Journal of Chromatography, 552 (1991) 563–573
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2161

Effects of β-cyclodextrin in the mobile phase on the retention and indirect detection of non-electrolytes in reversed-phase liquid chromatography

I. Study of aliphatic alcohols

MARTINE GOSSELET* and BERNARD SEBILLE
Laboratoire de Physico-Chimie des Biopolymères, Université Paris Val de Marne, Avenue du Général de Gaulle, 94 000 Créteil (France)

ABSTRACT

The influence of β-cyclodextrin–*p*-nitrophenol complexes in a *p*-nitrophenol-containing mobile phase on the chromatographic behaviour of aliphatic alcohols has been investigated. Depending upon the carbon chain length of the alcohol, the injected samples are detected before or after the first "system peak". The sample retention and the slope of the calibration curves depend upon the β-cyclodextrin concentration. The apparent molar absorptivity is maximal when the alcohol retention is close to the system peak. A comparison of these results to those obtained with an eluent devoid of β-cyclodextrin shows poor sensitivity for the indirect detection of alcohols, demonstrating that β-cyclodextrin inclusion complexes have some role in the mobile phase as detection enhancers.

INTRODUCTION

During the past few years several studies have shown that it was possible to detect and quantitate non UV-absorbing ionic compounds by the addition of a UV-absorbing ion in the aqueous mobile phase [1–6]. This method is applicable to both organic and inorganic ions using ion-exchange and reversed-phase chromatography. Recent reports have shown that this method can be extended to uncharged species [7–12]. Gnanasambandan and Freiser have used this method for the indirect UV detection of alcohols [7,8], ketones [7] and monosaccharides [9] with methylene blue in the eluent.

Similarly, Parkin and Lau [10,12] have detected alcohols by addition of UV-absorbing species such as benzamide, *n*-propyl-*p*-aminobenzoate uracil and theobromine in the mobile phase. The alcohols appeared as positive or negative peaks depending upon the chosen UV-detectable species and the respective capacity factors of UV-detectable species and alcohol.

In recent years, there has been considerable interest in the utilization of cyclodextrins (CDs) as stationary or as mobile phase in high-performance liquid

0021-9673/91/$03.50

chromatography (HPLC) [13–18]. CDs are toroidal-shaped, cyclic oligosaccharides made up of α-1,4-linked D-glucopyranose units and act as "hosts" to form stable inclusion complexes with a variety of "guest" species. The CD complexes form well defined species and 1:1, 1:2 and 2:1 guest–host inclusion complexes have been reported [19]. On the other hand Fujimura *et al.* [20] have studied the chromatographic properties of solutes (phenols, cresols and aniline derivatives) on reversed-phase RP-8 columns in the presence of β-CD in a water–organic solvent mobile phase.

The purpose of the present work was to examine the effects of the β-CD present in an eluent containing a UV–visible-absorbing compound, *p*-nitrophenol (PNP), in order to show evidence for an indirect detection of non-coloured solutes, such as aliphatic alcohols. We have studied the influence of β-CD concentration on calibration curves and apparent molar absorptivity of the different alcohols and stated the experimental conditions affording the highest detection sensitivity.

EXPERIMENTAL

Chemicals

Methanol, ethanol and propanol were of HPLC grade and were obtained from Prolabo (France). 1-Butanol, 2-butanol, 2-methyl-1-propanol and *p*-nitrophenol were from Aldrich (France). β-CD "Rhodocap" was a gift from Rhône-Poulenc (France).

Apparatus and conditions

The spectra were registered on a UV-visible spectrophotometer (Type 550 SE, Perkin-Elmer, Oakbrook, IL, U.S.A.) connected to a recorder (type R-100, Perkin-Elmer).

The chromatographic equipment consisted of a pump (Model 110A, Beckman Instrument, France), a variable-wavelength detector (LC-3, Pye-Unicam, Cambridge, U.K.) (cell length = 1 cm), monitored at 400 nm, a refractive index detector (Model R 401, Waters Assoc., Milford, MA, U.S.A.) and a 50-μl loop injector (Rheodyne 7120, Cotati, CA, U.S.A.).

The analytical column was 240 × 4.6 mm I.D. and the guard column 100 × 4.6 mm I.D.

Analytical columns were filled with LiChrosorb RP 18 (10 μm, 100 Å) (Merck, Darmstadt, Germany) by a slurry packing technique. The guard column was packed with LiChrosorb SI 60 (10 μm) (Merck).

The mobile phases were mixtures of variable composition consisting of 0.01 *M* potassium phosphate buffer (pH 7.85)–methanol (90:10, v/v) containing 10^{-5} *M* PNP and $0–5 \cdot 10^{-3}$ *M* β-CD. For comparison, buffered water–methanol mobile phases were used. The mobile phase was filtered through a 0.45-μm Sartorius filter membrane. The flow-rate was 0.95 ml/min.

The experiments were performed at room temperature (20°C), but the column was isolated by a casing to avoid temperature fluctuations.

Procedure

The column was equilibrated with the mobile phase containing PNP and CD, and the breakthrough curve was recorded. The steady-state conditions, corresponding to a constant absorbance at 400 nm, generally required 1 h. All the samples were previously dissolved in the mobile phase.

The capacity factors (k') were calculated from the equation $k' = (V_R - V_m)/V_m$, V_R being the sample retention volume and V_m the mobile phase volume of the column measured from the elution time of sodium nitrate. We have verified that the change of V_m during the experiments was negligible and have measured this value every ten runs. The peak areas were measured by planimetry.

RESULTS

As the mobile phase contains both PNP and β-CD, we first measured the absorbance of buffered water solutions containing different ratios of these compounds. We observed that the absorbance maximum of PNP solution is shifted by the addition of increasing amounts of β-CD (0–$5 \cdot 10^{-3}$ M) from 400 to 404 nm. An isobestic point is observed at 381 nm. The apparent molar absorptivity of PNP at 400 nm increases gradually from 15 600 M^{-1} cm^{-1} ([β-CD] = 0) to 17 870 M^{-1} cm^{-1} ([β-CD] = $5 \cdot 10^{-3}$ M).

The addition of β-CD to a potassium phosphate buffer–methanol (90:10, v/v) solution containing PNP led to the same shift of the absorbance maximum and to the same isobestic point. Moreover the apparent molar absorptivity of PNP increases from 17 400 M^{-1} cm^{-1} ([β-CD] = 0) to 19 250 M^{-1} cm^{-1} [(β-CD] = $5 \cdot 10^{-3}$ M) at 400 nm.

Chromatographic results

Fig. 1 shows typical chromatograms of alcohols injected separately on the chromatographic system used. 1-Propanol elutes as a positive peak followed by two "system peaks". The former is eluted at the same volume as a PNP sample injected in the same eluting conditions. The latter appears at a retention volume similar to that of β-CD eluted by a mobile phase containing only methanol and buffer (10:90, v/v) (refractive index detection). These two peaks are referred to as "system peaks" since

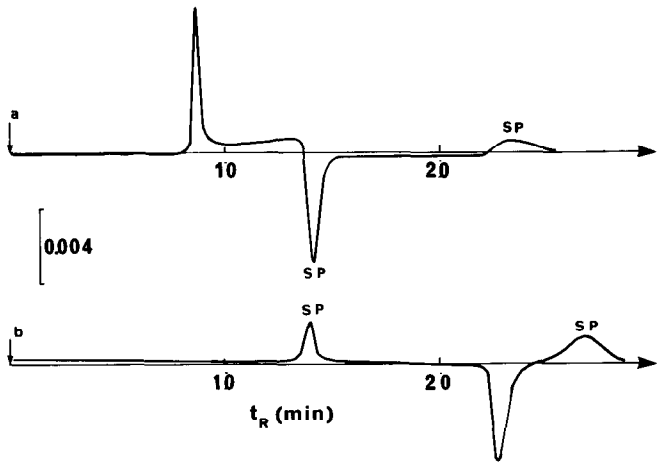

Fig. 1. Indirect detection of 1-propanol (a) and 2-butanol (b). Mobile phase: phosphate buffer–methanol (90:10, v/v), containing 10^{-3} M β-CD and 10^{-5} M PNP. Injected amounts: 80.34 μg for 1-propanol and 20.25 μg for 1-butanol. t_R = Retention time.

they correspond to species present in the eluent. The 1-butanol chromatogram shows a positive system peak corresponding to PNP, followed by a negative peak corresponding to the 1-butanol signal and a second positive system peak corresponding to β-CD.

A 15% methanol-containing mobile phase shows system peaks very close to the alcohol peaks in such a way that the detection of some alcohols becomes impossible (*e.g.* 1-propanol, 2-butanol). This effect of methanol in the eluent limits its effectiveness for these analyses.

In order to apply this system to the detection of a mixture of non visible-absorbing compounds, a sample containing aliphatic primary alcohols was injected (Fig. 2). It can be seen that the resulting peaks eluted at the retention volume of methanol, ethanol, 1-propanol and 2-propanol, before the first system peak, are positive while those eluted at the retention volume of 2-butanol, 2-methyl-1-propanol and 1-butanol, after the first system peak, are negative. Moreover, the sensitivity of the response depends on the alcohol retention time.

Influence of PNP on the capacity factors

A comparison (Fig. 3) of the capacity factors of the alcohols in the absence (k'_0) and the presence (k'_2) of PNP shows that the same sequence of retention is observed. It appears that the presence of PNP in the mobile phase slightly increases the retention of the C_1–C_3 alcohols but does not modify the retention of the C_4 alcohols. This result can be interpreted as a stronger interaction of C_1–C_3 alcohols with the stationary phase saturated by the PNP.

Influence of the addition of β-CD to the eluent

Fig. 4 compares the k' values of the C_1–C_4 alcohols in the presence of β-CD in the eluent to the k'_0 values measured without PNP and β-CD (refractive index detection) in the same eluent. For comparison the k' values of alcohols have been

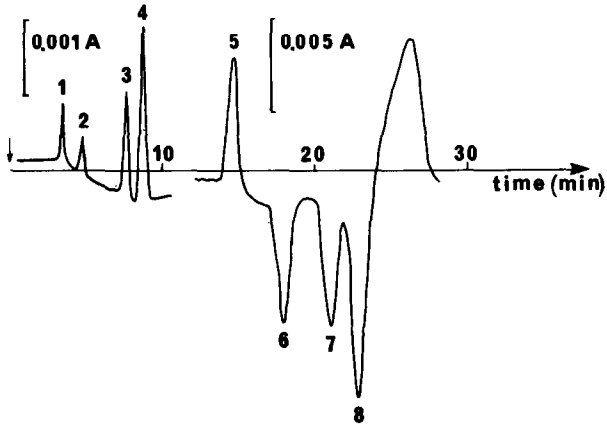

Fig. 2. Indirect detection of a 5‰ (v/v) aliphatic alcohols mixture. Peaks: 1 = methanol; 2 = ethanol; 3 = 2-propanol; 4 = 1-propanol; 5 = PNP (system peak); 6 = 2-butanol; 7 = 2-methyl-1-propanol; 8 = 1-butanol; 9 = β-CD (system peak). Mobile phase: phosphate buffer–methanol (90:10, v/v), containing 10^{-3} *M* β-CD and 10^{-5} *M* PNP. (From ref. 27, with permission).

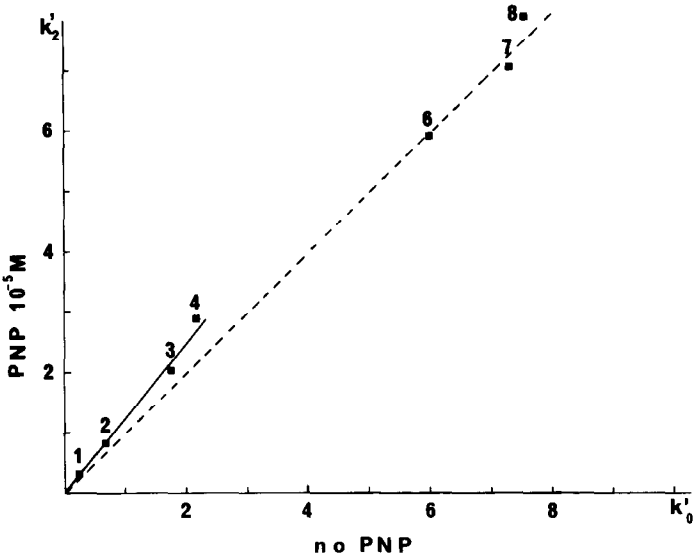

Fig. 3. Comparison of capacity factors of alcohols in the presence or the absence of PNP in the mobile phase. k'_0 values were determined by refractive index detection. The alcohols are referred by the same numbers as in Fig. 2. Mobile phase: phosphate buffer–methanol (90:10, v/v).

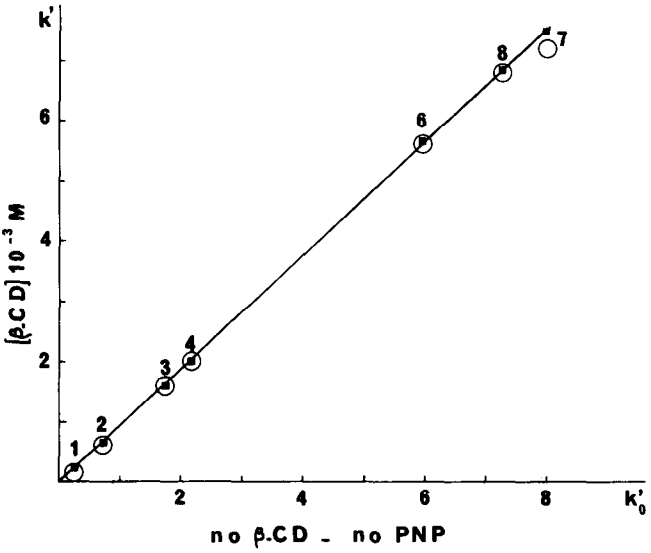

Fig. 4. Comparison of capacity factors of alcohols in different experimental conditions. ■ = 10^{-3} *M* β-CD, no PNP; ○ = 10^{-3} *M* β-CD and 10^{-5} *M* PNP. The alcohols are referred by the same numbers as in Fig. 2. Mobile phase: phosphate buffer–methanol (90:10, v/v).

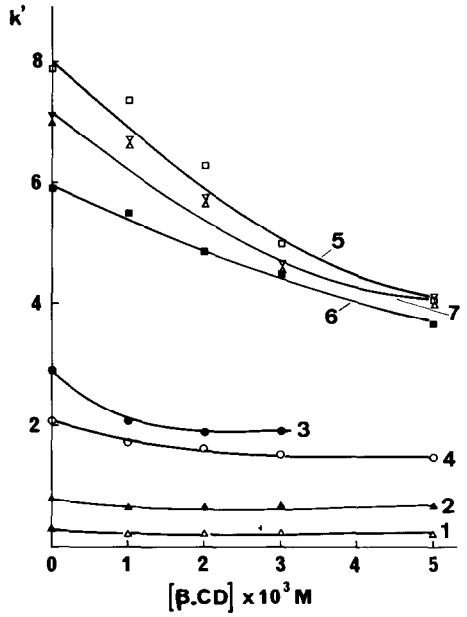

Fig. 5. Variation of capacity factors of aliphatic alcohols on β-CD concentration. 1 = Methanol; 2 = ethanol; 3 = 1-propanol; 4 = 2-propanol; 5 = 1-butanol; 6 = 2-butanol; 7 = 2-methyl-1-propanol. Mobile phase: phosphate buffer–methanol (90:10, v/v), containing 10^{-5} *M* PNP. The concentration of the alcohol solutions was 5‰ for methanol, 2‰ for ethanol and propanol and 0.5‰ for butanol (v/v).

measured with an indirect detection in the presence of PNP and β-CD in the eluent as a function of k'_0 defined above (Fig. 4). In both cases a linear variation is observed, the slope of which is 0.94, evidencing a slight alcohol–β-CD interaction in the mobile phase. It means that the previously observed short-chain alcohol–PNP interaction disappears.

The effect of β-CD at various concentrations in the mobile phase on the retention of aliphatic alcohols is reported on the Fig. 5. The variation of the capacity factor of all these alcohols with the concentration of β-CD is in agreement with the β-CD inclusion stability constants of these alcohols [24].

Calibration curves

We tested the validity of this indirect detection method in order to develop a quantitative analysis of these aliphatic alcohols. Fig. 6 shows results obtained with 1-propanol (a) and 2-butanol (b), which are eluted just before and after the first system peak, respectively. The detector response was linear and proportional to the amount of alcohol injected (from 10 to 200 μg). For several alcohols the peak area increases until $[\beta\text{-CD}] = 2 \cdot 10^{-3}$ *M* and decreases beyond this value.

Similar linear calibration curves were obtained with the other alcohols studied except for the low methanol concentrations for which a deviation was observed. However, the measurement accuracy is low when the sample emerges very near the system peak. For example with 1- and 2-propanol the vicinity of positive and negative peaks lowers the peak area measurement.

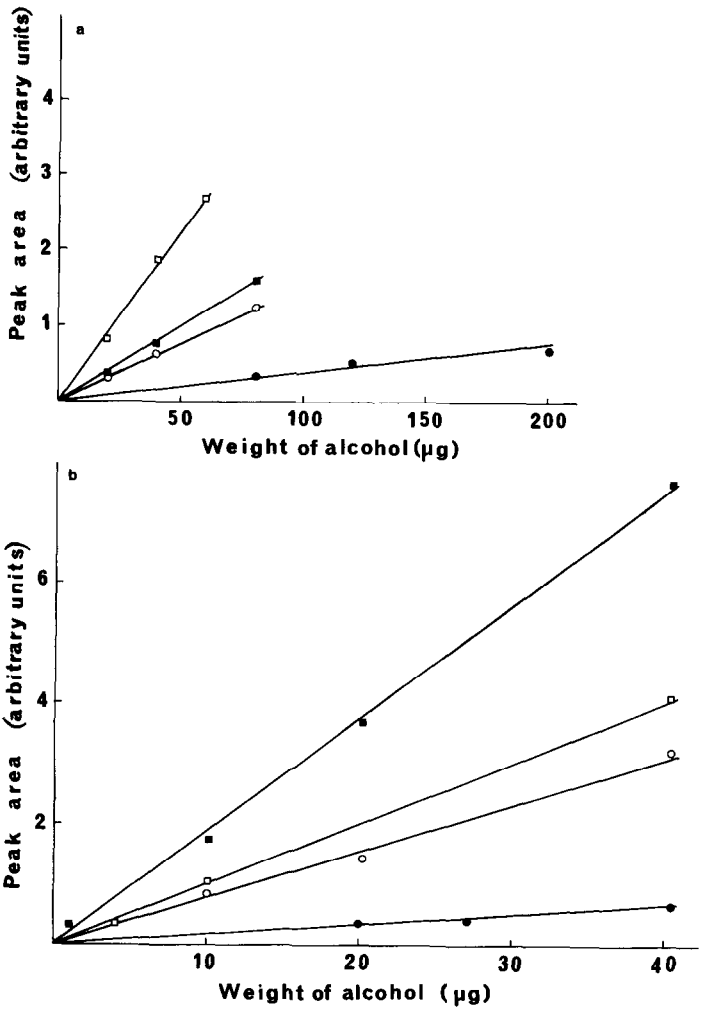

Fig. 6. Calibration curves of 1-propanol (a) and 2-butanol (b) at different β-CD concentrations. ● = [β-CD] = 0; ○ = [β-CD] = 10^{-3} *M*, ■ = [β-CD] = $2 \cdot 10^{-3}$ *M*; □ = [β-CD] = $3 \cdot 10^{-3}$ *M*. Mobile phase: phosphate buffer–methanol (90:10, v/v), containing 10^{-5} *M* PNP.

Detection sensitivity

We have determined the detection sensitivity obtained with the visible-absorbing PNP probe. The detection sensitivity for a given sample is expressed by the apparent molar absorptivity, ε*, in the chromatographic systems used. This ε* coefficient was defined by Hackzell and Schill [4]:

$$\varepsilon^* = Ysu/mdb$$

where Y is the sample peak area, s is the detector setting range, u is the flow-rate, m is the amount of the compound, d is the chart speed and b is the path length in the detector cell.

Fig. 7 shows that the addition of β-CD to an eluent containing 10^{-5} *M* PNP gives a convenient sample detection. The variation of the conditional molar absorptivity ε* is far more important (for 10^{-3} *M* and $2 \cdot 10^{-3}$ *M* β-CD concentrations) than the variation of the absorbance molar coefficient determined from static spectroscopic measurements in the presence of β-CD at the same concentration.

This point was verified from the spectra obtained by adding small amounts of each alcohol studied to a potassium phosphate buffer–methanol (90:10) solution containing 10^{-5} *M* PNP. No significant variation in absorbance was noticed.

On the other hand, Fig. 7 shows that the detection sensitivity reaches a maximum value when the relative retention of the sample (k'_{sample}/k'_{PNP}) is close to unity as previously described [3].

It must be emphasized that the addition of β-CD modifies the ratio k'_{sample}/k'_{PNP} for a given alcohol. In order to investigate the enhancing effect of β-CD on detection sensitivity we used a potassium phosphate buffer–methanol (85:15) solution containing 10^{-5} *M* PNP as mobile phase. This percentage was chosen so that the k'_{sample}/k'_{PNP} ratio was similar to that obtained in the presence of 10^{-3} *M* β-CD. In these conditions the ε* values obtained in all cases were from three to twenty times lower than those obtained with 10^{-3} *M* β-CD in the mobile phase, depending upon the hydrophobic properties of the studied alcohol (Fig. 8).

Fig. 9 shows the variation of the detection sensitivity (response) for each compound with β-CD concentration. For most alcohols studied, the response increases when the β-CD concentration varies from 10^{-3} *M* to $2 \cdot 10^{-3}$ *M*. Beyond $2 \cdot 10^{-3}$ *M* a decrease is observed. However, for the two propanol alcohols, eluted just before the system peak, the response increases continuously with the β-CD concen-

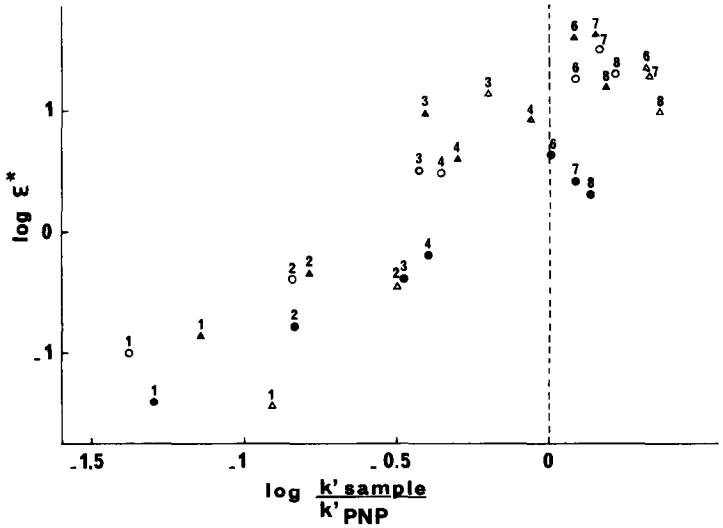

Fig. 7. Influence of relative alcohols retention on the detection sensitivity with different β-CD concentrations in the mobile phase. The alcohols are referred by the same numbers as in Fig. 2. Mobile phase: phosphate buffer–methanol (90:10, v/v) containing 10^{-5} *M* PNP. ● = [β-CD] = 0; ○ = [β-CD] = 10^{-3} *M*; ▲ = [β-CD] = $2 \cdot 10^{-3}$ *M*; △ = [β-CD] = $3 \cdot 10^{-3}$ *M*. (From ref. 27, with permission).

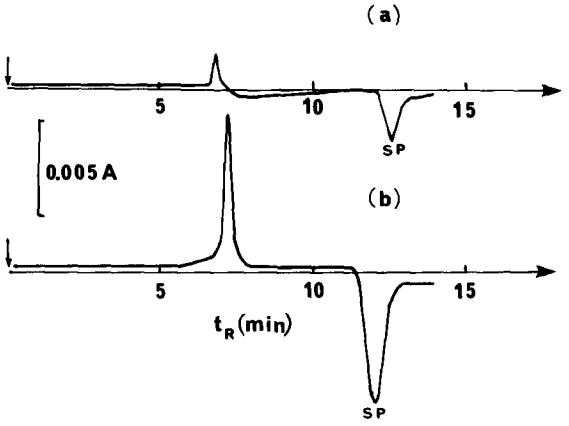

Fig. 8. Indirect detection of 2-propanol without β-CD (a) and with $2 \cdot 10^{-3}$ *M* β-CD (b). The experimental conditions were: (a): injected 2-propanol solution 5‰ (v/v), in a mobile phase of phosphate buffer–methanol (85:15, v/v), containing 10^{-5} *M* PNP; (b): injected 2-propanol solution 2‰ (v/v), in a mobile phase of phosphate buffer–methanol (90:10, v/v), containing 10^{-5} *M* PNP.

tration. The opposite response is obtained with 1-butanol which decreases continuously with increasing β-CD. Thus the 10^{-3} *M* β-CD concentration in the mobile phase is the best compromise between high detection sensitivity and satisfactory separation of the different compounds.

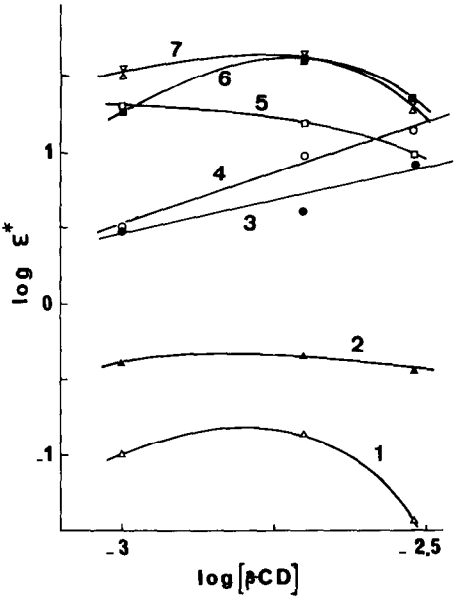

Fig. 9. Dependence of detection sensitivity on β-CD concentration. Mobile phase: phosphate buffer–methanol (90:10, v/v), containing 10^{-5} M PNP. 1 = Methanol; 2 = ethanol; 3 = 1-propanol; 4 = 2-propanol; 5 = 1-butanol; 6 = 2-butanol; 7 = 2-methyl-1-propanol.

DISCUSSION

The detection system described here is very complex since the detectable species PNP exists at pH 7.85 mainly in the ionized form PNP^- (pK_a = 7.15) and the ionization equilibrium can be displaced when methanol or β-CD are added to the mobile phase.

The indirect detection principle was given by Parkin [10]. As the eluting peak for all contains a large excess of compound over the UV-detectable species, the author has suggested that the presence of compounds modifies the partitioning characteristics of the UV-absorbing species during its passage through the column.

As a consequence, in all the elution zones the concentration of the eluent constituents, including the detectable species, is slightly different from those of the present eluent. Thus each constituent of the mobile phase of which concentration has been modified can give a "system peak".

Crommen [24] has developed a mathematical analysis in order to explain the sense and the area of the observed peaks. A more complete calculation of the chromatographic profile corresponding to indirect detection was theoretically achieved by Golshan-Shirazi and Guiochon [25].

The present case is different and more complex. Indeed, several species in the eluent can interact with the stationary phase and the alcohols. Different equilibriums must be considered:

In the mobile phase:

$$(PNP)_m + (CD)_m \rightleftarrows (CD\text{–}PNP)_m \quad (1)$$

$$(A)_m + (CD)_m \rightleftarrows (CD\text{–}A)_m \quad (2)$$

With the stationary phase:

$$(A)_m \rightleftarrows (A)_s \quad (3)$$

$$(PNP)_m \rightleftarrows (PNP)_s \quad (4)$$

$$(CD\text{–}PNP)_m \rightleftarrows (CD\text{–}PNP)_s \quad (5)$$

$$(CD\text{–}A)_m \rightleftarrows (CD\text{–}A)_s \quad (6)$$

$$(CD)_m \rightleftarrows (CD)_s \quad (7)$$

where A designates the injected alcohol.

Alcohol injection perturbs the partition equilibrium of all the constituents of the mobile phase. The presence of the CD–A complex in the mobile phase modifies the partitioning of CD–PNP and CD which are released in the mobile phase following the two equilibria 5 and 7, displaced to the left, and give rise to two "system peaks". The first peak corresponds to PNP, the second to CD. The intensity of the indirect detection signal essentially depends upon the competition between the reactions 5, 6 and 7.

The increase in β-CD concentration in the mobile phase leads to stronger

complexation of the alcohol and shifts reaction 2 to the right, involving a more important desorption of CD–PNP and CD.

The previously reported properties of the apparent molar absorptivity coefficient are verified in our case in the sense that it is higher when the capacity factors of injected alcohol and PNP are very close to each other. The presence of β-CD in the eluent plays a special role since the release of PNP following the injection of alcohol gives a greater signal than that obtained in the absence of CD, permitting an enhancement of the alcohol detection.

A similar technique applied to steroids has permitted us to increase the efficiency of their indirect detection [26]. A complete analysis of the phenomena involved in the formation of several complexes in the mobile phase is at present underway.

REFERENCES

1 N. Parris, *J. Liq. Chromatogr.*, 3 (1980) 1743.
2 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
3 M. Denkert, L. Hackzell, G. Schill and E. Sjögren, *J. Chromatogr.*, 218 (1981) 31.
4 L. Hackzell and G. Schill, *Chromatographia*, 15 (1982) 437.
5 A. Herrmann, E. Damawandi and M. Wagmann, *J. Chromatogr.*, 280 (1983) 85.
6 L. Hackzell, T. Rydberg and G. Schill, *J. Chromatogr.*, 282 (1983) 179.
7 T. Gnanasambandan and H. Freiser, *Anal. Chem.*, 53 (1981) 909.
8 T. Gnanasambandan and H. Freiser, *Anal. Chem.*, 54 (1982) 1282.
9 T. Gnanasambandan and H. Freiser, *Anal. Chem.*, 54 (1982) 2379.
10 J. E. Parkin, *J. Chromatogr.*, 287 (1984) 457.
11 S. Banerjee, *Anal. Chem.*, 57 (1985) 2590.
12 J. E. Parkin and H. T. Lau, *J. Chromatogr.*, 314 (1984) 488.
13 K. Uekama, F. Hirayama and S. Nasu, *J. Chem. Pharm. Bull.*, 26 (1973) 3477.
14 W. L. Hinze and D. W. Armstrong, *Anal. Lett.*, 13 (1980) 1093.
15 W. L. Hinze, *Sep. Purif. Methods*, 10 (1981) 159.
16 D. Sybilska, J. Lipkowski and J. Woycikowski, *J. Chromatogr.*, 253 (1982) 95.
17 K. Fujimura and T. Ueda, *Anal. Chem.*, 55 (1983) 446.
18 D. Sybilska, J. Debowski, J. Jurczak and J. Zukowski, *J. Chromatogr.*, 286 (1984) 163.
19 D. W. Armstrong, F. Nome, L. A. Spino and T. D. Golden, *J. Am. Chem. Soc.*, 108 (1986) 1418, and several references therein.
20 K. Fujimura, T. Ueda, M. Kitagama, H. Takayanagi and T. Ando, *Anal. Chem.*, 58 (1986) 2668.
21 A. Buvari, J. Szejtli and L. Barcza, *J. Incl. Phenom.*, 1 (1983) 151.
22 A. Buvari and L. Barcza, *J. Chem. Soc., Perkin Trans. II*, 4 (1988) 543.
23 K. A. Connors and J. M. Lipari, *J. Pharm. Sci.*, 65 (1976) 379.
24 J. Crommen, *Thèse d'Agrégation*, Liège University, Liège, (1989).
25 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 62 (1990) 923.
26 B. Agnus, M. Gosselet and B. Sebille, *J. Chromatogr.*, 552 (1991) 583.
27 M. Gosselet and B. Sebille, *Collection Minutes*, Edition de Santé, Paris, 1990, p. 269.