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CORRELATION OF SOLVATOCHROMIC EFFECTS WITH RETENTION IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

A study of mobile-phase intermolecular interactions is reported in which the solvatochromic technique is used for comparison with retention processes in supercritical fluid chromatography (SFC). The change in the solvation character of the cybotactic region of the selected test solutes was observed using solvatochromic probes and found to be dependent on the density of the solvent. The major solute-solvent interactions with carbon dioxide are expected to be from dipole-induced dipole interactions. The change in solvation with density was compared with solute retention under similar pressure and temperature conditions for capillary SFC.

INTRODUCTION

Retention in supercritical fluid chromatography (SFC) is dependent upon temperature, pressure, density, and solute concentration. Solute retention as a function of pressure or density at constant temperature has been described by Van Wasen and Schneider¹. Retention was shown to be dependent on the partial molar volume of the solute in the mobile and stationary phases and on the isothermal compressibility of the fluid solvent. Yonker *et al.*² have described the dependence of solute retention on temperature at constant pressure and have demonstrated a complex dependence on the partial molar enthalpy of transfer between phases, partial molar heat capacity and volume expansivity of the fluid. This complex dependence of solute retention on pressure or temperature can often obscure the mechanism of solute retention in SFC.

Studying mobile phase interactions between the solute and the solvent could lead to a better understanding of the fundamental intermolecular interactions occurring during the retention process. For supercritical fluids, one must study the effect of density as a function of temperature or pressure on solvation of the solute, independent of the stationary phase, to understand the role of the mobile phase during retention. There are many ways of probing the solute-solvent interactions in the cybotactic region for liquids (*i.e.*, the region of solvent molecules around the solute where the structural order of the solvent molecules has been influenced by the solute). These methods are dependent on the spectral shift in absorption maximum for selected chromophore molecules in the UV, visible, IR, electron spin resonance

(ESR) or nuclear magnetic resonance (NMR) spectra, a change in reaction rate, equilibrium constant, or fluorescence lifetime³.

The pioneering work of Kamlet and co-workers⁴⁻⁹, relating the spectroscopic shifts of the $\pi \rightarrow \pi^*$ transition of selected probe molecules in a wide range of solvents to their π^* polarity/polarizability solvent scale, allows one to study the effects of the solvent molecule on the cybotactic region of solutes. Some π^* values have been reported for carbon dioxide in the subcritical and supercritical region for limited densities^{10,11}. Yonker *et al.*¹², have reported spectral shifts and π^* values for carbon dioxide, nitrous oxide, ammonia and Freon-13 in the supercritical region over a wide range of densities (or pressures). They have also reported preliminary investigations on the effect of binary modifiers on the cybotactic region of the solute molecule¹², Carr and co-workers^{13,14} have applied the solvatochromic comparison method to the investigation of polarizable polymers used as gas-liquid chromatography stationary phases and to retention processes in reversed-phase high-performance liquid chromatography (HPLC).

In this article, we report on the use of the solvatochromic compression method to study the effect of density on the cybotactic region of select molecules in supercritical carbon dioxide. The solvatochromic observations are then compared to chromatographic retention for the same test molecules under similar pressure and temperature conditions for capillary SFC.

EXPERIMENTAL

A Model 8500 high-pressure syringe pump (Varian, Palo Alto, CA, U.S.A.) was used to maintain pressure and establish flow through the capillary column. The pump was operated under computer control which provided an accurate, pulse-free flow and control of fluid pressure. The temperature of the column was controlled with a constant-temperature air-bath oven within $\pm 0.3^\circ\text{C}$ (Model 5700A gas chromatograph, Hewlett Packard, Palo Alto, CA, U.S.A.). Solute samples were injected with a Valco C14W HPLC injection valve (0.2- μl rotor volume, Valco, Houston, TX, U.S.A.), which was mounted outside the oven and connected to the chromatographic column through a flow splitter. The split ratio was approximately 1:80. A flow restrictor, connected to the end of the column, controlled the linear velocity of the mobile phase and allowed decompression of the supercritical fluid before detection. Detection of the solutes was accomplished with a flame ionization detector. The open-tubular capillary column was coated and cross-linked with a phenyl-methyl dimethylpolysiloxane (5:95) stationary phase (SE-54, Alltech Assoc., Deerfield, IL, U.S.A.). Carbon dioxide was SFC-grade (Scott Speciality Gases, Plumsteadville, PA, U.S.A.) and was loaded directly into the pump. The solutes used in this study were 2-nitroanisole and 4-ethylnitrobenzene (Aldrich, Milwaukee, WI, U.S.A.). These solutes were two of the 45 test molecules used by Kamlet *et al.*⁴ in their studies.

Spectroscopic studies of the spectral shift of the test molecules were obtained with a Model 1605 spectrophotometer (Cary, Palo Alto, CA, U.S.A.) in the dual-beam mode. Supercritical fluid spectra at 35, 40, 50 and 60°C for 2-nitroanisole and 50°C for 4-ethylnitrobenzene were based on the use of air as a reference. The high-pressure sample cell was constructed in our laboratory from stainless steel (SS304) with an optical path of 1.7 cm \times 1.0 cm O.D. The overall sample cell dimensions

were 11.5 cm \times 7.0 cm O.D. High-pressure 1/8 in. compression fittings, mounted on the cell, allowed fluid to flow through and purge the cell. Each end of the cell has a seat for a 2.54 cm O.D. \times 1.25 cm thick quartz window. A high-pressure, high-temperature gas-tight seal was formed by compressing a 1.8-cm O.D. PTFE O-ring between the quartz window and the cell body on each end of the cell. The volume of the sample cell constructed in this way was *ca.* 1.3 ml. The absorption cell was wrapped with insulated nichrome wire and further insulated to minimize heat loss. The assembly was placed in the sample cell compartment, and the nichrome element was heated, using a single-mode Series 1400 temperature controller (West, East Greenwich, RI, U.S.A.), which provided temperature regulation to within $\pm 0.5^\circ\text{C}$. The solvent was delivered to the cell by a high-pressure syringe pump (High Pressure Equipment, Erie, PA, U.S.A.) which was connected to a HPLC sample valve (Rheodyne, Cotati, CA, U.S.A.) located between the pump and the sample cell. The valve, containing a 10- μl sample loop, facilitated introduction of the samples into the absorption cell. A Model 204 pressure transducer (Serta Systems, Acton, MA, U.S.A.), monitored the pressure of the entire system (± 10 p.s.i.).

The experimental procedure was as follows: the solute was loaded into the sample valve and flow through the cell was established. The sample loop was then switched into the line, and the absorbance at a predetermined wavelength was monitored to detect the presence of solute in the sample cell. The solute was diluted by introduction of additional fluid to obtain a final concentration of approximately $1.0 \cdot 10^{-4}$ M. Flow through the cell was then stopped, and the absorption spectrum was recorded. Subsequent scans at different pressures and temperatures were performed following appropriate equilibration times. Further details on the chromatographic system have been reported by Wright and co-workers^{15,16} and on the solvatochromic system by Yonker *et al.*¹².

RESULTS AND DISCUSSION

Solvatochromic investigations

The solvatochromic comparison method utilizes a linear solvation energy relationship to correlate solvent effects on a low concentration of a probe solute molecule. The general form of this relationship can be expressed as

$$XYZ = XYZ_0 + a\alpha + b\beta + \text{SPPE} \quad (1)$$

where XYZ, in this case, represents the frequency of the absorption maxima seen in the UV spectrum of the solute molecule, α is a solvent scale related to hydrogen-bond donor acidities (HBD), β is a scaler related to hydrogen-bond acceptor basicities (HBA), and SPPE is a parameter based on the polarity/polarizability effects of solvents⁴. Kamlet and co-workers⁴⁻⁹ have shown that for UV-VIS spectroscopic data the SPPE term in eqn. 1 can be correlated by a single parameter, π^* . This parameter correlates the effect of the solvent on the $p \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transition of the solutes, establishing a π^* scale of solvent polarities. Substituting π^* into eqn. 1 for SPPE results in

$$XYZ = XYZ_0 + a\alpha + b\beta + s\pi^* \quad (2)$$

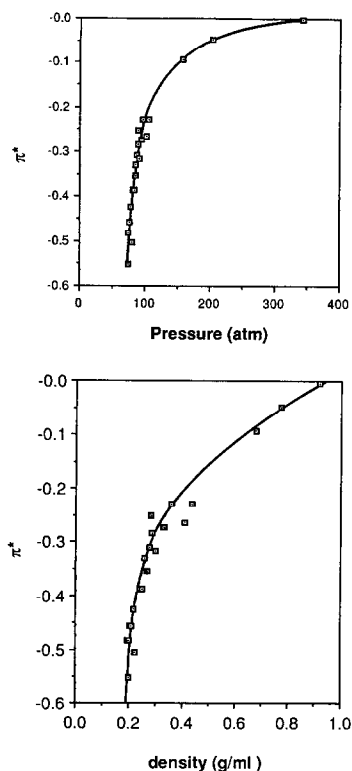


Fig. 1. Plots of π^* versus pressure (atm) and density (g/ml) for 2-nitroanisole at 50°C in carbon dioxide.

where s is the susceptibility of the XYZ of the solutes to changing SPPE.

With supercritical fluids one might expect π^* to be a function of fluid density. Therefore, both pressure and temperature should have an effect on the absorption maximum. The probe molecules chosen for study with carbon dioxide are expected to have minimum hydrogen-bond interaction¹¹, and therefore eqn. 2 can be reduced to

$$v_{\max} = v_0 + s\pi^* \quad (3)$$

where v_{\max} (kK) is the wave number of the solute absorption maximum and v_0 (kK) is the reference wavenumber, determined in a standard solvent (cyclohexane). Eqn. 3 will be used throughout this paper to determine the π^* value of the solvents. The s value for 2-nitroanisole is -2.428 , and for 4-ethylnitrobenzene it is -2.133 (ref. 4). The wavelength regions examined for the two test molecules were 32.47–34.01 kK (3080–2940 Å) for 2-nitroanisole and 37.45–39.22 kK (2670–2550 Å) for 4-ethylnitrobenzene. There was no background adsorption from carbon dioxide seen in these spectral regions. A change in v_{\max} of the solute can thus be interpreted as a change in the polarity/polarizability of the supercritical fluid with density. Therefore, one can investigate the cybotactic region of the solutes and study the solvating properties of neat or mixed-fluid systems as a function of fluid density.

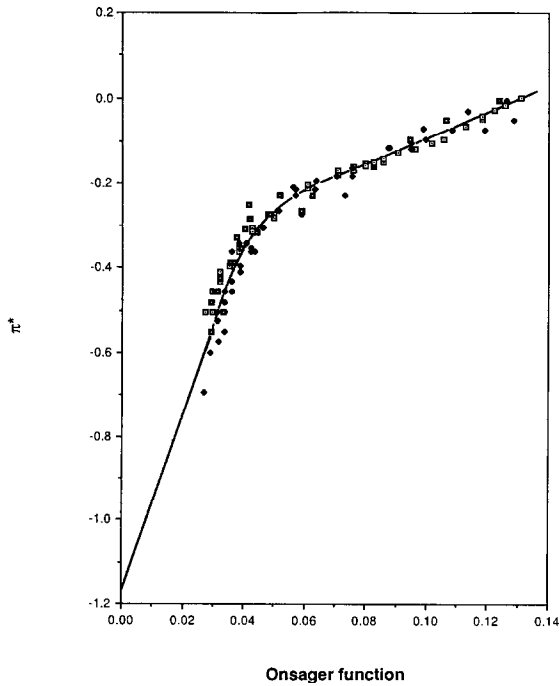


Fig. 2. Plot of π^* versus Onsager reaction field function for 2-nitroanisole at 35°C (\square), 40°C (\blacklozenge) and 50°C (\circ) in carbon dioxide.

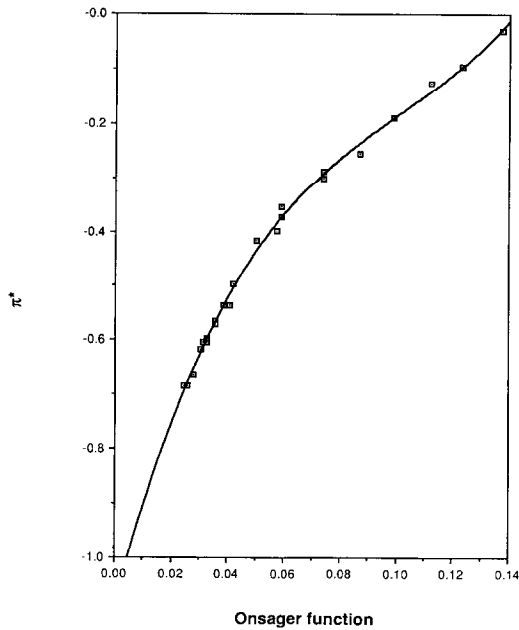


Fig. 3. Plot of π^* versus Onsager reaction field function for 4-ethylnitrobenzene at 50°C in carbon dioxide. The line is added for clarity.

Fig. 1 shows the dependence of π^* on pressure and density of carbon dioxide for 2-nitroanisole at 50°C. The major feature in these plots is the rapid change of π^* with pressure and density at low pressures and densities. There is less change at higher pressures and densities. An explanation for the effect seen in Fig. 1 is that the size of the solvation sphere of carbon dioxide molecules in the cybotactic region of the solute increases as density (pressure) increases. Therefore, the solute molecule experiences a wide range of solvation phenomena between the two limits of the gas phase behavior at low densities and "pseudo-liquid" phase behavior at higher densities. Thus, with a supercritical fluid solvent density (or solvent strength) can be continuously varied as a function of pressure to study the effect of density on the cybotactic region using the solvatochromic comparison method.

The π^* parameter in eqn. 3 contains both polarity/polarizability effects of the solvent. These effects entail dipole-dipole interactions, dipole-induced dipole interactions, and London-type interactions. Carbon dioxide has a zero dipole moment, based on molecular symmetry¹⁷, while the solute molecules have dipole moments of $>4.0 \text{ D}$ ¹⁷. Therefore, the main intermolecular interaction stabilizing the excited state of the electronic transmission of the sample molecule will be dipole-induced dipole interactions between carbon dioxide and the solute. This interaction is related to the polarizability of the carbon dioxide molecule in the presence of the polar solute molecule.

In any coherent explanation of the solvatochromic phenomenon applicable to non-polar (polarizable) liquids, solute transition energies or linear transformations of these (*e.g.* π^*) are expected to show a linear dependence on the Onsager reaction field function. This was demonstrated by Brady and Carr¹⁸ for a homologous series of *n*-alkanes and for polarizable polymeric liquids¹³. The Onsager reaction field function $[L(\eta^2)]$ can be related to the polarizability of the solvent and is dependent on the refractive index of the solvent

$$L(\eta^2) = (\eta^2 - 1)/(2\eta^2 + 1) \quad (4)$$

where η is the refractive index of the solvent. The dependence of the refractive index of carbon dioxide on pressure and temperature has been reported by various workers¹⁹⁻²². One expects a linear relationship between π^* and the Onsager reaction field function between $[L(\eta^2) = 0.0(\eta = 1.00, \text{ gas density})$ and $L(\eta^2) = 0.143(\eta = 1.26, \text{ liquid density})$ for carbon dioxide. Plots of π^* versus $L(\eta^2)$ for 2-nitroanisole in carbon dioxide at 35, 40 and 50°C are shown in Fig. 2. Fig. 2 shows a continuous solvation process exists between the two extremes of pseudo-gas behavior at low densities (small Onsager function values) and pseudo-liquid behavior at high densities with a transition occurring at *ca.* 0.40 g/ml for carbon dioxide at these temperatures. The shape of this curve is dictated by the extent of solvation of 2-nitroanisole, reflecting changes in the polarizability of the cybotactic region around the solute molecule. A similar phenomenon is seen for 4-ethylnitrobenzene at 50°C, as shown in Fig. 3. Therefore, from spectroscopic studies of supercritical fluids, one can learn about the solvent polarity/polarizability effects on a solute molecule in the mobile phase. In this particular case, carbon dioxide mimics a non-polar solvent, in that dipole-induced dipoles are the major intermolecular interactions occurring between the solute and the solvent. The polarizability of the cybotactic region is seen to change

as a function of the density of supercritical carbon dioxide for the selected test molecules. The detailed basis of these phenomena is the subject of continuing studies in our laboratory.

Chromatographic investigations

The study of mobile phase-solute interactions can lead to a better understanding of the retention mechanism for SFC. Relating solute retention (k') to π^* or $L(\eta^2)$ is necessary for the extension of the mobile phase studies to the overall retention process. Brady *et al.*¹³ have shown a linear correlation of the free energy of transfer (ΔG^0) of acetonitrile and nitromethane in polarizable polymeric gas chromatographic stationary phases with their π^* values. From general thermodynamic theory the free energy of solute transfer at equilibrium is,

$$\Delta G^0 = -RT \ln K_{eq} \quad (5)$$

where K_{eq} is the equilibrium distribution coefficient. This distribution coefficient is equal to $k'(V_m/V_s)$, where V_m and V_s are the volumes of the mobile and stationary phases respectively. From eqn. 5 and the relationship for K_{eq} , $\Delta G^0 \propto \ln k'$, one would expect a linear relationship between $\ln k'$ and π^* or $L(\eta^2)$ for a non-polar, polarizable solvent, such as carbon dioxide.

Plots of $\ln k'$ versus $L(\eta^2)$ for 2-nitroanisole at 40, 50 and 60°C and for 4-ethylnitrobenzene at 50°C are shown in Figs. 4 and 5. In both cases, a nearly linear

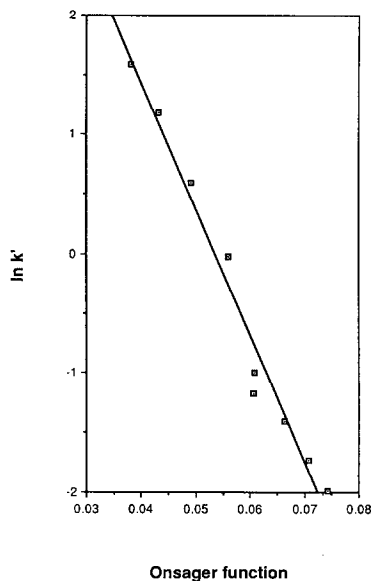
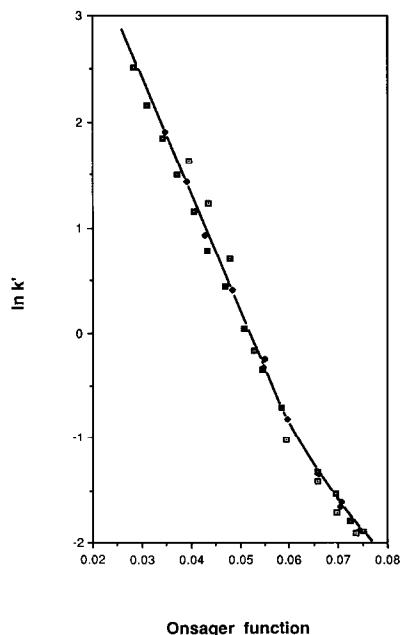


Fig. 4. Plot of $\ln k'$ versus Onsager reaction field function for 2-nitroanisole at 40°C (\square), 50°C (\blacklozenge) and 60°C (\square) in carbon dioxide.

Fig. 5. Plot of $\ln k'$ versus Onsager reaction field function for 4-ethylnitrobenzene at 50°C in carbon dioxide.

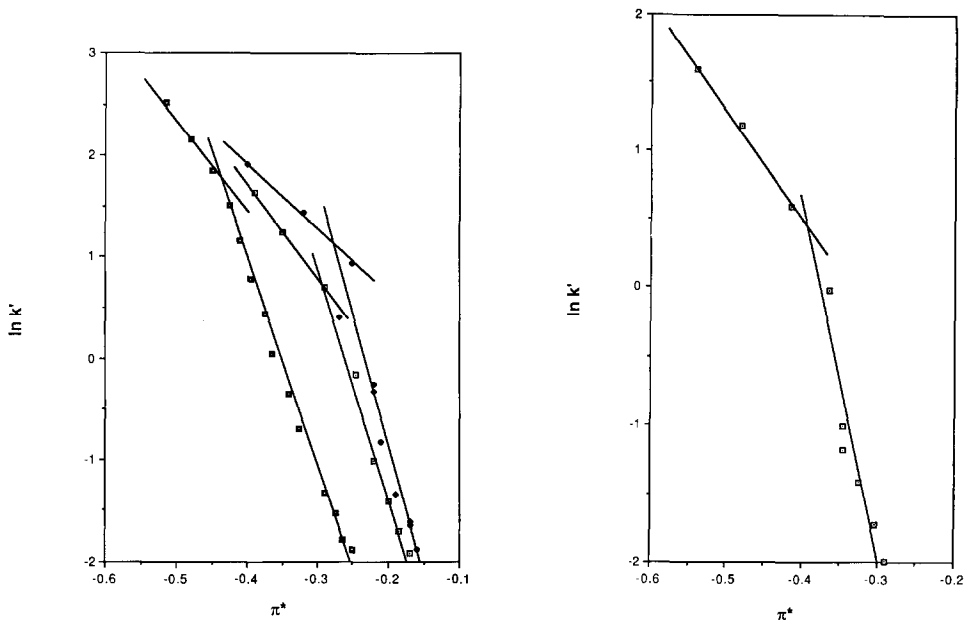


Fig. 6. Plot of $\ln k'$ versus π^* for 2-nitroanisole at 40°C (\square), 50°C (\blacklozenge) and 60°C (\square) in carbon dioxide.

Fig. 7. Plot of $\ln k'$ versus π^* for 4-ethylnitrobenzene at 50°C in carbon dioxide.

relationship between $\ln k'$ and density or $L(\eta^2)$ was observed, consistent with previous observations. One would expect a linear correlation with the optical Onsager reaction field function and carbon dioxide based on polarizability changes for carbon dioxide as the density is increased. Therefore, on the basis of chromatographic results, carbon dioxide behaves as a simple polarizable fluid under these conditions.

Figs. 6 and 7 show the plots of $\ln k'$ versus π^* for 2-nitroanisole at 40, 50 and 60°C and for 4-ethylnitrobenzene at 50°C, respectively. These figures show two linear regions for the temperatures studied with these test molecules. The solute retention in these regions is clearly dependent upon the solvation environment experienced under the given densities of carbon dioxide. At higher densities (less negative π^* values), the solute molecule experience a denser cybotactic region, approaching pseudo-liquid solvation. Therefore, solute retention is seen to assume a different slope in this region. At low densities (more negative π^* values) the cybotactic region of solute will experience pseudo-gas densities and therefore a smaller influence of solvation; the slope of retention reflects this. The origin of these observations, of course, is the change in solvatochromic behavior shown in Figs. 2 and 3. It is interesting that the change in solvatochromic behavior is *not* reflected in retention.

CONCLUSION

Our preliminary investigations of mobile phase-solute interactions and their effects on solute retention in SFC show promise for a better understanding of the fundamental processes determining retention. The solvatochromic comparison

method is a new method for studying the type of intermolecular interactions occurring between a solute and solvent in SFC. As illustrated in this paper, the solvatochromic technique can be used to distinguish between two solvation regions, one at low solvent densities and one in a different region, as pseudo-liquid densities are approached. The linear correlation between $\ln k'$ and the Onsager reaction field function $[L(\eta^2)]$ demonstrates the important role solvent density has in retention in SFC. The Onsager reaction field function is only dependent on the solvents' refractive index, which is directly related to fluid density. At this point, it is unclear why solvatochromic behavior (changes in the cybotactic region of the solute molecule) is not reflected in chromatographic retention. From our initial results, one might conclude, that in SFC specific mobile phase-solute interactions most likely play a role in retention, but the variation of these specific interactions as a function of density does not. A more definitive understanding of retention in SFC awaits the understanding of possible stationary phase solvation (swelling) by the solvent^{23,24}, and the role this plays in retention. Also, the complex interactions of the enthalpy and entropy of transfer of the solute between the mobile and stationary phases with density²⁵ must be better understood before a complete retention mechanism can be proposed. Detailed studies are now in progress to determine the origin of the changes in the cybotactic environment observed in this work and the possible role of solute concentration on this behavior²⁶.

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