we selected a BGE at pH

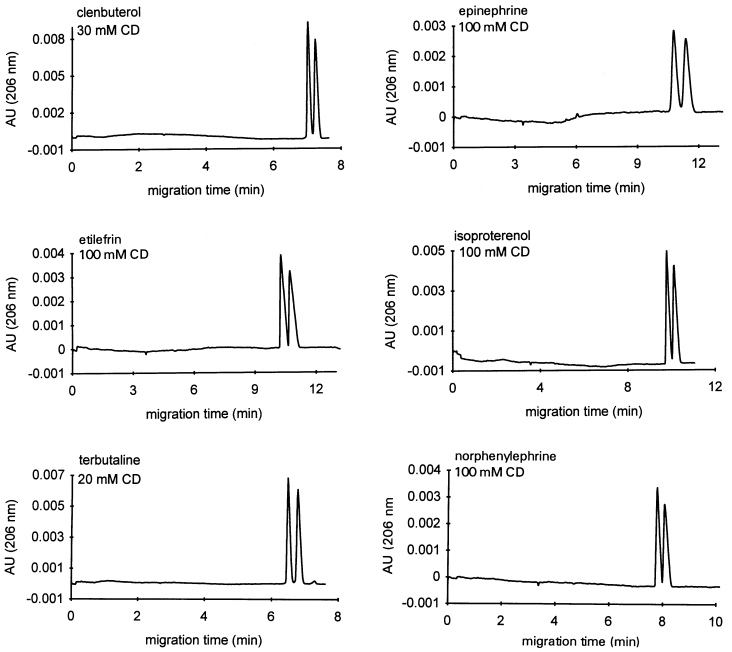


Fig. 2. Electropherograms of the chiral separation of racemic clenbuterol, epinephrine, etilefrin, isoproterenol, norphenyephrine and terbutaline at the optimum experimental conditions. For experimental conditions see Table 1.

Table 2
Effect of β-CD-CN concentration on chiral resolution (R) and migration separation factor (α) of basic compounds of pharmaceutical interest[a]

Compounds	Concentration of β-CD-CN (mM)																			
	0			10			20			30			70			100			150		
	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t
Bupivacaine	–	–	4.6	–	–	5.5	–	–	5.3	–	–	5.9	0.58	1.020	5.9	0.76	1.026	11.0	0.88	1.024	22.1
Chlorpheniramine	–	–	2.5	0.50	1.020	5.2	0.50	1.014	5.9	0.56	1.018	7.1	<0.5	1.012	11.3	–	–	16.4	n.m.		
Clenbuterol	–	–	4.5	0.96	1.028	5.6	1.05	1.031	6.7	1.21	1.031	7.2	0.92	1.027	11.4	0.72	1.034	16.5	n.m.		
Ephedrine	–	–	3.5	–	–	4.6	<0.5	1.006	5.9	<0.5	1.008	6.5	<0.5	1.010	9.8	<0.5	1.012	12.8	<0.5	1.014	14.4
Epinephrine	–	–	3.7	<0.5	1.016	4.2	<0.5	1.048	4.7	0.68	1.020	5.0	1.12	1.029	7.6	1.14	1.056	11.4	1.18	1.033	11.6
Etilefrin	–	–	3.8	<0.5	1.016	4.3	0.51	1.026	5.2	0.70	1.030	5.8	0.68	1.041	8.5	0.88	1.040	10.7	0.80	1.068	25.9
Ketamine	–	–	3.7	–	–	5.7	–	–	7.2	–	–	7.7	–	–	11.7	–	–	20.1	n.m.		
Isoproterenol	–	–	4.2	<0.5	1.007	4.7	<0.5	1.017	5.1	0.50	1.021	5.7	0.93	1.032	8.1	0.97	1.032	10.1	1.02	1.059	19.8
Metoprolol	–	–	4.7	–	–	8.0	–	–	8.4	–	–	9.4	–	–	12.8	–	–	16.6	n.m.		
Norepinephrine	–	–	3.6	–	–	3.7	–	–	4.9	<0.5	1.011	4.6	<0.5	1.022	6.3	0.55	1.028	7.0	0.62	1.022	8.4
Norphenylephrine	–	–	3.4	<0.5	1.013	3.8	0.60	1.022	4.6	0.80	1.021	4.9	0.84	1.034	7.0	0.92	1.039	8.1	1.03	1.016	8.5
Oxprenolol	–	–	4.4	–	–	5.7	–	–	6.4	–	–	7.2	–	–	10.4	–	–	13.3	N.m.		
Pindolol	–	–	4.2	<0.5	1.008	6.2	<0.5	1.008	6.1	<0.5	1.007	7.0	<0.5	1.012	10.2	<0.5	1.014	14.9	N.m.		
Promethazine	–	–	4.0	–	–	11.5	–	–	9.9	–	–	10.5	–	–	14.7	–	–	25.8	N.m.		
Propranolol	–	–	4.4	<0.5	1.009	9.2	–	–	8.7	–	–	9.5	–	–	18.2	N.m	N.m.				
Terbutaline	–	–	4.4	1.11	1.050	6.7	1.37	1.049	6.8	1.22	1.046	7.6	1.35	1.045	11.2	1.33	1.060	16.8	N.m.		

[a] Capillary; 35 (30.5 cm)×50 μm I.D. polyacylamide coated; BGE, 75 mM B.R.B., pH 2.5 with different concentrations of β-CD-CN; applied voltage, 20 kV (27–40 μA); injection 5 p.s.i., 2 s of racemic standard $10^{-4}/5\cdot10^{-5}$ M at the anodic end.
n.m.=not measured; t=migration time of the second enantiomer (min).

Table 3
Effect of β-CD-CN concentration on chiral resolution (R) and migration separation factor (α) of acidic compounds of pharmaceutical interest

Compounds	β-CD-CN (mM)																			
	0			2.5			5			7.5			10			20			30		
	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t	R	α	t
Carprofen	–	–	4.8	0.71	1.029	11.7	1.04	1.023	13.5	0.86	1.022	13.9	0.86	1.021	15.1	<0.5	1.009	17.0	–	–	19.3
Cicloprofen	–	–	4.7	1.25	1.033	13.0	1.55	1.033	15.1	1.30	1.033	15.5	1.31	1.031	16.7	1.28	1.027	18.6	1.14	1.024	20.8
Fenoprofen	–	–	4.9	<0.5	1.004	11.2	<0.5	1.016	12.8	0.81	1.017	13.6	0.85	1.022	14.5	0.97	1.019	15.0	1.03	1.016	15.9
Flurbiprofen	–	–	4.7	–	–	11.6	–	–	12.2	–	–	12.5	–	–	12.7	–	–	13.5	–	–	14.2
Ibuprofen	–	–	5.2	<0.5	1.017	14.2	<0.5	1.010	14.8	<0.5	1.004	15.0	<0.5	1.012	15.3	<0.5	1.016	16.3	0.54	1.015	16.8
Indoprofen	–	–	5.2	0.50	1.010	8.7	0.68	1.023	11.2	0.70	1.017	12.0	0.74	1.019	13.7	<0.5	1.008	16,7	<0.5	1.027	18.9
Ketoprofen	–	–	4.9	–	–	8.4	–	–	9.6	–	–	10.0	–	–	10.9	–		12.4	–	–	13.2
Naproxen	–	–	4.5	1.65	1.057	11.6	2.06	1.059	14.8	2.18	1.060	15.6	2.39	1.060	17.4	2.39	1.016	19.9	2.34	1.059	22.1
Suprofen	–	–	4.8	–	–	9.7	–	–	10.6	–	–	11.0	–	–	12.0	–	–	12.9	–	–	13.5
Warfarin	–	–	8.5	1.31	1.054	18.8	1.52	1.031	22.0	1.79	1.078	25.7	n.m.	n.m.	n.m.			nm			
Acenocoumarol	–	–	6.7	–	–	10.9	–	–	11.2	–	–	13.2	–	–	14.0	–	–	–	16.4	–	

BGE, 75 mM B.R.B., pH 5 with different concentrations of CD; injection at the cathodic end. Injected samples, $10^{-4}/5 \cdot 10^{-5}$ M. For other experimental conditions see Table 2.
n.m. = not measured.

5, in the absence of the EOF, for the electrophoretic study of acidic compounds. The studied analytes include carprofen, cicloprofen, fenoprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, naproxen, suprofen, warfarin and acenocoumarol. In the absence of CD all compounds moved as negatively charged ions and reached the detector in a relatively short time (4.8–8.5 min). β-CD-CN was added to the BGE at different concentrations in the range 2.5–30 m*M* and the electrophoretic runs performed after injecting, separately, the racemic samples. The results are shown in Table 3. Enantiomeric resolution was obtained for all the studied anionic analytes except for flurbiprofen, ketoprofen, suprofen and acenocoumarol at any CD concentration investigated.

The increase of the chiral selector concentration caused a general increase of migration time as a consequence of both complexation with the CD and increase of viscosity.

Increasing the CD concentration caused an increase of resolution (*R*) for fenoprofen and warfarin, which showed the highest value of *R* at 30 and 7.5 m*M* CD, respectively. Warfarin enantiomers were studied in the range 2.5–7.5 m*M* due to the strong complexing effect of the chiral selector. For carprofen, cicloprofen, indoprofen and naproxen a maximum of resolution was obtained at different concentrations of CD (5, 5, 10 and 10 m*M* respectively). These results are in accordance with the theoretical studies concerning the existence of a maximum resolution between two extreme concentrations of CD [35].

The CD concentration also influenced the migration separation factor for acidic compounds and the highest values were obtained for naproxen and warfarin (1.060 and 1.078, respectively) when 7.5 m*M* of β-CD-CN was added to the BGE at pH 5. The value of α for anionic compounds was generally higher than that obtained for cationic compounds analyzed at pH 2.5.

A mixture (2:1, v/v) of *R*-(−)-naproxen and *S*-(+)-naproxen was run in the BGE at pH 5 containing 5 or 30 m*M* of β-CD-CN in order to verify the migration order. *S*-(+)-naproxen was more retarded showing that it complexes with the CD used more readily. These results are the opposite of those obtained using trimethylated-β-CD as the chiral selector [29]. Fig. 3 shows the electropherograms of the chiral separation of warfarin, carprofen, cicloprofen and naproxen at pH 5.

3.2. Effect of pH of the buffer on resolution

Three buffer systems of pH 2.5, 4.5 and 6.5 supplemented with 40 or 70 m*M* of β-CD-CN were tested in order to study the effect of the pH of the BGE on the chiral resolution of propranolol, oxprenolol, metoprolol, chlorpheniramine, clenbuterol, etilefrin, pindolol, isoproterenol, norepinephrine, epinephrine, bupivacaine, terbutaline, ketamine, ephed-

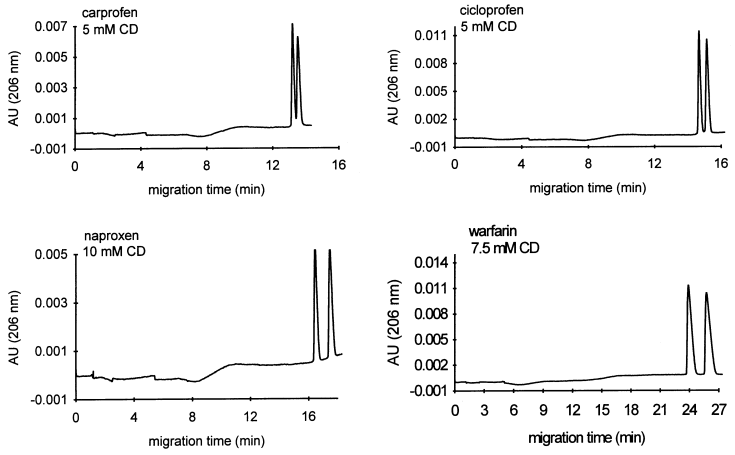


Fig. 3. Electropherogram of the enantiomeric separation of racemic warfarin, naproxen, carprofen and cicloprofen; for experimental conditions see Table 3.

Table 4
Effect of buffer pH on resolution of basic drugs using two CD concentrations[a]

Compounds	*R*					
pH	2.5		4.5		6.5	
CD concentration (m*M*)	40	70	40	70	40	70
Bupivacaine	0.3	0.6	0.6	1.0	–	–
Chlorpheniramine	0.6	0.1	–	–	0.2	–
Clenbuterol	1.1	0.9	1.8	1.1	1.6	1.5
Ephedrine	0.1	0.1	0.2	0.2	0.8	0.9
Epinephrine	0.9	1.1	1.1	1.3	–	–
Etilefrine	0.7	0.7	1.0	1.0	0.9	1.1
Ketamine	–	–	–	–	0.7	1.9
Isoproterenol	0.6	0.9	1.0	1.3	–	–
Metoprolol	–	–	–	–	–	–
Norepinephrine	0.2	0.4	0.5	0.5	–	–
Norphenylephrine	0.8	0.8	0.9	1.0	0.9	1.1
Oxprenolol	–	–	–	–	–	–
Pindolol	0.1	0.1	0.3	0.3	0.2	0.2
Promethazine	–	–	–	–	0.9	0.9
Propranolol	–	–	–	–	–	–
Terbutaline	1.3	1.3	1.7	1.9	1.9	1.8

75 m*M* B.R.B., pH 2.5, 4.5 and 6.5 containing 40 or 70 m*M* of β-CD-CN; other experimental conditions as in Tables 2 and 3.

rine, promethazine and norphenylephrine. Table 4 shows the resolution of basic compounds obtained at different pH values when 40 or 70 m*M* of CD were used.

The resolution increased with increasing pH of the BGE for terbutaline, promethazine and ketamine while for clenbuterol, etilefrin, epinephrine isoproterenol, bupivacaine and norepinephrine a maximum value of *R* was achieved at pH 4.5. The chiral resolution of metoprolol, oxprenolol and propranolol was not achieved at any pH and no remarkable effect was recorded for pindolol. For bupivacaine, isoproterenol, epinephrine and norepinephrine the increase of pH from 2.5 to 4.5 caused an increase of resolution that was completely lost at pH 6.5. At pH 6.5 some analytes were not resolved at all while ketamine and promethazine exhibited chiral resolution only at this pH. Fig. 4a and b show the electropherograms of the enantioseparation of ketamine and isoproterenol at different pH values.

The chemical structure of the studied basic compounds influenced the chiral resolution due to differences in the inclusion complexation with the CD derivative. For instance comparing three analytes with similar chemical structure, epinephrine, norepinephrine and isoproterenol, the amino group (pri-

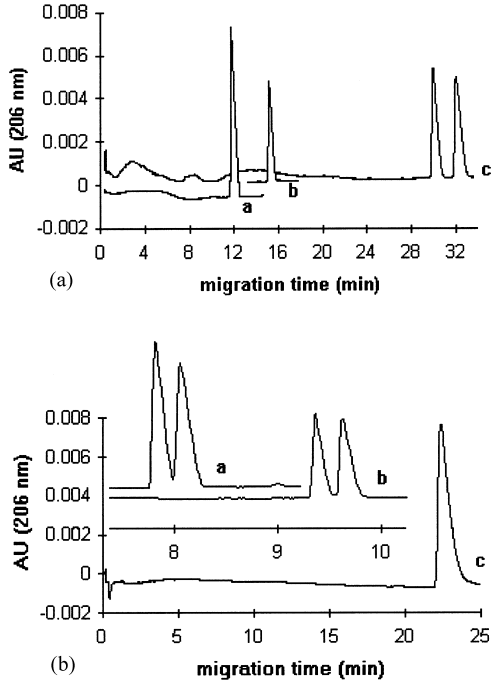


Fig. 4. Electropherograms of the enantioseparation of (a) ketamine and (b) isoproterenol at different pH values in 75 m*M* B.R.B. containing 70 m*M* of β-CD-CN; pH: (a) 2.5, (b) 4.5 and (c) 6.5; for other experimental conditions see Table 4.

mary for norepinephrine and secondary for the others) is responsible for the different stereoselectivity. In fact the three compounds were not resolved at pH 6.5 and poor resolution was achieved for norepinephrine at pH 2.5 and 4.5 while epinephrine and isoproterenol were both resolved at these pH, showing the highest values of R at pH 4.5 (70 mM of CD with $R=1.3$). Observing propranolol, oxprenolol and metoprolol we can suppose that the position of the chiral center is important in the chiral recognition process. The three analytes were not resolved at all at any studied pH and possess the chiral center in the β position. A hindrance effect can be expected for oxprenolol due to the substituent at the *ortho* position.

The effect of the pH on chiral resolution of acidic compounds was done at pH 5, 6 and 7 in a BGE containing 5 or 30 mM of β-CD-CN. The increase of pH caused a general decrease of resolution probably due to the higher dissociation of the acidic group of analytes at pH 6 and 7 than at pH 5. Very poor resolution was recorded for flurbiprofen and naproxen (at pH 6) and for warfarin (at pH 6 and 7) using 5 mM of CD (data not shown).

3.3. Effect of organic modifier

The organic modifier can affect both migration time and chiral resolution when the inclusion-complexation mechanism is involved in the enantiorecognition process. Usually the presence of an organic additive to the aqueous BGE causes a reduction of the inclusion complexation due to competition between the analyte and the organic solvent in fitting the CD cavity. However, in several cases improvement of chiral resolution has been achieved modifying the BGE with organic solvents such as methanol, acetonitrile etc. [36]. Recently it has been shown that good enantiomeric resolution can be achieved in pure organic solvents (formamide, dimethylformamide) probably due to adsorption mechanisms [37,38].

The effect of the organic modifier on the chiral resolution of racemic bupivacaine, chlorpheniramine, epinephrine, isoproterenol, norphenylephrine, propranolol and pindolol was investigated running, separately, the racemic mixtures in a BGE 75 mM B.R.B., pH 2.5–organic modifier containing 50 mM β-CD-CN. Acetonitrile, methanol and 2-propanol (10 or 30% v/v) were the organic solvents tested for these experiments.

The use of the three organic additives caused a general decrease of resolution for all the studied compounds except for chlorpheniramine. In fact an improvement of resolution (poor in aqueous buffer) was recorded with 10% of both acetonitrile ($R=0.9$) or 2-propanol ($R=1.0$).

3.4. Effect of capillary temperature

The change in capillary temperature can influence several parameters such as sample stability, buffer viscosity, pH of the BGE, interaction equilibrium etc. [39]. It has been reported that by decreasing the temperature of the capillary, an improvement of chiral separation can be achieved [9,40–44]. The effect of capillary temperature on enantiomeric resolution was studied for several basic drugs including bupivacaine, chlorpheniramine, isoproterenol, norphenylephrine, propranolol and pindolol. The experiments were run in the BGE at pH 2.5 containing 50 mM of β-CD-CN at different capillary temperatures in the range 15–35°C.

Fig. 5 shows the plot of temperature versus resolution (R). The increase of capillary temperature caused a general decrease of migration time for all the studied drugs (results not shown) and a decrease of enantiomeric resolution. This effect can be due to the strong influence of column temperature on the kinetics of the inclusion mechanism between CD and

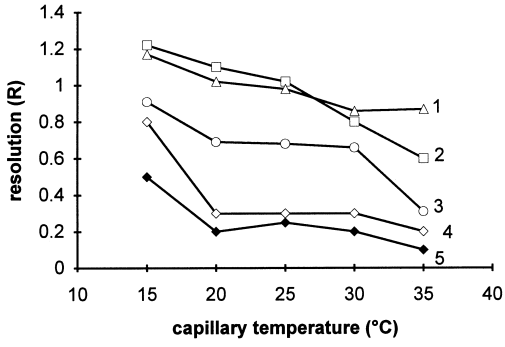


Fig. 5. Effect of capillary temperature on chiral resolution of racemic (1) bupivacaine, (2) chlorpheniramine, (3) epinephrine, (4) isoproterenol and (5) norphenylephrine. Capillary, 35 (30.5) cm×50 μm I.D. polyacrylamide coated; 75 mM B.R.B. (pH 2.5) and 50 mM β-CD-CN; 20 kV; injection, 5 p.s.i.×2 s of $10^{-4}/5\cdot 10^{-5}$ M of racemic samples.

enantiomers. The best chiral resolutions were achieved, for all studied analytes, when the capillary temperature was kept at 15°C.

3.5. NMR study of selector–analyte interactions

Fig. 6 shows the ^{1}H NMR spectra in aqueous solvent of the aromatic region of: *R,S*-naproxen, *R,S*-naproxen with CD and *S*-naproxen with CD. In the ^{1}H NMR spectrum of *R,S*-naproxen with CD all resonance signals are shifted (see Fig. 6 and Table 4) due to the complexation with the chiral selector.

The different structures of the complexes formed by the enantiomeric forms and the CD induces chemical shift nonequivalence between the signals of the two enantiomeric forms: this difference of chemical shift is most pronounced for H-3, H-5 and H-7 (see Fig. 6). The double-doublet of H-3 of *R,S*-naproxen with CD is downfield shifted. For the two enantiomeric complexes this signal is well resolved; in fact the ^{1}H NMR spectrum shows two partially overlapped double-doublets whose chemical shift difference is 2.4 Hz.

The doublet due to H-5 is upfield shifted: for the two enanantiomeric complexes the ^{1}H spectrum shows two partially overlapped doublets whose chemical shift difference is 3.0 Hz Table 5. The double doublet due to H-7 is downfield shifted: for the two enanantiomeric complexes the ^{1}H spectrum shows two partially overlapped double doublets whose chemical shift difference is 2.4 Hz. The doublet at 1.544 ppm due to the methyl of the *R,S*-naproxen (see Fig. 7) is downfield shifted: for the two enanantiomeric complexes the ^{1}H spectrum shows two well resolved doublets whose chemical shift difference is 3.0 Hz. The ^{1}H spectrum of the *S*-naproxen–CD complex (see Figs. 6 and 7) is coincident with one set of resonances and allows the chemical assignment.

Thus, based on the above reported ^{1}H NM data we can suppose that naproxen fits the β-CD-CN cavity interacting with the H proton at positions 3, 4, 5 and 7 of the aromatic moiety as represented in Fig. 8. Strong interaction was also recorded for the methyl group of naproxen, however the bonds between the substituent groups on the asymmetric carbon of the analyte and those (cyanoethyl or hydroxyl) on the CD rim are under investigation in order to understand the stereoselective resolution mechanism.

4. Conclusions

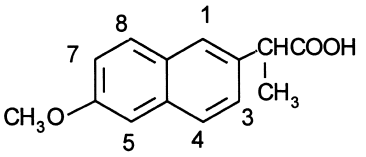
The use of β-CD-CN as chiral selector in CE allowed the enantiomeric separation of several basic and acidic racemic compounds of pharmaceutical interest. Resolution and enantioselectivity were influenced by the concentration of the CD as well as by the pH of the buffer.

Acidic compounds were more strongly complexed than the basic analytes and a lower CD concentration was necessary in order to achieve their chiral resolution (2.5–30 m*M* and 10–150 m*M* for acidic and basic analytes, respectively).

Table 5
^{1}H Chemical shifts (ppm) of *R,S*-naproxen, *R,S*-naproxen + β-CD-CN, *S*-naproxen + β-CD-CN in aqueous solvent[a]

Proton	Type	*R,S*-Naproxen	*R,S*-Naproxen + CD	*S*-Naproxen + CD
1	CH	7.822	7.752	7.750
3	CH	7.540	7.612	7.611
			7.616	
4	CH	7.885	7.827	7.829
			7.322	
5	CH	7.420	7.317	7.320
			7.342	
7	CH	7.279	7.346	7.341
8	CH	7.909	7.857	7.829
			1.608	
	CH_3	1.543	1.613	1.612
	CH	Hidden		
	OCH_3	Hidden		

[a] Concentration was 10^{-4} *M* for *R,S*-naproxen and *S*-naproxen and 10^{-3} *M* for CD.



Naproxen

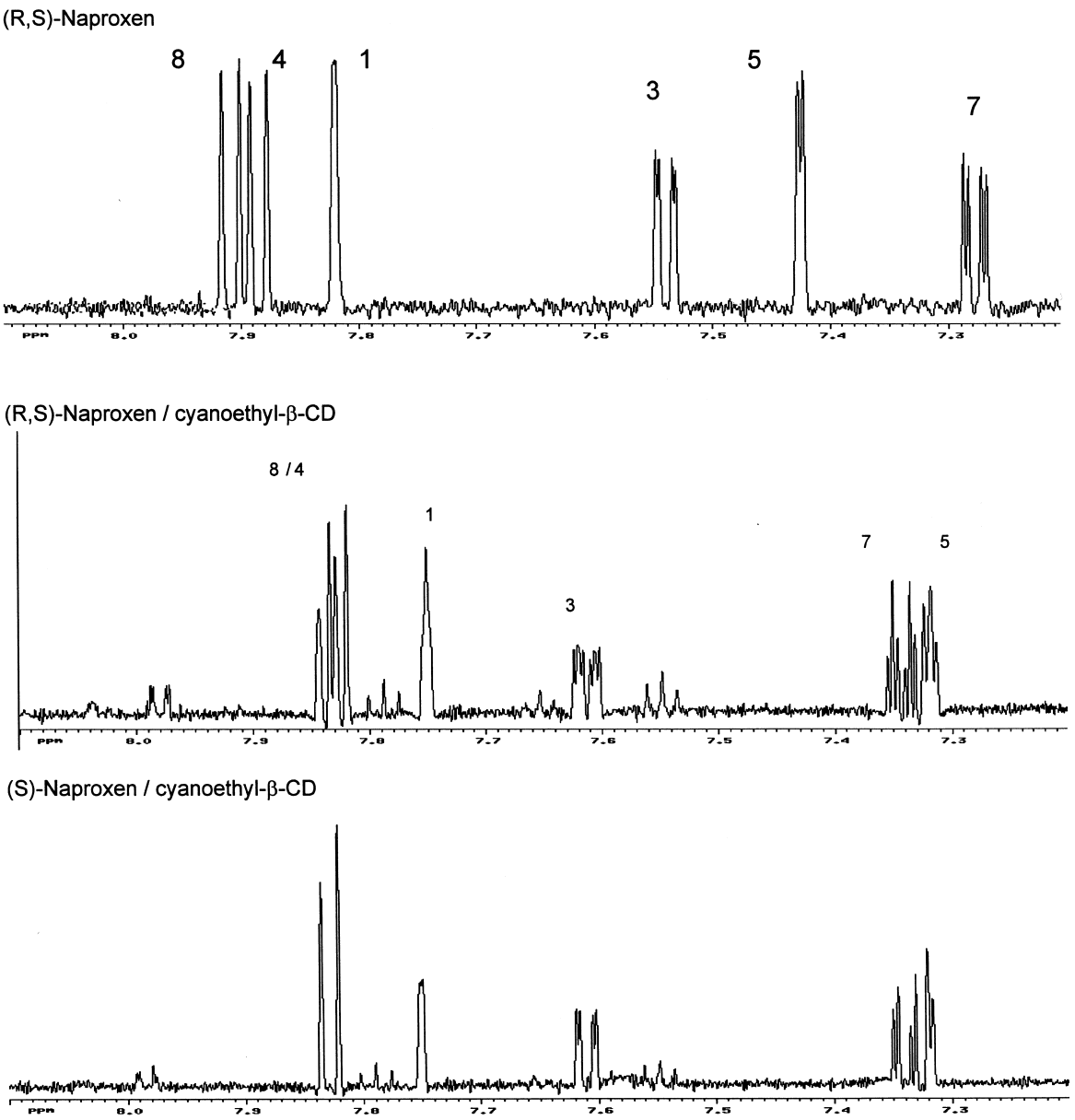


Fig. 6. ^{1}H NMR spectra (aromatic region) of *R,S*-naproxen, *R,S*-naproxen–β-CD-CN and *S*-naproxen–β-CD-CN. For experimental conditions see text.

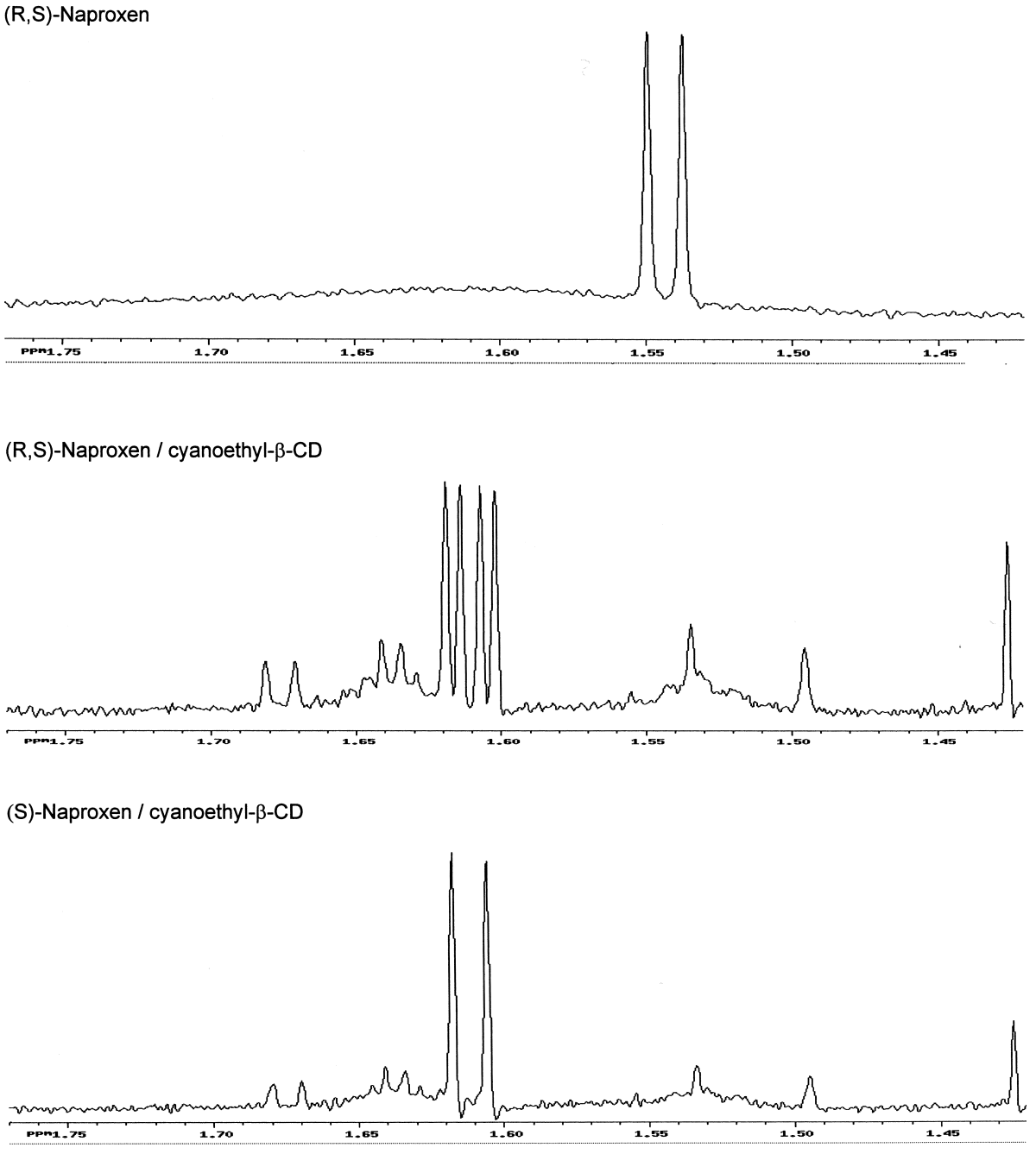


Fig. 7. ^{1}H NMR spectra (methyl region) of *R,S*-naproxen, *R,S*-naproxen–β-CD-CN and *S*-naproxen–β-CD-CN. For experimental conditions see text.

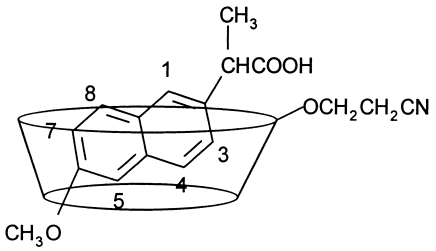


Fig. 8. Scheme of the supposed inclusion complex between naproxen and β-CD-CN.

β-CD-CN possesses a higher solubility than the parent β-CD in both aqueous and nonaqueous solvents. The studied CD exhibited different behaviour towards acidic compounds compared to the uncharged trimethylated-β-CD, e.g., both resulted to be good stereoselective agents for the enantiomeric separation of NSAIDs but with a different separation mechanism. In fact an inversion of migration order was obtained for naproxen [*R*-(−) faster than *S*-(+)] when using β-CD-CN as the chiral selector. NMR studies revealed that naproxen forms an inclusion complex.

Acknowledgements

The authors are grateful to Cyclolab R & D Lab. for the gift of the cyanoethylated-β-cyclodextrin used in this work and the NMR Service, Area della Ricerca di Roma, Montelibretti (Rome, Italy) for the ^{1}H NMR spectra. The authors would also like to thank Mr. G. Caponecchi and Mr. M. Cristalli for technical assistance.

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