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Glycerol as a new dissolution medium for α-, β- and γ-cyclodextrins for preparing stereoselective stationary phases for gas–liquid chromatography

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Abstract

Glycerol was applied as the dissolution medium for α, β- and γ-cyclodextrins and its gas–liquid chromatographic performance was compared with that of formamide. Various stationary glycerol-based chiral phases for classical gas–liquid chromatography were examined. The columns containing glycerol appeared relatively stable in comparison with those prepared with formamide. Glycerol-based phases functioned over the temperature ranged 80–100°C for about 400 h without any changes indicating losses of glycerol or activity. Owing to the lower stability of cyclodextrin complexes in glycerol medium relative to those in formamide, and the fact that glycerol-based columns allow operation ca. 40°C higher, many separations which were previously unreported at higher temperatures were achieved. The model compounds tested were (+/−)-α-pinene, *cis*/*trans*-decalin, *cis*/*trans*-anethole, *cis*–*trans*-isosafrol, *cis*–*trans*-isoeugenol, (+/−)-camphor, (+/−)-fenchone, (+/−)-isomenthone, (+/−)-isopinocampheol, (+/−)-borneol and (+/−)-isoborneol.

1. Introduction

Cyclodextrins (CDs) are known to form stereoselective inclusions complexes with a variety of substances of either an acidic, basic or neutral nature [1]. This property has been used to advantage in gas chromatography, especially during recent years as they have appeared on the market as commercial products. To date hundreds of publications, reviews [2,3] and a monograph [4] have appeared demonstrating their utility in many fields of fundamental and applied research.

For a long time it was generally believed that CDs form inclusion complexes only in pure aqueous solutions [5,6]. For that reason, many attempts to modify gas chromatographic systems by free cyclodextrins for analytical purposes failed, mainly because the partition gas chromatographic mode (gas–liquid chromatography) remained unachievable.

In the early 1980s we obtained a satisfactory gas–liquid chromatographic system by successful application of a formamide medium with dissolved β-cyclodextrin[7], γ-cyclodextrin and α-cyclodextrin[8], the last solution after addition of some auxiliary compound ($LiNO_3$) [9].

It appeared that β-CD permits highly selective separations of structural and geometrical isomers whereas α-CD is especially useful for the chiral

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recognition of terpenic compounds (see Ref. [6] and references citated therein). However, owing to the physical properties of formamide, i.e. its very polar nature and relatively low boiling point, the area of applications of this method mainly involved lower hydrocarbons, i.e., volatile hydrophobic compounds.

It has become clear that, despite the remarkably high separation factors achieved with packed columns with CDs dissolved in formamide stationary phase, the efficiency of packed columns is poor. Some other limitations should also be mentioned, primarily the short lifetime of the columns and the fact that the method could not be adapted for work using capillary columns. Further, the analysis of higher molecular mass hydrocarbons or more polar compounds, which are more easily dissolved in formamide medium, requires another matrix solvent of higher boiling point and lower polarity. We have found that such requirements, to a limited extent at least, are met by glycerol, which has a boiling point of 290°C and a dielectric constant of 42.98 in comparison with the 210°C and 109.5, respectively, for formamide.

This paper presents our first results obtained using packed-column systems with glycerol in place of formamide as the CD-dissolving stationary phase. Finally, it is noted that improved analysis performance was achieved by König and co-workers [10,11] and subsequently by Armstrong et al. [12] by the introduction CD derivatives, which are liquid or waxy at relatively low temperatures, as the stationary phases in capillary columns. CD derivatives used in capillary GC are characterized by a relatively poorer enantioselectivity but a higher efficiency of the columns than obtained with packed CD columns. Both capillary and packed approaches are still undergoing refinement and development. An additional alternative, approached by Schurig and co-workers [13,14] uses high-melting cyclodextrin derivatives dissolved or suspended in polysiloxane stationary phases, while Smolkova-Keulemansova et al. [5] adapted formamide modified with β-cyclodextrin to micropacked columns.

2. Experimental

2.1. Chemicals

α-, β- and γ-cyclodextrins were supplied by Chinoin (Budapest, Hungary). Chromosorb W NAW (0.18–0.25 mm) for GC was a product of Johns-Manville (Litho, USA). All other substances were of analytical-reagent grade and were used without further purification.

Table 1
Column characteristics

Column code	Cyclodextrin	Concentration of CD in stationary phase (m)[a]	Stationary phase
M	–	0.000	Glycerol
A1	α-CD	0.041	Glycerol
A2	α-CD	0.082	Glycerol
A3	α-CD	0.163	Glycerol
A4	α-CD	0.163	Formamide
B1	β-CD	0.070	Glycerol
B2	β-CD	0.175	Glycerol
B3	β-CD	0.175	Formamide
G1	γ-CD	0.082	Glycerol
G2	γ-CD	0.184	Glycerol

[a] m = Molal.

2.2. Apparatus and procedures

Chromatographic studies were performed using a Hewlett-Packard (Waldbronn, Germany) Model 5890 series II gas chromatograph equipped with dual flame ionization detectors. The peak areas and retention times were measured by means of a Hewlett-Packard Model 3396 series II integrator.

2.3. Columns

Glass columns with dimensions 2 m × 4 mm I.D. (2 m × 2 mm I.D. in the case of a semi-micropacked column) were filled with Chromosorb W NAW, which was coated with either α-, β-, or γ-CD dissolved in either glycerol or formamide as shown in Table 1.

2.4. Procedures

The stabilities of CD complexes were compared using the following simplified equations [6,7]:

$$t'_{R(CD,Gly)} = t'_{R(Gly)}(1 + K_G[CD]) \quad (1)$$

where $t'_{R(CD,Gly)}$ and $t'_{R(Gly)}$ are the adjusted retention times of a solute on the column containing a given CD in glycerol and of the solute on the control column containing glycerol alone, respectively, $K_G(m^{-1})$ is the stability constant of the complex and [CD] is the molal concentration of a given CD in glycerol:

$$K_G = \frac{[G \cdot CD]}{[G][CD]} \quad (2)$$

Thus Eq. 1 is true only if a CD complex of 1:1 stoichiometry is formed, and under comparable conditions when the only varying parameter is the CD concentration. For this reason, a constant inlet pressure (2.75 ± 0.05 atm) and helium flow-rate (40 ± 0.5 ml/min) were carefully maintained. Under these conditions, it was possible to compare the stability constants of various CD · G complexes although their exact values could not be determined [16,17]. More general equations taking into account complexes of 1:1 and 1:2 stoichiometry are being formulated.

3. Results and discussion

The main topic of this study was to expand the area of application of cyclodextrins in gas–liquid chromatography by using glycerol instead of formamide as the dissolution medium. Gas–liquid chromatography is characterized by high reversibility and a large range of linear isotherms that allows recognition of the regio- and stereoselectivity imparted to the liquid stationary phase by complexation with CDs. However, the use of this approach requires the influence of the solvent matrix–medium to first be considered.

Examination of Eq. 1 leads to the conclusions that the dissolution medium plays a predominant role. In fact, it dictates the initial parameters of the system under investigation, and subsequently influences the inclusion processes between the CD and analyte molecules.

3.1. Comparison of formamide and glycerol behaviour

Solubility

The physical properties of glycerol differ substantially from those of formamide. Their polarities and boiling points were given above. The more limited working temperature range possible with formamide versus glycerol was also discussed in the Introduction. The relative solubility of glycerol and formamide will be considered next.

In a preliminary study, we found that various cyclodextrin species exhibit differing solubility in the solvents considered in the study, formamide, glycerol and water, namely α-CD is relatively soluble in water but is almost insoluble in either formamide or glycerol, β-CD is very soluble in formamide (like sugar in water), whereas its solubility in either water or glycerol is very limited, and γ-CD exhibits moderate solubility in all three solvents. Hence to prepare β-CD–glycerol, α-CD–formamide and α-CD–glycerol columns the use of some solubilizers was necessary.

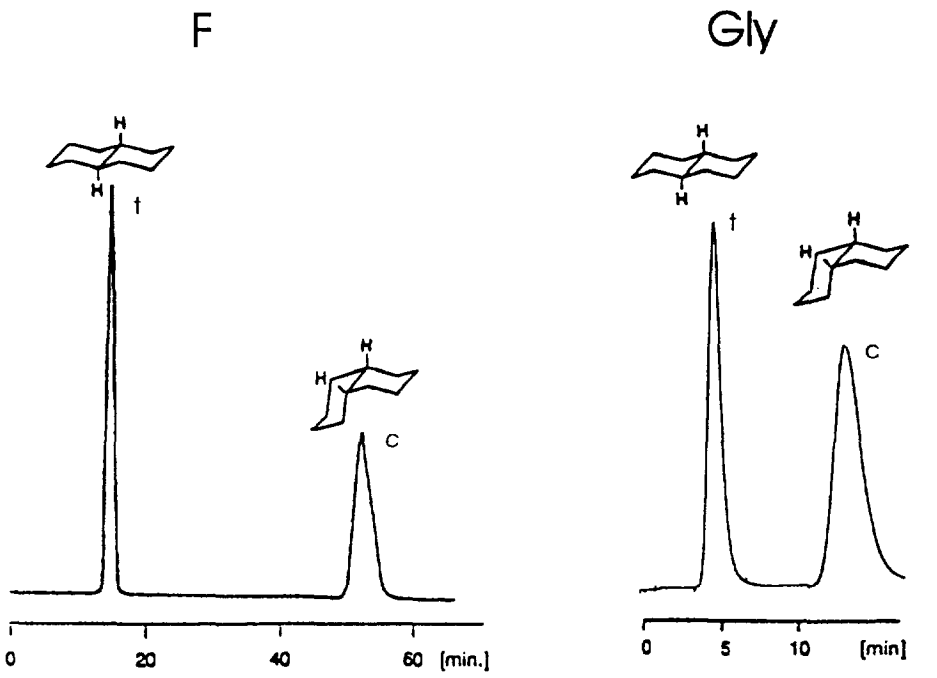

Fig. 1. Separations of decalins obtained on formamide B3 (F) and glycerol B2 (Gly) columns modified with β-CD. Temperature, 70°C; flow-rate, 60 ml/min.

The best appeared to be H_2O and $LiNO_3$ for α-CD and urea for β-CD. The respective roles of H_2O, $LiNO_3$ and urea have not yet been clarified, but may involve structural changes in α- and β-CD molecules, or may involve simple solubilization processes. In the α-CD–form-

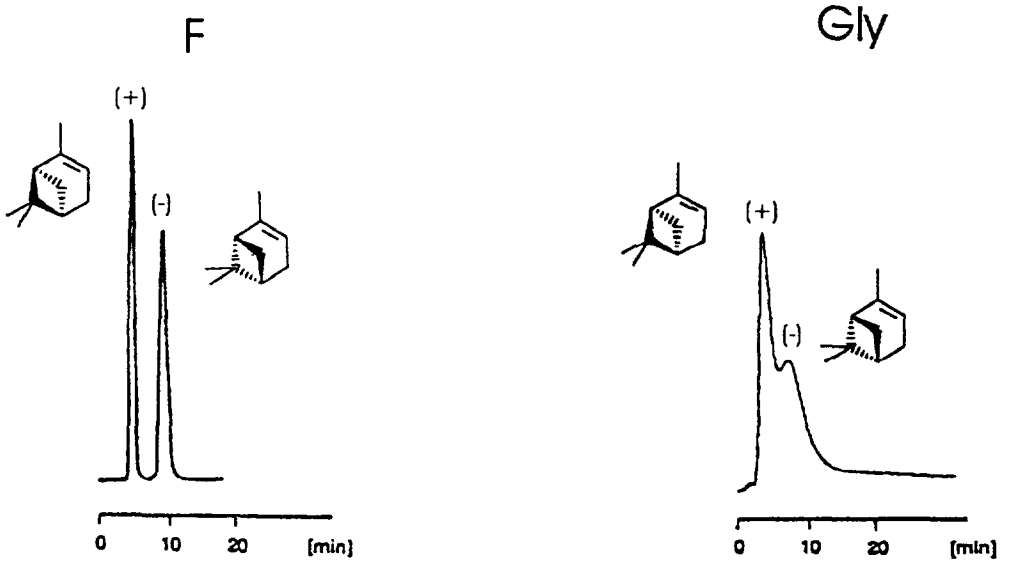

Fig. 2. Separations of (+/−)-α-pinenes obtained on formamide A4 (F) and glycerol A3 (Gly) columns modified with α-CD. Temperature, 40°C; flow-rate, 40 ml/min.

amide system we used $LiNO_3$ for stabilization the column, whereas Lindström et al. [18] successfully applied a wetting procedure of the carrier gas.

Durability

We found that the columns prepared with glycerol are relatively durable in comparison with those prepared with formamide. The glycerol columns were operated at temperature from 80 to 100°C for about 400 h without any changes indicating losses in either glycerol or column activity.

Chromatographic properties of β- and α-CD dissolved in glycerol and formamide media

The model compounds examined were *cis–trans*-decalins and (+/−)-α-pinenes. They were chosen for this investigation since it has been found previously that β-CD is a very efficient selector for decalins, and α-CD is known to be remarkably enantioselective towards (+/−)-α-pinenes. These two compounds allow the experiments to be carried out under very similar conditions. In practice, a 70–75°C temperature range is the upper limit possible for formamide, and at the same time it is very close to the lower limit for the convenient use of glycerol.

As can be seen from Fig. 1, which shows separations of decalins, using β-CD the selectivity factors $\alpha_{(cis/trans)}$ are very similar on both glycerol and formamide modified with β-CD, i.e., ca. 3. In contrast the retention data (adjusted retention times, t') differ substantially, indicating that the stabilities of β-CD complexes with decalins in glycerol are about three times lower than those in formamide. This behaviour agrees with theory, since the expected stability of the complexes should increase with increasing polarity of the matrix–solvent.

The results for α-pinenes are shown in Fig. 2; it seems that the stabilities of α-CD complexes with α-pinenes at temperatures from 75 to 80°C are too low to give evidence of the true enantioselectivity of the column. For this reason the separations shown in Fig. 2 were performed at a lower temperature, 40°C, which is far below that recommended for glycerol media. Nevertheless, the same tendency has been confirmed, the separation factors by $\alpha_{(-/+)}$ are very similar and the retention times of pinenes on glycerol modified with α-CD are shorter than those observed on formamide. The low-temperature experiment demonstrates the low efficiency of glycerol columns when working at 40°C.

3.2. Examples of resolution

Geometrical isomers

The examples given below related to various separations achievable only on the glycerol column.

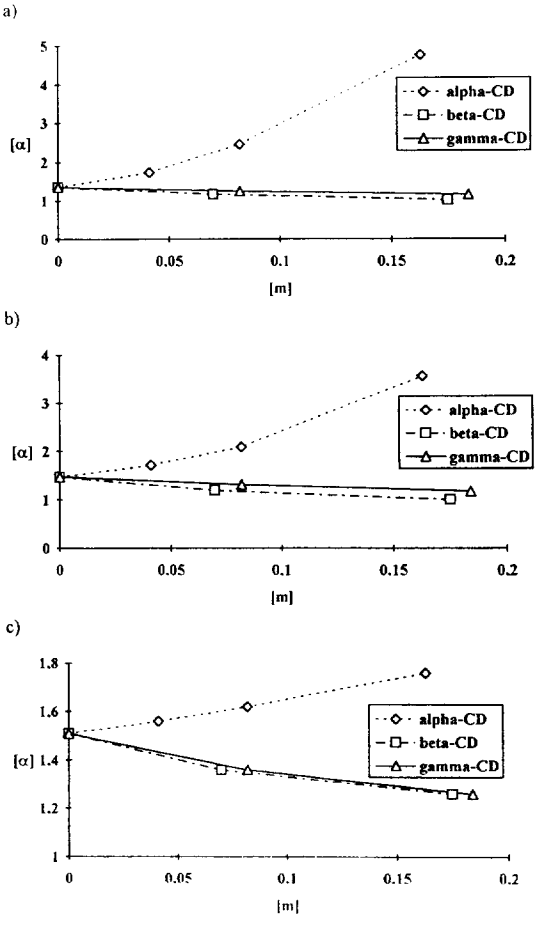

Fig. 3. Separation factor α for (a) *cis–trans*-anethole, (b) isosafrol and (c) isoeugenol versus α-, β- and γ-CD concentration. Temperature (a) and (b) 80 and (c) 100°C; flow-rate, 40 ml/min.

Fig. 3 presents the separation factor α for *cis–trans*-anethole, -isosafrol and -isoeugenol versus α, β- and γ-CD concentration. It can be seen that α-CD exhibits a remarkable stereoselectivity towards *trans–cis* positions. Much more stable complexes are possible with *trans* isomers. The direction of this stereoselectivity follows that of the starting material, i.e., for glycerol (α-*trans*/*cis* ≈ 1.5).

Using α-CD modifier, a relatively high stereoselectivity ($\alpha \approx 4$) could be reached for anethole and isosafrol. β-CD aand γ-CD behave in a reverse manner; they exhibit moderate stereoselectivity and form more stable complexes with a *cis* configuration. In effect, the overall stereoselectivity decreases as the β- or γ-CD concentration increases and under the same conditions one process in nullified by the other ($\alpha \approx 1.00$).

The similar observed complexation of *trans–cis* isomers by CDs (especially by α-CD) may suggest that the insertion of a $-CH=CHCH_3$ group in the CD cavity is a major factor in the stereospecific recognition of these geometrical isomers, almost independently of the other dissimilar molecular features between these three compounds. It is noted that the relationship between the separation coefficient and the α-CD concentration at 80°C is distinctly non-linear. This may indicate that at higher α-CD concentration, in addition to complexes of 1:1 stoichiometry, also 1:2 complexes richer in α-CD are formed.

Isoeugenol was seen to behave differently, maybe because the temperatures of the investigations were higher (by about 20°C) than those applied to anethole and isosafrol. The temperatures were chosen in order to reach reasonable elution times of isoeugenol from the glycerol-containing columns. Nevertheless, it is seen that the tendencies seen in Fig. 3 are similar to those observed for both anethole and isosafrol.

Enantiomners

Fig. 4 shows the separation obtained for enantiomers of selected terpene ketones, (+/−)-camphor, (+/−)-fenchone and (+/−)-isomenthone, which were obtained on glycerol columns modified with α-CD.

Fig. 5 shows the separations of enantiomers of selected terpene alcohols, (+/−)-isopinocampheol, (+/−)-borneol and (+/−)-isoborneol, obtained on glycerol columns modified with α-CD.

As mentioned earlier (see Introduction), there are great difficulties in adapting formamide

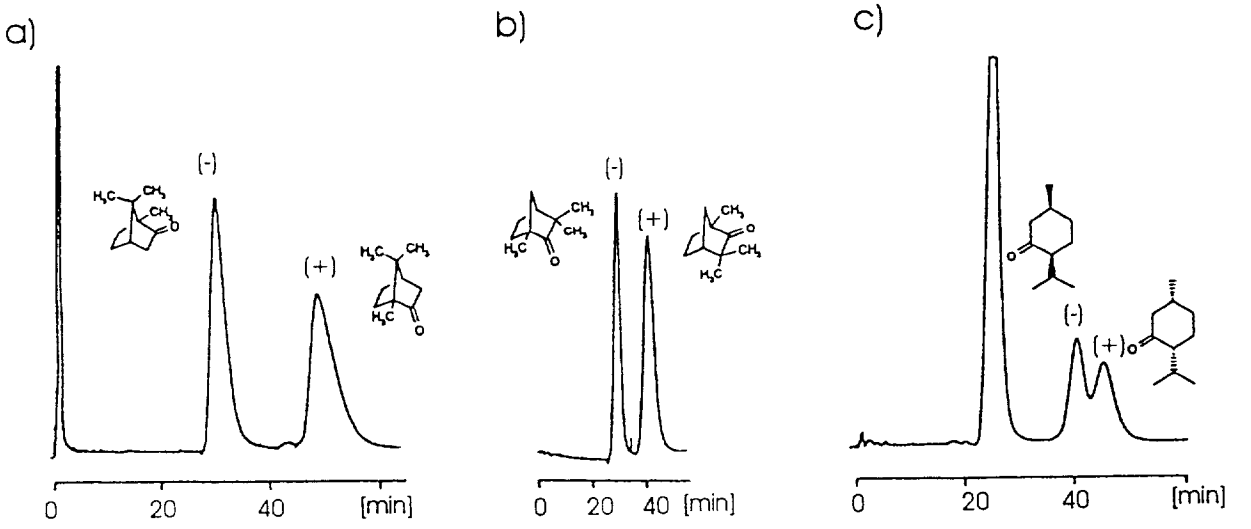

Fig. 4. Separations of enantiomers of terpene ketones: (a) (+/−)-camphor, column A1, 90°C; (b) (+/−)-fenchone, column A1, 60°C; (c) (+/−)-isomenthone, column A2, 60°C. Flow-rate, 40 ml/min.

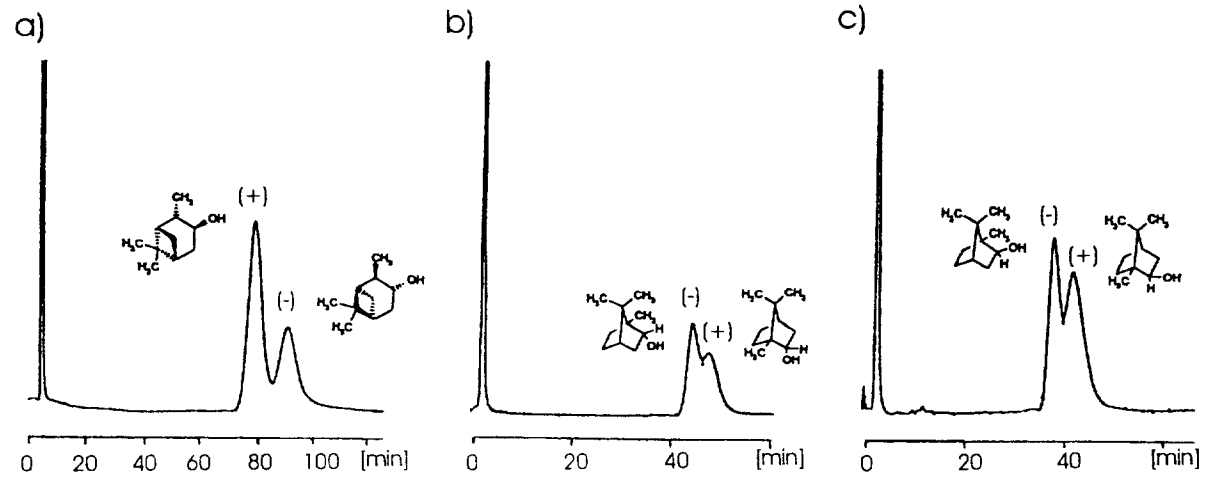

Fig. 5. Separations of enantiomers of terpene alcohols: (a) (+/−)-isopinocampheol, column A3, 80°C; (b) (+/−)-borneol, column A1, 90°C; (c) (+/−)-isoborneol, column A1, 90°C. Flow-rate, 40 ml/min.

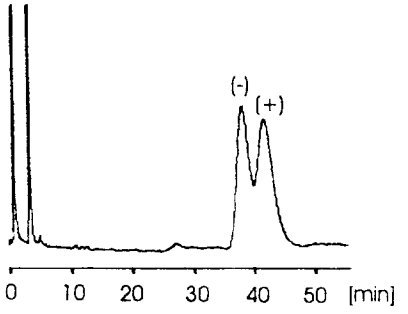

Fig. 6. Separation of (+/−)-isoborneol obtained on a semi-micropacked Al column. Temperature, 90°C; flow-rate, 15 ml/min.

phases modified by cyclodextrins, especially α-CD, to work in capillary column techniques, with micropacked columns and semi-micropacked (2 mm I.D.) columns. Our latest results for a preliminary investigation, shown in Fig. 6, suggest that the problem with capillary columns also should find some progress through application of glycerol (compare with Fig. 5c).

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