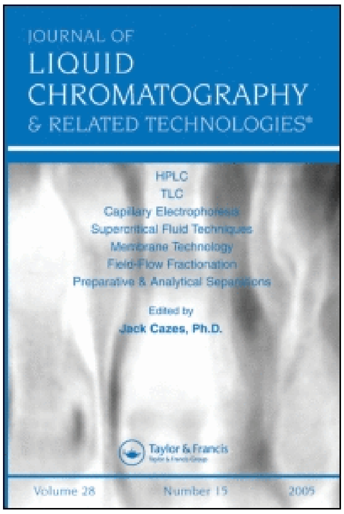

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UNUSUAL SOLVENT AND TEMPERATURE EFFECTS WITH A POLY(TRIPHENYLMETHYL METHACRYLATE) COLUMN FOR CHIRAL LIQUID CHROMATOGRAPHY (1)

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ABSTRACT

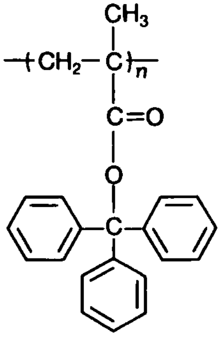
The structure of the alkane component in alkane/ 2-propanol (10:1) eluents was found to have a great influence on chiral resolution on a commercial poly(triphenylmethyl methacrylate) (Chiralpak OT(+)) column. Both increased and decreased separation of the enantiomers of the phthalimide 2 in different eluents was seen on increasing the temperature in the range 13 to 75 °C.

Furthermore, a considerably improved separation of the enantiomers was observed after a resting period of the column of ten months following the temperature experiments. Thus, an almost 50 % increase in the α-value of the phthalimides in a hexane/ 2-propanol eluent was gained. When cyclohexane was used in the eluent, part of the coating material was washed off the column. This, however, led to a further improvement in the separation of 2. Possible explanations for the anomalous phenomena observed are given.

INTRODUCTION

Optically active (+)-poly(triphenylmethyl methacrylate) (PTrMA), coated on silylated macroporous silica (Chiralpak OT(+), Daicel Chemical Industries, Japan), is a useful stationary phase for chiral liquid chromatography (2,3). Since the chirality of the polymer is due to helicity, it has been possible to resolve optically active hydrocarbons and other substances of low polarity that are difficult to separate on other types of chiral phases.

Both nonpolar (hexane/ 2-propanol mixtures) and polar (aq. methanol) eluents can be used with the sorbent. Generally, low temperatures (15 °C) are recommended by the manufacturer. One reason is that resolution generally improves at lower temperature, another is to prevent cleavage of the triphenylmethyl group by alcohols in the eluent.



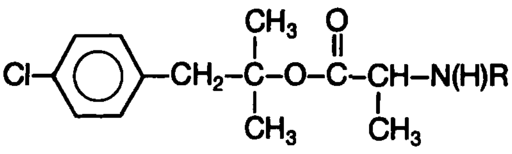
PTrMA

Methanol appears to be the solvent of choice for most of the compounds separated with this type of phase, although hexane/ 2-propanol eluents gave improved separation in a few cases (4,5). From a mechanistic point of view, Okamoto et al., has suggested that, in polar media, hydrophobic interactions between nonpolar sites of the analyte and the triphenylmethyl groups of the polymer chains are important in the chiral discrimination process. In less polar media, the ester group may more effectively participate in chiral discrimination. In certain cases, only one single TrMA unit was found necessary for resolution (4).

The influence of column temperature on resolution with this sorbent has been reported previously (5,6). In a few cases, e.g. trans-cyclohexane-dicarboxylic acid dianilide, higher resolution was observed at higher column temperatures (5). A conformational change of the analyte was thought to account for this effect. In an other study (6) it was suggested that the chiral spaces between the polymer chains change with temperature such that in certain cases the solute fits better to the macromolecular structure.

Racemic alaproclate 1, a central nervous system depressant, is a derivative of the amino acid alanine and should therefore be easy to resolve in different chiral LC systems. The dinitrobenzamides of 1 for example, are separated on a R-N-(α)-phenethyl-N-propylurea column (7). However, the phthalimide derivative 2, which is a synthetic intermediate, could not be separated on that or other, similar, chiral stationary phases.

With a new Chiralpak OT(+) column, almost baseline resolution of 2 ($\alpha = 1.12$) was obtained in a hexane/ 2-propanol (100:5, v/v) eluent. However, when the temperature was lowered from ambient to 11 ^{0}C, a decreased resolution ($\alpha = 1.06$) was noticed. Since, in this case, the effect was observed in an eluent where alcoholysis is less likely to occur, it was of interest to study temperature effects for a larger temperature range.



1 R = H

2 R = Phthalimidoyl

Usually hexane is the solvent of choice in straight-phase chiral separations. However, due to its toxic effects, other alkanes were tried. As will be shown here, considerable improvements in chiral discrimination could be obtained with the Chiralpak OT(+) column on changing the alkane component of the eluent. To test the generality of this finding, benzoin, which has been separated before (4), was included in this study for the purpose of comparison.

EXPERIMENTAL

Apparatus

Chromatography was performed with a Model 510 pump, a Lambda-Max Model 480 variable-wavelength UV detector (both from Waters Associates, Milford, MA, USA), a Model 231 sample injector (Gilson, Villiers Le Bel, France) and a SP 4270 integrator (Spectra Physics, San Jose, CA , USA). The two columns used in this study were CHIRALPAK OT(+), 25 cm x 4.6 mm I.D. (Daicel GmbH, Düsseldorf, Germany, purchased in 1987 and in 1991) equipped with a water jacket for thermostating. The column temperature was regulated with a thermostat (Heto, Birkeröd, Denmark). The eluent flow-rate was 0.9 ml/min and the detection wavelength 254 nm. All solvent mixtures were filtered through a 0.4 µM filter before use. Sample solutions (0.25 mg/mL in hexane/2-propanol (100:7.5) of 20 µL were injected. t_0-values were determined from the appearance of the hexane peak in all eluents used.

Chemicals

The racemic analyte 2 was obtained by preparing the phthalimide of alaproclate 1 (Astra Arcus AB, Södertälje, Sweden) by standard methods. Racemic benzoin was purchased from Fluka

Chemie AG (Buchs, Switzerland). The eluents were made from analytical-grade solvents (Merck, Darmstadt, Germany).

RESULTS AND DISCUSSION

The two Chiralpak OT(+) columns used showed excellent agreement in retention characteristics initially. Column 1 was used for low-temperature studies (13.5 - 20 °C) and column 2 for higher temperature experiments (up to 75°C, see below).

The change in separation factors (alpha-values) and retention factors (k′-values) of the phthalimide 2 and benzoin with temperature at 13.5 and 20.0 °C and alkane content in the alkane/2-propanol eluents are shown in Table 1. Dramatic differences can be seen, particularly for 2, in the series cyclohexane, hexane, pentane, heptane and isooctane (2,2,4-trimethylpentane). The α-value was 1.10 in the hexane eluent and 1.74 in isooctane (at 13.5 °C). The magnitude of the α-values roughly follows the molecular weight of the alkane component of the eluent. With cyclohexane in the eluent no separation at all of 2 was obtained.

For benzoin these differences in α are less pronounced but still notable. There is a general trend that the separation factor incrases on lowering the temperature for benzoin, whereas the opposite is true for the phthalimide. In pentane, however, 2 shows different behavior as does benzoin in cyclohexane.

The enantioselectivity of the column was altered after the use of cyclohexane in the eluent (this eluent was used last in the series as presented in Table 1). Thus, when another eluent was used following the cyclohexane eluent, a further increase in the α-values of 2 was observed. In heptane/2-propanol α was now 2.09 at 13.5 °C. Benzoin, however, with an α of 1.22 was less affected.

A gradual change of the baseline with cyclohexane in the eluent indicated that part of the column material was dissolved. All eluent used (ca 200 mL) was collected and evaporated. The

TABLE 1

Chromatographic Data of Alaproclate Phthalimide 2 and Benzoin in Different Eluents and at Different Temperatures.

		Phthalimide 2			Benzoin		
Solvent[1]	Temp.(°C)	k'_1	k'_2	α	k'_1	k'_2	α
hexane	13.5	1.78	1.62	1.10	1.45	1.77	1.22
	20.0	1.38	1.57	1.14	1.26	1.54	1.23
heptane	13.5	1.85	2.82	1.53	1.81	2.20	1.22
	20.0	1.57	2.55	1.62	1.55	1.88	1.21
isooctane	13.5	3.41	5.93	1.74	3.62	4.73	1.31
	20.0	2.78	4.92	1.77	2.94	3.77	1.28
pentane	13.5	1.56	2.16	1.39	1.50	1.86	1.24
	20.0	1.53	1.85	1.21	1.32	1.61	1.22
c-hexane	13.5	0.57	0.57	-	0.54	0.69	1.28
	20.0	0.45	0.45	-	0.50	0.58	1.16
hexane[2]	13.5	1.46	1.83	1.25	1.41	1.74	1.23
	20.0	1.26	1.66	1.32	1.25	1.52	1.22
heptane[2]	13.5	1.72	3.60	2.09	1.82	2.23	1.22
	20.0	1.53	3.44	2.25	1.59	1.93	1.21

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[1] Mixtures with 2-propanol 10:1; [2] Data obtained after passing the cyclohexane/2-propanol eluent (10:1) through the column.

residue amounted to ca 5%, based on published data for the preparation of the column (5). Carbon 13 and proton NMR and mass spectrometry indicated that the material consisted of oligomers of PTrMA and not of the polymer itself (8). This loss of material lowered the retention of 2 but increased enantioselectivity whereas benzoin was neglibly affected.

One possible explanation for the solvent dependent phenomena observed, is that the polymeric PTrMA displays different solvation in different eluents and that this leads to altered spatial requirements for chiral discrimination.

The second Chiral OT(+)-column was used for studies at somewhat higher temperatures (up to 75 °C). In this case it was assumed that cleavage of the triphenylmethyl-group of the PTrMA polymer would be less extensive with the alkane/ 2-propanol eluents compared to when pure methanol is being used. Indeed no change in the baseline as a result of cleavage (the triphenylmethyl group displays high absorbance) could be seen.

Fig. 1 shows the temperature dependent change in resolution of the racemate 2 with a new CHIRALPAK OT (+) column in three different eluents (isooctane/ 2-propanol, hexane/ 2-propanol and heptane/ 2-propanol, all 10:1, v/v). With hexane/ 2-propanol (10:1) (Fig. 1B) an increase of ca. 10% for the alpha value up to 55 °C was observed. When hexane was replaced by isooctane an opposite effect was seen (Fig. 1A). In this eluent, the α value was initially higher (1.8) but decreased to 1.3 in the interval 23 - 75 °C (almost 30 % decrease). With heptane/ 2-propanol (10:1) α increased in the interval 15-35 °C and then decreased in the interval 35 - 55 °C (Fig. 1C). In all cases, the R(+) isomer was most strongly retained. There was a general trend for higher efficiency (N values) at higher temperatures. With hexane/ 2-propanol (10:1), N increased from 3200 to 4700 on going from 30 - 45 ^{o}C (second eluted isomer).

Furthermore, when the column was allowed to rest for 10 months after the heat-treatments, an increase in the α-value of 2

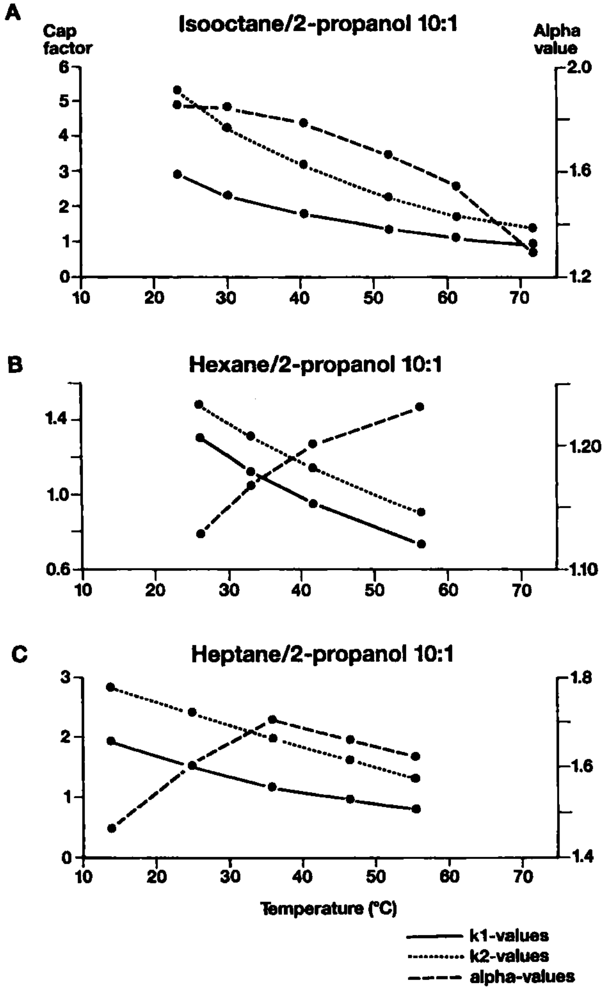

FIGURE 1. Temperature dependent change of capacity factors and separation factors of 2 in different eluents on a new Chirakpak OT(+) column. Conditions: Eluents A) isooctane/2-propanol (10:1); B) hexane/2-propanol (10:1) and C) heptane/2-propanol (10:1). Flow-rate 0.9 mL/min; Detection at 254 nm.

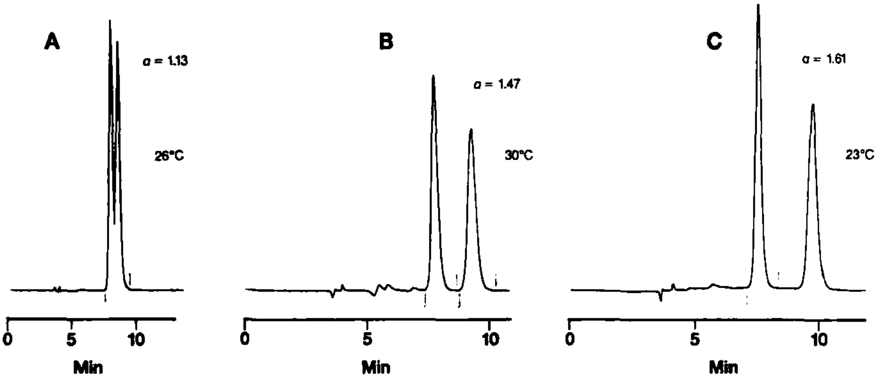

FIGURE 2. A) Separation of 2 in hexane/2-propanol (10:1) on a new Chiralpak OT(+) column. B) Same column after heat treatment and a 10 months' resting period. C) Same column as in B) after passing a cyclohexane/2-propanol (10:1) eluent through the column. Flow-rate 0.9 mL/min. Detection at 254 nm.

from 1.13 to 1.47 with the hexane/ 2-propanol eluent was observed (Fig. 2A,B) after resumed use of the column. When this column was washed with cyclohexane/ 2-propanol (10:1), a further increase in the α-value to 1.61 in the hexane eluent was observed (Fig. 2C).

Table 2 shows chromatographic data for 2 and benzoin in different eluents on a two year old column (column 2). To allow comparison, data for ethanol and butanol mixtures of hexane have been included, as well as data for pure methanol. As can be seen, there was no increase in selectivity using other alcohols as modifiers. With pure methanol, a comparatively low separation factor (1.29) was obtained. Contrary to what was observed for 2, the α value of benzoin was almost constant (1.17 - 1.18) in most eluents. A somewhat higher value (1.22) was observed in isooctane/ 2-propanol and in cyclohexane/ 2-propanol a value of 1.09 was obtained.

There is no straightforward explanation of the various temperature effects observed in the chiral separations described for column 2. Since the PTrMA columns are prepared by evaporating

TABLE 2

Comparison of Chromatographic Data at 22.5 °C for Alaproclate phthalimide 2 and Benzoin on a Two Year Old Column with Different Eluents. Flow-rate 0.9 mL/min

Eluent[1]	$k'_2(2)$	k'_2(benzoin)	α_2	α_{benzoin}
hexane/ 2-propanol	1.70	1.36	1.43	1.18
heptane/ 2-propanol	2.48	1.57	2.00	1.18
pentane/ 2-propanol	1.81	1.41	1.38	1.17
isooctane/ 2-propanol	4.82	3.00	2.26	1.22
cyclohexane/ 2-propanol	0.72	0.92	1.07	1.09
hexane/ ethanol	1.17	1.26	1.23	1.17
hexane/ t-butanol	1.30	1.40	1.19	1.17
methanol	1.14		1.29	

1) All mixed eluents are 10:1, v/v.

a solution of the the polymer onto deactivated silica (5), one would anticipate that a mild heat treatment (up to ca. 75 °C) of such a coating material might bring about a more evenly spread layer of the polymer, and therefore form a more efficient column. Obviously 2 is more sensitive to changes in the over-all structure of the PTrMA polymer on the silica particles. This polymer has a high degree of crystallinity (4). It is possible that the heat-treatments reported here, in combination with a resting period, would give a column with higher structural regularity and with more sites available for specific interaction. The solvent polarity difference for the alkanes used (p'= -0.2 - 0.2) (9) is small and does not reflect the results obtained in a straightforward manner.

The possibility that the conformation of the phthalimide 2 changes with temperature and therefore leads to altered discrimination, would explain the substrate dependent behaviour and cannot be excluded but should be less important since very different results for 2 were seen in different solvents. Further experiments would be needed to study the physico-chemical properties of the neat PTrMA polymer in different solvents.

CONCLUSIONS

This work has shown that chiral resolution on a PTrMA polymer is more complicated than previous work has indicated. The solvent effects observed are noticable and should be further evaluated. The great differences in selectivity using hexane, pentane, isooctane and heptane (and possibly other alkanes) can safely be used to influence selectivity. Rinsing the column with a cyclohexane eluent appears to give a column of improved performance due to removal of interfering oligomeric impurities.

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