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INVESTIGATION OF POLAR MODIFIERS IN CARBON DIOXIDE MOBILE PHASES FOR CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The effect of low concentrations of polar modifiers on the selectivity of a non-polar carbon dioxide mobile phase in capillary supercritical fluid chromatography (SFC) was determined. The selectivity of mixtures of carbon dioxide–water (approximately 0.3–0.9 mole%) and carbon dioxide–methanol (approximately 0.8 mole%) were compared to the selectivity of pure carbon dioxide using polarity-test mixtures. Under the conditions of this study, water modifiers did not significantly affect selectivity or retention. Slight changes in selectivity and decreased retention, however, were observed for the methanol modifier. These data contrast with those obtained for packed-column SFC in which similar concentrations of modifiers produced significant changes in selectivity and greatly reduced retention, and are consistent with results indicating these previous results were due to modification of the stationary phase rather than changes in fluid phase solubilities.

INTRODUCTION

Capillary supercritical fluid chromatography (SFC) is becoming recognized as a viable analytical methodology. Supercritical fluid mobile phases offer advantages over conventional gas and liquid phases in that their densities approach those of liquids, yet solute diffusivities are up to two orders of magnitude greater and viscosities significantly less than the liquid[1]. The lower viscosities and higher diffusion coefficients result in enhanced chromatographic efficiency and shorter analysis times compared to liquid chromatography. The liquid-like densities give favorable mass transfer properties and allow the separation of less volatile materials than is possible by gas chromatography. In addition, the solvating power of a supercritical fluid is dependent on density and can be controlled as a function of pressure. The advantages of capillary columns in SFC include the low pressure drop which allows pressure programming techniques to be effectively used[2], the ability to produce highly deactivated column surfaces and inert stationary phases[3,4], and the compatibility of flow-rates with gas phase detectors (*e.g.*, flame ionization and mass spectrometric)[5-7].

Pressure programming in SFC is somewhat analogous to temperature programming in gas chromatography or gradient elution in liquid chromatography. The low pressure drop also allows long column lengths to be used to achieve large numbers of theoretical plates.

In addition to the variable solvating power achieved by controlling the pressure (or density) of a supercritical fluid, selectivity of the mobile phase can also be obtained by altering the chemical properties of the fluid. This is accomplished by using either chemically different fluid systems or by low concentrations of fluid modifiers[8,9]. Usually, low concentrations of a polar or chemically active modifier are added to a less polar and chemically inert fluid system (e.g., methanol to carbon dioxide). The use of a more polar fluid system and/or a polar modified fluid system also increases the maximum solvent power of the mobile phase and provides the potential for elution of more polar and less soluble analytes. The use of modifiers in packed-column SFC has been more extensive than in capillary SFC and has demonstrated that very low concentrations of an appropriate modifier (< 1%) can drastically change retention and selectivity[8-11]. Such effects have been accounted for by increased solubility of the solute in the mobile phase and/or modification of the stationary phase or silica surface. However, recent chromatographic evidence[9,11] suggests that deactivation of active column sites by the polar modifiers may be the dominant mechanism.

In this study the influence of low concentrations of water and methanol modifiers on a supercritical carbon dioxide mobile phase in capillary supercritical fluid chromatography was investigated. Polarity-test mixtures containing alkanes and hydroxy-substituted components (to provide both non-specific and specific mobile phase-solute interactions) and a relatively non-selective, 5% phenyl polymethylphenylsiloxane (SE-54) stationary phase were used to monitor mobile phase selectivity effects. Since it is possible essentially to eliminate residual silanol groups on fused-silica capillary column surfaces and significant modification of the polysiloxane stationary phase is unlikely, any changes in selectivity should be accounted for by changes in the mobile phase polarity. The results of this study complement data obtained recently on the solvating power of supercritical fluids and fluid mixtures[12,13] and support the conclusion that modifier effects in previous packed column studies were due to modification of the stationary phase.

EXPERIMENTAL

The primary polarity-test mixture consisted of the following compounds (Chem Service, West Chester, PA, U.S.A.) at approximately 1 mg/ml concentration in methylene chloride: n-decane, phenol, 1-octanol, n-dodecane, 1-decanol and n-tetradecane. A second polarity-test mixture containing the following heavier molecular weight probes was also used: n-triacontane (C_{30}), diacetoxyscirpenol (DAS), deoxynivalenol (DON) and n-dotriacontane (C_{32}).

The instrumentation used for capillary SFC is similar to that previously described[14,15]. The apparatus utilized a modified Varian 8500 syringe pump to generate a high-pressure and pulse-free flow of mobile phase and a Hewlett-Packard 5700 gas chromatograph to provide constant temperature conditions and flame ionization detection. Constant-pressure operation was controlled and maintained to within 0.2 atm with a microcomputer. Sample introduction was accomplished using a Valco 0.2-

μl C14W HPLC injection valve. The injection valve was connected to the chromatographic column through a splitter device which was adjusted to give approximately a 1:20 flow into the chromatographic column. The chromatographic column was prepared from a length of 12 m × 50 μm I.D. fused-silica tubing (Spectran Corp., Sturbridge, MA, U.S.A.) and coated with a 0.20-μm film-thickness of 5% phenyl polymethylphenylsiloxane stationary phase (SE-54) that was rendered non-extractable by extensive cross-linking with azo-*tert.*-butane[16]. Prior to detection the supercritical mobile phase was decompressed and the linear velocity controlled to approximately 1.5 cm/s by connecting the end of the chromatographic column to a short length (*ca.* 5 cm) of 5 μm I.D. fused-silica restrictor tubing.

Binary fluid mixtures of carbon dioxide–water were produced by equilibrating the carbon dioxide with water in a gas–liquid equilibrium cell placed in-line immediately prior to the injection port. The transfer line and injection port were then maintained at the same temperature as the equilibrium cell to assure a homogeneous fluid mixture. Under a defined set of pressure and temperature conditions the solubility of water in carbon dioxide is accurately known[17-19] (typically ranges between 0.3 and 1.4 mole%) and provides a convenient method of generating a binary mixture. When pure carbon dioxide was used for the mobile phase the fluid was carefully dried prior to loading in the syringe pump by distilling it through activated silica gel. As a further safeguard to ensure that absolutely dry carbon dioxide was delivered to the chromatographic column, an additional drying column, also packed with silica gel, was placed between the syringe pump and injection port. Care was taken to ensure that the pressure drop across this column was negligible. Carbon dioxide–methanol mixtures were prepared by preloading the syringe pump with the proper volume of methanol and then filling the remaining volume with carbon dioxide and allowing the mixture to equilibrate. Isothermal and isobaric chromatographic operating conditions were chosen to provide capacity ratios (k') between 0.5 and 5 and to provide the appropriate equilibrium conditions to generate the carbon dioxide–water mixtures. The void or hold-up times (t_0) used in the k' calculations were obtained from the elution time of the leading edge of the solvent peak which was verified to be a good approximation for the present chromatographic conditions.

RESULTS AND DISCUSSION

The use of fluid modifiers in SFC has tremendous potential for generating highly selective and specific mobile phases. However, it is necessary to understand the modifier interaction mechanism(s) to take full advantage of this potential. By using a capillary chromatographic column with an inert and well-defined surface and stationary phase, the influence of modifiers on the mobile phase can be probed more directly. Two polarity mixtures were used to monitor retention (k') as a function of fluid system. Each mixture contained components that would be expected to exhibit both specific interactions (hydroxy compounds) and non-specific interactions (alkanes) with the polar modifiers. One mixture contained lower-molecular-weight·materials that eluted at lower pressures (reduced pressures of 1.1–1.5) and the other mixture contained heavier molecular weight materials that eluted at higher pressures (reduced pressures > 2). DAS and DON are trichothecene mycotoxins (sesquiterpenoids) of molecular weights 366 and 296 daltons, respectively, and contain free
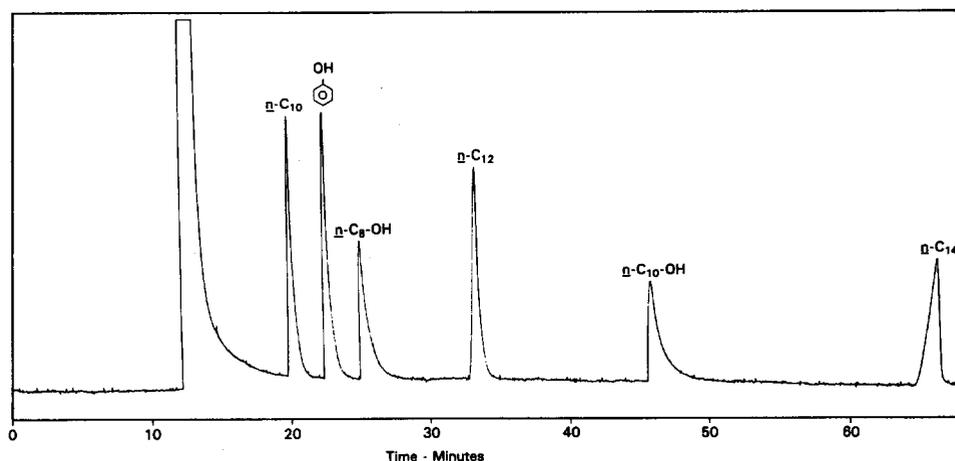
Fig. 1. Typical capillary supercritical fluid chromatogram of a polarity-test mixture. Chromatographic conditions: 12 m × 50 μm fused-silica column coated with 0.20-μm film of cross-linked 5% phenyl polymethylphenylsiloxane (SE-54). Supercritical carbon dioxide mobile phase at 60°C and 80 atm.

hydroxyl and keto groups[20]. A typical supercritical fluid chromatogram of the lower-molecular-weight polarity mixture is shown in Fig. 1. This separation was obtained at 60°C and 80 atm with pure carbon dioxide as the mobile phase. Although the more polar components exhibited slight peak tailing, the column deactivation was acceptable and allowed reproducible elution of these components.

Data comparing the retention (k') for a pure carbon dioxide fluid and a water-modified carbon dioxide fluid system are listed in Table I. Identical chromatographic conditions were employed and at least five replicate runs were made for each fluid system to evaluate reproducibility. To generate the water-modified fluid system the equilibrium cell was heated to 50°C, which at 84 atm produced a fluid mixture of approximately 0.35 mole % water[18]. The chromatographic operating temperature was maintained at 60°C to prevent the possibility of any water condensing from the fluid mixture. Within the limits of the standard deviations of each capacity ratio measurement, the pure carbon dioxide and the water-modified carbon dioxide displayed identical retention for the components in the polarity mixture. This is the expected behavior for the non-polar alkanes since the low modifier concentration

TABLE I

CAPACITY RATIO DATA USING WATER-MODIFIED CARBON DIOXIDE

Chromatographic conditions: 60°C and 84 atm.

Fluid system	k'					
	$n\text{-}C_{10}$	Phenol	$n\text{-}C_8\text{-}OH$	$n\text{-}C_{12}$	$n\text{-}C_{10}\text{-}OH$	$n\text{-}C_{14}$
CO_2	0.49 ± 0.00	0.70 ± 0.01	0.79 ± 0.01	1.26 ± 0.01	1.95 ± 0.02	3.03 ± 0.02
$CO_2\text{-}H_2O^*$	0.49 ± 0.01	0.70 ± 0.01	0.79 ± 0.01	1.26 ± 0.01	1.98 ± 0.04	3.05 ± 0.05

* Equilibrium cell at 50°C; approximately 0.35 mole% water mixture.

TABLE II

CAPACITY RATIO DATA USING METHANOL-MODIFIED CARBON DIOXIDE

Chromatographic conditions: 100°C and 78 atm.

Fluid system	k'					
	$n\text{-}C_{10}$	Phenol	$n\text{-}C_8\text{-}OH$	$n\text{-}C_{12}$	$n\text{-}C_{10}\text{-}OH$	$n\text{-}C_{14}$
CO_2	0.41 ± 0.01	0.52 ± 0.01	0.66 ± 0.01	1.09 ± 0.01	1.68 ± 0.02	2.72 ± 0.03
CO_2– $CH_3OH^\star$	0.42 ± 0.01	0.54 ± 0.01	0.67 ± 0.01	1.08 ± 0.01	1.70 ± 0.02	2.67 ± 0.03

    $\star$ 0.77 mole% methanol mixture.

would not appreciably change the fluid density and competition for active sites is not important. However, it is significant that the retention of the more polar alcohols was not affected.

    This is consistent if the modifier only affects the solvent power of the fluid, since its low concentration would not significantly change the fluid solvating properties. It is also unlikely that the hydrophobic stationary phase was modified by the water. The small change in fluid properties is reasonable if the Hildebrand solubility parameter[21] concept is used to describe the solvent power of the fluid mixture. Recent studies in this laboratory have shown that solubilities change only slightly with the addition of a fluid modifier, which is consistent with the small changes in solvating powers noted from solvatochromic studies of supercritical fluids[12,13].

    Data for the same polarity mixture comparing the retention in pure carbon dioxide and in a 0.77 mole% methanol-modified carbon dioxide mixture (Table II) also support these conclusions. Slightly different operating conditions of 78 atm and 100°C were utilized in this comparison. The higher temperature was used to ensure the mixture was above the critical point and in the single-phase equilibrium region[22]. Again, within the limits of the standard deviations of k', no differences in retention existed for the two fluid systems. Although the concentration of the methanol modifier was over twice that of the water modifier, it was insufficient to alter the solvent power of the mobile phase significantly. It is interesting to note that at these conditions of lower pressure and higher temperature, which created a lower density (0.14 versus 0.22 g/ml) with a corresponding decrease in mobile phase solvent power, the test probes eluted with lower k' values. This infers that at higher temperatures where solute volatility increases the nature of the fluid becomes less important in determining retention and a gas chromatographic retention mechanism becomes dominant.

    Consequently, the second test mixture incorporating larger molecules with lower volatilities was also used to evaluate retention. The k' values of these compounds obtained with pure carbon dioxide, water-modified carbon dioxide and methanol-modified carbon dioxide fluid systems are listed in Table III. In all cases at least five replicate separations were obtained to calculate the capacity ratio data. Operating conditions of 100°C and 168 atm were utilized. At the higher chromatographic operating temperature, two water equilibrium cell temperatures of 50°C and 75°C were utilized which at 168 atm generated water mixtures of approximately 0.55 mole% and 0.90 mole%, respectively[18]. These fluid mixtures, however, did not show any

TABLE III

CAPACITY RATIO DATA FOR TRICHOTHECENE POLARITY MIXTURE

Chromatographic conditions: 100°C and 168 atm.

Fluid system	k'			
	$n\text{-}C_{30}$	DAS	DON	$n\text{-}C_{32}$
CO_2	0.49 ± 0.01	0.66 ± 0.02	1.02 ± 0.03	3.64 ± 0.08
$CO_2\text{-}H_2O^*$	0.48 ± 0.00	0.66 ± 0.00	1.04 ± 0.01	3.60 ± 0.03
$CO_2\text{-}H_2O^{**}$	0.47 ± 0.01	0.65 ± 0.01	1.02 ± 0.01	3.58 ± 0.04
$CO_2\text{-}CH_3OH^{***}$	0.45 ± 0.00	0.58 ± 0.01	0.89 ± 0.01	3.25 ± 0.03

* Equilibrium cell at 75°C; approximately 0.90 mole% water mixture.
** Equilibrium cell at 50°C; approximately 0.55 mole% water mixture.
*** 0.77 mole% methanol mixture.

significant retention differences compared to the pure carbon dioxide fluid system. But, with the methanol-modified fluid system significant changes in retention were apparent. Retention decreases of 8–13% for the methanol-modified fluid system occurred, with the more polar DAS and DON showing the greatest decreases. However, these changes were small compared to retention changes (factors of 2–5) observed on packed columns with similar methanol concentrations[9,10]. The small decreases in retention observed with the capillary columns supports the idea that the modifier is changing the solvent power of the mobile phase, with increased selectivity for the more polar compounds. It also supports the conclusion that the drastic changes in retention observed for packed columns are a result of surface and stationary phase modification.

ACKNOWLEDGEMENTS

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