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Density dependence of retention factors of trans-stilbene oxide for chiral separation by supercritical fluid chromatography

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ABSTRACT

Retention factors for two enantiomers of trans-stilbene oxide, k_1 and k_2, were measured with a chiral AD-H column using two syringe pumps to feed CO_2 and methanol as a co-solvent at various temperatures, pressures and co-solvent mole fractions to determine the effects of these operating conditions on the retention factors. The retention factors k_1 and k_2 are for the (*R*,*R*)- and (*S*,*S*)-forms, respectively. When the isothermal compressibilities of a mixture of CO_2 and MeOH were lower than 0.01, far from the critical locus of the CO_2 and methanol mixture, both retention factors were well expressed with the solvent density and temperature with an average absolute relative deviation of 1–2%. In the vicinity of the critical locus, however, where the isothermal compressibilities were much larger than 0.01, the relationship between retention factor and density was complicated. Both retention factors were proportional to the isothermal compressibility, irrespective of methanol mole fraction at each temperature.

1. Introduction

Supercritical fluid chromatography is an attractive tool for analyzing and separating both chiral and achiral compounds because supercritical fluid or that with a co-solvent as a mobile phase has advantages such as low viscosity, tunable physicochemical properties, and easy separation of the solute from the solvent [1–4]. In addition to the operating variables in HPLC, pressure is also a key parameter. However, the increased number of operating parameters in SFC makes it difficult to find optimum analytical conditions, as pointed out by Lesellier and West [4]. Since density affects peak retention time significantly, the effects of solvent properties on peak retention times at certain temperatures and pressures must be determined to optimize the analytical/separation conditions. Various approaches to estimate retention factors for chiral and achiral compounds have been investigated with either packed or open capillary columns: (1) by expressing *k* values by a polynomial function of temperature, pressure/density, or properties related to chemical structure [5–15], determining the constants involved statistically [16], and with an artificial neural network [17], (2) by a function of thermodynamic properties such as heat of vaporization and entropy [8,18–25], e.g., the van't Hoff Equation, (3) with fugacity coefficients and an equation of state under equilibrium conditions between a mobile phase and a stationary phase [26], and (4) by a mathematical model describing solute concentration in packed [27,28] and open columns [15,29]. However, the effects of operating conditions on retention factors are not well understood, and an effective predictive correlation for retention factors over a wide range of operating conditions has not been proposed. In addition to the complexity of SFC separation mechanism as well as that of HPLC, one of the difficulties in developing the correlations for retention factors in SFC is that it is commonly operated near the critical locus of the mixture, where the solute partial molar volume is known to possess negative large values, which are derived from the solute retention factors [30,31]. Under such conditions, small changes in density or solvent composition significantly affect the retention factor.

The objective in the present study was to study the effects of operating conditions on retention factors of racemic trans-stilbene oxide (tSO) samples in supercritical fluid chromatography, across two regions in particular for comparison purposes: in the vicinity of critical points of a mixture of CO_2 and methanol, and far from the critical points. Moreover, we also aimed to develop a predictive correlation of retention factor.

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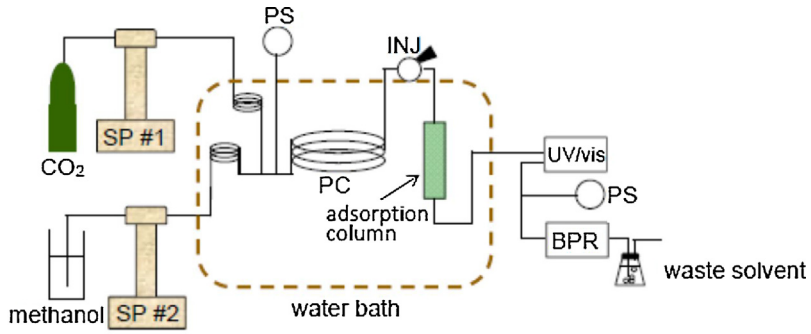

BPR: back pressure regulator
INJ: injector
PC: preheating tube
PS: pressure sensor
SP: syringe pump
UV/Vis: UV/Vis multi-detector
VC: view cell

Fig. 1. Schematic diagram of the experimental apparatus.

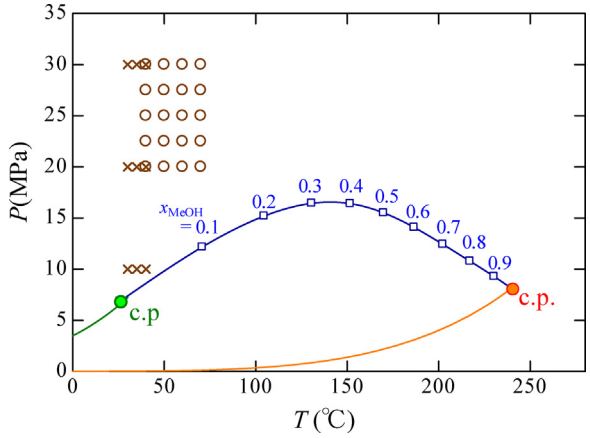

Fig. 2. The present measurement conditions (×, ○) and critical locus of a mixture of CO_2 and methanol. The numerals shown are methanol mole fractions x_{MeOH}.

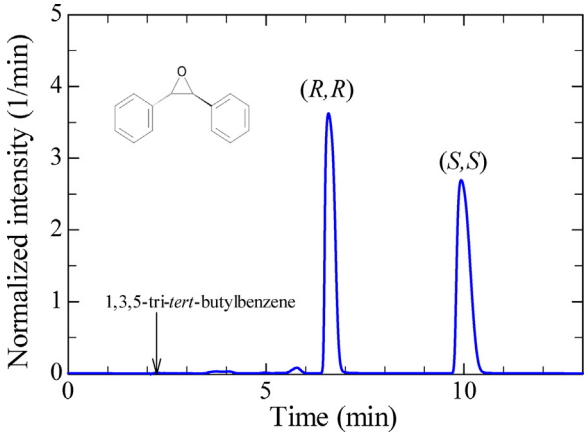

Fig. 3. Chromatogram of tSO at 40 °C; 20 MPa; Feed rate: CO_2, 1.35; methanol, 0.15 mL/min. The former and latter peaks are the (*R*,*R*) form and (*S*,*S*) form, respectively.

2. Materials and methods

2.1. Apparatus

Fig. 1 shows a schematic diagram of the experimental apparatus. Two syringe pumps (260D and 100DM, ISCO) were employed to feed CO_2 and methanol separately. The fluids were first mixed, then the mixture of CO_2 and methanol was introduced to a preheating tube (0.8 $mm^{I.D.}$ × 6 m), which was immersed in a water bath with a temperature maintained at a prescribed value within a fluctuation of ±1 °C. The temperature of the mixture of CO_2 and methanol attained the intended temperature during passage through the preheating tubing, and was fed into an adsorption column (Chiral AD-H column, Daicel, Co. Japan, 4.6 $mm^{I.D.}$ and 20 cm packed with 5 μm particles) placed vertically in the water bath via an injector (Model 7520, Rheodyne). The chromatogram was monitored by the UV/Vis multi photodiode array detector (MD-1510, JASCO) placed downstream at the outlet of the column. The system pressure was regulated at the prescribed pressure within a fluctuation of mainly ± 0.1 MPa, sometimes ± 0.2 MPa, by a back pressure controller (Model 880-81, JASCO), which was electromagnetically operated by a high-frequency open-shut valve, and placed at the end of the line. All connecting lines were stainless steel tubing with 0.8 $mm^{I.D.}$. The pressures were monitored at the pressure sensors equipped with the syringe pumps, the pressure sensor (Huba pressure transmitter, range up to 40 MPa, accuracy: within 0.1% full scale, Switzerland) installed just upstream of the preheating tube and the pressure sensor equipped with the back pressure controller. The calibrations of these pressure sensors were made using a Heise pressure gauge with ranges up to 50 MPa. The pressure drop from the position upstream the preheating tube to that at the back pressure controller, almost equal to pressure drop between the AD-H column inlet and outlet, were at most 0.7 MPa at all conditions studied. Note that since the pressure drop gradually increased with increasing the injection number of times, the column was washed by flowing liquid methanol periodically.

Prior to a run, either CO_2 or methanol was filled in each cylinder of each ISCO pump, and maintained at room temperature for at least an hour. The temperature of the cylinder was monitored at the attached thermometer. Once the baseline was stable, trans-stilbene oxide dissolved in methanol at a concentration of 5 mM was introduced to the adsorption column. Response signals, monitored with the UV–vis photodiode array detector, were measured by scanning from 190 to 600 nm at increments of 1 nm and intervals of 1.6 s. At least three measurements for each condition were made to confirm the reproducibility. 1,3,5-Tri-*tert* butylbenzene was used to determine the retention time t_0 for a compound with no adsorption according to the literature [8,32]. Moreover, almost no differences of the retention times of 1, 3, 5-tributylbenzene were confirmed for the AD-H column and the same size column packed with the same deactivated particles without the ligand.

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