Refractive Index Detection Using an Ultraviolet Detector with a Capillary Flow Cell in Preparative SFC

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Abstract

An ultraviolet detector with a capillary flow cell is evaluated for use as a refractive index detector in supercritical fluid chromatography (SFC) using a CO_2 mobile phase. The temperature or pressure control or both of the cell is critical to minimize density-related refractive index changes. Using CO_2, which has a refractive index value that is significantly smaller than that of common organic solvents, leads to higher detector response. Based on a long-term noise level of 2.3×10^{-5} refractive index units, the detection limit for *n*-hexadecyl alcohol at a signal-to-noise ratio of 2 is 30 µg on a 4.6-mm i.d. packed column. This corresponds to the actual detection limit of 30 ng in the capillary cell (at a split ratio of 1:1000). The linear dynamic range of the detector is more than 2 orders of magnitude. This detection method can readily be used for preparative SFC.

Introduction

Supercritical fluid chromatography (SFC) has many advantages as a preparative separation technique over high-performance liquid chromatography (HPLC). The high diffusivity and low viscosity of supercritical fluids allow both highly efficient and rapid separations. Many supercritical fluids are volatile or gaseous at ambient conditions, so solutes can easily be recovered, solvent free, from the collected fractions by depressurization. To establish SFC as a preparative technique for industrial purposes, however, various problems must be solved, including sample injection, and detection, fraction collection, and solute recovery.

An ultraviolet–visible (UV–vis) detector is the most commonly used detector in preparative SFC as well as in analytical packed-column SFC. Because carbon dioxide (CO_2), which is the most common mobile phase in SFC, allows the use of short wavelengths down to 190 nm, many types of compounds can be detected. However, sensitivities are largely dependent on the compound types, and non-UV-absorbing species cannot be detected. A universal detector with similar sensitivities for all types of compounds is needed for collecting solutes of interest in preparative separations. The first choice may be a flame-ionization detector (FID). The destructive characteristics of FID do not matter because a small part of the column effluent can be introduced into the detector by splitting (1). However, one of the major problems is restrictor plugging, which occurs when the highly concentrated effluent is introduced. In addition, organic modifiers cannot be used with FID. The evaporative light-scattering detector (ELSD) is also a universal detector, but the sensitivity is dependent on the nebulization conditions and solute properties (2).

A conventional refractive index (RI) detector, to our knowledge, has not been utilized for SFC. This may be due to the difficulties of constructing a high-pressure cell or operating the cell at strictly regulated temperature and pressure. A promising RI detector consists of a capillary flow cell and laser optical systems (3–5) that have been applied for capillary electrophoresis and microbore HPLC. Excellent sensitivity of an order of 1×10^{-6}–10^{-7} refractive index units (RIU) and a linear range of more than 3 orders of magnitude can be achieved. A high-pressure version of this type is required for use in SFC.

A method to control the temperature and pressure of the capillary cell was reported (6) and was applied for UV detection in capillary SFC. RI detection was also demonstrated with relatively good sensitivity. This work describes systematic studies on this RI detector with a capillary cell and its application in preparative SFC. The effect of density of the mobile phase on the detector response was investigated, and the detection limit and linearity were evaluated.

Experimental

Experiments were performed with a Super 200 supercritical fluid chromatograph (Jasco; Tokyo, Japan) using the detection system shown in Figure 1. The system consisted of two UV detectors: a UVIDEC 100-V UV spectrophotometer (Jasco) with a conventional 8-µL high pressure flow cell and a modified

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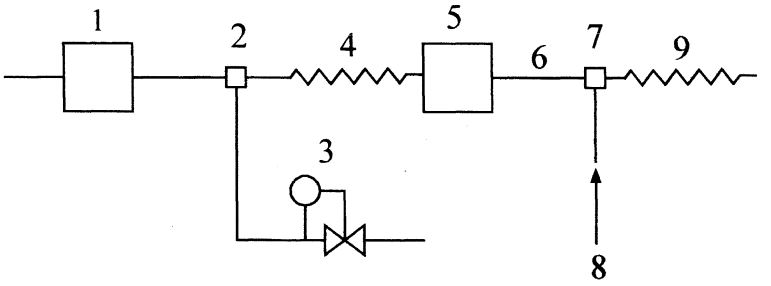

Figure 1. Schematic diagram of the detection system: 1, conventional UV detector; 2, split tee; 3, back-pressure regulator; 4, fused-silica restrictor (30 cm × 15 µm); 5, UV–RI detector with capillary cell; 6, fused-silica tubing (30 cm × 0.1-mm i.d.); 7, make-up tee; 8, nitrogen gas; 9, fused-silica column (15 cm × 0.53-mm i.d.) packed with 5-µm silica.

UV-970 UV–vis spectrophotometer (Jasco) with a capillary flow cell. Modification of the UV detector was described in detail previously (6), but a general description is presented here. The cell block with the same size as the original was constructed by sandwiching copper tubing between copper plates and then soldering them. The capillary UV cell consisted of quartz glass tubing (0.6-mm o.d., 0.22-mm i.d.) prepared by drawing in an oxygen–hydrogen flame to achieve an effective cell volume of 0.06 µL. A masking slit (1.5 mm × 0.2 mm) was placed close to the cell at the side of lamp. Fused-silica tubing (two) (15- and 100-µm i.d., respectively) were cemented into the capillary cell with epoxy glue. The cell temperature was controlled by passing water through the copper tubing in the cell block. The temperature of water was regulated within ±0.1°C with a T-22L water bath (Thomas; Tokyo, Japan). The cell pressure was controlled by applying pressurized nitrogen gas (70 to 100 atm) to the make-up tee and calibrated using a pressure gauge from a model 880-81 back pressure regulator (Jasco).

The columns (25 cm × 4.6-mm i.d., 5-µm L-column ODS and 15 cm × 4.6- or 10-mm i.d., 5-µm Superpak Crest SIL) were from Chemicals Inspection & Testing Institute (Tokyo, Japan) and Jasco, respectively. The mobile phase was food additive grade (99.99%) carbon dioxide (Showa-Tansan Co.; Yokkaichi, Japan). The flow rate of liquid CO_2 was 3 mL/min for the 4.6-mm i.d. column or 10 mL/min for the 10-mm i.d. column. The column temperature was 60°C for the ODS column and 45°C for the silica gel column. Samples were alkylbenzenes *n*-alcohols, kerosene, and fatty acid methyl esters derived from perrila oil dissolved in methylene chloride or *n*-hexane. Samples were injected with a model 7125 injector (Rheodyne; Cotati, CA) with a 20-µL loop. For preparative separations, samples were injected with a Rheodyne 7000 valve with an 1-mL loop. In this case the sample solvent was vented, and the solutes were trapped on the trap column and then refocused onto the separation column. Details of the injection method for preparative SFC were reported (1).

Results and Discussion

Figure 2 shows the simultaneous detection of alkylbenzenes. Figure 2A was obtained with the conventional UV detector set at 254 nm, whereas Figures 2B and 2C were obtained with the modified UV detector set at 254 and 600 nm, respectively. The column effluent at a flow rate of 3 mL/min was divided at the split tee after passing through the conventional UV detector, as shown in Figure 1. The main flow was vented from the back pressure regulator at 140 atm, and the split flow entered the modified UV detector through a restrictor (30 cm × 15-µm i.d.). When the nitrogen gas pressure of 80 atm was applied to the capillary cell, the split flow passed through the capillary cell at approximately 3 µL/min (a split ratio of 1:1000). The sensitivity in Figure 2B was about 20 times less than in 2A because of the shorter path length. The value did not agree with the ratio of path lengths of the two detectors, which is about 45. This may be due to the deviation of the capillary cell position from the optics. When the detection was made at 600 nm with the modified UV detector, all peaks including the solvent peak had negative responses, as shown in Figure 2C. Because these compounds have no UV absorption at 600 nm, these responses can be attributed to RI changes caused by solutes. This is supported by the fact that the negative baseline shift has usually been observed during pressure programming in SFC with UV detection, where the cell pressure is also increased (7).

To examine the band broadening in this detection system,

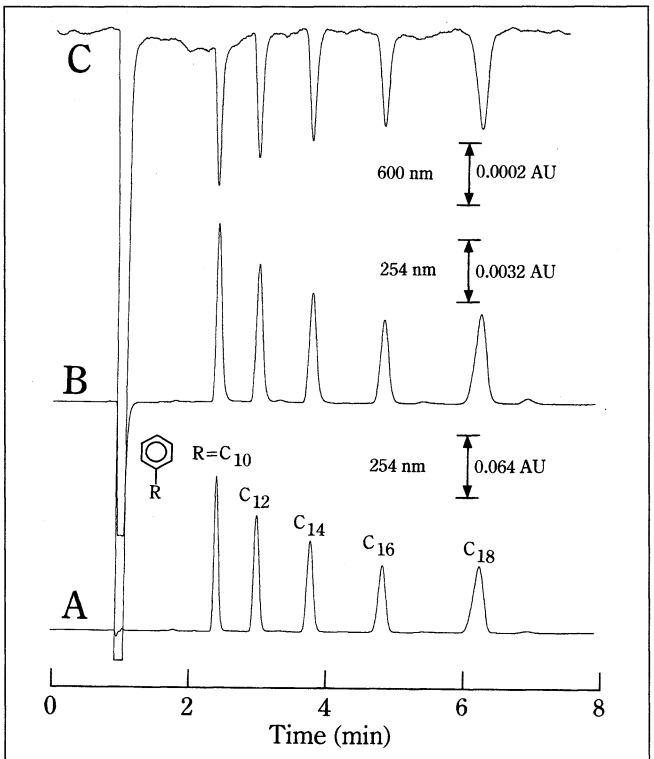

Figure 2. Chromatograms obtained with conventional (A) and modified (B and C) UV detectors. An ODS L-column (25 cm × 4.6-mm i.d., 5-µm thickness) was used. The conditions were as follows: mobile phase, CO_2; flow rate, 3 mL/min; temperature, 60°C; pressure, 140 atm; sample, 5% alkylbenzenes in methylene chloride; sample volume, 5 µL.

peak widths in Figure 2B were compared with those in Figure 2A. Peak widths increased about 20% for C_{10} and 6% for C_{16}. The flow rate entering the capillary cell was varied by changing the pressure of the capillary cell in the range of 70 to 100 atm and maintaining the column outlet pressure at 140 atm. However, no noticeable change in peak width was observed, indicating that the contribution of the dead volume in the modified UV detector to the band broadening is negligibly small. Therefore, this broadening may be caused by the dead volume of the connection tubing between the conventional UV detector and the split tee. Although a relatively long connection tubing (50 cm × 0.5-mm i.d.) was used in this study for experimental convenience, its length can easily be decreased. Even with the present set-up, this effect should be less than 1% for all peaks under the comparable conditions for the 10-mm i.d. column.

Figure 3 shows the relationship between the RI of CO_2 and pressure at different temperatures. RI was calculated using the Lorentz–Lorenz equation:

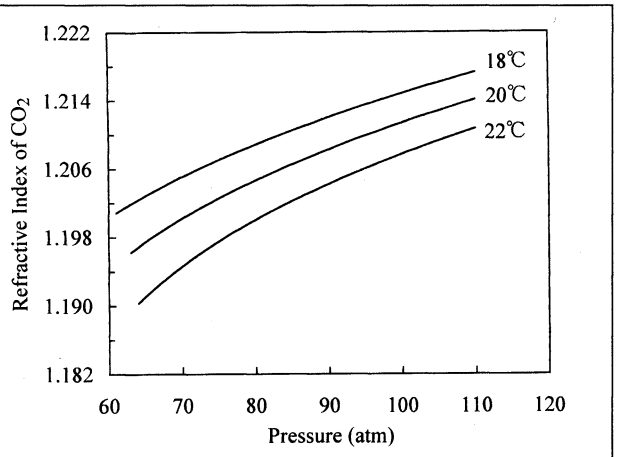

Figure 3. Plots of the refractive index values of carbon dioxide versus pressure.

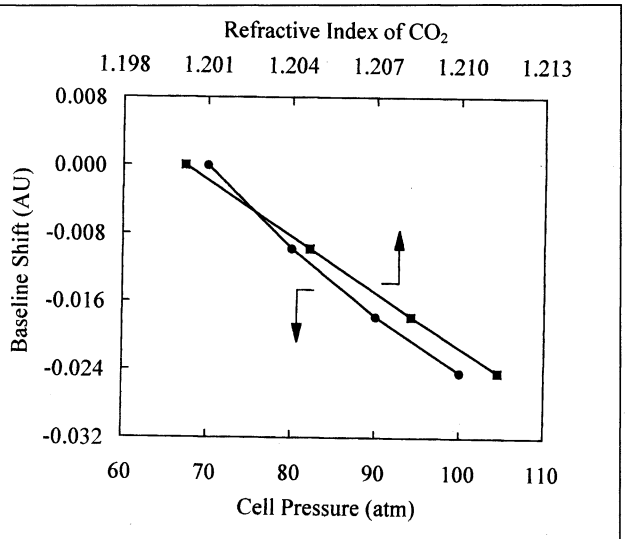

Figure 4. Plots of baseline shifts against the cell pressure and refractive index values of CO_2. The cell temperature was 20°C, and detection was at 600 nm.

$$R_m = \frac{n^2-1}{n^2+2}\frac{MW}{\rho} \qquad \text{Eq 1}$$

where R_m is molar refraction (6.84 for CO_2), n is the refractive index, MW is molecular weight, and ρ is density. Density was calculated using the Lee–Kesler equation (8). The results clearly indicate that RI is very sensitive to temperature and pressure changes. At 20°C and 80 atm, RI changes were estimated to be 1.1×10^{-3} RIU/°C and 3.7×10^{-4} RIU/atm. These values are far greater than those of common LC solvents, for example, 5.5×10^{-4} RIU/°C and 1.4×10^{-5} RIU/atm for methanol. Thus, in SFC both the temperature and pressure of the cell must be very accurately regulated to maintain a stable baseline. This is one of the disadvantages of using an RI detector in SFC. However, CO_2 has a significantly small RI value compared with the common HPLC solvents. Typical values of the RI are approximately 1.2 for CO_2 (see Figure 3) and in the range of 1.3–1.5 for the HPLC solvents. This smaller RI of CO_2 can be expected to result in a higher response when it is used as the mobile phase.

Figure 4 shows the plots of baseline shift against the cell pressure and RI of CO_2 obtained with the modified UV detector. Measurements were performed at 600 nm and 20°C by changing the cell pressure from 70 to 100 atm. The plot of baseline shift against RI shows good linearity (r = 0.9999) in the range of $\Delta n = 1.1 \times 10^{-2}$ RIU, corresponding to 2.4×10^{-2} absorbance units (AU). Hence, 1 AU corresponded to 0.46 RIU. The response slightly decreased with increasing wavelength, approximately 10% in the range of 200 to 700 nm. The results indicate that this RI detection mode can be used for quantitative analyses.

Figure 5 shows the baselines obtained at 300 and 600 nm. The long-term noise levels are comparable with each other, which are approximately 5×10^{-5} AU. The short-term noise level is smaller at 600 nm than at 300 nm. This may be

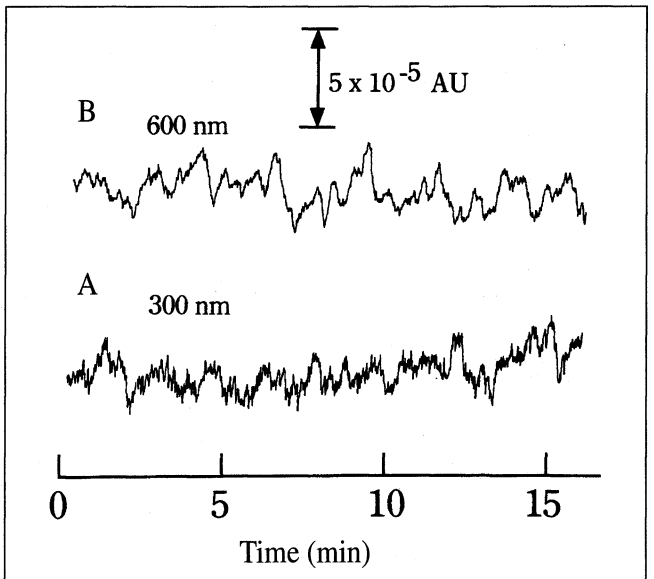

Figure 5. Baselines obtained at different wavelengths. The cell pressure was 80 atm, and cell temperature was 20°C.

attributed to the differences in stability and light intensities between the two light sources: deuterium and a tungsten lamp. The long-term noise, which seems to be caused by the temperature or pressure fluctuations or both, corresponded to $\Delta n = 2.3 \times 10^{-5}$ RIU. Thus, the linear dynamic range was more than 2 orders of magnitude. If the long-term noise level is decreased to the level of short-term noise, an improvement in sensitivity of approximately 10 times can be expected.

Figure 6 shows the chromatograms obtained with RI detection for different injected amounts of *n*-hexadecyl alcohol. The plot of peak area against injected amount showed good linearity ($r = 0.9999$). The detection limit based on the long-term noise level was approximately 30 µg at a signal-to-noise ratio of 2. In this case, the split ratio was 1:1000, implying that the detection limit of this detector is about 30 ng. Although the noise level of this RI detector is 2 orders of magnitude higher than a laser-based RI detector used for microbore HPLC (4), the detection limits are comparable. This may be due to the smaller RI of CO_2 compared with those of solvents used in HPLC.

Figures 7 and 8 shows the applications of RI detection. The main peak in the UV chromatogram in Figure 7A corresponds to the dimethylnaphthalenes. Paraffins (the main peak in Figure 7B) were clearly detected with RI detection. Figure 8 shows the preparative SFC separation of fatty acid methyl esters. The mix-

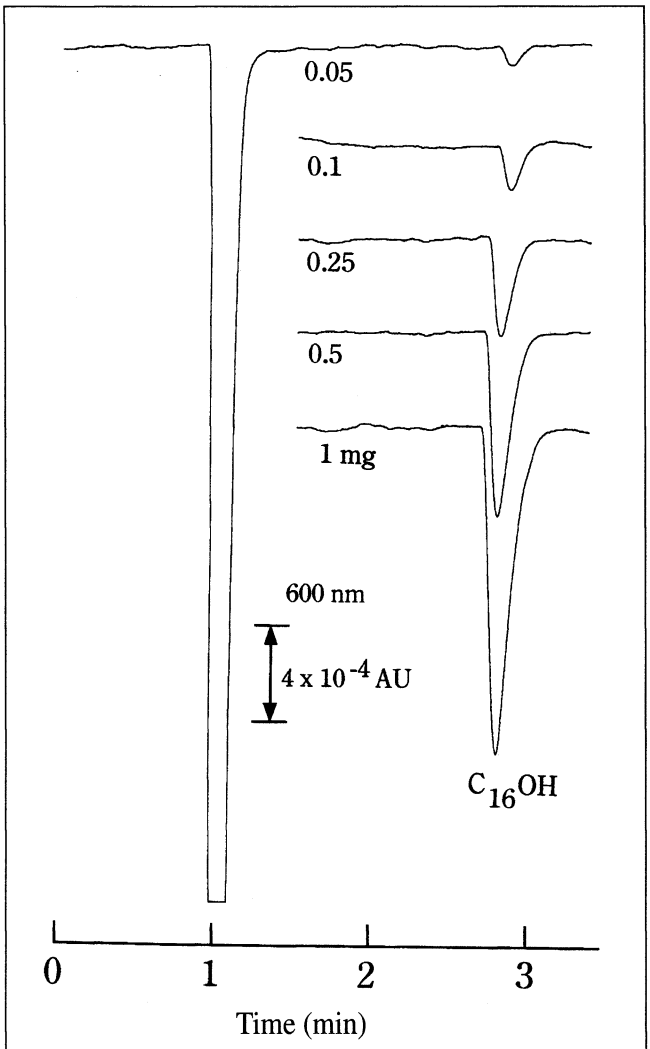

Figure 6. RI detection of *n*-hexadecyl alcohol. Conditions were as follows: mobile phase, CO_2; flow rate, 3 mL/min; temperature, 60°C; pressure, 140 atm.

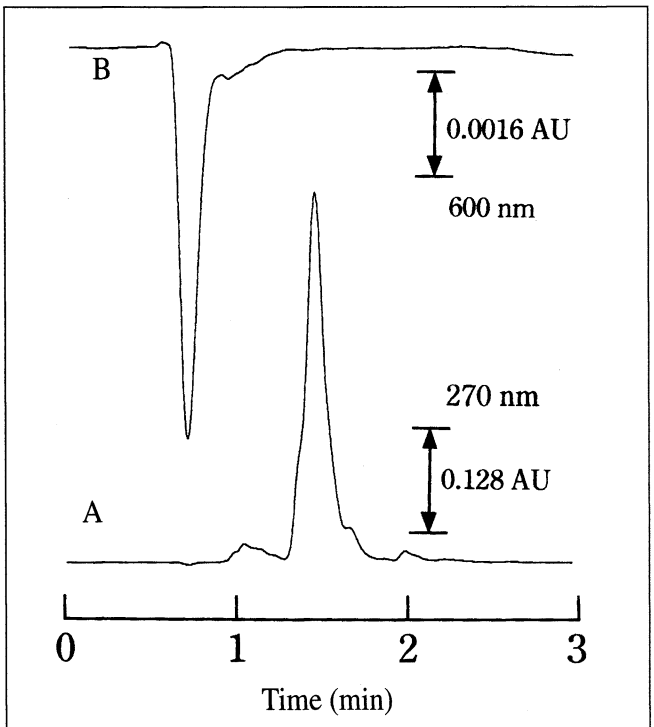

Figure 7. SFC chromatograms of kerosene obtained with UV (A) and RI (B) detection. A Superpak Crest SIL column (15 cm x 4.6-mm i.d., 5-µm particles) was used. Conditions were as follows: mobile phase, CO_2; flow rate, 3

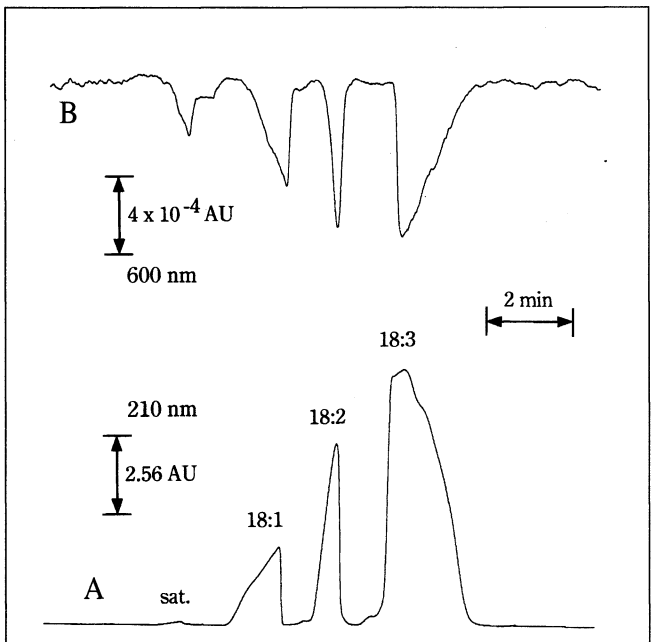

Figure 8. Preparative SFC chromatograms of fatty acid methyl esters from perrila oil obtained with UV (A) and RI (B) detection. A Superpak Crest SIL column (15 cm x 10-mm i.d., 5-µm particles) was used. Conditions were as follows: mobile phase, CO_2; flow rate, 10 mL/min; temperature, 45°C; pressure, 140 atm; sample amount, 20 mg.

ture of 20 mg was loaded onto a 10-mm i.d. column. With UV detection (Figure 8A), the response was highly dependent on the number of double bonds in the compound and was out of the linear range, especially for the 18:3 solute mixture. On the other hand, the response with RI detection remained within the linear range (see Figure 4). Therefore, this RI detection method can be used for monitoring highly concentrated effluents encountered in preparative SFC separations.

Conclusion

A method to control the temperature and pressure of the capillary cell of a UV detector was developed. This technique allowed the monitoring of RI changes in column effluent caused by solutes in SFC. RI detection has great potential for preparative SFC.

Acknowledgment

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