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Resolution of Enantiomers by Supercritical Fluid Chromatography with Cellulose Tris(phenylcarbamate) Coated on Silica Gel

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The solvent characteristics of super- and subcritical carbon dioxide for the chromatographic resolution of enantiomers (*trans*-stilbene oxide) by chiral cellulose tris(phenylcarbamate) were investigated by altering the pressure (10, 15, 20, and 40 MPa), the temperature (0, 25, and 40 °C), and the concentrations of the modifiers. Among methanol, ethanol, and 2-propanol used as modifiers, methanol is the most effective in terms of the resolution of enantiomers, though 2-propanol gives a slightly high value for the separation factor. When the concentration of alcohol increases, the capacity ratio decreases substantially, but the separation factor decreases only a little. At 40 °C, an increase in pressure results in a decrease in the capacity ratio and a slight increase in the separation factor. These pressure effects may be attributed to the enhanced solubilities of both the solute and the modifier with an increase in the mobile-phase density. The temperature is the most effective variable for the separation factor of enantiomers; it increases as the temperature lowers. Therefore, the resolution becomes higher at 0°C (subcritical conditions) than at 40 °C on the basis of the same analysis time.

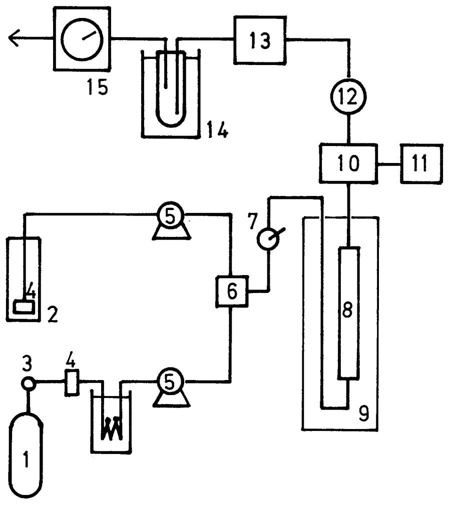
A supercritical fluid has interesting solvent characteristics: low viscosity, high diffusivity, and a drastic change of solubility upon alteration of the temperature and the pressure. These properties are suitable for a mobile phase in chromatography both for analytical and preparative purposes. A monograph on supercritical fluid chromatography (SFC) published recently[10)] reviewed the relevent theories, instrumentation, and applications of the new method. Though many articles on SFC have been published, applications to the chromatographic resolution of enantiomers have been scarce and have been limited to columns with chemically bonded phases.[2,3,6,7)] Very recently Macaudière et al.[5)] reported their experimental results on the effects of CO_2 SFC on chiral polymer phases: cellulose tribenzoate and poly(triphenylmethyl methacrylate).

Däppen et al.[1)] classified the chiral stationary phases into five types: (a) chiral ligand-exchange phase, (b) chiral affinity phase, (c) helical polymer phase, (d) cavity phase, and (e) brush-type phase; they pointed out that the chiral polymer stationary phases show an extraordinary selectivity for many enantiomers.[1,4)]

In the present work, cellulose tris(phenylcarbamate)[8,9)] coated on silica gel particles is used as a chiral stationary phase. The retention characteristics of racemic trans-stilbene oxide, as determined by carbon dioxide super- and subcritical fluid chromatography, are investigated by varying the pressure, the temperature, and the concentrations of the alcohol modifiers. Some comparisons are made with the results of liquid chromatography (LC).

Experimental

Supercritical Fluid Chromatograph. A schematic diagram of the apparatus is shown in Fig. 1. Liquefied gas from a cylinder with a dip tube and a liquid modifier are introduced into a mixing chamber; the mixture flows up the column. The two pumps and the UV detector are from the Jasco Spectroscopic Co. (pumps: model BIP-1; detector: UVIDEC-100-V). The output signal from the detector is



1 gas cylinder (dip tube)
2 solvent bottle
3 valve
4 filter
5 pump
6 mixer
7 injector
8 column
9 column jacket
10 UV detector
11 data analyzer
12 pressure gage
13 back pressure regulator
14 liquid collector
15 gas flowmeter

Fig. 1. Schematic diagram of apparatus for supercritical fluid chromatography.

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resolved by means of a data analyzer (Shimadzu, CHROMATOPAC C-R6A). The line pressure is regulated by means of a manual back-pressure regulator (TESCOM, model 26-3200). The mobile phase is depressurized after the regulator, and it passes through the liquid collector housed in an ice-bath to a dry-type gas flowmeter. The flow-rate of the liquid modifier was determined from a digital scale of the pump since the flow-rate was kept unchanged at various elevated pressures. To keep the gas flow-rate constant, the pump head must be maintained at a low and constant temperature.

Column and Reagents. The cellulose tris(phenylcarbamate) was coated by 20 wt% on macroporous silica gel particles (20 µm—800 Å, treated by (3-aminopropyl)triethoxysilane) as has been described by Okamoto et al.[8] The silica gel was then packed in a stainless-steel column (250X4.6 mm i.d.) by a slurry method. The racemic compound resolved is *trans*-stilbene oxide (Aldrich Chem. Co.). 1,3,5-Tri-*t*-butylbenzene (Aldrich Chem. Co.) was used as a marker (non-retained) substance. Methanol, ethanol, and 2-propanol (reagent grade; Wako Pure Chemical Co., Ltd.) were fractionated by the use of a laboratory packed column. Carbon dioxide (99.9%, Nichigo Acetylene Co., Ltd.) was used without further purification.

Chromatographic Parameters. To describe the chromatographic separation behavior, the capacity factor of a substance i, k_i', the separation factor, α, the number of theoretical plates, N, and the resolution, R_s, are defined as follows:

$$k_i' = \frac{t_{Ri} - t_0}{t_0} \tag{1}$$

$$\alpha = \frac{k_2'}{k_1'} = \frac{t_{R2} - t_0}{t_{R1} - t_0} \tag{2}$$

$$N = 16(t_R/W)^2 \tag{3}$$

$$R_s = \frac{2(t_{R2} - t_{R1})}{(W_1 + W_2)} \tag{4}$$

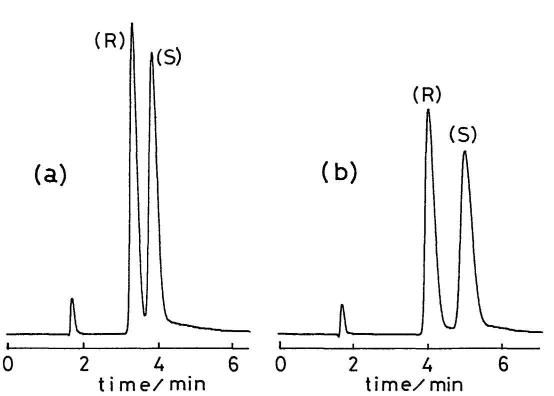

Fig. 2. Separation of racemic *trans*-stilbene oxide on the cellulose tris(phenylcarbamate) (20 wt%) coated on silica gel in SFC. Column: 25 cmX4.6 mm i.d., 20 µm; solvent: CO_2+methanol (mole fraction x_c=0.063); flow rate: 2 ml·min^{-1} (liq. CO_2); (a) 40 °C, 20 MPa; (b) 25 °C, 10 MPa.

where t_0 is the retention time of a non-retained substance; t_{R1} and t_{R2} are the retention times of the first- and the second-eluted substances respectively, and W is the peak width. When a peak shape is regarded as symmetric, the peak width is evaluated from the peak area, A, and the peak height, h, by assuming the Gaussian distribution function:

$$W = 1.596\,A/h. \tag{5}$$

The data analyzer used in the present work gives outputs of t_R, A, and h for each peak, from which all the chromatographic parameters, k_i', α, N, and R_s, were evaluated. Some typical chromatograms are shown in Fig. 2.

Results and Discussion

Capacity Ratio and Separation Factor. The first-eluted component of racemic trans-stilbene oxide is the R-enantiomer for a mobile phase of supercritical carbon dioxide modified by alcohols, which is the same as in liquid chromatography (LC) with a hexane–alcohol mobile phase. Figure 3 shows the influence of the mole fraction of alcohols (methanol, ethanol, 2-propanol), x_c, on the capacity factor, k_1', and the separation factor, α, at 40 °C and 10 MPa, which exceed the critical temperature (31 °C) and the critical pressure (7.39 MPa) of carbon dioxide. The capacity factor decreases rapidly with an increase in x_c for the three alcohols. The order of decreasing k_1' values is methanol, ethanol, and 2-propanol, which is the same order as the increase in molecular weight. *trans*-Stilbene oxide was not eluted in the mobile phase of pure carbon dioxide as in pure hexane. Therefore, the retention mechanism in both mobile phases may be a competitive adsorption between solute and

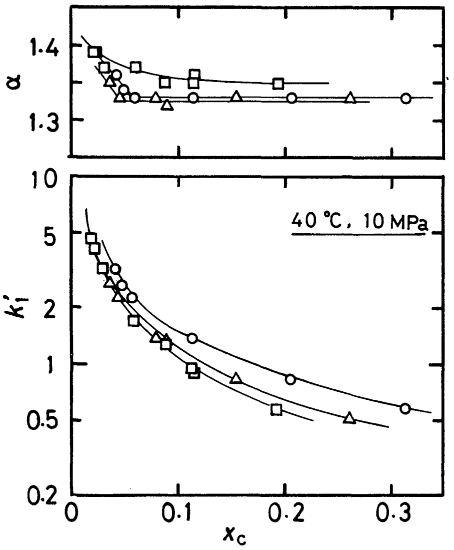

Fig. 3. Influence of alcohol composition on the capacity ratio k_1' and the separation factor α for racemic *trans*-stilbene oxide (40 °C, 10 MPa); solvent: CO_2+alcohol (C); modifier: (○) methanol, (△) ethanol, (□) 2-propanol.

alcohol molecules onto active sites of the chiral polymer.

The separation factor for the enantiomers, α, decreases a little with an increase in x_c in a region of low alcohol concentration, but it remains constant when x_c exceeds 0.08. 2-Propanol is the modifier giving a higher value for α than methanol and ethanol. The effective action of 2-propanol as a modifier for chiral recognition has been reported for the cellulose tribenzoate phases by Macaudiére et al.[5] and also for brush-type bonded phases by Macaudiere et al.,[6] Mourier et al.,[7] and Dobashi et al.[2] It may be deduced from Fig. 3 that the increase of α corresponds to the decrease in the alcohol concentration in the stationary phase, since the chiral recognition is made only at the stationary phase.

Figure 4 shows the influence of the column pressure on k_1' and α at 40 °C and 0.1 in x_c. When the pressure increases from 10 MPa to 25 MPa, k_1' decreases and α increases slightly for the three alcohols. The decrease in k_1' and the increase in α are contradictory to the results shown in Fig. 3; however, the increase in α may lead to the conclusion that the relative concentration of alcohol in the stationary phase dercreases with an increase in the pressure. The decrease in k_1' with an increase in the pressure results from two causes: the solubility increase in the mobile phase of supercritical carbon dioxide and the decrease in adsorptive sites available for the solute. The major reason for the pressure effects on α and k_1' may be the enhanced solubilities of both the solute and the alcohol into the mobile phase with an increase in the density.

Figure 5 shows the change in k_1' with the mole fraction of methanol at three temperatures (0, 25, and 40 °C) and at 10 MPa. The data points of k_1' in LC

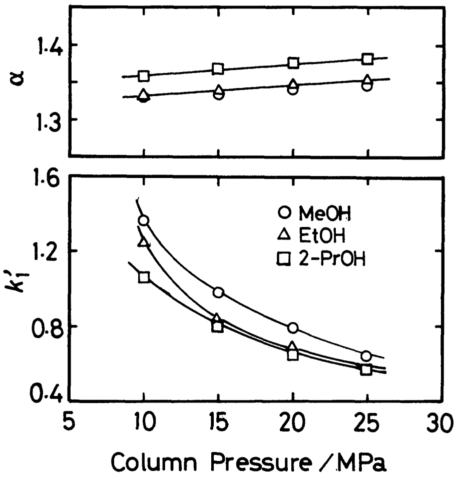

Fig. 4. Influence of column pressure on the capacity ratio k_1' and the separation factor α for racemic *trans*-stilbene oxide (40 °C); solvent: CO_2+alcohol (C) (x_c=0.1); modifier: (O) methanol, (Δ) ethanol, (□) 2-propanol.

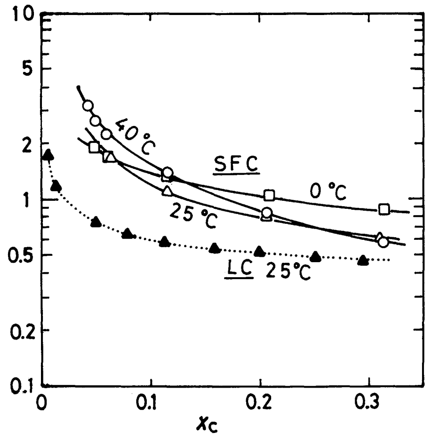

Fig. 5. The capacity ratio of *trans*-stilbene oxide against mole fraction of alcohol in SFC and LC; SFC: CO_2+methanol at 10 MPa and 0 °C (□), 25 °C (Δ), 40 °C (O); LC: hexane+2-propanol at 25 °C (▲).

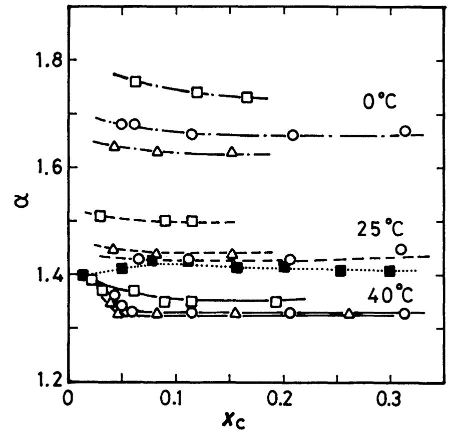

Fig. 6. Effect of alcohol composition and temperature on the separation factor α for racemic *trans*-stilbene oxide in SFC and LC; SPC: solvent: CO_2+alcohol (C), column pressure: 10 MPa, column temperature: (———) 40 °C, (------) 25 °C, (-·-·-·-) 0 °C, modifier: (O) methanol, (Δ) ethanol, (□) 2-propanol; LC: hexane+2-propanol at 25 °C (■).

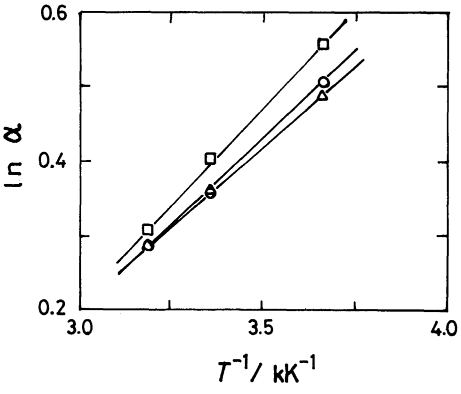

Fig. 7. Temperature dependence of the separation factor α for racemic *trans*-stilbene oxide at 10 MPa; solvent: CO_2+alcohol (x_c=0.1); modifier: (O) methanol, (Δ) ethanol, (□) 2-propanol.

(hexane+2-propanol eluents at 25 °C) are also shown for the sake of comparison. *trans*-Stilbene oxide is retained longer with CO_2+methanol eluents than with hexane+2-propanol; this may be attributed to the low solubility of the solute in the expanded fluids.

Figure 6 shows the plots of α against the alcohol mole fraction for the three alcohols at the three temperatures (0, 25, and 40 °C). The experimental data for α in LC are also plotted in the figure. The separation factor changes with the mobile fluid as well as with the modifier. Carbon dioxide modified by 2-propanol gives the highest values for α at 25 °C. The temperature is the variable most sensitive to the separation factor α; that is, the lower the temperature, the larger the separation factor. In Fig. 7 the logarithm of α at 10 MPa and 0.1 in x_c is plotted against the reciprocal temperature for the three alcohols. The differences in the adsorption enthalpy of the enantiomers, $\Delta H[=R\,\mathrm{d}\ln\alpha/\mathrm{d}(1/T)]$, as determined from the slopes, are as follows: 3.9 kJ mol^{-1} (methanol), 3.6 kJ · mol^{-1} (ethanol), and 4.5 kJ mol^{-1} (2-propanol). These results suggest that the chiral recognition of cellulose tris(phenylcarbamate) is mainly based on the difference in the adsorption ethalpy of the enantiomers.

Resolution. Figure 8 shows the effect of the alcohol mole fraction in a mobile phase, x_c, on the resolution, R_s. The column pressure is 10 MPa, while the flow-rate of liquid carbon dioxide is kept at approximately 2 ml · min^{-1}; therefore, the mass flow-rate in a column increases by the amount of alcohol added to carbon dioxide. The resolution decreases with an increase in x_c, while it increases with a decrease in the temperature for all the alcohols. In the same figure, the filled squares stand for the data points for R_s in LC (hexane+2-propanol eluents at 25 °C and 0.5 ml · min^{-1}). Though the flow-rate in LC is four times smaller than in SFC, the resolution is still inferior to those in SFC with a methanol modifier. Therefore, carbon dioxide SFC has the advantage that it can reduce analysis times compared with hexane LC. It is noteworthy that methanol is the most effective modifier of the three in terms of the resolution, though 2-propanol becomes more effective when the x_c value is less than 0.03.

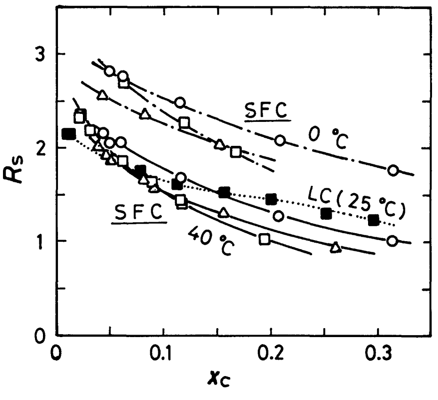

Fig. 8. Effect of alcohol composition on the resolution R_s of racemic *trans*-stilbene oxide in SFC and LC; SFC: column pressure: 10 MPa, column temperature: ——— 40 °C, ········· 0 °C, modifier: (○) methanol, (△) ethanol, (□) 2-propanol, flow rate: 2 ml·min^{-1} (liq. CO_2); LC: column temperature: ------ 25 °C, solvent: hexane+2-propanol (▲), flow rate: 0.5 ml·min^{-1}.

The experimental results for R_s can be explained in terms of the different contributions of the thermodynamic properties (k_1' and α) and the kinetic properties (N_1 and N_2). Equation 4 is rewritten as:

$$R_s = \frac{1}{4}\sqrt{N_{av}}(\alpha - 1)k_1'/(1 + k'_{av}) \tag{8}$$

where k'_{av} and N_{av} are the average quatities defined below:

$$k'_{av} = (k_1' + k_2')/2 \tag{9}$$

$$\frac{1}{\sqrt{N_{av}}} = \left(\frac{t_{R1}}{\sqrt{N_1}} + \frac{t_{R2}}{\sqrt{N_2}}\right)/(t_{R1} + t_{R2}). \tag{10}$$

The most effective factor in Eq. 8 to raise R_s for chiral separation is the parameter (α−1), which increases at a lower temperature. At the same temperature and a nearly constant flow-rate, R_s increases with an increase in k_1', since α and $\sqrt{N_{av}}$ change only a little. The reason for the superiority of methanol to other modifiers in terms of R_s may be its high diffusivity, which produces the high efficiency of a column (a large N_{av}).

In summary, the solvent characteristics of super- and subcritical carbon dioxide are superior to those of liquid hexane as a chromatographic mobile phase for the optical resolution of enantiomers (racemic *trans*-stilbene oxide) with a column of chiral polymer (cellulose tris(phenylcarbamate)) coated on silica gel. Comprehensive SFC data have been presented here on k_1', α, and R_s for various pressures, temperatures, and modifier compositions.

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