



# Permethylated $\beta$ -cyclodextrin in liquid poly(oxyethylene) as a stationary phase for capillary gas chromatography

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## ABSTRACT

This paper reports the study of poly(oxyethylene) as a solvent for heptakis(2,3,6-tri-*O*-methyl)- $\beta$ -cyclodextrin (PM- $\beta$ -CD) and the potential of these mixtures for constructing enantioselective gas chromatography columns. Enantioseparations of volatile racemic mixtures using capillaries coated with 10–50% PM- $\beta$ -CD diluted in Carbowax 20M were evaluated. The influence of the polymer type on retention, separation and reproducibility over a period of time is critically discussed. The poly(oxyethylene)-based columns were also compared to columns prepared by dilution of PM- $\beta$ -CD in two polysiloxanes, SE-30 and SE-54, at several temperatures. Finally, we evaluated a new stationary phase prepared by crosslinking the chiral selector to the poly(oxyethylene) matrix.

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## 1. Introduction

Capillary columns based on cyclodextrin derivatives (CDs) are widely used for enantioselective separations by high-resolution gas chromatography (HRGC). These chiral selectors were first introduced by Koscielsky et al. [1] for use in packed columns. Since then, several other authors have systematically investigated the performance of chiral capillary columns coated with a large number of modified CDs [2–12]. Most of these stationary phases, derived from  $\alpha$ -,  $\beta$ - or  $\gamma$ -CDs, were prepared by diluting the CD derivative in a polysiloxane to obtain selective enantioseparations at relatively low temperatures [3]. A few stationary phases derived from  $\beta$ -CD were prepared by chemically bonding permethylated alkenyl- $\beta$ -CD to a polysiloxane backbone, leading to columns characterized by good chiral recognition abilities when used in capillary GC and in supercritical fluid chromatography [13,14]. Since then, a wide variety of derivatives have been used to coat capillary columns. A review of the reported results shows that the enantioselective ability of a given CD derivative is not limited to certain class of compounds; their chiral recognition abilities are observed toward an extremely wide variety of chemical families [3,11,15].

The dilution of cyclodextrin derivatives in a polysiloxane stationary phase has proven to have several advantages. First, the thermodynamic data that characterize the enantioselectivity can be

obtained from the retention data of the two enantiomers being separated in columns containing different concentrations of the chiral selector. The second reason for diluting the chiral selector is a wider range of the column temperature; that is, it is possible to work at temperatures below the usually high melting points of the pure derivatives. Finally, improved column efficiencies can be achieved due to the favorable coating properties of polysiloxanes. For these phases, the amount of chiral selector in the polymeric stationary phase has a large influence on enantioselectivity. As expected, Schmarr et al. reported that low cyclodextrin concentrations reduce enantioresolution [16]. Hardt and König [17] also reported a significant increase in selectivity as the amount of octakis(3-*O*-butyryl-2,6-di-*O*-pentyloxy)- $\gamma$ -cyclodextrin in OV-1701 increased, but the separation factor leveled off when the concentration of the chiral selector neared 50% (w/w). Bicchi et al. achieved separation of 1,8-epoxy-9-(3-methyl-6-buten-1-yl)-*p*-methane using 30% permethylated  $\beta$ -CD in OV-1701 but failed when the permethylated  $\beta$ -CD was at a concentration of 10% (w/w) [10]. Jung et al. demonstrated both theoretically and experimentally that different solutes exhibited different behaviors; that is, the apparent enantioselectivity factor for each racemate reached a plateau at different amounts of chiral selector [18]. In general, however, it is expected that the highest possible concentration of the CD derivative compatible with its solubility in the polymeric solvent should be used to achieve the highest enantioselective factor for most solutes. On the other hand, several experiments conducted by using the same concentration of chiral CD diluted in different polymeric stationary phases demonstrated the significant influence of the polysiloxane chosen as the solvent on the enantioselectivity. As a general rule,

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it has been stated that less polar polymers lead to enhanced enantioselectivity. However, the conclusions of those experiments do not seem to be conclusive. Thus, the main dilemma is that the solubility of the most polar CD derivatives in weakly or moderately polar polysiloxanes is rather limited at relatively low temperatures, while low elution temperatures are required to enhance the molecular interactions that lead to enantioseparation. Therefore, conditions that favor low chromatographic temperatures should be selected to improve the resolution of enantiomers. The maximum solubility that allows gas–liquid chromatographic separations at low temperatures has to be critically studied for each mixture of the specific cyclodextrin derivative and the achiral stationary phase in which it is diluted.

Heptakis(2,3,6-tri-*O*-methyl)- $\beta$ -cyclodextrin (PM- $\beta$ -CD) is one of the CD derivatives most often used as the chiral phase in GC [8,19]. PM- $\beta$ -CD is most commonly diluted in moderately polar polysiloxanes such as OV-1701 [3]. These chiral columns have given very good results, and this type of chiral stationary phase has been shown to be highly universal [15]. The solubility of PM- $\beta$ -CD in moderately polar OV-1701 is, however, limited to about 10–14% (w/w) (~0.11 molal). To increase the relative amount of the chiral selector to 0.17 molal, Schurig chemically immobilized mono-6-*O*-octamethylenepermethy- $\beta$ -cyclodextrin on poly(dimethylpolysiloxane) [20]. Chiral GC columns using this stationary phase are under the trade name of Chirasil-DEX.

Capillary columns based on Carbowax 20M, the more common name for poly(oxyethylene) [21], are used for the retention of compounds of medium and high polarities. These phases exhibit good selectivity toward alcohols, diols and aromatic isomeric molecules. The structure of the polymer, H-(OCH<sub>2</sub>CH<sub>2</sub>)<sub>*n*</sub>-OH, makes it especially interesting as a solvent for relatively polar molecules, such as cyclodextrin derivatives. Despite the potential usefulness of Carbowax, their use as a solvent of PM- $\beta$ -CD has been very scarce [22,23].

This paper aims to study the use of poly(oxyethylene) (Carbowax 20M) as the diluting polymer for PM- $\beta$ -CD. We have evaluated the selector solubility, chromatographic performance and enantioselective ability of the capillary columns constructed with these systems. The physical properties of this polymer do not allow the column temperature to drop below its melting point (about 60 °C) [24]. Below this temperature, adsorption, and no partition, does occur. We have also crosslinked the poly(oxyethylene) after coating the inner wall of the capillary with the phase solution to allow lower chromatographic temperatures. Different relative amounts of crosslinking reagents were tested with the aim of optimizing the capillary column performance.

## 2. Experimental

### 2.1. Materials

Permethy- $\beta$ -cyclodextrin was obtained from Cyclolab (Budapest, Hungary). Fused-silica capillaries were obtained from Microquartz Electronics (Münich, Germany). Poly(oxyethylene) (Carbowax 20M) was purchased from Alltech (Deerfield, IL, USA). SE-30, SE-54 and OV-1701 were obtained from Supelco (Bellefonte, PA, USA). Benzoyl peroxide, poly[oxy(methyl-1,2-ethanediy)] (PPGDA, CAS number 25322-694) and all other materials including the racemic mixtures were obtained from Aldrich (Milwaukee, WI, USA), Sigma (St. Louis, MO, USA) or Fluka (Ronkonkoma, NJ, USA). All solvents used in the construction of the capillary columns were spectroscopic grade and were obtained from Merck (Darmstadt, Germany). A 25 m Hydrodex  $\beta$ -PM column (250  $\mu$ m I.D.) was purchased from Macherey-Nagel (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

### 2.2. Capillary gas chromatography

Chromatographic runs were carried out on a HP6890 chromatograph with flame ionization detection and manual injection. The solutes were injected in the vapor phase by applying the “wet-needle” technique, using split ratios greater than 100:1. The instrument was connected to a computer, and data acquisition was done through Clarity software (DataApex, Czech Republic). Chromatographic grade dinitrogen was used as the carrier gas, and the flow was controlled by keeping the pressure constant at the column inlet. Injector and detector temperatures were 220 and 250 °C, respectively. All chromatographic measurements were isothermal, ranging from 25 to 100 °C.

### 2.3. Preparation of the coated capillary columns

Silica capillary tubes of about 20 m in length with an internal diameter of 250  $\mu$ m were heated to 200 °C in a slow stream of dinitrogen for several hours and then statically coated with the stationary phases. In all experimental procedures, great care was taken to avoid introducing dioxygen into the capillary columns.

A fast static evaporation method at a high temperature was used. We designed a special oven for this purpose. The static method has the advantage of reducing the evaporation time required compared to the time needed for the usual vacuum procedure. This method has been successfully applied to the preparation of several capillary columns in our laboratory [25,26]. Thus, mixtures of PM- $\beta$ -CD and Carbowax 20M were prepared and then dissolved in dichloromethane. The columns filled with these solutions were heated to allow evaporation of the solvent. After complete solvent evaporation, the columns were conditioned by raising the temperature to 150 °C. The columns were left overnight at 120 °C under a dinitrogen stream, then were cooled and transferred to the HP6890 gas chromatograph. The film thickness was kept at approximately 0.1  $\mu$ m. The columns constructed are described in Table 1.

Poly(oxyethylene) and poly(oxypropylene) diacrylate (PPGDA) were crosslinked during the coating of some columns (see Table 1).

**Table 1**  
Columns<sup>a</sup> and stationary phases.

| Denomination     | Column length (m) and film thickness ( $\mu$ m) | Stationary phase                          |
|------------------|---|---|
| WX               | 20 $\times$ 0.2                                 | Carbowax 20M (C20M)                       |
| $\beta$ PWX10    | 23 $\times$ 0.1                                 | 10% Permethy- $\beta$ -CD/C20M            |
| $\beta$ PWX20    | 36 $\times$ 0.1                                 | 20% Permethy- $\beta$ -CD/C20M            |
| $\beta$ PWX30    | 20 $\times$ 0.1                                 | 30% Permethy- $\beta$ -CD/C20M            |
| $\beta$ PWX50    | 20 $\times$ 0.2                                 | 50% Permethy- $\beta$ -CD/C20M            |
| $\beta$ PXL10-10 | 20 $\times$ 0.1                                 | 10% Permethy- $\beta$ -CD/C20M/10% PPG DA |
| $\beta$ PXL20-10 | 20 $\times$ 0.1                                 | 20% Permethy- $\beta$ -CD/C20M/10% PPG DA |
| $\beta$ PXL10-20 | 20 $\times$ 0.2                                 | 10% Permethy- $\beta$ -CD/C20M/20% PPG DA |
| $\beta$ PXL10-30 | 20 $\times$ 0.25                                | 10% Permethy- $\beta$ -CD/C20M/30% PPG DA |
| $\beta$ PXL30-50 | 20 $\times$ 0.17                                | 30% Permethy- $\beta$ -CD/C20M/50% PPG DA |
| $\beta$ PXL30-10 | 20 $\times$ 0.14                                | 30% Permethy- $\beta$ -CD/C20M/10% PPG DA |
| $\beta$ PSE54-30 | 20 $\times$ 0.1                                 | 30% Permethy- $\beta$ -CD/SE54            |
| $\beta$ PSE30-30 | 17 $\times$ 0.1                                 | 30% Permethy- $\beta$ -CD/SE30            |

<sup>a</sup> Capillary diameter was 250  $\mu$ m id for all columns.

Benzoyl peroxide (0.1%) was used as the radical initiator. Concentrations of PPGD from 10% to 50% (w/w) were tested. Capillary coating efficiencies were checked by estimating column plate numbers for *n*-alkanes; the typical values were greater than 2000 plates per meter.

#### 2.4. SEM Imaging of the coated capillary column

The images were obtained using a Philips SEM 505 instrument with image digitalization ADDA II and the software Imaging System. Two random pieces of two capillary columns containing pure Carbowax 20M and 20% PM- $\beta$ -CD/Carbowax 20M stationary phases were mounted on a septa and then coated with a thin gold layer. The voltage applied was de 25 kV.

#### 2.5. Differential scanning calorimetry

A Shimadzu DSC-50 thermal analyzer system was used. 3 g samples of mixtures of 30% PM- $\beta$ -CD in SE 30, in SE54 and in Carbowax 20M were sealed in aluminum cells and scanned from room temperature to 200 °C at a heating rate of 5 °C/min. The cooling was done at -5 °C/min during 30 min, followed by two other heating/cooling cycles.

### 3. Results and discussion

The capillary columns constructed in this work, including their geometrical characteristics, are detailed in Table 1. A pure Carbowax 20M column and capillaries coated with two non-polar phases containing PM- $\beta$ -CD in SE-30 and in SE-54 were used for comparison purposes.

Although the coating method has been proven effective when using both polar and non-polar phases [27,28], the effectiveness of this fast static technique in the preparation of the chiral columns was checked. Using SEM analysis, the presence of a uniform film within the capillary GC column could be confirmed (Fig. 1). Stationary-phase thickness was measured in two random positions, and the average of these values was 0.27  $\mu$ m. Visually, there was little variation around the capillary circumference, and no bare areas were observed.

In Fig. 2, the plate numbers for some chiral analytes in one of these home-made columns were compared with those obtained using a commercial column of the same dimensions. Unfortunately, the film thickness of the commercial column is not available. The

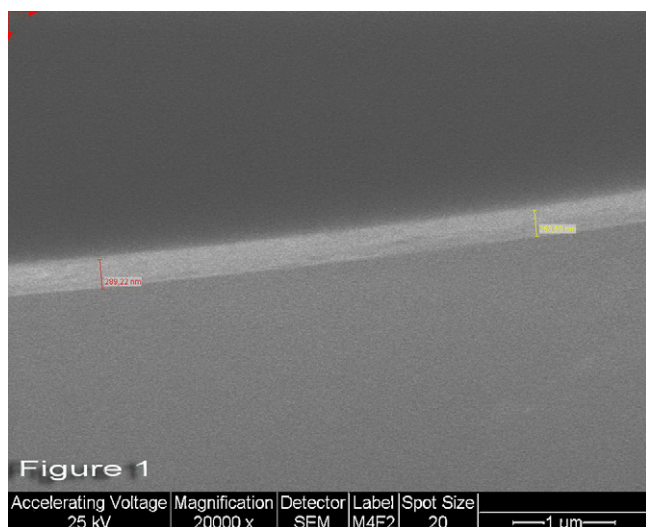


Fig. 1. SEM image of the 20% PM- $\beta$ -CD in Carbowax 20M.

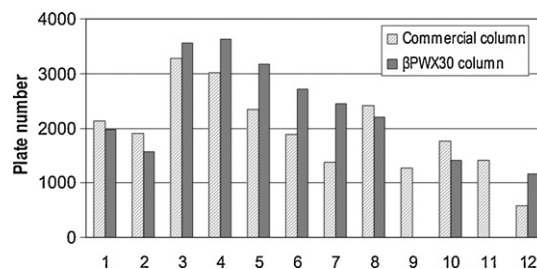


Fig. 2. Comparison of plate numbers obtained with  $\beta$ PWX30 and Hydrodex  $\beta$ -PM chiral column (Macherey-Nagel). Solutes—1 and 2: enantiomers of 1-phenyl-ethanol at 120 °C; 3 and 4: 2,5-dimethyltetrahydrofuran at 80 °C; 5, 6 and 7: isomers of 2,5-dimethoxytetrahydrofuran at 80 °C; 8 and 9: 2-pentanol at 80 °C; 10 and 11: 4-methyl-2-pentanol at 80 °C; 12: 2-heptanol at 80 °C.

efficiencies of both columns at the same temperature and linear velocities were comparable. Improved plate numbers would be possible if dihydrogen or helium were used as the carrier gas.

PM- $\beta$ -CD is one of the most extensively used chiral selectors. From the exhaustive work of Schurig and co-workers [8,15,20], it is evident that a wide range of chiral analytes can be enantioseparated with this selector. Usually, the selector is dissolved in the moderately polar polysiloxane OV-1701 [3] or in SPB-35 (35% diphenyl, 65% dimethylpolysiloxane); columns using this kind of stationary phase are commercially available. However, the solubility of PM- $\beta$ -CD in the OV-1701 polymer is limited. According to the study by Bicchi et al. [29], the minimum operating temperature for columns containing PM- $\beta$ -CD were 110 °C for the column coated with undiluted PM- $\beta$ -CD, 75 °C for the column coated with 30% PM- $\beta$ -CD in OV-1701, 55 °C for the column coated with 10% PM- $\beta$ -CD in OV-1701, and 0 °C for the column coated with 5% PM- $\beta$ -CD in the same polysiloxane. Research has been carried out with several other commonly used polymers with the aim of obtaining a higher PM- $\beta$ -CD solubility and, as a consequence, better enantioseparations. Table 2 summarizes the diluting solvents that have been used for this CD derivative along with their GC polarities as measured by the McReynolds numbers.

From Table 2, it is evident that high polarity stationary phases have rarely been reported in the literature even though solubility of the chiral selector is expected to increase as the polarity of the polymer increases.

The polarity of Carbowax 20M, which is also included for comparison in Table 2, is approximately three times greater than the polarity of the more polar polysiloxanes. Previously, Szejtli and co-workers dissolved randomly methylated  $\alpha$ -cyclodextrin (degree of substitution between 1.8 and 2.0) at 50% in Carbowax 400, indicating that this CD is completely dissolved even at this high concentration [22]. Moreover, earlier studies demonstrated that ethylene glycol and formamide, both of which are very polar, were good solvents for native  $\alpha$ -CD and  $\beta$ -CD, and columns packed with  $\alpha$ -CD dissolved in these solvents led to highly enantioselective systems [1,30]. The last column of Table 2 presents solubility limit data at a given column temperature that was gathered from previous studies.

#### 3.1. Comparison of C20M with SE-30 and SE-54

In Table 3, we compare the retention and separation of the enantiomers of two racemates in the columns based on SE-30, SE-54 and C20M, which were all prepared with the same film thickness and an identical amount of chiral selector. These two racemates exhibited resolution factors that were significantly larger in the column coated with PM- $\beta$ -CD in SE-54. The enantioselectivity for dimethoxytetrahydrofuran was larger in the SE-30

**Table 2**  
Nature and polarity<sup>a</sup> of the achiral polymers used to dilute permethyl- $\beta$ -cyclodextrin.

| Commercial denomination | Chemical nature   | Polarity | % (w/w) of PM- $\beta$ -CD and lowest column temperature | References      |
|-------------------------|---|----------|--|-----------------|
| SE-30/OV-101            | 100% Dimethylpolysiloxane                                   | 216/229  | –  | [3], This study |
| SE-54                   | 5% Phenyl, 1% vinyl, 94% dimethylpoly-siloxane              | 337      | 30% (>100 °C)  | This study      |
| DC-710 <sup>b</sup>     | Polydimethylsiloxane:polydiphenylsiloxane 1:1               | 727      | 10% (40–50 °C)   | [35]            |
| OV-1701                 | 86% Dimethyl, 7% cyano, 7% phenylpolysiloxane               | 790      | 10% (23 °C) <sup>c</sup>                                 | [3,36]          |
| SPB-35 (OV-11)          | 35% Phenyl, 65% dimethylpolysiloxane                        | 786      | 20% (>30 °C)   | Supelco         |
| OV-225                  | 25% Cyanopropyl, 25% phenyl, 50% dimethylpolysiloxane       | 884      | 25% (80 °C)  | [37]            |
| DC-550                  | 50% Phenyl, 50% methylpolysiloxane                          | 884      | –  | [3]             |
| Carbowax 20M            | Poly(oxyethylene)   | 2208     | <50% (60 °C)   | This study      |
| PS 086                  | 85–88% Dimethyl, 12–15% diphenylpoly siloxane OH terminated | –        | –  | [18]            |

<sup>a</sup> As measured by McReynolds indices [38].

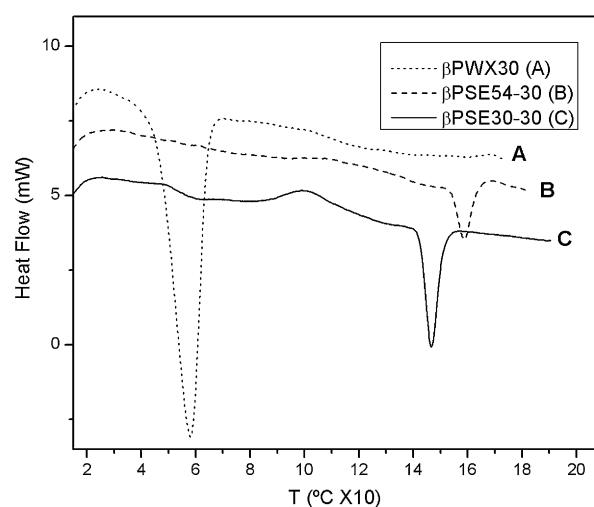
<sup>b</sup> Packed column with the phase coated on silanized Chromosorb W [35].

<sup>c</sup> Bicchi et al. reported the use of 30% PM- $\beta$ -CD in OV-1701-OH [10].

( $\beta$ PSE30-30) column, whereas trimethylcyclohexanone was better separated by the C20M ( $\beta$ PWX30) column. These results are in agreement with several previous studies. Dietrich et al. compared OV-1701-vi with SE-30 and SE-52 as achiral solvents of 2,3-dimethyl-6-tert-butyl-6-(dimethylsilyl)- $\beta$ -CD and concluded that the less polar SE-52 was better in terms of the achieved resolution [12]. NMR experiments carried out by König [11] with heptakis(3-O-acetyl-2,6-di-O-pentyl)- $\beta$ -cyclodextrin in the presence of chiral esters showed important differences in the chemical shifts due to diastereomeric associations when non-polar solvents were used. These chemical shift differences disappeared when more polar solvents were used. Similar experiments conducted using dimethyl-6-tert-butyl-6-(dimethylsilyl)- $\beta$ -CD, however, did not show differences in the chemical shifts with any of the solvents used.

The reason for the commonly observed higher enantioselectivity of the PM- $\beta$ -CD chiral selector in less polar polysiloxanes as compared to Carbowax 20M is not obvious. It is clear, however, that the low enantioselectivity observed for the  $\beta$ PWX30 column cannot be attributed to non-stereoselective retention of the analytes since retention in this column was much lower than in the other two weakly polar and non-polar columns. We also discarded the hypothesis that inclusion complexes were formed between PM- $\beta$ -CD and poly(oxyethylene) based on the strong experimental evidence that poly(ethylene glycol) is not easily included into the native  $\beta$ -CD cavity [31,32]. Although the permethylation of the  $\beta$ -CD significantly changes the physicochemical properties of the molecule, the cavity diameter is practically unaffected by this reaction [33].

On the other hand, comparison of the plate numbers provides strong evidence suggesting that an adsorption mechanism takes place at lower temperatures ( $\leq 100$  °C) in the  $\beta$ PSE54 and  $\beta$ PSE30



**Fig. 3.** Differential scanning calorimetric thermograms of 30% PM- $\beta$ -CD in Carbowax 20M (A), SE-54 (B) and SE-30 (C).

columns. Similarly, the peak broadening (decrease in the number of plates) observed below 60 °C when using PM- $\beta$ -CD in C20M indicate the formation of a heterogeneous phase.

To study the thermal behavior of these phases, differential scanning calorimetric (DSC) analysis of these mixtures was conducted. The results of these experiments are shown in Fig. 3. The first peak at 61.9 °C in thermogram A corresponds to an endothermic fusion; this temperature is close to the melting temperature of pure C20M [24]. A small endothermic effect was observed at 71–72 °C, a temperature range that is close to the glass transition temperature

**Table 3**  
Retention and selectivity for two racemates in columns containing 30% PM- $\beta$ -CD in three polymers.

| Solute                       | T (°C) | SE30    |            |       |        | SE54  |          |      |      | C20M  |          |      |      |
|------------------------------|--------|---------|------------|-------|--------|-------|----------|------|------|-------|----------|------|------|
|                              |        | $k_1^a$ | $\alpha^b$ | $N^c$ | $Rs^d$ | $k_1$ | $\alpha$ | $N$  | $Rs$ | $k_1$ | $\alpha$ | $N$  | $Rs$ |
| 2,5-Dimethoxytetrahydrofuran | 90     | –       | –          | –     | –      | 1.37  | 1.20     | 220  | 2.4  | 0.39  | 1.04     | 3100 | 0.7  |
|                              | 100    | 0.61    | 1.15       | 220   | 0.9    | 0.86  | 1.18     | 700  | 2.9  | –     | –        | –    | –    |
|                              | 110    | 0.41    | 1.13       | 620   | 1.1    | 0.55  | 1.16     | 1400 | 2.7  | 0.20  | 1.0      | 4600 | –    |
| 3,3,5-Trimethylcyclohexanone | 60     | –       | –          | –     | –      | –     | –        | –    | –    | 5.43  | 1.08     | 600  | 1.6  |
|                              | 80     | –       | –          | –     | –      | –     | –        | –    | –    | 2.06  | 1.04     | 1800 | 1.1  |
|                              | 90     | 4.30    | 1.0        | <10   | –      | 5.03  | 1.051    | 130  | 0.8  | 1.35  | 1.02     | 1700 | 0.7  |
|                              | 100    | 2.95    | 1.0        | 30    | –      | 2.86  | 1.044    | 230  | 0.8  | –     | –        | –    | –    |
|                              | 110    | 1.76    | 1.0        | 100   | –      | –     | –        | –    | –    | –     | –        | –    | –    |

<sup>a</sup> Retention factor for the first eluted enantiomer.

<sup>b</sup> Enantioselectivity factors.

<sup>c</sup> Theoretical plate numbers.

<sup>d</sup> Enantioresolution factors between the enantiomers.

observed for pure PM- $\beta$ -CD [5]; this peak was not observed during the subsequent heating cycles. Finally, a large endo–exothermic effect was observed at 123.7 °C. This effect did not disappear during the subsequent heating cycles. This signal can be attributed specifically to this mixture; however, the nature of this transition is not clear. Similarly to the results reported by Sandra with pure C20M [24], the reversible cooling transition for the mixture occurred at a lower temperature, 46.6 °C, during the cooling cycle. During the second and third heating cycles, the glass transition that was observed at about 70 °C during the first cycle was not present. The chromatographic performance of this stationary phase agreed with the behavior observed in the thermogram. The efficiency of the column was greatly reduced at temperatures close to 60 °C, and this loss of efficiency was indicative of a change in the physical properties of the stationary phase in this temperature region. Similar DSC experiments were carried out with a mixture of 30% PM- $\beta$ -CD in SE-54 and in SE-30, and the thermograms showed strong endothermic peaks around 150 °C. These peaks were attributed to pure PM- $\beta$ -CD aggregates that were not soluble in the polymer (thermograms B and C). These peaks did not disappear during the subsequent cycles. However, the peak was not observed in the thermograms recorded for the 10% (w/w) PM- $\beta$ -CD in the SE-54 polysiloxane mixture (plot not shown).

The complete solubility of chiral selectors in polysiloxane is proving to be of paramount importance for separation quality.

Homogeneous solvation prevents self-association between selectors, which leads to a heterogeneous system and has detrimental consequences for the resolution in terms of plate numbers and peak shapes. The concentration of the selector in the chosen polysiloxane can be increased only if the temperature of the column is increased. However, this has negative effects on enantioseparation factors.

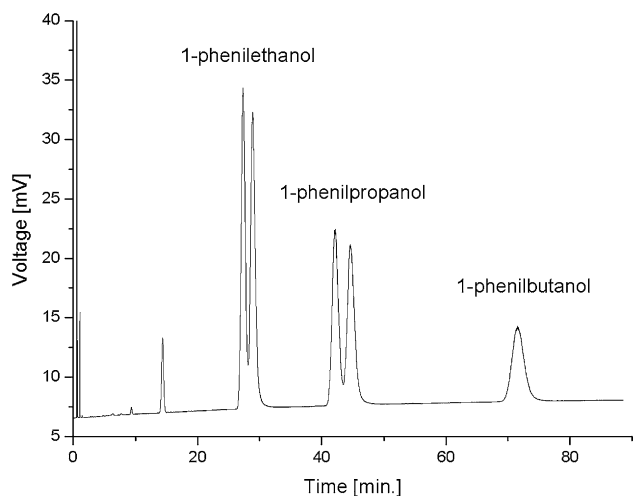
### 3.2. PM- $\beta$ -CD dissolved in C20M as the chiral stationary phase

The chromatographic results corresponding to several solutes analyzed in columns containing 20%, 30% and 50% (w/w) chiral selector are presented in the first three columns of Table 4. The number of racemic mixtures tested was greater than 25, but we have only reported the most significant results obtained with these systems, as well as a few results that were exceptions to the general behavior. It is clearly noted that the enantioselectivity increases with an increase in the amount of chiral selector dissolved in the polymer independent of the polarity or chemical structure of the analyte. It is also evident that decreases in temperature have favorable effects on enantioseparation. Camphene could be separated with the  $\beta$ PWX30 and  $\beta$ PWX50 columns, but no separation was observed for other terpenes, including limonene and  $\alpha$ -pinene, with any of the columns tested. These racemic terpenes can be separated with a commercial column containing 20% PM- $\beta$ -CD. Similarly, isopinocampheol was resolved in columns containing

**Table 4**  
Chromatographic results on permethyl- $\beta$ -cyclodextrin in poly(oxyethylene).

| Solute                       | Chiral stationary phases |                      |                           |                         |        |                      |                           |                         |        |                      |                           |                  |           |                      |                           |                         |
|------------------------------|--------------------------|----------------------|---------------------------|-------------------------|--------|----------------------|---------------------------|-------------------------|--------|----------------------|---------------------------|------------------|-----------|----------------------|---------------------------|-------------------------|
|                              | 20% CS                   |                      |                           |                         | 30% CS |                      |                           |                         | 50% CS |                      |                           |                  | 20% CS/XL |                      |                           |                         |
|                              | T (°C)                   | k                    | $\alpha$                  | Rs                      | T (°C) | k                    | $\alpha$                  | Rs                      | T (°C) | k                    | $\alpha$                  | Rs               | T (°C)    | k                    | $\alpha$                  | Rs                      |
| Dimethyltetrahydrofuran      | 80                       | 0.12<br>0.14         | 1.14 <sup>a</sup>         | 0.9 <sup>a</sup>        | 80     | 0.08<br>0.09         | 1.15 <sup>a</sup>         | 0.8 <sup>a</sup>        | 80     | 0.31<br>0.35         | 1.13 <sup>a</sup>         | 1.1 <sup>a</sup> | 60        | 0.19<br>0.22         | 1.21 <sup>a</sup>         | 2.2 <sup>a</sup>        |
| Dimethyltetrahydrofuran      | 50                       | 2.84<br>3.00<br>4.10 | 1.06<br>1.37 <sup>a</sup> | 1.0<br>3.7 <sup>a</sup> | 30     | 0.49<br>0.58<br>0.60 | 1.18 <sup>a</sup><br>1.04 | 1.6 <sup>a</sup><br>0.7 | 30     | 1.54<br>1.83<br>1.86 | 1.18 <sup>a</sup><br>1.02 | 1.0              | 23        | 0.74<br>0.90<br>0.92 | 1.22 <sup>a</sup><br>1.02 | 1.4 <sup>a</sup><br>0.7 |
| Dimethoxytetrahydrofuran     | 80                       | 0.82<br>0.84<br>1.12 | 1.03<br>1.33              | 0.6<br>5.2 <sup>a</sup> | 80     | 0.53<br>0.56<br>0.79 | 1.05<br>1.42 <sup>a</sup> | 1.1<br>8.2 <sup>a</sup> | 80     | 1.74<br>1.86<br>2.49 | 1.07<br>1.34 <sup>a</sup> | 0.7              | 60        | 1.77<br>1.82<br>2.69 | 1.03<br>1.48 <sup>a</sup> | 0.6<br>3.3 <sup>a</sup> |
| 2,6-Dimethylcyclohexanone    | –                        | –                    | –                         | –                       | –      | –                    | –                         | –                       | 80     | 3.77<br>4.55         | 1.21                      | 1.7              | –         | –                    | –                         | –                       |
| 3,3,5-Trimethylcyclohexanone | 80                       | 2.93<br>3.07         | 1.05                      | 1.1                     | 80     | 2.06<br>2.13         | 1.06                      | 1.05                    | 80     | 7.51<br>7.72         | 1.03                      | 0.9              | 60        | 3.51<br>3.64         | 1.04                      | 0.9                     |
| 3,3,5-Trimethylcyclohexanone | 60                       | 6.22<br>6.71         | 1.08                      | 0.9                     | 30     | 33.1<br>40.3         | 1.22                      | 1.6                     | 60     | 17.5<br>18.5         | 1.06                      | 1.0              | 50        | 5.85<br>6.19         | 1.06                      | 1.0                     |
| Camphene                     | –                        | –                    | –                         | –                       | 60     | 1.03<br>1.07         | 1.04                      | 0.9                     | 120    | 2.25<br>2.45         | 1.09                      | 1.0              | –         | –                    | –                         | –                       |
| 2,3-Butanediol               | 90                       | 3.89<br>4.37         | 1.12 <sup>a</sup>         | 1.2 <sup>a</sup>        | 80     | 5.07<br>6.51         | 1.28 <sup>a</sup>         | 1.9                     | 90     | 3.46<br>4.15         | 1.20 <sup>a</sup>         | 2.1 <sup>a</sup> | 60        | 12.8<br>17.2         | 1.35 <sup>a</sup>         | 2.0 <sup>a</sup>        |
| 2-Heptanol                   | 90                       | 1.18                 | 1.0                       | –                       | 80     | 1.89                 | 1.0                       | –                       | 40     | 10.7<br>11.4         | 1.05                      | 1.1              | 25        | 7.01                 | 1.0                       | –                       |
| 4-Methyl-2-pentanol          | 90                       | 0.46                 | 1.0                       | –                       | 80     | 0.72                 | 1.0                       | –                       | 60     | 3.63<br>3.77         | 1.04                      | 0.9              | 25        | 2.23                 | 1.0                       | –                       |
| 2,4-Pentanediol              | –                        | –                    | –                         | –                       | –      | –                    | –                         | –                       | 110    | 6.75<br>7.45         | 1.10 <sup>a</sup>         | 1.8 <sup>a</sup> | –         | –                    | –                         | –                       |
| Isopinocampheol              | –                        | –                    | –                         | –                       | 70     | 25.1<br>26.1         | 1.04                      | 1.0                     | 80     | 49.5<br>52.4         | 1.06                      | 0.9              | 60        | 29.7<br>30.7         | 1.02                      | 0.8                     |
| 1-Phenyl-ethanol             | –                        | –                    | –                         | –                       | 110    | 3.56<br>3.61         | 1.01                      | 0.6                     | 120    | 6.84<br>7.06         | 1.03                      | 0.7              | 100       | 2.83                 | 1.0                       | –                       |
| 1-Phenyl-propanol            | –                        | –                    | –                         | –                       | 90     | 9.09<br>9.29         | 1.02                      | 0.7                     | 80     | 75.7<br>80.2         | 1.06                      | 1.2              | –         | –                    | –                         | –                       |
| 1-Phenyl-butanol             | –                        | –                    | –                         | –                       | –      | –                    | –                         | –                       | 120    | 14.7                 | 1.0                       | –                | –         | –                    | –                         | –                       |

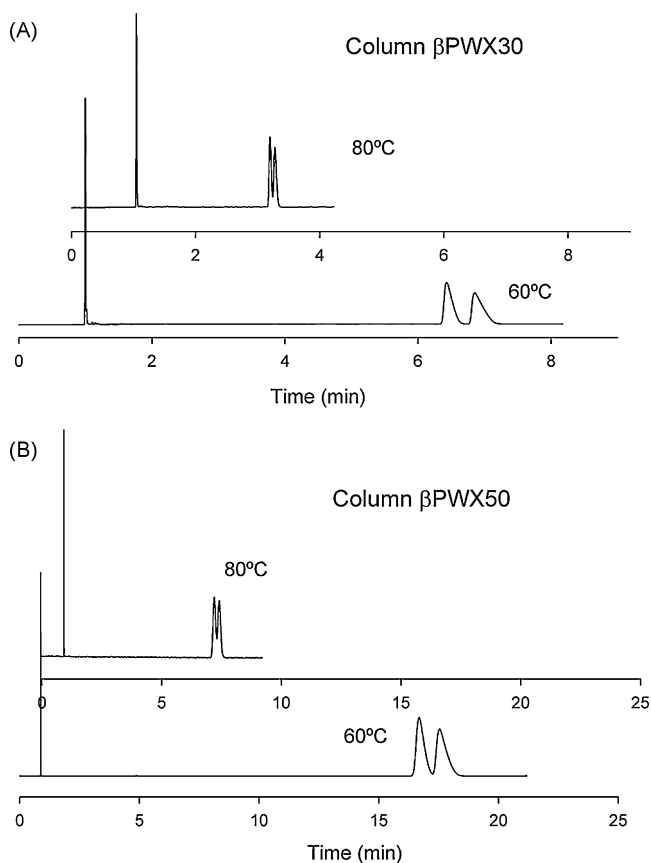
<sup>a</sup> Separation factor of geometric isomers.



**Fig. 4.** Chromatographic separation of 1-phenyl-1-ethanol, 1-phenyl-1-propanol and 1-phenyl-1-butanol at 80 °C on the  $\beta$ PWX50 column (see Table 1 for column nomenclature).

30% and 50% PM- $\beta$ -CD; however, menthol was not separated under any conditions. A representative chromatogram of a mixture of 1-phenyl-1-alcohols run at 80 °C on a  $\beta$ PWX50 column is shown in Fig. 4. Separation of 1-phenyl-1-ethanol and 1-phenyl-1-propanol was quite good; however, no enantioseparation was observed for 1-phenyl-1-butanol.

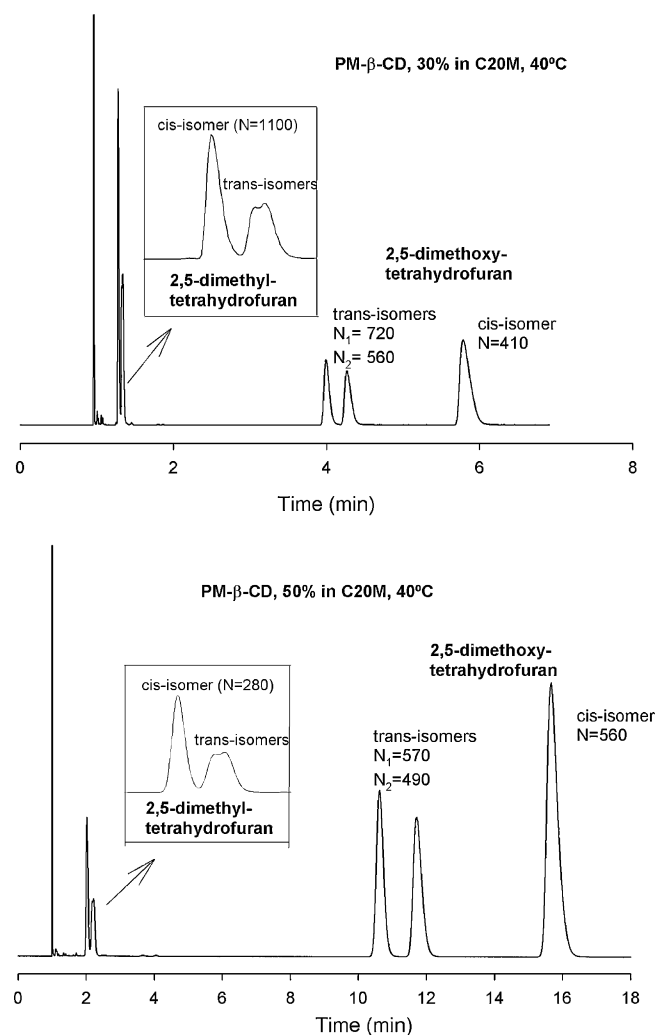
While the attempt to increase the enantioselectivity by increasing the amount of chiral selector was successful for most compounds, it failed for 3,3,5-trimethylcyclohexanone; increas-



**Fig. 5.** Enantiomeric separations of 3,3,5-trimethylcyclohexanone on the  $\beta$ PWX30 (A) and  $\beta$ PWX50 (B) columns at 60 and 80 °C (see Table 1 for column nomenclature).

ing the amount of PM- $\beta$ -CD from 30% to 50% did not improve the enantioseparation of this compound. In Fig. 5, the GC chromatograms for racemic mixtures of 3,3,5-trimethylcyclohexanone separated at 80 and 60 °C on capillary columns coated with 30% PM- $\beta$ -CD in Carbowax 20M (Plot A) is shown. The same solute was run on a column containing 50% PM- $\beta$ -CD at the same temperatures (Plot B). For this mixture, better enantioseparation was achieved with the 30% PM- $\beta$ -CD column (Plot A) for both column temperatures. A similar observation has been reported by Bicchi et al. [34]. They found that increasing the amount of 2,6-dimethyl-3-pentyl- $\beta$ -cyclodextrin from 10% to 30% in OV-1701 increased enantioresolution of some racemates but caused complete loss of resolution for the menthol racemate. On the other hand, the separation of another group of analytes was not affected by increasing the concentration of the chiral selector in OV-1701 [34].

In our study was observed that the column efficiency was strongly dependent on the column temperature and that the enantioresolution factor was very sensitive to column conditioning. This chromatographic behavior suggested that the chiral PM- $\beta$ -CD molecules were only dispersed in C20M at temperatures below 60 °C; thus, it was assumed that chiral discrimination at temperatures below 60 °C occurred via enantioselective adsorption interactions between the enantiomers and the solid chiral selector present at the surface of polymeric matrix. Fig. 6 shows



**Fig. 6.** Chromatograms of 2,5-dimethyltetrahydrofuran and 2,5-dimethoxytetrahydrofuran enantioseparated on the  $\beta$ PWX30 and  $\beta$ PWX50 columns at 40 °C (see Table 1 for column nomenclature).

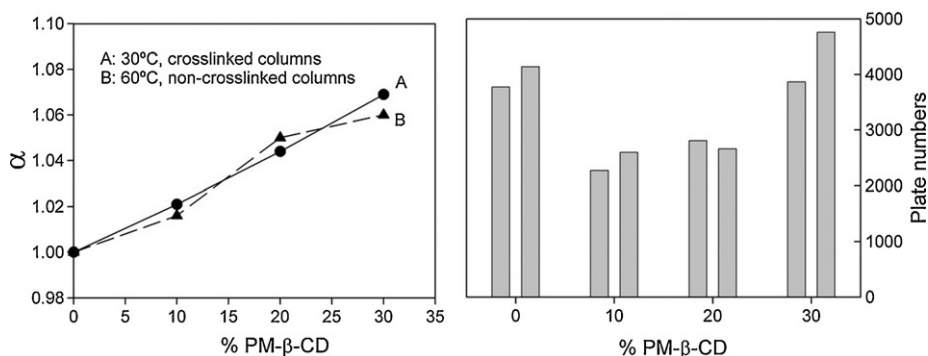


Fig. 7. Enantioselectivity and plate numbers for columns obtained after crosslinking of the Carbowax polymer as a function of the amount of PM-β-CD.

the chromatograms of a mixture of 2,5-dimethyltetrahydrofuran and 2,5-dimethoxytetrahydrofuran run on βPWX30 and βPWX50 columns at 40 °C. Separation of the *cis/trans*-diastereomers of both compounds was excellent on both columns. Indeed, very good enantioseparation of 2,5-dimethoxytetrahydrofuran was also obtained, and better selectivity factors were obtained with the column containing 50% PM-β-CD. However, the efficiencies at this temperature were rather low (see the plate numbers).

### 3.3. Chiral phases obtained by crosslinking Carbowax 20M

To improve the efficiencies at low temperatures (below 60 °C), the poly(oxyethylene) was crosslinked. Crosslinking was also intended to produce beds that are more stable over time. Columns coated with stationary phases obtained after crosslinking the polymer with PPGDA include βPXL in their names (Table 1). Details on the crosslinking reagent concentrations and the columns obtained are also presented in Table 1. After the crosslinking reaction, all the columns were conditioned at 120 °C under a flow of dinitrogen.

Although the increase in the amount of PM-β-CD resulted in an increase in the enantioselectivity, as shown in Fig. 7, all of the crosslinked stationary phases exhibited lower enantioresolution factors for all assayed analytes as compared with the analogous column containing the same amount of chiral selector dissolved in the Carbowax matrix. Some of these results can be compared in Table 4 (first and last columns). Notably, retention times were only slightly influenced by the crosslinking. It seems reasonable to think that the enantio-recognition ability of PM-β-CD was partially affected by the crosslinking reaction, whereas the main inter-

actions responsible for retention were not affected. Fig. 7 also shows the plate numbers achieved with these columns containing increasing amounts of chiral selector when operated at 30 °C. Fig. 8 depicts the chromatograms of 2,5-dimethyltetrahydrofuran and 2,5-dimethoxytetrahydrofuran obtained using the βPXL30-10 column at 20 °C. The main feature of these crosslinked columns is that retention and selectivity were reproducible over a period of several months, but they were unstable and exhibited noisy baselines at temperatures above 90 °C, independent of the preparation conditions (crosslinker and initiator amounts).

## 4. Conclusion

The gas chromatographic performance of columns containing increasing amounts of PM-β-CD in Carbowax 20M was evaluated. The main purpose of the study was to increase the enantioselectivity of PM-β-CD capillary columns by increasing the amount of the chiral selector and to explore the use of columns at the lowest possible temperature within the region of the gas-liquid partitioning process. The main disadvantage of working at relatively low temperatures is longer analysis times.

In most cases, it was shown that heterogeneity of the mixtures resulted in rather poor column efficiencies. Column efficiencies at low temperatures were significantly improved by using a stationary phase in which the chiral selector was fixed by crosslinking of the poly(oxy)ethylene solvent. These new columns were very stable; however, they exhibited a lower enantioselectivity.

It was also demonstrated that the enantioselectivity was superior when SE-54 was used as the solvent. Increasing the amount of the chiral selector by more than a factor of three in the range studied had a smaller effect on enantioresolution than using a less polar diluting phase did. Retention times were much less influenced by a change in polymer polarity.

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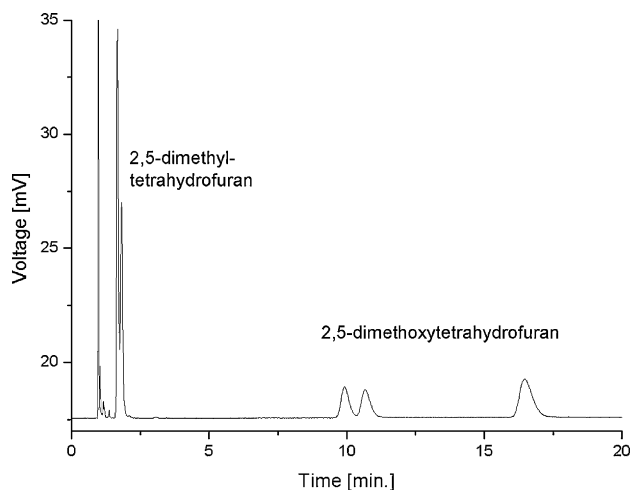


Fig. 8. Enantioseparation of 2,5-dimethyltetrahydrofuran and 2,5-dimethoxytetrahydrofuran obtained on the βPXL30-10 column at 20 °C.

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