

Enhanced Fluidity Liquid Chromatography

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Introduction

High performance liquid chromatography (HPLC) is often the method of choice for the separation of nonvolatile solutes. LC commonly exhibits longer analysis times, larger pressure drops and lower efficiency than either supercritical fluid chromatography (SFC) or gas chromatography (GC). The greater viscosities and lower diffusivities of liquids compared to those of supercritical fluids or gases are the primary cause of these differences in the three types of chromatography. The ideal mobile phase for a chromatographic separation of nonvolatile solutes would combine the positive attributes of liquids and supercritical fluids. That is, a solvent with low viscosity, high diffusivity and high solvent strength is desirable. Enhanced-fluidity liquid mixtures are solvents that have these attributes. These solvents are prepared by dissolving large proportions of liquefied gases, such as CO₂, in commonly associated liquid solvents such as methanol.

To date, enhanced-fluidity mixtures such as methanol-CO₂, methanol-CO₂-H₂O, tetrahydrofuran-CO₂, hexane-CO₂, methanol-fluoroform and methanol-fluoroform-H₂O have been used to improve the performance in a range of different types of liquid chromatographies.

Properties of Enhanced-Fluidity Liquid Mixtures

When a liquefied gas is added to an associated solvent, the viscosity of the resultant mixture decreases. For example, Figure 1 shows the variation in the viscosity of methanol-CO₂ and solute diffusivity as a function of the proportion of added CO₂ at 25°C. The addition of 50% v/v CO₂ causes the viscosity of the mixture to lower to a value that is approximately 70% of the viscosity of methanol. The viscosity, η , of a liquid is typically inversely related to the diffusion coefficient of a solute at infinite dilution, D_{12} , as described in eqn [1]:

$$D_{12}\eta^p = AT \quad [1]$$

where A and p are constants that are characteristic of the solute and T is the absolute temperature. There-

fore, Figure 1 shows that the addition of 50% v/v CO₂ causes the diffusion coefficient of benzene to increase by approximately a factor of 2.

If liquefied gas addition and temperature elevation are combined, improvements in mass transport are substantial. For example, at 58°C with a 0.49 : 0.21 : 0.30 mol fraction methanol-H₂O/CO₂ mixture, the diffusion coefficient of benzene increases ninefold relative to that in the same methanol-H₂O mixture at 26°C. Without temperature elevation, the diffusion coefficient of benzene increases by a factor of 2 by adding 0.30 mol fraction CO₂ to the 0.70 : 0.30 mol ratio methanol-H₂O mixture (Figure 2).

These improvements in the transport properties of the liquid mixtures are only advantageous if the solvent strength of the mixtures remains high when the liquefied gas is added. Figures 3 and 4 show the variation of the hydrogen bond acidity, hydrogen-bond basicity and dipolarity for methanol-CO₂ mixtures as measured by Kamlet-Taft α , β , and π^* solvent strength parameters. Clearly the hydrogen bond acidity and basicity of the methanol-CO₂ mixtures is similar to that of methanol, even with 70% added CO₂. The dipolarity of the mixture decreases faster than the hydrogen bond acidity or basicity with added CO₂. However, the dipolarity remains relatively high, even with 50% added CO₂. Data collected on a number of enhanced-fluidity mixtures have shown that often as much as 50% liquefied gas can be added to the organic solvent without significantly affecting the solvent strength.

Theory

To demonstrate the importance of mass transfer coefficients in liquid chromatography, the van Deemter equation (eqn [2]) is used to model the variation of the chromatographic band dispersion, H , in packed column as a function of linear velocity, u . A is a constant that measures the contribution to band dispersion caused by multiflow paths, B describes the band dispersion caused by longitudinal diffusion and C describes the band dispersion caused by nonequilibrium in both the stationary and mobile phases:

$$H = A + \frac{B}{u} + \sum C_i u \quad [2]$$

By taking the derivative of the van Deemter equation with respect to u , equating the derivative to zero, and substituting in appropriate definitions for A , B and C ,

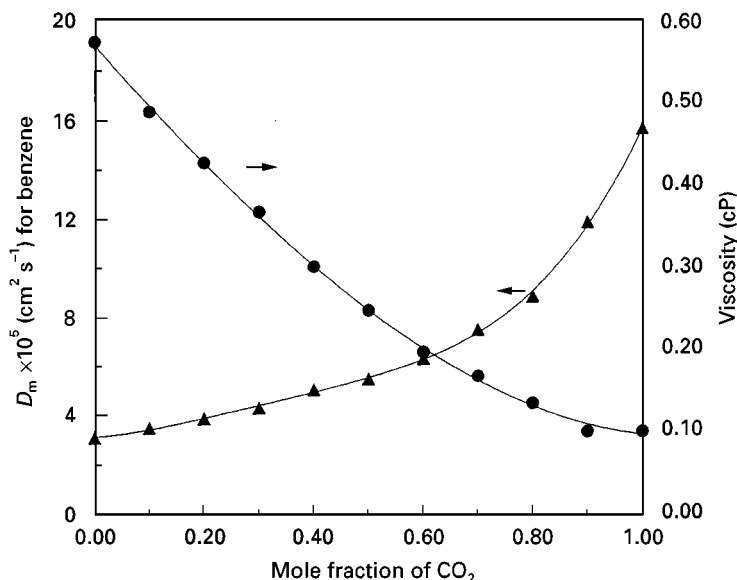


Figure 1 Variation of the viscosity (●) of methanol–CO₂ mixtures and the diffusion coefficient (▲) of benzene at 25°C and 170 atm as a function of mixture composition. (Reproduced with permission from Cui and Olesik (1991).)

the optimum velocity, u_{opt} , is described by eqn [3]: C_{stag} (eqn [4]):

$$u_{\text{opt}} \propto \frac{D_m}{\sqrt{f(k')}} \quad [3]$$

$$C_{\text{stag}} = \frac{f(k)}{D_m} \quad [4]$$

The use of enhanced-fluidity mobile phases is expected to increase the diffusion coefficients of the solute and therefore shift u_{opt} to larger values.

For most HPLC separations that involve packed columns containing porous particles and function at linear velocities greater than u_{opt} , the predominant contribution to band dispersion is the diffusion in the stagnant mobile phase inside the porous packing,

k is the retention factor and D_m is the diffusion coefficient of the solute in the mobile phase. Therefore, since solutes in enhanced-fluidity liquid mixtures have significantly higher diffusion coefficients than in the organic solvent, chromatographic band dispersion decreases (as will be illustrated later). In addition to higher diffusion rates in enhanced-fluidity solvents, the addition of the liquefied gas often lowers

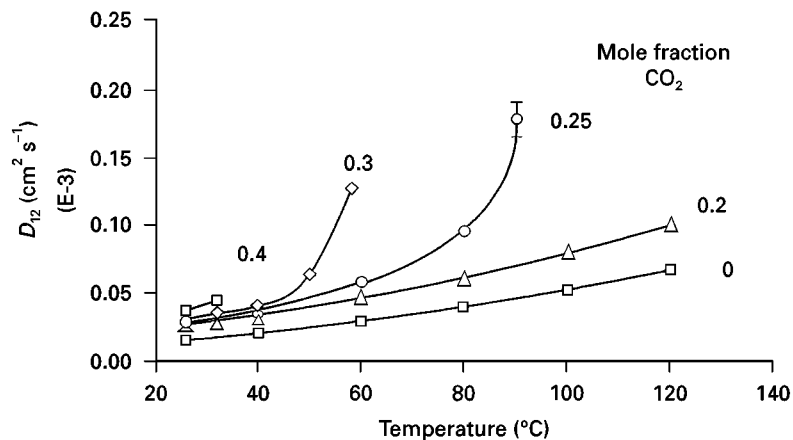


Figure 2 Variation in the diffusion coefficient of benzene for: squares, 0.70:30 mol fraction methanol–H₂O; triangles, 0.56:0.24:0.20 mol fraction methanol–H₂O–CO₂; circles, 0.49:0.21:0.30 mol fraction methanol–H₂O–CO₂; diamonds, 0.42:0.18:0.40 mol fraction methanol–H₂O–CO₂ as a function of temperature. (Reproduced with permission from Lee and Olesik (1994).)

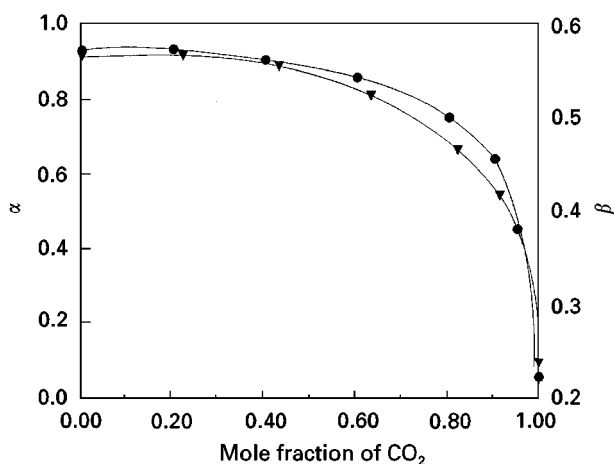


Figure 3 Variation of Kamlet-Taft α (●) and β (▼) parameter as a function of mixture composition for methanol-CO₂ mixtures at 25°C and 170 atm. (Reproduced with permission from Olesik (1991).)

the capacity factor of solutes in some liquid chromatographies. Significant improvements in efficiency can result from the combination of the two effects.

The separation time for a chromatographic analysis is proportional to the slope of a plot of H versus u . Therefore the separation time is also shortened when using enhanced-fluidity solvents. Finally, Darcy's law (eqn [5]) shows that the pressure drop across a packed chromatographic column is linearly related to the product of the linear velocity, u , and the mobile-phase viscosity, η :

$$\Delta P = \frac{u\eta\varepsilon L}{B^0} \quad [5]$$

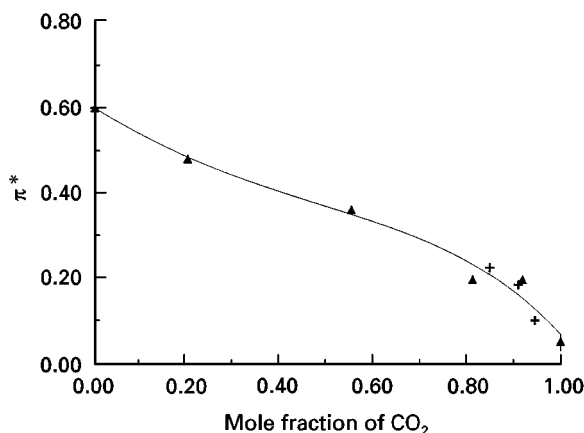


Figure 4 Variation of Kamlet-Taft π^* as a function of mixture composition for methanol-CO₂ mixtures at 25°C and 170 atm. (Reproduced with permission from Cui and Olesik (1991).)

where B^0 is the specific permeability, ΔP is the pressure drop across the column, ε is the interparticle porosity, η is the viscosity and L is the length of the column.

Experimental Methods and Instrumentation

The instrumentation required to do enhanced-fluidity liquid chromatography is very similar to a standard HPLC with two exceptions. Firstly, the components of the mobile phase (liquefied gas and associated liquid) are mixed under pressurized conditions. Therefore two pumps are required to make the mixtures. Either syringe pumps or reciprocating pumps may be used. Pumps that are commonly used for SFC are optimum for pumping the liquefied gases. Secondly, the outlet of the column must be pressurized to maintain single-phase conditions for the mobile-phase mixture at least until after the solutes are detected. Therefore a flow restrictor similar to those used in SFC is used in enhanced-fluidity LC. However, the problems which are often experienced in SFC with restrictor plugging do not occur in enhanced-fluidity LC because phase changes are not occurring in the restrictors and the solvent strength of the enhanced-fluidity liquids is greater than that of commonly used supercritical solvents. Finally, phase diagram information can be used to determine what the minimum operating pressure at the end of the column should be, to ensure that the mobile phase is operating under single-phase conditions. This information for the mixtures described herein is readily available in the literature.

Reversed-Phase Chromatography

When methanol-H₂O-CO₂ mixtures are used in reversed-phase separations with an octadecyl polysiloxane stationary phase, the efficiency of the separation increases significantly compared to the same conditions without the addition of CO₂. For example, the reduced plate height for naphthalene at a reduced velocity of 10 decreased from 11 to 4 for a mobile-phase composition change involving the addition of 0.50 mol fraction CO₂ to a 0.7 : 0.3 mol ratio methanol-H₂O mixture. The addition of 0.5 mol fraction CO₂ to the 0.7 : 0.3 mol ratio methanol-H₂O shortens the analysis time of a separation of polynuclear aromatic hydrocarbons by factors of 2.5 and 8 at linear velocities of 0.15 (reduced velocity = 5) and 0.35 (reduced velocity = 17) cm s⁻¹, respectively, compared to that for the methanol-water mixture at the same linear velocities.

Figure 5 shows the variation in the band dispersion of pyrene using the 0.70 : 0.30 mol ratio methanol-

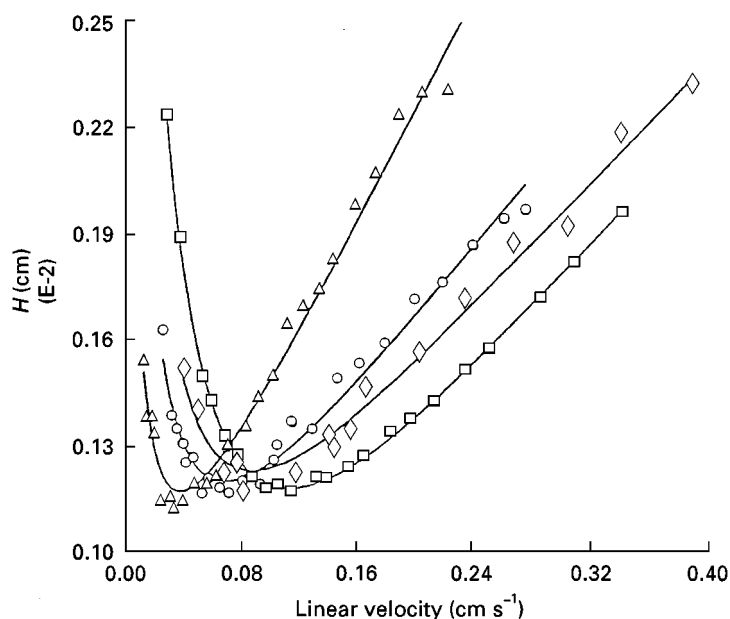


Figure 5 Variation of plate height with mobile-phase linear velocity for pyrene at 204 atm for different mobile-phase conditions: triangles, 0.70 : 0.30 mol ratio methanol-H₂O at 26°C, $k' = 3.77$; circles, 0.70 : 0.30 mol ratio methanol-H₂O at 60°C, $k' = 2.02$; diamonds, 0.49 : 0.21 : 0.30 mol ratio methanol-H₂O-CO₂ at 26°C, $k' = 1.82$; squares, 0.49 : 0.21 : 0.30 mol ratio methanol-H₂O-CO₂ at 60°C, $k' = 0.92$. (Reproduced with permission from Lee and Olesik (1994).)

H₂O mixture, with 0.30 mol fraction CO₂ added at room temperature and with 0.30 mol fraction CO₂ added at 60°C. As expected, the band dispersion decreased and the optimum linear velocity increased when CO₂ was added to the mobile phase. The increase in temperature also yields substantial gains in efficiency, especially at high linear velocities.

Figure 6 shows the improved separation time which is possible by adding CO₂ and increasing the temperature to 60°C at the same linear velocity for the separation of 16 polynuclear aromatic hydrocarbons. The separation required approximately 40 min with 0.70 : 0.30 methanol-H₂O approximately 15 min when 0.30 mol fraction CO₂ was added and less than 7 min when this mixture was heated to 60°C. However, when 30% CO₂ was present in the mobile phase and the temperature was 60°C, the selectivity of the separation degraded substantially.

Also, due to the low viscosity of the methanol-H₂O-CO₂ multiple columns can be placed in series to increase the overall efficiency of a chromatographic separation. For the same linear velocity, Figure 7 compares the separation of a coal tar sample using one and four ODS columns. Substantial gains in efficiency are obtained by placing the columns in series.

However, the addition of liquefied CO₂ is not optimal to improve the performance of every separation. When CO₂ is mixed with H₂O, carbonic acid is for-

med. The acidic nature of this mobile phase can have a significant impact on the retention factor of ionizable compounds. Also CO₂ and H₂O are not highly miscible at room temperature without the addition of a co-solvent. Even with the addition of co-solvent, the total amount of CO₂ that is miscible in the mixture is controlled by the amount of H₂O present. For example, a 0.70 : 0.30 mol ratio methanol-H₂O mixture is immiscible with more than 0.50 mol fraction CO₂. To eliminate many of these problems, other liquefied gases can also be used to modify the fluidity of the mixture.

Normal-Phase Chromatography

The addition of a liquefied gas is even beneficial when mixed with nonpolar solvents commonly used in normal-phase LC. For example, when 50 mol% CO₂ is combined with 50 mol% hexane, the efficiency increases by at least a factor of 2 across the entire range of linear velocities studied and the mobile phase viscosity also decreases by a factor of approximately 2. However, the addition of proportions of CO₂ greater than 50 mol% causes significant peak asymmetry and decreased efficiency.

Size Exclusion Chromatography

In size exclusion chromatography (SEC), the selectivity of the separation is not affected significantly by the

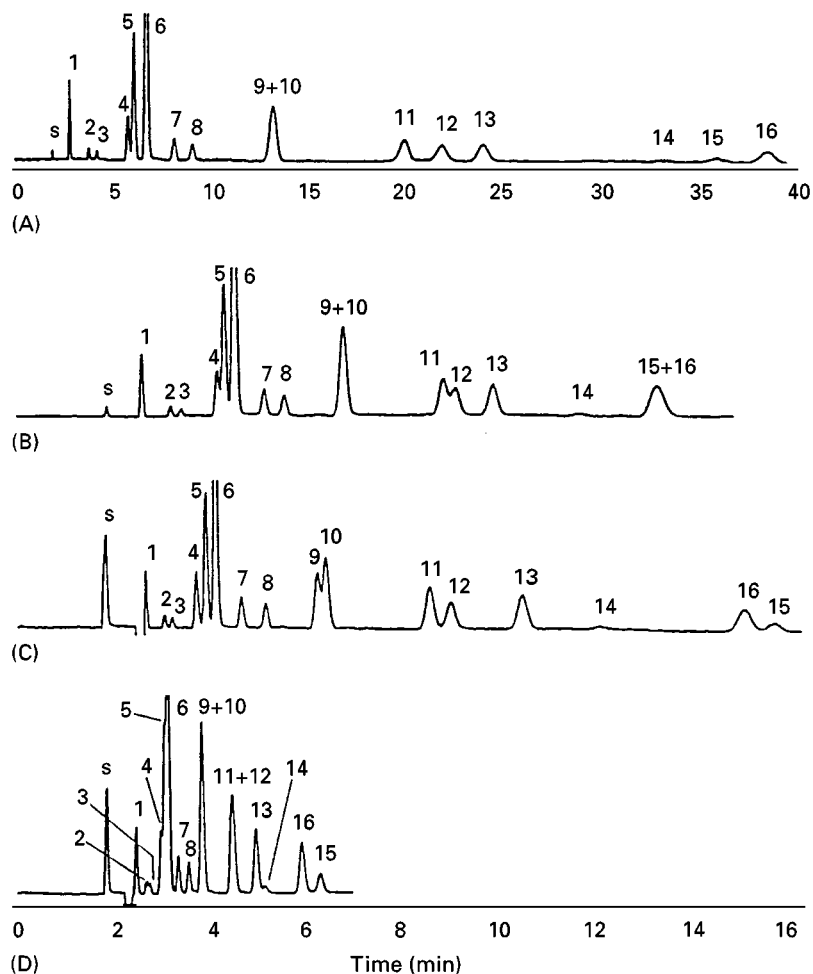


Figure 6 Chromatograms of 16 polynuclear aromatic hydrocarbons at 204 atm for different mobile-phase conditions: (A) 0.70 : 0.30 mol ratio methanol-H₂O at 26°C; (B) 0.70 : 0.30 mol ratio methanol-H₂O at 60°C; (C) 0.49 : 0.21 : 0.30 mol ratio methanol-H₂O-CO₂ at 26°C; (D) 0.49 : 0.21 : 0.30 mol ratio methanol-H₂O-CO₂ at 60°C. 1, Benzene; 2, naphthalene; 3, acenphthalene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[a]anthracene; 10, chrysene; 11, benzo[b]fluoranthene; 12, benzo[k]fluoranthene; 13, benzo[a]pyrene; 14, dibenzo[a,h]anthracene; 15, benzo[ghi]perylene; 16, indeno[1,2,3-cd]pyrene. (Reproduced with permission from Lee and Olesik (1994).)

choice of mobile phase because the separation is based solely on the entropic partitioning of the solute between the bulk mobile phase and the stagnant solvent in the pores. Therefore, efficient separations are highly desirable in SEC. Often, to increase the total efficiency of a separation, analytical columns are connected in series until the maximum pressure of the chromatographic pumping system is approached. SEC is frequently used at elevated temperatures to improve the solubility of high MIC samples and this also lowers the mobile-phase viscosity and allows more columns to be linked together to increase the total efficiency of the separation.

Enhanced-fluidity liquid mixtures can improve the chromatographic performance of SEC without need-

ing to increase the temperature. When CO₂ was added to tetrahydrofuran (THF) for the separation of polystyrene standards, improved efficiency and decreased separation time resulted. For a mobile-phase velocity of approximately 0.8 cm s⁻¹, the reduced plate height for polystyrene (*M_w* 12 600) decreased by a factor of 2 by adding 0.40 mol% CO₂ to the THF. In addition, the 40 mol% CO₂ : 60 mol% THF mixture had a viscosity that was approximately 50% that of pure THF, which resulted in an approximate 50% decrease in the separation time for the separation. However, when greater proportions of CO₂ were added to the THF, the solvent strength diminished significantly, which caused adsorptive interactions to compete with the exclusion mechanism.

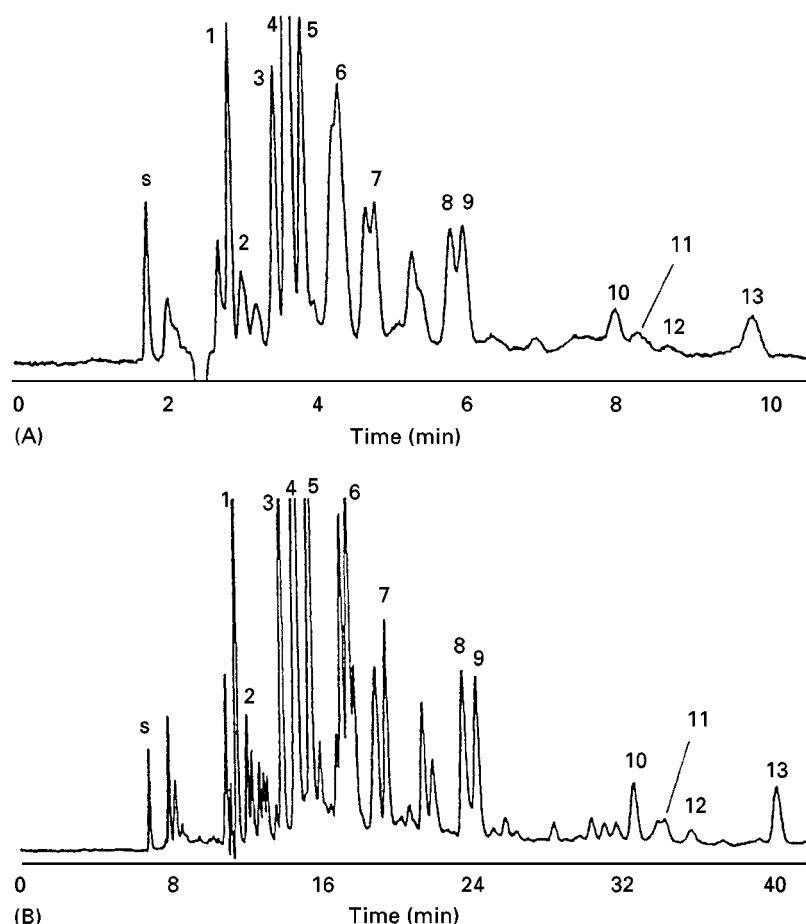


Figure 7 Chromatograms of the NIST SRM 1597 coal tar standard using 0.49 : 0.21 : 0.30 mol ratio methanol-H₂O-CO₂ at 26°C and 204 atm for (A) one and (B) four columns (A) $u = 0.143 \text{ cm s}^{-1}$; $\Delta P = 22.0 \text{ atm}$; (B) $u = 0.145 \text{ cm s}^{-1}$; $\Delta P = 127.5 \text{ atm}$. (Hypersil C₁₈ 150 × 1 mm packed with 5 μm diameter particles.) 1, Naphthalene; 2, acenaphthalene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benz[a]anthracene; 9, chrysene; 10, benzo[b]fluoranthene; 11, benzo[k]fluoranthene; 12, perylene; 13, benzo[a]pyrene. (Reproduced with permission from Lee *et al.* (1995).)

Both enhanced-fluidity SEC and high temperature SEC have limits to the scope of their application. Enhanced-fluidity SEC is limited by the solvent strength of the high fluidity mixtures and high temperatures can cause decomposition of the polymer. Therefore the combination of increased temperature and use of enhanced-fluidity SEC might often be the best choice to improve the chromatographic performance in exclusion separations.

By adding 30 mol% CO₂ to THF and increasing the temperature from 24 to 80°C, the slope of a plot of reduced plate height versus linear velocity decreases substantially. For example, with styrene as the solute, the *C* coefficient in the van Deemter equation decreases to a value that is only 17% of the *C* value when THF at 24°C is the mobile phase (Figure 8). With such a flat slope, SEC separations can be accomplished at high velocities with minimal loss in efficiency.

Liquid Chromatography at Critical Condition

Liquid chromatography at the critical condition is a separation method that allows the analyst to decide what portion of the molecule will control the separation. For example, at the critical condition for polystyrene polymers, all polystyrene oligomers will elute at the same elution volume, while functionalized polystyrene will be separated based solely on the functionality distribution within the polymer. This method can also be used to make a component of a copolymer chromatographically invisible and then obtain separation based on the other components of the copolymer.

At the critical condition, the change in free energy associated with the transfer of the polymer (in this case, polystyrene) to the stationary phase is zero. That is, at the critical condition, the enthalpic interaction and the entropic interaction of the polymer with

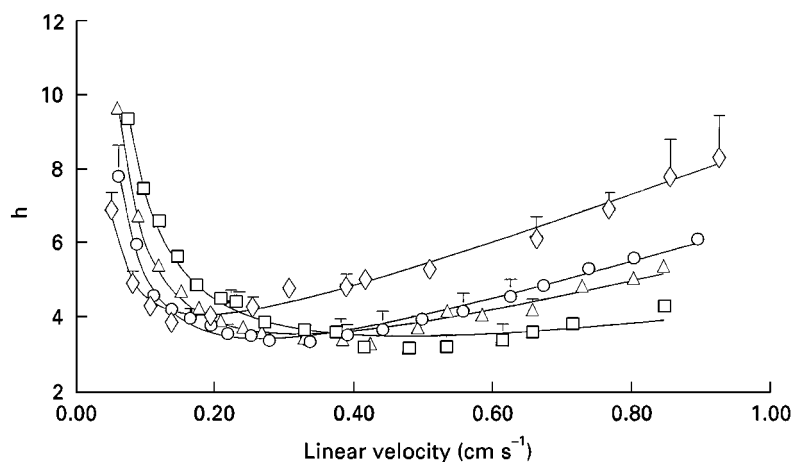


Figure 8 Variation of reduced plate height of styrene using a Betasil silica column with mobile-phase linear velocity using different mobile-phase compositions: diamonds, pure THF at 24°C; circles, 70 : 30 mol% THF-CO₂ at 24°C; triangles, pure THF at 80°C; squares, 70 : 30 mol% THF-CO₂ at 80°C. (Reproduced with permission from Yuan and Olesik (1997b).)

the stationary phase are exactly balanced. This chromatographic condition is typically found by carefully changing the mixture composition used as the mobile phase until this thermodynamic condition is met. This specific composition is sometimes difficult to determine experimentally.

Because the solvent strength of enhanced-fluidity liquid mixtures changes with varying pressure and,

temperature, as well as mixture composition, the critical condition is more readily found using these liquid mixtures than common liquids. **Figure 9** shows a molecular weight calibration plot for polystyrene polymers. With enhanced-fluidity liquid mixtures, by

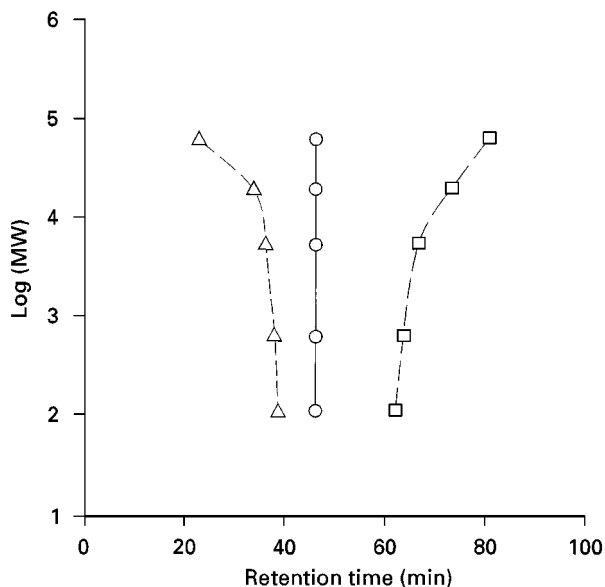


Figure 9 Plots of polystyrene molecular weight versus retention time at different mobile-phase conditions. Conditions: 1.8 m × 250 μm-i.d. silica (20 nm pore size, 5 μm particle size) packed column, column pressure 260 atm: triangles, 50% CO₂ in THF; circles, 54% CO₂ in THF; squares 60% CO₂ in THF. (Reproduced with permission from Yuan and Olesik (1998a).)

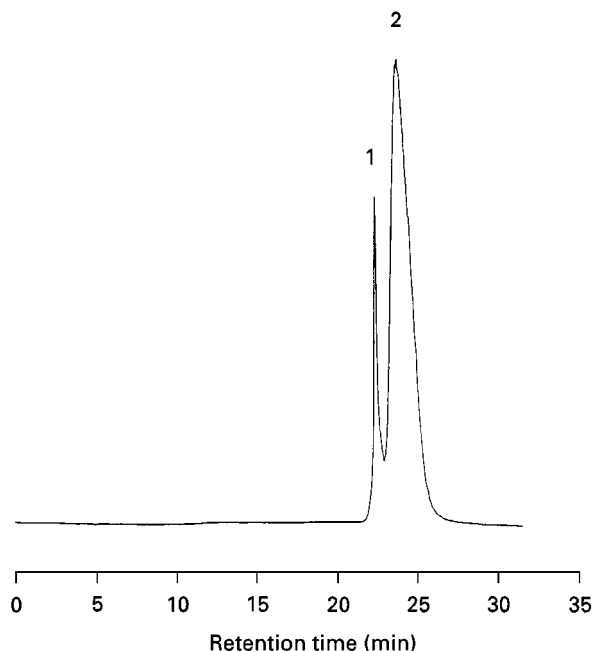


Figure 10 Chromatogram of polystyrenes with mono- and dicarboxylic terminal groups. Conditions: 1.8 m × 250 μm i.d. silica (20 nm pore size 5 μm particle size) packed column; 54% CO₂ in THF mobile phase at 260 atm; column at 70°C using evaporative light-scattering detection. Peaks: 1, polystyrene with dicarboxylic terminal groups, approximate MW 50 000; 2, polystyrene with monocarboxylic terminal group, approximate MW 50 000. (Reproduced with permission from Yuan and Olesik (1998a).)

changing the temperature and or pressure, all three modes of separation (size exclusion, critical chromatography and adsorption chromatography) of polystyrene polymers are possible using the same mixture as the mobile phase. Increased efficiency is also beneficial at the critical condition because only the weak interactions of the functionalities or of the other components of a copolymer control the separation. The low viscosity of enhanced-fluidity liquid mixtures allows significantly improved efficiency. Long (2 m) packed capillary columns have been produced that have efficiencies as high as 100 000 theoretical plates. Figure 10 shows an example of the critical condition separation of mono- and dicarboxy-terminated polystyrene polymers separated at the polystyrene backbone with a long packed capillary column.

Use of Alternative Liquefied Gases

While CO₂ was the first liquefied gas used for enhanced-fluidity liquid chromatography, other gases may also be useful to increase the fluidity of a liquid mixture. CO₂ is limited in its applications in that, when it is included in mixtures with water, carbonic acid is produced. Acidic buffer conditions can be generated for the reversed-phase separations but not basic buffers.

For a gas to be useful as a fluidity modifier, the gas must be highly miscible with the organic liquid solvent and have a low viscosity. Fluoroform is an example of a liquified gas that is highly soluble under similar pressure and temperature conditions to CO₂. Accordingly, the efficient separation of basic solutes using basic buffered mobile phases is possible when fluoroform is used to decrease the viscosity of the mobile-phase mixture instead of CO₂. For example, Figure 11 shows a comparison of the separation of some triazine herbicides and a common metabolite, hydroxyatrazine, using 64 : 36 mol% methanol–10 mmol L⁻¹ phosphate buffer (Figure 11A) with 51 : 29 : 20 mol% methanol–10 mmol L⁻¹ phosphate buffer–CO₂ (Figure 11B) and 51 : 29 : 20 mol% methanol–10 mmol L⁻¹ phosphate buffer–fluoroform (Figure 11C) as the mobile phase with the same flow restrictor. The addition of CO₂ clearly causes the co-elution of the more polar solutes. When the chromatogram using the methanol–buffer mixture is compared to that containing the same mole ratio methanol–buffer but with the addition of 0.20 mol fraction fluoroform, use of fluoroform is clearly optimal. The linear velocity and the efficiency increased with the addition of fluoroform. At constant linear velocity, the addition of 20 mol% fluoroform to the methanol–buffer solution increased the efficiency by approximately 30%.

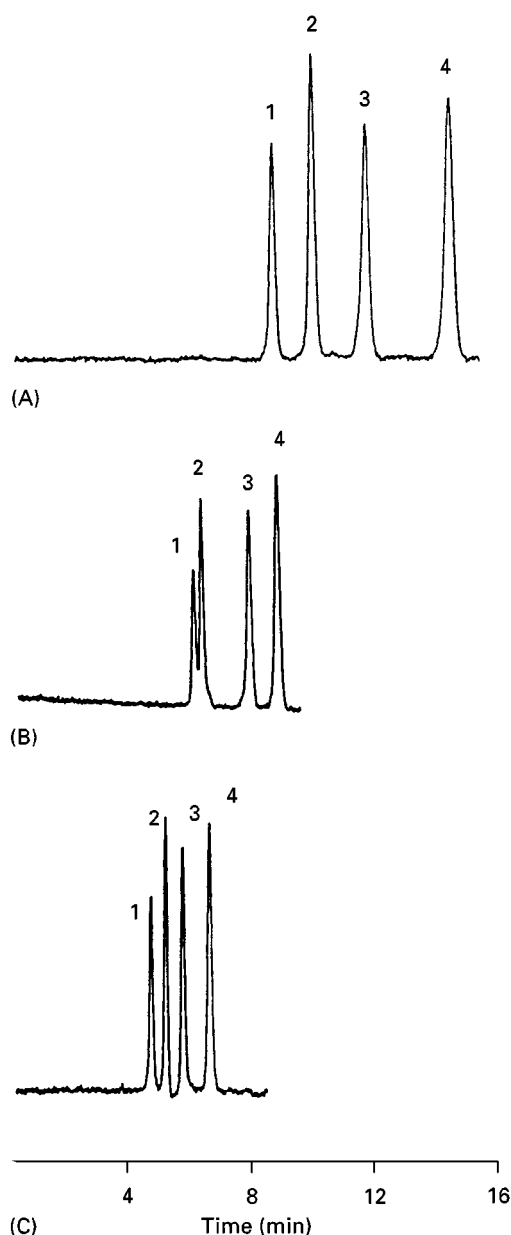


Figure 11 Chromatogram at same restrictor length and pressure drop of 16.3 atm with different mobile-phase conditions (A) 64 : 36 mol% methanol–10 mmol L⁻¹ phosphate buffer; (B) 51 : 29 : 20 methanol–10 mmol L⁻¹ phosphate buffer–CO₂; (C) 51 : 29 : 20 methanol–10 mmol L⁻¹ phosphate buffer–CHF₃. Peaks: 1, ammeline; 2, hydroxyatrazine; 3, atrazine; 4, terbutryne. (Reproduced with permission from Yuan and Olesik (1998b).)

Other Separations Using Enhanced-Fluidity Liquid Mixtures

The extraction of polar pollutants from very adsorptive matrices is a difficult task. A large range of polar pollutants can be effectively removed from adsorptive matrices such as dust and fly ash, by using enhanced-fluidity liquid mixtures. Solutes as polar as phenols,

triazine herbicides, and as large as 3–4 ring polynuclear aromatic hydrocarbons are effectively removed using enhanced-fluidity liquid mixtures, such as methanol–H₂O–CO₂ and methanol–H₂O and are superior to the use of other standard methods such as Soxhlet extraction.

Conclusions

Improved performance has been observed using enhanced-fluidity liquid mobile phases in every type of liquid chromatography studied to date. As mentioned earlier, typically as much as 50 mol% liquefied gas can be added to a polar solvent while still maintaining much of the solvent strength of the original organic solvent. As the solvent strength of the mixture decreases for mixtures containing more than 50 mol% liquefied gas, the chromatographic performance decreases. Extraction techniques for highly polar substances that would otherwise be very difficult to extract have been developed using enhanced-fluidity liquid mobile phases. The use of enhanced-fluidity liquids as mobile phases has also made viable a powerful technique termed critical chromatography. This separation method allows careful characterization of functionality distributions in polymers and oligomer distributions in copolymers. The precise control of solvent strength that is possible with enhanced-fluidity liquids will allow critical chromatography to be more thoroughly studied.

Reversed-phase liquid chromatography is presently the most commonly used HPLC method. pH variation is often used to control retention in reversed-phase HPLC. While the use of enhanced-fluidity mixtures significantly improves the chromatographic performance in reversed-phase HPLC, more information is needed on pH control in these mixtures to evaluate the potential of enhanced-fluidity liquid mixtures.

Chiral chromatography is another area of separation science where enhanced-fluidity liquid mixtures may find applications in the future. Increasingly, SFC is under consideration for chiral separations. However, solvent strength often limits the utility of SFC. Enhanced-fluidity liquid mixtures have high solvent strength and therefore may be applicable to separations that SFC cannot handle.

See also: II/Chromatography: Liquid: Mechanisms: Reversed Phases; Size Exclusion Chromatography; Theory of Liquid Chromatography. **Chromatography: Supercritical Fluids:** Theory of Supercritical Fluid Chromatography.

Further Reading

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Evaporative Light Scattering Detectors in Liquid Chromatography

See II/CHROMATOGRAPHY: LIQUID/Detectors: Evaporative Light-Scattering