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STUDY OF RETENTION PROCESSES IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY WITH BINARY FLUID MOBILE PHASES

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SUMMARY

The ability to alter retention and selectivity through organic modifiers in capillary supercritical fluid chromatography (SFC) has been studied. The role of methanol, acetonitrile, and 2-propanol solvent modifiers on retention with supercritical carbon dioxide was investigated for selected solutes. The retention of these probe molecules was seen to decrease with increasing mole fraction of the solvent modifier in the fluid phase. Selectivity of the column for polar analytes was shown to change while selectivity remained constant for non-polar probe molecules. Binary fluids in capillary SFC appear promising for extending the range of amenable separations, as with packed column SFC, but also retain advantages related to the ease of density programming.

INTRODUCTION

The study of the effect of solvent modifiers upon retention in supercritical fluid chromatography (SFC) has gained impetus in the past few years due to the need for more polar fluids at relatively mild temperatures¹⁻¹¹. The ability to modify the solute-fluid mobile phase intermolecular interactions, while maintaining the critical conditions for the binary solutions comparable to those of the pure fluid, can be very important in the separation of thermally labile polar compounds. Chemical tailored binary or ternary mobile phases have an important advantage in the separation of complex sample mixtures by producing increased selectivity and decreased overall separation time. Retention in SFC is governed by a combination of fluid density, composition of the mobile phase, stationary phase type, and temperature¹². The addition of a solvent modifier to the supercritical fluid can produce enhanced selectivity, altered retention, and improved peak shape¹⁻¹¹. Polar solvent modifiers have been used in the separation of polystyrenes³⁻⁶, metal chelates¹⁰ and compounds of varying polarity^{1,2,9,11}. Most studies to date have involved packed columns using either normal-phase or reversed-phase chemically modified silica^{1,3-11}. However, recently Wright *et al.*² have reported limited data for capillary columns using low concentration solutions of methanol and water as polar modifiers in carbon dioxide.

In this paper we have studied the effects on retention of polar modifiers in carbon dioxide with capillary SFC. The range of solvent modifier concentration studied is larger than reported earlier², resulting in a correspondingly greater effect on solute retention and selectivity. The results support the phase partitioning mechanism and show that for the less active capillary column stationary phases the major changes due to solvent modifiers may be ascribed to the altered solvating power of the mobile phase.

EXPERIMENTAL

The polar solvent modifiers methanol, acetonitrile and 2-propanol mixed in carbon dioxide at a mole fraction (χ) of 0.20 were obtained from Scott Specialty Gases. Varying mixtures of 2-propanol in carbon dioxide were also made by weighing a known amount of 2-propanol into a lecture bottle which contained a PTFE coated stir bar and then filling the lecture bottle with carbon dioxide. By weighing the lecture bottle before and after addition of carbon dioxide and 2-propanol the mole fraction could be accurately calculated. A high-pressure syringe pump (Varian 8500) was loaded directly from the compressed gas cylinder and was used to maintain pressure and establish flow through the capillary column. The column used in this study was a 100- μm I.D., fused-silica capillary coated with cross-linked 50% phenylmethylpolysiloxane (OV-17) stationary phase. A small section of the polyimide coating of the column was removed and was used as the UV absorbance cell with a ISCO V⁴ variable-wavelength detector. This on-column detection scheme eliminated extra-column contributions to band broadening. Further details on the experimental system has been reported by Wright and co-workers^{13,14}. The column was conditioned for *ca.* 8 h after a change in solvent modifier type or concentration to ensure complete column equilibration. The selected analytes were myristophenone, phenanthrene, decylbenzene, phenanthridine and perinaphthenone. The analytes were chosen for their polar functionalities and for comparison with non-polar solutes. The analytes were dissolved in methylene chloride, which was used as the void volume marker for capacity factor determinations. The capacity factors (k') were calculated from the retention time of the analyte (t_R) and the retention time of the void volume peak (t_0) using $k' = (t_R - t_0)/t_0$.

RESULTS AND DISCUSSION

Binary supercritical fluids

Potential binary fluid mixtures for SFC must be carefully considered to ensure they remain as a single phase at the temperature and pressures used during the separation. For the simplest class of Type 1 binary fluid systems¹⁵, an increase in the mole fraction of a polar modifier generally increases the critical pressure and critical temperature of the binary system. This presents the possibility of inadvertently operating in the two-phase vapor-liquid region, where either the lower density vapor or denser liquid phase is used as the mobile phase. The minimum temperature and pressure needed to remain in a supercritical fluid region can be estimated using phase equilibria calculated using various numerical techniques. These numerical methods can be used to extrapolate from limited experimental data or to approximate the

critical loci for a binary system when experimental data is non-existent. Crowther and Henion¹⁶ have discussed methods they have applied for determination of the critical loci for the methanol-carbon dioxide system.

The method we prefer in estimating the critical loci of binary fluids was outlined by Prausnitz and co-workers^{17,18}. For methanol-carbon dioxide the critical loci as a function of pressure and temperature estimated by the Prausnitz method, is shown in Fig. 1. Fig. 1 also gives the data of Brunner¹⁹ and McHugh and Seckner²⁰ for comparison. A characteristic of such Type 1 systems is the continuous mixture critical points between the critical points of the pure fluids^{15,19}. The fit between the estimation of the critical loci and the experimental data is quite reasonable. Calculated critical pressures and critical temperatures for binary mixtures of acetonitrile and 2-propanol in carbon dioxide are shown in Fig. 2. For these calculations we have assumed that carbon dioxide-acetonitrile and carbon dioxide-2-propanol are Type 1 binary fluid systems. In this study experimental conditions were chosen to assure that supercritical conditions were maintained for all binary mixtures.

Modifier effects

The modifiers used in this study were chosen for their hydrogen-bonding capability (methanol, 2-propanol) and their range of solubility parameters (polarity) 2-propanol < acetonitrile < methanol²¹. This combination allowed the study of specific and non-specific interactions between the fluid and the probe molecules.

The capacity factors of the five probe molecules in pure carbon dioxide with OV-17 are shown in Table I. The retention times for phenanthridine and perinaphthenone with pure carbon dioxide were so large that they precluded the accurate determination of the capacity factors. The addition of polar solvent modifier to the carbon dioxide mobile phase not only decreased solute retention by an appreciable amount, but also caused a reversal in the elution order of myristophenone and phenanthrene. This is due to the greater hydrogen-bonding interaction with myristophenone than phenanthrene. The polar modifiers are good solvents for all the probe molecules and their introduction into the fluid mobile phase produces the large decrease

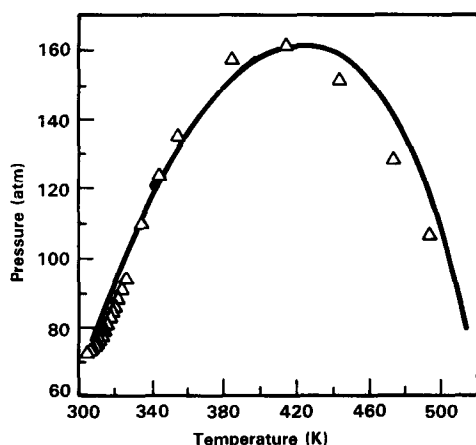


Fig. 1. Critical loci (—) for methanol-carbon dioxide calculated by the Prausnitz method^{17,18}; (Δ) Brunner data and (\bullet) McHugh and Seckner data.

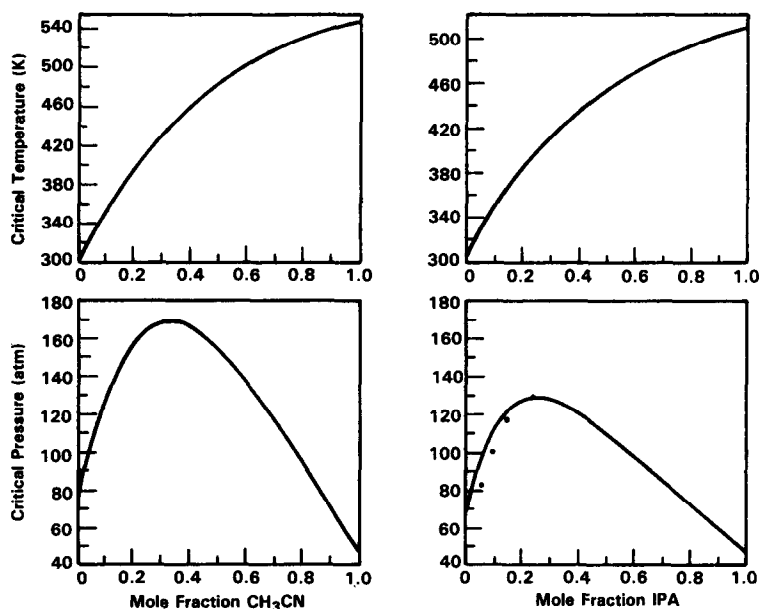


Fig. 2. Calculated critical temperature and pressure for binary mixtures of acetonitrile (CH_3CN) and 2-propanol (IPA) in carbon dioxide as a function of mole fraction^{17,18}. ● represents critical pressure data for IPA estimated from the vapor-liquid equilibrium data of Radosz³⁰.

in retention seen in Table I. Furthermore, as seen in Table I, relative k' values with solvent modifiers are in the order: methanol > acetonitrile > 2-propanol. Since the mole fraction of the solvent modifier was held constant in these studies, one can compare the solvent strengths of the various binary supercritical fluids. Therefore, 2-propanol-carbon dioxide seems to be the strongest solvent for these analytes.

The retention reversal seen between myristophenone and phenanthrene illustrates the benefits in chemically tailored mobile phases for increased selectivity with binary or ternary mixed solvents for SFC. Capillary columns also have an advantage compared to micro-particle packed columns for altering solvent strength, due to the

TABLE I

EFFECT OF POLAR MODIFIER ON THE CAPACITY FACTOR (k') FOR OV-17 AT 120°C AND 168 atm

All solvent modifiers were present at a mole fraction (χ) of 0.20. All values are mean \pm S.D.

Solute	k'			
	Carbon dioxide	Carbon dioxide-methanol	Carbon dioxide-acetonitrile	Carbon dioxide-2-propanol
Decylbenzene	0.98 \pm 0.04	0.13 \pm 0.02	0.09 \pm 0.01	0.05 \pm 0.01
Phenanthrene	4.89 \pm 0.20	0.57 \pm 0.02	0.32 \pm 0.01	0.25 \pm 0.01
Myristophenone	5.38 \pm 0.47	0.21 \pm 0.02	0.15 \pm 0.01	0.13 \pm 0.01
Phenanthridine	> 10	0.44 \pm 0.03	0.33 \pm 0.02	0.26 \pm 0.01
Perinaphthenone	> 10	0.51 \pm 0.01	0.35 \pm 0.01	0.29 \pm 0.01

increased permeability and the resulting ease of density programming²². Therefore SFC with capillary columns allows the combination of programming density and solvent modifier concentration concurrently to enhance the selectivity and efficiency of complex mixture separations^{23,24}.

As the mole fraction (χ) of solvent modifier increases, the fluid mobile phase becomes a stronger solvent for specific solute molecules resulting in decreased retention. The mole fraction of 2-propanol in carbon dioxide was varied over the range $\chi = 0.0-0.20$ and the retention data for the probe molecules are shown in Fig. 3. At low concentrations of 2-propanol in carbon dioxide, a retention reversal of myristophenone and phenanthrene was observed as compared to pure carbon dioxide as the mobile phase. This mole fraction value corresponds to *ca.* 2.1% (w/w) of 2-propanol in carbon dioxide. The weight percent of modifier required to produce a significant change in solute retention for capillary SFC is larger than that reported for packed column studies¹¹. This could be due to the more effective solvation of the bonded phase by the solvent modifier for the packed columns than for the cross-linked polymer capillary column coating or the elimination of active sites by the polar modifier. Enhanced solvation of bonded phase column material by organic solvent modifiers is well established in liquid chromatography²⁵⁻²⁸.

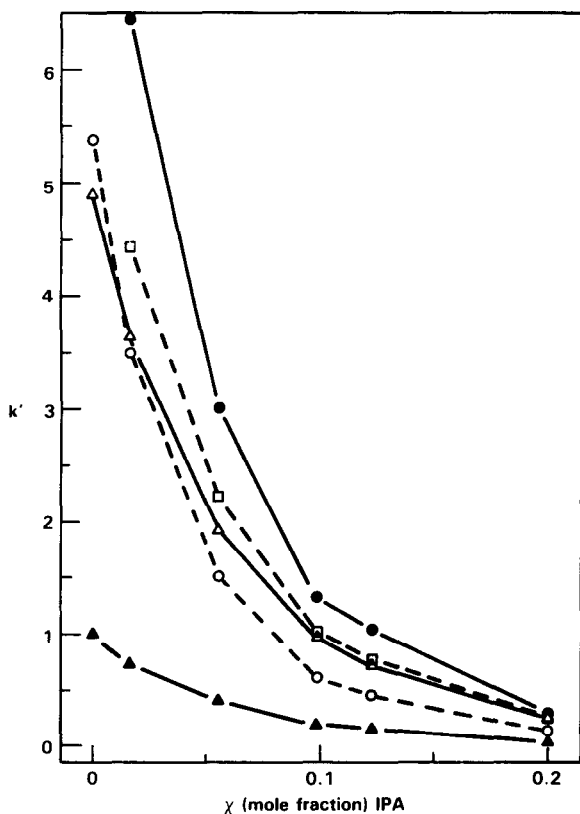


Fig. 3. Capacity factors (k') for (●) perinaphthenone, (□) phenanthridine, (▲) decylbenzene, (△) phenanthrene and (○) myristophenone versus mole fraction of 2-propanol (IPA) in carbon dioxide. Experimental conditions: 120°C, 168 atm, OV-17.

The selectivity of the separation for the probe molecules relative to decylbenzene is plotted in Fig. 4. The selectivity (α) of the separation process decreases with increasing concentration of 2-propanol in the fluid mobile phase under the experimental conditions. This could be explained by increased solvent strength of the binary fluid phase as the concentration of 2-propanol is increased and possible solvation by 2-propanol of the cross-linked phenylmethylsiloxane polymer phase. It is of interest to note that the addition of 2-propanol in the fluid mobile phase has the largest effect on selectivity for the polar analytes perinaphthenone, phenanthridine and myristophenone. This is understandable in terms of the greater intermolecular interactions anticipated with 2-propanol. In contrast, the relative retention (α) of phenanthrene to decylbenzene appeared independent of 2-propanol concentration in carbon dioxide. Fig. 4, once again shows the ability of chemically tailoring the binary fluid mobile phase for the specific retention and selectivity needed in a separation process.

Bilie and Greibrokk¹ have studied the effect of solvent modifier on peak shape for elution from packed columns and concluded that peak shape improved on the addition of polar modifiers. The postulated reason being the coverage of surface active sites on the silica by the polar modifiers. We also compared peak shape as a

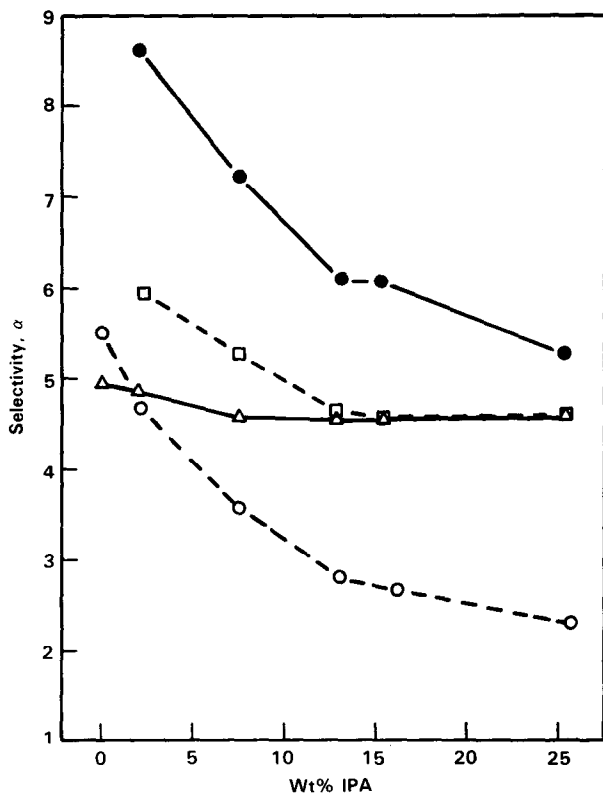


Fig. 4. Selectivity (α) as compared to decylbenzene for (●) perinaphthenone, (□) phenanthridine, (○) myristophenone and (△) phenanthrene versus weight percentage (Wt%) 2-propanol (IPA) in carbon dioxide. Experimental conditions: 120°C, 168 atm, OV-17.

function of modifier concentration in carbon dioxide and found qualitative results similar to those reported by Blilie and Greibrokk¹. As 2-propanol was added to the fluid mobile phase the peak tailing was seen to decrease. This is most probably caused by the coverage of residual active sites in the polymer phase by the 2-propanol.

CONCLUSION

Solvent modifiers can be used to modify the retention and selectivity of separations in capillary SFC. We have noted retention reversals and selectivity changes with both the type and mole fraction of solvent modifier. Similar effects have been reported with packed columns used for SFC separations^{1,11}. The advantage of capillary columns over packed columns arises from the differences in permeability. Comparison of typical 50- μm I.D. capillary columns and 5- μm microparticle packed columns shows that the pressure drop per plate is *ca.* 10^4 greater for packed columns at their optimum linear velocities²⁹. Pressure ramps are much easier to use in capillary columns to modify the solvent strength (density) as compared to packed columns²⁹. Therefore, it should be entirely feasible in capillary SFC to combine the benefit of solvent density (pressure) programming with simultaneous modification of the solvent strength, by dynamically changing the modifier solvent concentration^{23,24}. This type of separation scheme should be useful for complex sample mixtures when simple density ramps do not afford sufficient selectivity or when retention is prohibitively long.

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