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Journal of Chromatography A, 724 (1996) 91–96

JOURNAL OF
CHROMATOGRAPHY A

Counter-current chromatography using supercritical fluid carbon dioxide as mobile phase

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First received 28 February 1995; revised manuscript received 14 August 1995; accepted 22 August 1995

Abstract

Supercritical fluid carbon dioxide was employed in solar eccentric counter-current chromatography (SECCC) to replace the liquid mobile phase in the traditional arrangement. The fundamental difference from the conventional liquid–liquid system is the requirement for high-pressure operation. The experimental set-up is presented to illustrate the necessary modifications that accommodate operation with a supercritical fluid. Studies on the stationary phase retention due to various experimental conditions are discussed. A mixture of acetophenone and benzophenone was chromatographed using mixtures of water and methanol as the stationary solvent system to demonstrate the feasibility of this technique. At present the efficiency of the separation is not satisfactory owing to the phase separator inserted in the system to remove liquid droplets which contributed to considerable peak broadening.

Keywords: Counter-current chromatography; Supercritical fluids; Mobile-phase composition; Acetophenone; Benzophenone

1. Introduction

Counter-current chromatography (CCC) has been recognized as a useful preparative separation technique [1–3]. Solutes are separated due to the different partition coefficients in two liquid systems, i.e., mobile and stationary phases. Without support from the solid packing material, liquid stationary phases are usually retained in the separation tubing through the application of gravitational or centrifugal force [4]. Sample capacity and several other separation parameters

are believed to be affected by the phase retention ratio [5]. A complete description of theory and application of CCC can be found in two books [6,7].

Although the separation speed has been improved significantly in recent years, CCC basically still is a relatively slow process with mediocre efficiency. Chromatography using supercritical fluids, which have relatively high diffusivity, low viscosity and liquid-like solvent strength, has emerged as an alternative to liquid and gas chromatography in many respects [8,9]. We attempted to replace the liquid mobile phase with supercritical fluid carbon dioxide (SF CO₂).

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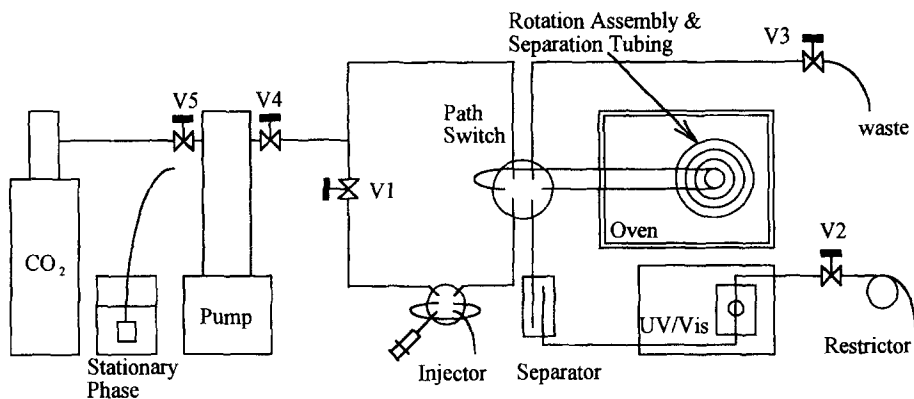


Fig. 1. Schematic diagram of the experimental set-up.

Since the stationary phase volume in CCC can be as large as 70% of the total volume of the column, mass transfer during the chromatographic process should play a crucial role in the separation efficiency. The higher diffusivity and lower viscosity of SF CO₂ may increase the rate of dynamic equilibrium of the solute distribution in both phases, thereby resulting in a faster speed. However, band broadening due to the same factors should not be neglected. In addition, SF CO₂ vaporizing into a gas under atmospheric pressure at the outlet may help concentrate the solutes during collection. The potential applications of this technique may include the preparative separation of natural products, such as polyphenols in *Ginkgo biloba* leaves [10], the

purification of pharmaceuticals [11] and large-scale separations of chiral compounds [12]. In addition, SF CO₂ may replace organic solvents in the extraction of pesticides at very low concentrations [13] in waste water using CCC. In this paper, we demonstrate the feasibility of this novel technique.

2. Experimental

2.1. Set-up

The instrumental layout is shown in Fig. 1. The rotation assembly shown in Fig. 1 as concentric rings is depicted in Fig. 2 in greater detail.

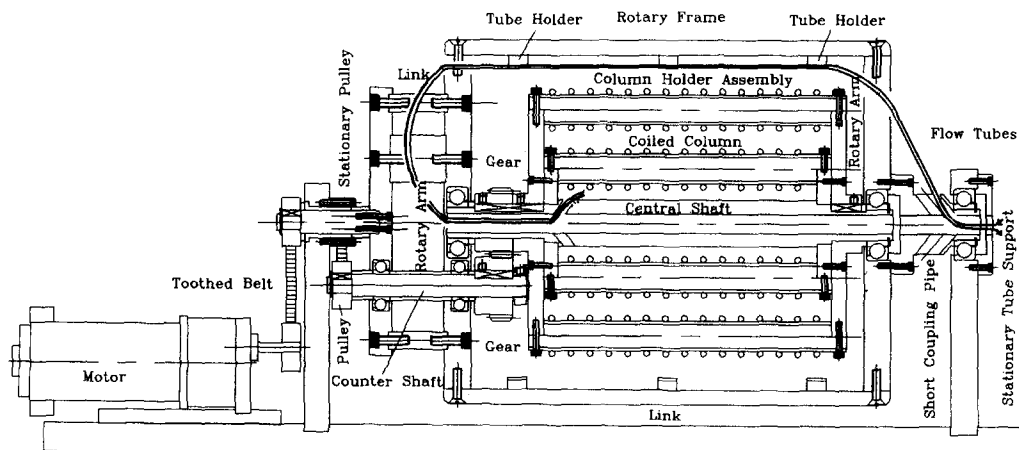


Fig. 2. Schematic diagram of the rotating assembly.

Owing to the requirement for high pressure in the system, an anti-twisting mechanism for the rotation is preferable to rotation seals. This laboratory-made rotation assembly is a solar eccentric type of device originally designed by Ito and Bhatnagar [14]. This device affiliates the Adams anti-twisting mechanism that makes it possible to exert centrifugal force on the tubing without breaking it. When it runs, the column holder assembly revolves at a speed twice the rotary frame that prevents the tubings from twisting. As shown, the tubings can be placed at outer and inner positions on the column holder assembly. The radii of the inner and outer positions are 3.5 and 7.5 cm, respectively. The first column holder assembly on our device consists of sixteen aluminum rods (1.78 cm O.D., 20 cm long) held on the outside of the rotary assembly. The second column holder assembly contains eight rods on the inside with the same dimensions. In this work only eight evenly spaced rods on the outside were used. These rods were wound from PEEK tubing (3.18 mm O.D., 1.57 mm I.D.) that could withstand pressures up to 20 MPa. It totalled 320 turns with a length of 30 m. The total volume of the separation column was 46 ml. All the other connecting tubes were made of PEEK tubing of 1.59 mm O.D. and 0.25 mm I.D. in order to reduce the dead volume. In addition, the smaller PEEK tubing endured stress much better than the larger tubing. One must handle the PEEK tubing with care when placing the inlet and outlet tubings in position because optimum stress on the tubing at the curved position is necessary to help prevent it from breaking. Reducing the stress at the contact points where the tubing touches the aluminium assembly also proved to be indispensable. The PEEK tubing was lubricated with silicone grease wherever necessary to reduce friction during rotation. The rotor was powered by a 40-W d.c. motor (Model TWT-06SGN) supplied by Chi-Wang Inc. (Taipei, Taiwan). The highest speed was 450 rpm. The whole assembly except the motor was housed in an oven that gave a $\pm 1.0^\circ\text{C}$ tolerance.

The high-pressure pump used in this study was an ISCO (Lincoln, NE, USA) Model 260D

syringe pump. An ISCO V4 UV-Vis detector was employed for monitoring the absorbance of the solutes. Two Valco (Houston, TX, USA) six-port valves were utilized in the system; one was for sample injection and the other for flow path switching. A 30 cm \times 20 μm I.D. stainless-steel flow restrictor supplied by ISCO was placed at the end of the system to provide the back-pressure. A phase separator which removed solvent droplets occasionally carried out by the SF CO₂ was installed before the detector. All tubings and valves were connected by Swagelok fittings to sustain high pressure.

In addition to the on-line UV-Vis detector, fractions of the effluent were also examined with an off-line Beckman (Fullerton, CA, USA) DU 7400 UV-Vis spectrophotometer.

2.2. Procedures

2.2.1. Phase retention study

The syringe pump was first filled with the desired stationary phase. With valves V3 (a Swagelok SS-22RS2 metering valve) and V4 open while V1 was closed, the separation tubing was filled with stationary solvent with suitable operation of the six-port valve (labelled path switch in Fig. 1). The pump was stopped when the solvent flow was seen at the waste tubing connected to valve V3. Subsequently the pump was connected to a CO₂ cylinder, charged with CO₂ and compressed to the desired pressure. After the oven had reached the desired experimental temperature, the rotation assembly was turned on and set at desired rotation speed and CO₂ was pumped through the coiled column. With suitable control of V3, the flow-rate was set at about 0.2 ml/min. At the beginning, part of the aqueous stationary phase was gradually removed by the CO₂ flow. After a period of time a strong spray of liquid was squeezed out by CO₂ gas at the waste tubing. Eventually only a CO₂ flow was "observed" (dry-ice formed in the spraying jet and sublimed) at the waste tubing. Valve V3 was closed at this time and the rotation assembly was stopped. Subsequently the stationary solvent was slowly displaced by SF CO₂ flow with V3 open and collected at the waste tubing.

2.2.2. Chromatographic separation study

The counter-current equilibrium of the liquid and the supercritical fluid was formed in the same manner as described in the above section. With appropriate arrangement of the path switch (the six-port valve), and with valves V1, V2 and V4 open, CO₂ flowed through the sample injector valve and the separation tubing, then toward the phase separator and the UV–Vis absorbance detector. Samples were then ready to be injected from the sample injector.

2.3. Reagents

Analytical-reagent grade acetophenone and benzophenone were purchased from Sigma (St. Louis, MO, USA), HPLC-grade methanol from Mallinckrodt (Paris, KY, USA) and SFC-grade CO₂ from Scott Specialty Products (Plumsteadville, PA, USA).

3. Results and discussion

Phase retention has been considered an important parameter in the operation of CCC. Variables such as rotation speed, solvent composition, tubing diameter and temperature are some of the major factors that could affect the S_F value (Fractional volume of column occupied by stationary phase) [6,7]. In this study, we briefly examined the S_F values at various pressures, rotation speeds and solvent compositions. In the early stages of the study, pure water was utilized as the stationary phase. Under various conditions, the S_F values are always less than 10%, if any. Water and SF CO₂ are reported to be only sparingly soluble in each other [15]. A modification of the solvent system was therefore considered. Methanol was added to the stationary phase to increase the mutual solubility of the two phases. As shown in Fig. 3, the phase retention was significantly improved after this modification. Each point in the plot was the average of three measurements. The phase retention ratio for both methanol–water (30:70) and (50:50) solvents declined when the pumping pressure was raised. Both experiments were

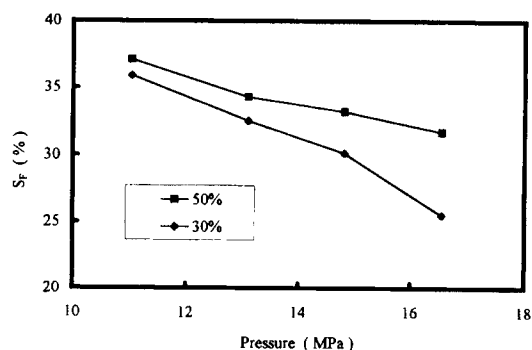


Fig. 3. Phase retention ratio measured in two stationary phases under various applied pressures at 435 rpm and 50°C. ■ = Methanol–water (50:50); ◆ = methanol–water (30:70).

conducted at 435 rpm and 50°C. As can be seen, when the pressure decreases the S_F values become very close to each other between these two compositions. Since the SF CO₂ is more compressible than common liquids, the density change of neat CO₂ in this experiment ranged from 0.491 to 0.734 g/ml [16] as the pressure increased from 11.03 to 16.55 MPa. Note that the content of methanol in the SF CO₂ flow was not considered in the above density calculations. However, the trend of density change would be still the same if the contribution from methanol was included. The reason for the higher S_F value at lower applied pressure is probably the greater density difference between the stationary and mobile phases [17].

In a typical conventional CCC operation, the stationary and mobile phases are mutually saturated in a separating funnel to minimize the homogeneous bleeding of the stationary phase caused by being dissolved in the mobile phase during the elution. Second, it also keeps the composition of both phases from changing. In practice, however, this exercise is very difficult to perform for the liquid–SF CO₂ pair. The operation was therefore ignored temporarily in the preliminary work. Accordingly, the phase retention ratio in this system should be time dependent. Both water and methanol would be dissolved and carried out the SF CO₂ flow in the phase retention and chromatographic measurements. The solubility of water in SF CO₂ is only 0.44 and 0.67 mol% at applied pressures of 11.03

and 16.55 MPa, respectively [15]. However, the solubility of methanol in SF CO₂ is fairly high. For example, the CO₂–methanol mixture is reported to be in a homogeneous supercritical condition at 68.4°C and 9.653 MPa when the methanol mole fraction is as high as 0.6 [18]. The composition of the stationary phase would therefore change to a certain extent during the process. One way to approach the conventional solvent treatment would be to incorporate a saturator, such as a liquid extraction cell [19], or like the phase separator shown in Fig. 1, between the pump and the separation column. The SF CO₂ passes through the saturator which is filled with the desired stationary phase before flowing into the column. We believe that the composition change and also bleeding of the stationary phase would be reduced owing to this pretreatment of the mobile phase.

Phase retention was also investigated at different rotation speeds, as shown in Fig. 4. As expected, a higher speed resulted in a higher retention volume owing to the larger centrifugal force that held the stationary phase. Again, three measurements were made for each point. As can be seen in Fig. 4, the S_F value shows a tendency to level off as the methanol content reaches 50%. As the content of methanol increased, the viscosity of the stationary phase would decline and result in higher retention [16].

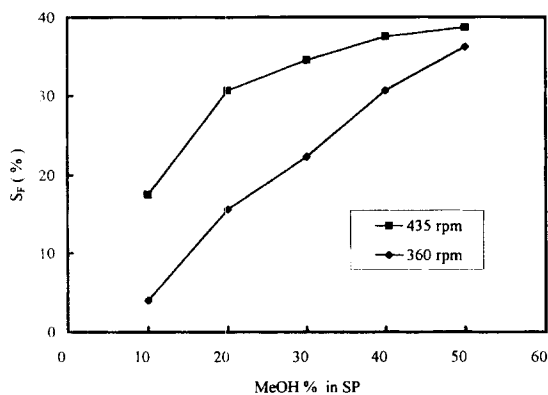


Fig. 4. Phase retention ratio measured in aqueous stationary phases with various contents of methanol in the stationary phase at two different rotation speeds at 50°C and 13.1 MPa. ■ = 435 rpm; ◆ = 360 rpm.

The phase retention ratio of our CCC system was considered acceptable compared with conventional systems [6,7]. A sample solution of a mixture of acetophenone (AP) and benzophenone (BP) in methanol was prepared and chromatographed using this system. The concentrations of both compounds were 1000 ppm and the volume of the injection loop was 100 μ l. The results are illustrated in Fig. 5. Both runs were performed at 435 rpm, 50°C and 13.1 MPa. The stationary phase composition was methanol–water (30:70) and methanol–water (40:60) in Fig. 5a and b, respectively. The monitoring wavelength was set at 247 nm. The high-pressure pump was first operated in the constant-flow mode. However, the pressure fluctuations under these conditions led to considerable baseline noise. The constant-pressure mode was therefore applied for all subsequent chromatographic runs. The flow-rate readings oscillated around ca. 0.2

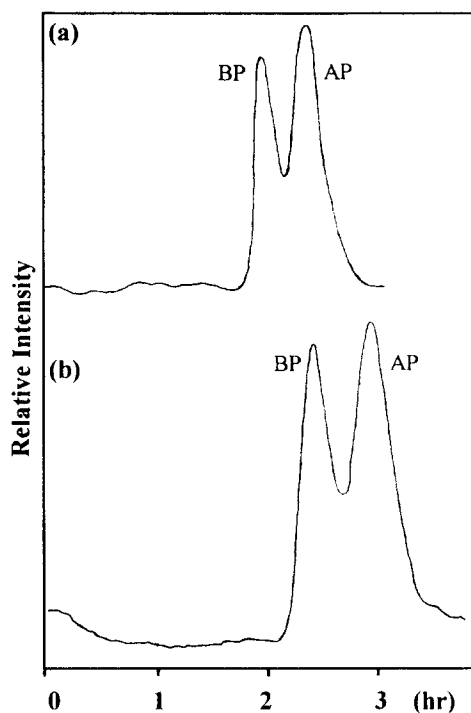


Fig. 5. Chromatograms of a mixture of acetophenone (AP) (1000 ppm) and benzophenone (BP) (1000 ppm) in methanol with a 100- μ l injection at 435 rpm, 13.1 MPa and 50°C. Stationary phase: (a) methanol–water (30:70); (b) methanol–water (40:60).

ml/min. The chromatogram of the stationary solvent with a higher content of methanol showed a longer retention time. Since the flow-rate could not be measured accurately, the longer retention time may not represent a higher retention volume. Otherwise, the higher solubility of both compounds in the stationary phase with a higher content of methanol (Fig. 5b) should result in a longer retention, as seen in the figure. Collected fractions (every 10 min, in methanol) were investigated using the off-line UV-Vis spectrophotometer to confirm the composition of the peaks.

As indicated earlier, a phase separator was introduced to protect the detector from aqueous droplets carried by SF₆ CO₂ that created enough noise at the UV-Vis detector to bury the signal. Inevitably it increased the dead volume of the system. The total volume of the separator determined was about 0.5 ml, only a small proportion of the total mobile phase volume in the separation tubing. A test run was carried out by directly placing the separator between the sample injector and the detector without passing the separation tubing. The eluted peak demonstrated some tailing with a width about 5 min at the peak half-height. It made a considerable contribution to the band broadening of real separation signals. This could result from the space in the separator that was not entirely swept by the mobile phase. Improvements are apparently needed to stabilize the system and eventually eliminate the phase separator in order to reduce the band broadening.

In conclusion, we have demonstrated the possibility of applying a supercritical fluid as the mobile phase in counter-current chromatography. Several experimental parameters have been investigated to monitor the phase retention ratio. Currently this system is under more comprehensive study in order to enhance the separation efficiency.

Acknowledgements

The authors thank Mr. Tung-Yu Chen for this efforts in the earlier stages of this work and Mr.

Shin-Shyang Liang (Precision Instrument Development Centre, National Science Council, Republic of China) for assistance in making the rotating assembly. Financial support (grant no. NSC 82-0421-M-009-072-Z) from the National Science Council of the Republic of China is also gratefully acknowledged.

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