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Stationary phase effect on enantioselectivity of dansyl phenylalanine in microcolumn liquid chromatography with γ-cyclodextrin as mobile phase additive

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ABSTRACT

The effects of the stationary phase on enantioselectivity were examined in microcolumn liquid chromatography using γ-cyclodextrin as a mobile phase additive. The stationary phases examined were octadecyldimethylsilica, octyldimethylsilica and trimethylsilica. Dansyl phenylalanine enantiomers were employed as test analytes. For the octadecyldimethylsilica stationary phases the effect of surface coverage on enantioselectivity was not significant. The retention behaviours observed for the octadecyldimethylsilica and octyldimethylsilica were similar, but different from those observed for the trimethylsilica stationary phase.

INTRODUCTION

Cyclodextrin-bonded stationary phases were investigated to determine the resolution of optical isomers in liquid chromatography (LC) [1–4]. The use of cyclodextrins as mobile phase additives in LC is another approach to determining chiral resolution. Three kinds of cyclodextrins are commercially available, which differ in the number of the glucose unit, *viz.* α-, β- and γ-cyclodextrin. γ-Cyclodextrin has so far been applied to the enantiomeric resolution of norgestrel [5], 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate [6] and dansyl amino acids [7]. In a previous study [7], it was found that enantioselectivity was affected by the concentrations of γ-cyclodextrin and acetonitrile in the mobile phase. In this paper, the effect of the stationary phase on enantioselectivity has been examined in microcolumn LC using γ-cyclodextrin as the mobile phase additive.

EXPERIMENTAL

Apparatus

The microcolumn LC system was assembled in the laboratory and comprised an MF-2 microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS GAN-050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan), an ML-522 microvalve injector with an injection volume of 20 nl (Jasco, Tokyo, Japan), a separation column, a UVIDEC 100V UV detector (Jasco) with a laboratory-made microflow cell, and a Chromatopac C-R4AX data processor (Shimadzu, Kyoto, Japan). The separation column was prepared from fused-silica tubing of 15 cm × 0.35 mm I.D. The flow-rate of the mobile phase was 2.8 μl/min. The separation was carried out at room temperature, *i.e.* 25°C.

The stationary phases employed were Develosil octadecyldimethylsilica (ODS), octyldimethylsilica (C_8) and trimethylsilica (TMS) (Nomura Chemical,

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TABLE I

PHYSICAL DATA OF THE PACKING MATERIALS EMPLOYED IN THIS WORK

Packing materials	Functional group	C[a] (%)	Surface coverage[b] (μmol/m^2)
Develosil TMS-5	Trimethyl	5	5.3
Develosil C_8-5	Octyldimethyl	13	3.6
Develosil ODS-P-5	Octadecyldimethyl	11	1.4
Develosil ODS-N-5	Octadecyldimethyl	16	2.1
Develosil ODS-5	Octadecyldimethyl	20	3.1

[a] The values represent the carbon contents and contain the amount of trimethylsilane used for end capping.
[b] The values show the surface coverage before end capping.

Seto, Japan), and the surface properties of these packing materials are compared in Table I. In the text, surface coverage refers to the coverage of the silica by the bonded phase. The various ODS packings are seen to vary widely in their coverage by bonded phase. All of the packing materials are end capped with trimethylsilane. The original silica gel used for the preparation of these packing materials has the following dimensions and surface properties: specific surface area 340 m^2/g; average particle diameter 5 μm; average pore diameter 120 Å; specific pore volume 1.0 ml/g.

Reagents

High-performance liquid chromatography (HPLC) grade distilled water and reagent-grade γ-cyclodextrin were obtained from Wako (Osaka, Japan). Dansyl derivatives of phenylalanine (Phe) were supplied by Sigma (St. Louis, MO, USA). Other reagents were obtained from Wako, unless otherwise stated. The reagents were used without further purification.

RESULTS AND DISCUSSION

The dependence of the retention behaviours of the analytes on the acetonitrile concentration was examined. The logarithm of the capacity factor (k') was plotted as a function of the acetonitrile concentration, as shown in Figs. 1 and 2, in which linear relationships between the two parameters are observed. In this work, the concentration of γ-cyclodextrin was kept constant (30 mM). The capacity factors were calculated by assuming that nitrate was

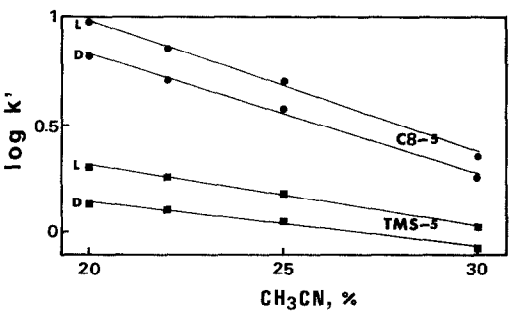

Fig. 1. Relationships between the logarithm of the capacity factor (k') and the acetonitrile concentration for the TMS and C_8 stationary phases. Columns: TMS-5 and C_8-5. Mobile phase: acetonitrile–water mixture containing 30 mM γ-cyclodextrin and 50 mM ammonium acetate. Flow-rate: 2.8 μl/min. Analytes: D = dansyl-D-Phe; L = dansyl-L-Phe.

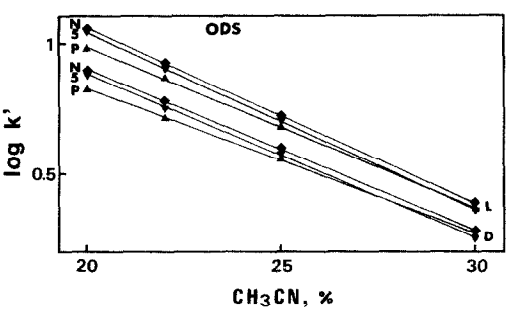

Fig. 2. Relationships between the logarithm of the capacity factor and the acetonitrile concentration for the ODS stationary phases. Columns: P = ODS-P-5; N = ODS-N-5; 5 = ODS-5. Other operating conditions as in Fig. 1.

not retained on the stationary phase. In all cases dansyl D-Phe was eluted before dansyl L-Phe. Among the ODS stationary phases examined, ODS-N-5 gave the largest capacity factor. The C_8 stationary phase gave nearly the same capacity factor as those observed with the ODS stationary phases.

Fig. 3 shows the separation factor (α) as a function of the acetonitrile concentration. The separation factor increases with decreasing acetonitrile concentration in the mobile phase, which suggests that the enantioselectivity can be improved by decreasing the acetonitrile concentration but with an increase in the analysis time. It was found that the TMS stationary phase provided the largest separation factor when the acetonitrile concentration was kept constant. The varying surface coverage has no effect on the separation factor for the ODS stationary phases.

The separation factor is plotted as a function of the capacity factor of the L-isomer in Fig. 4. Much larger values of the separation factor were achieved for the TMS stationary phase in comparison with the other stationary phases. It is seen again that varying the surface coverage of the ODS stationary phases has no effect on the relationship between the two parameters.

The resolution (R_s) is commonly expressed as follows:

$$R_s = \frac{\alpha - 1}{4} \cdot \frac{k'}{1 + k'} \cdot N^{1/2} \quad (1)$$

where N is the theoretical plate number. Here, we define the resolution coefficient (R_s^*) as follows:

$$R_s^* = \frac{\alpha - 1}{4} \cdot \frac{k'}{1 + k'} \quad (2)$$

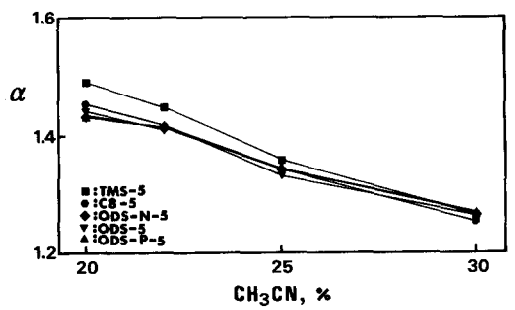

Fig. 3. Separation factor (α) as a function of the acetonitrile concentration. Operating conditions as in Figs. 1 and 2.

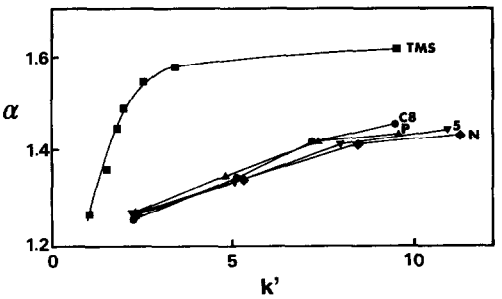

Fig. 4. Separation factor as a function of the capacity factor of the L-isomer. Operating conditions as in Figs. 1 and 2.

If the same theoretical plate numbers are achieved, better resolution can be achieved under the conditions which generate a larger R_s^* value.

Fig. 5 shows the relationships between R_s^* and acetonitrile concentration. At the same acetonitrile concentration, the TMS stationary phase gave smaller R_s^* values than the other stationary phases, because the capacity factor produced by the TMS stationary phase is much smaller than that of the other stationary phases, as demonstrated in Figs. 1 and 2. However, by using a mobile phase containing a lower concentration of acetonitrile, the capacity factor for the TMS stationary phase can be increased, which in turn improves the R_s^* value. This is because, if the same capacity factor is achieved for the TMS stationary phase as the other stationary phases, the separation factor of the former stationary phase is larger than that of the latter, as demonstrated in Fig. 4. On the other hand, the ODS and C_8 stationary phases showed almost the same

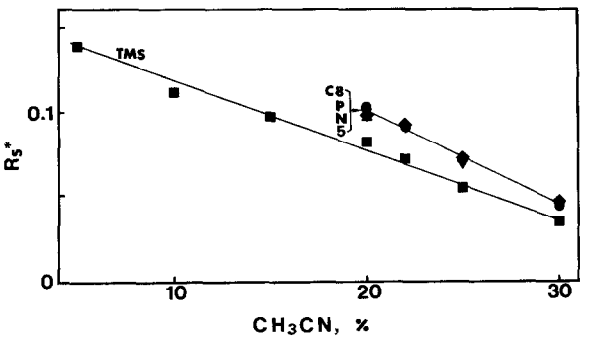

Fig. 5. Relationships between the resolution coefficient (R_s^*) and the acetonitrile concentration. Operating conditions as in Figs. 1 and 2.

relationships between the two parameters. Unfortunately, since the TMS column prepared in this work exhibited a somewhat poorer column efficiency than the other columns, the resolution (R_s) obtained with the TMS column was less than that with the other columns.

In conclusion, enantioselectivity in the present separation mode can be improved by the careful selection of the stationary phase as well as the mobile phase additives.

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