The Effect of Dissolved Helium on the Density and Solvation Power of Supercritical Carbon Dioxide

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Abstract

Carbon dioxide (CO_2) cylinders pressurized with helium in their headspace are widely used in supercritical fluid chromatography and extraction (SFC and SFE). A few percent of helium are dissolved in the liquid CO_2 phase of the cylinder, which can result in such problems as retention time shift and poor reproducibility in SFC as well as reduced solubility and extraction rate in SFE. In this study, a high precision density meter and a gas chromatograph equipped with a thermal conductivity detector are used to monitor the density and composition of the supercritical fluid generated from a helium headspace CO_2 cylinder over the duration of its use. These measurements are related to the solubility of soybean oil and cholesterol in the fluid. The density measurements (accurate to 10^{-4} g/mL) show that the density of the fluid is linearly proportional to the helium content of the CO_2. However, the significant drop of solute solubility in helium–CO_2 mixtures cannot be explained by the reduction in fluid density alone and may involve a disruption of the solvent (CO_2) shell around the dissolved solute.

Introduction

Carbon dioxide (CO_2) is probably the most often used fluid in supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE). The compressibility of CO_2 is quite high when it is delivered from a cylinder to a pump at room temperature, resulting in poor pumping efficiency. To reduce compressibility and improve pumping efficiency, CO_2 must be delivered to the pump either at high pressure (about 2000 psi) or at a temperature much lower than 25°C. The latter requires the cooling of the pump head or pump cylinder. The former is often realized by using a helium headspace (HHS) inside the CO_2 cylinder. The use of helium headspace CO_2 in SFC, however, leads to poor reproducibility in peak retention times and peak areas (1–3). In recent years, researchers have also noticed that HHS CO_2 produces adverse effects in SFE as well. For example, the solubility of soybean oil in HHS CO_2 is substantially

lower than that in pure CO_2 (4). HHS CO_2 also reduces the extraction rate of cholesterol (5) and affects polymerization reactions in supercritical CO_2 (6). The problems associated with HHS CO_2 are attributed to a small percentage of helium dissolved in liquid CO_2, which has been confirmed by recent studies (3,7). How dissolved helium affects the fluid density and solute solubility in a supercritical fluid is still not clear. In fact, to our knowledge, the density of the HHS CO_2 has only been theoretically calculated (2) but not experimentally measured.

Our interest in HHS CO_2 stems from the observation that the solubility of soybean oil is 50–70% lower in HHS CO_2 than that in pure CO_2 at the same temperature and pressure (4). To better understand the reason behind this observation, we utilized an Anton Parr high-pressure density meter capable of measuring density accurate to 10^{-4} g/cm^3, and a gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) to monitor the composition and density of the supercritical fluid from an HHS cylinder during SFE until depletion of the cylinder contents. These data were then related to the solubilities of soybean oil and cholesterol in the binary fluid as well as to those observed in pure CO_2.

Experimental

A Spe-ed SFE unit (Applied Separation, Allentown, PA) equipped with a liquid booster pump and a pump head chiller were used for this study. The densities of supercritical fluids were measured by a model DMA 48 Anton Parr density meter in combination with a DMA 512P high-pressure measuring cell (Anton Parr, Graz, Austria). The density meter was calibrated by a Haskel gas booster pump (Haskel, Burbank, CA) and an ISCO syringe pump (ISCO, Lincoln, NE) using nitrogen and water, respectively. After the calibration utilizing the known densities of nitrogen and water at selected temperatures and pressures, the density of pure CO_2 was measured and compared with the data reported in the literature (8). This difference was found to be less than 0.2%, indicating that the accuracy of the density meter was quite satisfactory. The precision of the density meter was better than 0.02% from seven replicate runs.
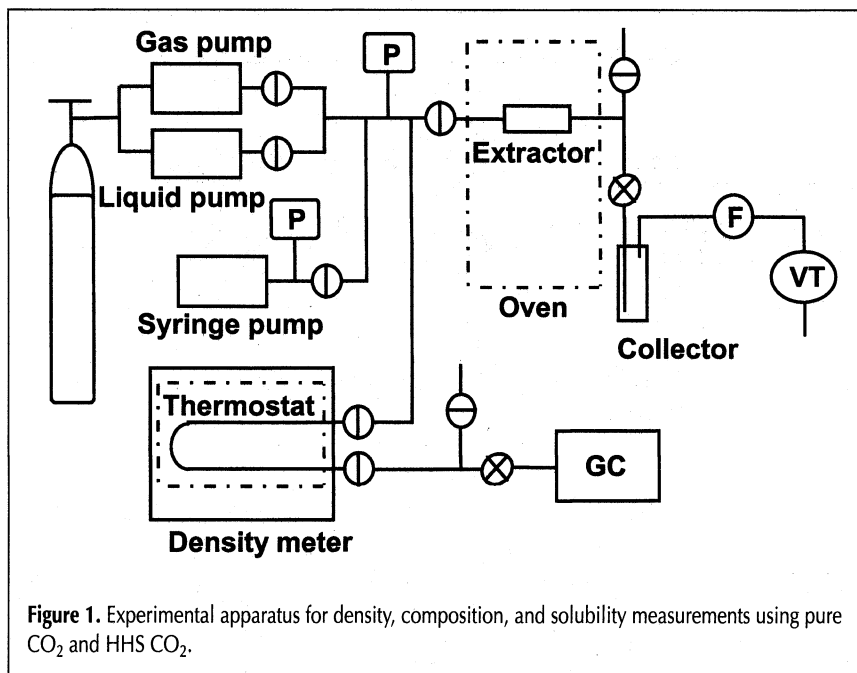

Figure 1. Experimental apparatus for density, composition, and solubility measurements using pure CO_2 and HHS CO_2.
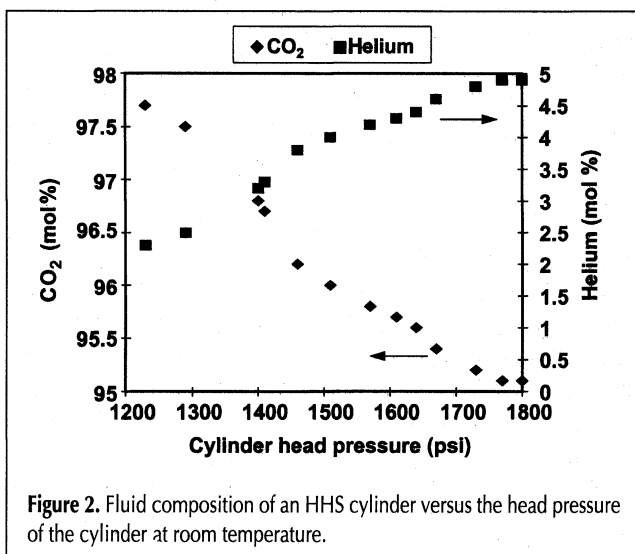

Figure 2. Fluid composition of an HHS cylinder versus the head pressure of the cylinder at room temperature.

The experimental apparatus used for making the solubility measurements is illustrated in Figure 1. Here, the gas booster pump and syringe pump were only used for calibration purposes. The pressurized fluid coming out of the liquid pump was split into two streams. One stream flowed toward the extraction cell housed in an oven and then exited through a micrometering valve to a collection vial before passing through a flow meter and a dry test meter (American Meter, Philadelphia, PA). The other stream was connected to the thermostated DMA 512P high-pressure density measuring cell before exiting through a micrometering valve. It then flowed through a 2-mL gas sampling loop into a GC utilizing a 20 wt% DC-200 on Chromasorb P, an AW-DMCS column, and a TCD (GOW-MAC, Lehigh Valley, PA). High-purity nitrogen (99.9995%) was used as the carrier gas for GC. The TCD signal of the GC was calibrated with pure CO_2 and pure helium. During extraction experiments, the temperature and pressure of the extraction cell

and density cell were identical, and the density and composition of the fluid used for each extraction was monitored and recorded.

The method for determining soybean oil solubility in supercritical fluids has been reported previously (9,10). In this study, about 10 g of soybean flakes were packed in a 25-mL extraction cell (Keystone, Bellefonte, PA). The fluid passing through the cell was controlled at the selected temperature and pressure. After a static hold for 30 min, a dynamic extraction was commenced at a flow rate of 2 L/min (expanded gas). The oil solubility was then calculated from the linear portion of the extraction curve, in which the weight of extracted oil was plotted versus the volume of supercritical CO_2 passed through the extraction cell.

For measurements of cholesterol solubility, 1.5 g of cholesterol (J.T. Baker, Philipsburg, NJ) and 4 g of Hydromatrix, a diatomaceous earth-based sorbent (Varian, Harbor City, CA), were thoroughly mixed and then packed into a 15-mL extraction cell. The fluid in the cell was equilibrated at a selected temperature and pressure for 30 min, and the extraction was then carried out under the same selected temperature and pressure at a flow rate of 1 L/min (expanded gas). In the case of the cholesterol extractions, the tubing between the exit of the extraction cell and the collection vial was washed with 10 mL acetone to assure complete removal of the solute after the extraction was completed. This extract was then dried under nitrogen and weighed. For solubility determinations, the extraction was not terminated until at least 80 mg of cholesterol had been extracted.

The SFC–SFE grade CO_2 and HHS CO_2 (> 99.9999% purity) used in these experiments were purchased from Air Products (Allentown, PA) and used without further purification.

Results and Discussion

The HHS CO_2 cylinder used in this study had an initial head pressure of 2000 psi. Because gas was used for extraction, the cylinder pressure dropped. At about 1200 psi, the CO_2 content inside the cylinder became too low for conducting additional extractions. Figure 2 shows the molar percentages of CO_2 and helium in the liquid phase of the HHS cylinder as the cylinder head pressure decreased. The fluid composition was measured by a GC and a TCD with a precision of about 3% relative standard deviation (RSD) for helium and 1% RSD for CO_2 (based on three replicates). This difference in precision indices was due to the fact that helium had a smaller peak area in the GC analysis than carbon dioxide. The composition of the fluid changed relatively slowly as the cylinder head pressure decreased. The fluid was considered as having a constant composition if the variation of the helium molar percent was not outside the

Table I. Density of Pure CO_2 and Helium-Entrained CO_2 at Three Different Temperatures and Selected Pressures

| Temperature | Fluid | Composition (mol %) | | Pressure (psi) | | | |
		CO_2	He	5,000	7,000	8,000	9,950
				Density (g/mL)			
50°C	Pure CO_2	100	0	0.8968	0.9564	0.9795	1.0164
	CO_2–He	95.1	4.9	0.8457	0.9159	0.9420	0.9827
	Difference in density (%)			5.7	4.2	3.8	3.3
60°C	Pure CO_2	100	0	0.8609	0.9276	0.9526	0.9926
	CO_2–He	95.2	4.8	0.8135	0.8882	0.9153	0.9593
	Difference in density (%)			5.5	4.2	3.9	3.4
70°C	Pure CO_2	100	0	0.8242	0.8981	0.9254	0.9686
	CO_2–He	95.4	4.6	0.7752	0.8598	0.8898	0.9372
	Difference in density (%)			5.9	4.3	3.8	3.2

Figure 3. Density of helium–CO_2 with different helium contents at 70°C and three selected pressures.

Figure 4. Soybean oil solubility in helium–CO_2 having different helium content at 70°C and selected pressures.

above RSDs. The composition of the fluid changed more slowly when the cylinder was full and more rapidly when the cylinder was almost empty. As Figure 2 shows, the helium molar percent in the fluid decreased from 4.9% with a cylinder head pressure of 1800 psi (almost full) to 2.3% at 1230 psi (near depletion). The measured helium content was quite consistent with those reported by Leichter et al. (3). Clearly, the fluid came out of an HHS CO_2 cylinder as a mixed CO_2–helium fluid that changed composition over time instead of pure CO_2. The ratio of CO_2–helium in the fluid did not change after being pressurized by the pump. The helium content measured directly from the cylinder was exactly the same as the fluid that had been pressurized to various pressures ranging from 2,000 to 10,000 psi by the booster pump. The observed adverse effects on SFC and SFE using HHS CO_2 were obviously caused by the dissolved helium in the extraction fluid.

One of the most obvious effects of dissolved helium in liquid CO_2 was the reduction of fluid density. Table I shows the density differences between pure CO_2 and helium–CO_2 at selected temperatures and pressures. The density for the latter was reduced by about 3–6% compared with pure CO_2. It appears that temperature did not affect the magnitude of density drop as significantly as pressure. For the fluid with the same helium content, the density drop at high pressure (about 3%) was much less than that at lower pressure (about 6%).

Figure 3 shows the density of the fluid at 70°C and three selected extraction pressures as a function of the fluid composition. The CO_2 content can easily be obtained from Figure 3 by subtracting helium content from 100%. The data at 100% CO_2 (0% helium) were from the experiments using a pure CO_2 cylinder. As Figure 3 demonstrates, the density of the fluid decreased linearly as helium content increased under constant pressure. Because the solvation power of supercritical fluid is strongly related to its density, the drop in fluid density affects the solubility of a solute.

Figure 4 shows the soybean oil solubility in fluids with varying helium content at 70°C and three selected pressures. The decrease of oil solubility with the increased helium presence in the extraction fluid is very pronounced for all three extraction pressures. However, this relationship is not linear. It appears that at low helium content, the solubility of soybean oil decreased more rapidly for the same molar percentage increase in helium. Because the density changed linearly with helium content, this result suggests that the density of the fluid alone does not explain the solubility drop in helium–CO_2 mixtures.

Table II summarizes the solubility of cholesterol in both pure CO_2 and helium–CO_2 mixtures. The experimental uncertainty is based on three replicate determinations. At two different extraction temperatures and pressures, the solubility
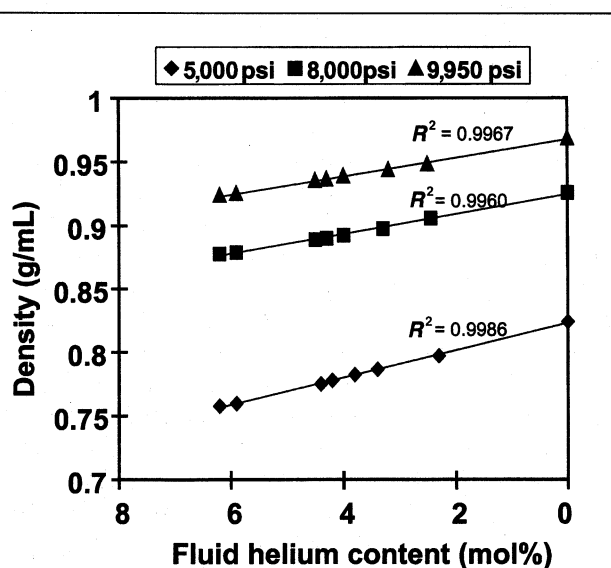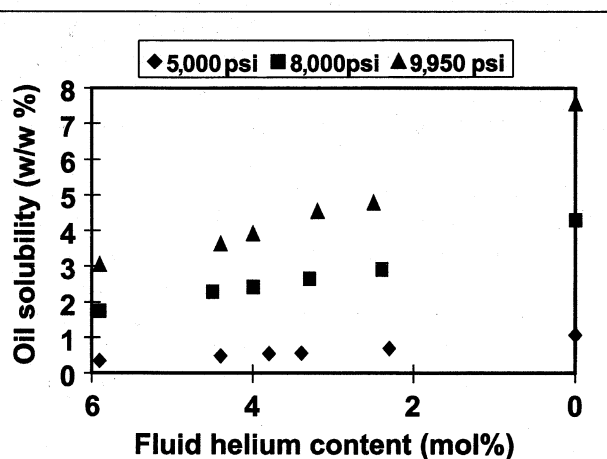
Table II. Solubility of Cholesterol in Pure CO_2 and Helium-Entrained CO_2 Based on Three Replicates

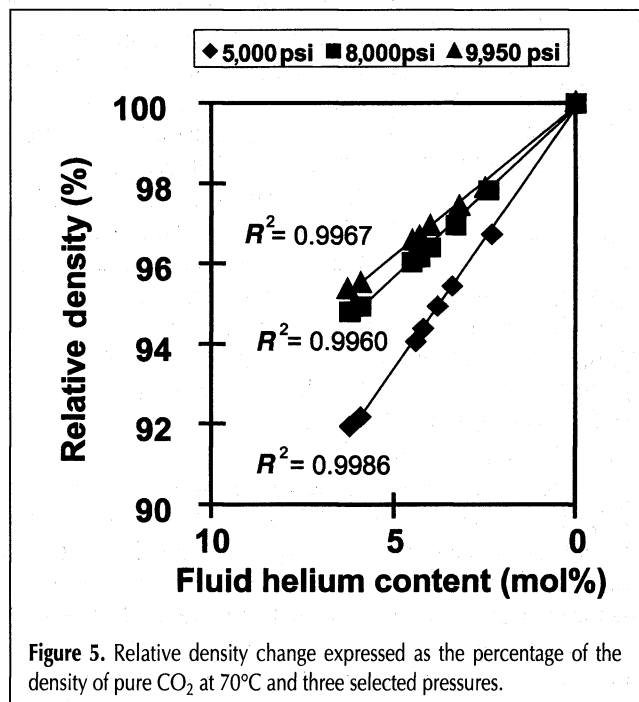| Fluid composition	50°C and 4,000 psi		40°C and 3,500 psi	
	Pure CO_2	CO_2 (96%)–He (4%)	Pure CO_2	CO_2 (96%)–He (4%)
Solubility (w/w%)	0.074 ± 0.001	0.053 ± 0.002	0.055 ± 0.001	0.034 ± 0.001

Figure 5. Relative density change expressed as the percentage of the density of pure CO_2 at 70°C and three selected pressures.
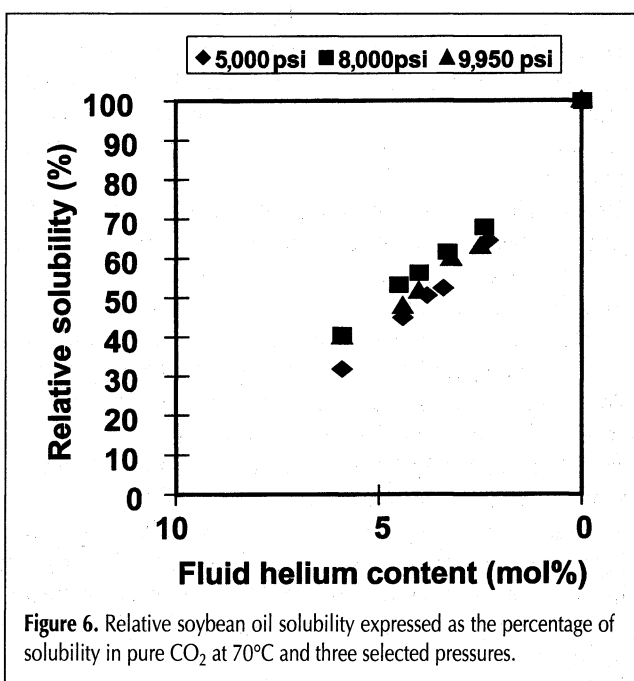

Figure 6. Relative soybean oil solubility expressed as the percentage of solubility in pure CO_2 at 70°C and three selected pressures.

drop in helium–CO_2 mixtures is very significant. There is no doubt that the helium presence in supercritical CO_2 depresses the solubility of cholesterol. These results are also consistent with the observation by Gahrs (11) that when CO_2 is mixed with nitrogen, the solubility of caffeine and nicotine in the mixed fluid decreases substantially (addition of 5 mol % of nitrogen in CO_2 reduces the solubility of caffeine by 50%). This verifies Brunner's thesis (12) that a modifying agent (such as helium or nitrogen) with a lower critical temperature than that of the extraction fluid (CO_2) causes a decrease in the solubility of a low-volatility solute; also, a modifying agent with a higher critical temperature (such as methanol or acetone) causes an increase in solubility (12).

Figure 5 shows the density of the helium–CO_2 mixtures as a percentage of pure CO_2 density at 70°C and three selected pressures. It is clear that the percentage of the density drop in the helium–CO_2 mixtures is almost the same as the molar percentage of helium in the fluid. For example, 6 mol % helium caused the density to drop by 8% at 5,000 psi and by about 5% at 8,000 psi.

Figure 6 compares the solubility of soybean oil in helium–CO_2 mixtures with that in pure CO_2 at 70°C and three selected pressures. The solubility in pure CO_2 (0% helium) was taken as 100%. These results are quite different from those shown in Figure 5. With 6 mol % helium in supercritical CO_2, the solubility of soybean oil decreased by 70% at 5,000 psi and 60% at 8,000 and 9,950 psi. Although the pressure had a very clear effect on the relative density of the fluid, it did not have the same strong influence on the relative solubility of soybean oil. For the fluid with the same helium content, the change in relative solubility at 8,000 psi was often the largest, whereas the largest change in relative density occurred at 9,950 psi. Considering that the solubility of soybean oil dropped by 70% and its density dropped by only 8% at 5,000 psi for 6 mol % of helium in the fluid, these results together clearly suggest that the solubility decrease in helium–CO_2 mixtures cannot be explained by a decrease in density alone. As further evidence of this observation, the solubility of soybean oil at 8,000 psi and 70°C was 4.51 wt% in pure CO_2 at a density of 0.9254 g/mL, whereas the solubility of the same oil was 2.91 wt% for CO_2–6 mol % helium at a density of 0.9253 g/mL at 9,950 psi and 70°C. In fact, as Table III shows, the soybean oil solubilities in CO_2–helium fluids with higher density were smaller than that in pure CO_2 and had lower density at the same extraction temperature.

The solvation power of a solvent can be characterized by solubility parameter, a concept first developed by Hildebrand (13) and later extended to the supercritical fluid state by Giddings et al. (14). The solubility parameter takes account of the cohesive energy of the solvent and is a much better description of the solvation power of a solvent than density. For supercritical fluids, the solubility parameter as defined by Giddings is (14):

$$\delta = 1.25 P_c^{1/2} \times \frac{\rho_r}{\rho_r \text{ (liq)}} \qquad \text{Eq 1}$$

where δ is the fluid solubility parameter, P_c is the critical pressure of the fluid, ρ_r is the reduced density of the fluid, and ρ_r (liq) is the reduced density for the fluid at infinite compression

Table III. Soybean Oil Solubility and Fluid Density and Composition at 70°C Based on Three Replicates

Extraction pressure (psi)	8,000	9,950	9,950	9,950
Helium (mol %) in CO_2	0	5.9 ± 0.2	4.4 ± 0.1	4.0 ± 0.1
Density (g/mL)	0.9254	0.9253	0.9355	0.9391
Oil solubility (w/w%)	4.3 ± 0.2	2.9 ± 0.1	3.6 ± 0.2	3.9 ± 0.2
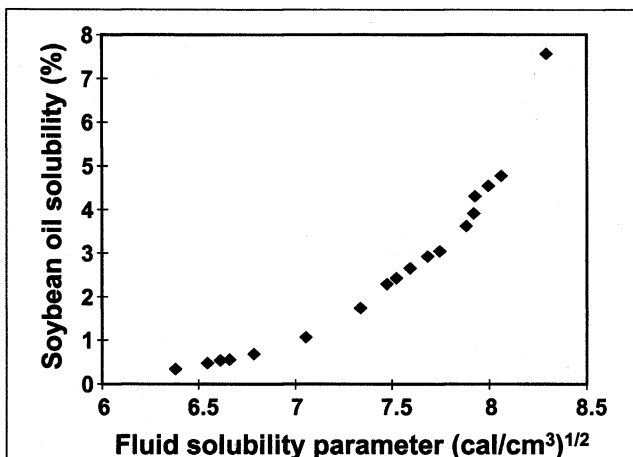
Figure 7. Soybean oil solubility versus the solubility parameters of the fluids.
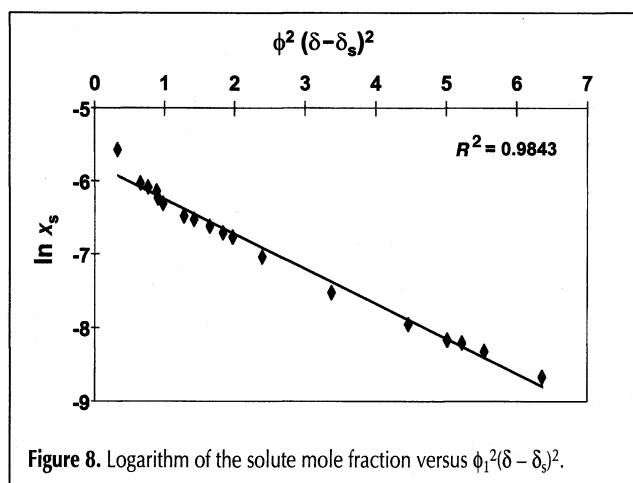
Figure 8. Logarithm of the solute mole fraction versus $\phi_1^2(\delta - \delta_s)^2$.

(approximately 2.66). For a binary fluid composition, the geometric mean approximation for δ is often used to estimate the δ value of the mixture (15). The solubility parameter is then calculated using the following equation:

$$\delta = \frac{\phi_1\delta_1 + \phi_2\delta_2}{\phi_1 + \phi_2} \qquad \text{Eq 2}$$

where δ_1 and δ_2 are the solubility parameter (Equation 1) of the two fluids, and ϕ_1 and ϕ_2 are the volume fractions of the two fluids. The required density data used for pure CO_2 were measured using the density meter, as noted previously. The density of helium was calculated using the generalized Benedict-Webb-Rubin (BWR) equation of state for helium (16). The solubility parameters of two fluids at selected temperatures and

pressures were thus calculated separately using Equation 1. Then the solubility parameter of the binary fluid mixture was calculated using Equation 2.

Figure 7 shows the supercritical fluid solubility parameter versus the soybean oil solubility. All of the solubility data from Figure 4 were used here, including data for both pure CO_2 and CO_2–helium compositions at different pressures (from 5,000 to 9,950 psi) at 70°C. As shown in Figure 7, the solubility of soybean oil increased exponentially as the fluid solubility parameter increased from 6.38 to 8.29 $(cal/cm^3)^{1/2}$. The pressure and composition of the supercritical fluids themselves did not have a very obvious effect on solubility, as shown in Table III, but the calculated solubility parameters, which are dependent on pressure and composition, demonstrated an unambiguous influence on the solubility of the soybean oil at various fluid compositions.

The Hildebrand-Scatchard equation can be used to relate the solute solubility to solubility parameters of the solute and solvent by (13):

$$\ln x_s = \frac{\Delta H_f}{R} \left(\frac{1}{T_f} - \frac{1}{T}\right) - \frac{V_s\phi^2}{RT}(\delta - \delta_s)^2 \qquad \text{Eq 3}$$

where x_s and V_s are the molar fraction and molar volume, respectively, of the solute, ΔH_f is the fusion heat of the solute, R is the gas constant, T_f is the melting point of the solute, ϕ is the volume fraction of the solvent, and δ and δ_s are the solubility parameters of the fluid and the solute, respectively. Because the only variable in Figure 7 is the solubility parameter of the solvent (supercritical fluid), the results in Figure 7 suggest that the solubility parameter of soybean oil (δ_s) at 70°C must be larger than 8.29 $(cal/cm^3)^{1/2}$; otherwise, the soybean oil solubility would not have increased exponentially with the fluid solubility parameter (δ). The δ_s value for soybean oil calculated from Fedors' structural group contribution method (17) was 8.91 $(cal/cm^3)^{1/2}$. With the δ_s value known, the solubility of soybean oil in the supercritical fluid $(\ln x_s)$ could be plotted against the values of $\phi^2(\delta - \delta_s)^2$. If the Hildebrand-Scatchard equation is valid for soybean oil in CO_2 and CO_2–helium supercritical fluids, the results should be a straight line. Figure 8 shows the diagram of $\ln x_s$ versus $\phi^2(\delta - \delta_s)^2$. Just as the equation predicts, $\ln x_2$ increased linearly as $\phi^2(\delta - \delta_s)^2$ values became smaller $(R^2 = 0.9843)$. Hence, the solubility parameter is a very good indicator for the solvation power of a supercritical fluid (18). The substantial reduction in soybean oil solubility in helium–CO_2 mixtures was the result of the decrease in the fluid solubility parameter, as Figures 7 and 8 clearly demonstrate.

However, the solubility parameter is a macroscopic parameter. On a molecular level, the effect of helium on solubility may also have been related to a disruption of the CO_2 clustering associated with the solute molecules, as reported from fundamental studies on solute solubilities in recent years (19–21). Helium may thus disrupt the intermolecular association between a chosen solute molecule and CO_2, thereby weakening the solvation power of supercritical CO_2, resulting in a lower solute solubility in the supercritical fluid. A more detailed study will be needed to understand if this molecular mechanism influences the solvation process in helium–CO_2 mixtures.

Conclusion

This study confirmed experimentally that liquid CO_2 from an HHS CO_2 cylinder contained a substantial amount of helium, which decreased as the cylinder pressure was reduced. The entrained helium in HHS CO_2 caused a significant reduction of solubility in supercritical fluids by as much as 70% for soybean oil. The reduction of solute solubility in HHS CO_2 cannot be accounted for simply by a reduction in the density, as shown in Table III. Because helium solubility in CO_2 is affected by both temperature and pressure (i.e., the fluctuating room temperature and constant declining cylinder head pressure during extraction will cause the helium content to change constantly, resulting in substantial variation in the solubility of a solute in the fluid mixture), the results in this study strongly suggest that the use of HHS CO_2 should be avoided whenever possible. For improvement of pumping efficiency, the cooling of the pump head is a much better approach.

The observation that lower solubilities of fat or oil occur in helium–CO_2 mixtures can be used to an advantage in the SFE of trace chemicals in lipid-rich samples. Because of the high solubility of lipid materials (e.g., soybean oil) in supercritical CO_2, the SFE analysis of lipid-rich samples can contain coextracted lipids, which interfere with the chromatographic analysis of trace compounds. Therefore, by using a binary mixture of CO_2 with helium or nitrogen, the solvation power of the fluid can be significantly reduced, and coextraction of lipid moieties can be substantially reduced. Because the recovery of trace compounds by SFE does not require high solute solubilities, the use of such a binary fluid will have little adverse impact on the recovery of trace levels of solutes, thereby allowing the preparation of lipid-free extracts for direct chemical analysis.

References

1. S.H. Page, S.R. Sumper, and M.L. Lee. Fluid phase equilibrium in supercritical fluid chromatography with CO_2-based mixed mobile phases: A review. *J. Microcol. Sep.* **4:** 91–122 (1992).
2. T. Gorner, J. Dellacherie, and M. Perrut. Comparison of helium head pressure carbon dioxide and pure carbon dioxide as mobile phases in supercritical fluid chromatography. *J. Chromatogr.* **514:** 309–16 (1990).
3. E. Leichter, J.T.B. Strode, L.T. Taylor, and F.K. Schweighardt. Effect of helium in helium headspace carbon dioxide cylinders on packed-column supercritical fluid chromatography. *Anal. Chem.* **68:** 894–98 (1996).
4. J.W. King, J.H. Johnson, and F.J. Eller. Effect of supercritical carbon dioxide pressurized with helium on solute solubility during supercritical fluid extraction. *Anal. Chem.* **67:** 2288–91 (1995).
5. D.E. Raynie and T.E. Delaney. Effect of entrained helium on the kinetics of supercritical fluid extraction with carbon dioxide. *J. Chromatogr. Sci.* **32:** 298–300 (1994).
6. Y.L. Hsiao and J.M. DeSimone. Effect of added helium on particle size and particle size distribution of dispersion polymerizations of methyl methacrylate in supercritical carbon dioxide. *Polym. Mater. Sci. Eng.* **74:** 260–61 (1996).
7. A. Kordikowski, D.G. Robertson, and M. Poliakoff. Acoustic determination of the helium content of carbon dioxide from He head pressure cylinders and FT–IR studies of the density of the resulting supercritical CO_2: Implications for reproducibility in supercritical experiments. *Anal. Chem.* **68:** 4436–40 (1996).
8. S. Angus. *IUPAC International Thermodynamical Tables of the Fluid State — Carbon Dioxide.* Pergamon, Oxford, England, 1974.
9. J.P. Friedrich, G.R. List, and A.J. Heakin. Petroleum-free extraction of oil from soybean with supercritical CO_2. *J. Am. Oil Chem. Soc.* **59:** 288–92 (1982).
10. S.L. Taylor, J.W. King, and G.R. List. Determination of oil content in oilseeds by analytical supercritical fluid extraction. *J. Am. Oil Chem. Soc.* **70:** 437–39 (1993).
11. H.J. Gahrs. Applications of atmospheric gases in high pressure extraction. *Ber. Bunsenges. Phys. Chem.* **88:** 894–97 (1984).
12. *Ion Exchange and Solvent Extraction.* J.A. Marinsky and Y. Marcus, Eds. Marcel Dekker, New York, NY, 1988, pp. 132–33.
13. J.H. Hildebrand and R.L. Scott. *The Solubility of Nonelectrolytes,* 3rd ed. Dover Publications, New York, NY, 1964.
14. J.C. Giddings, M.N. Myers, L. McLaren, and R.A. Keller. High pressure gas chromatography of nonvolatile species. *Science* **162:** 67–73 (1968).
15. A.F.M. Barton. *Handbook of Solubility Parameters and Other Cohesion Parameters,* 2nd ed. CRC Press, Ann Arbor, MI, 1991, p. 61.
16. R.C. Reid, J.M. Prausnitz, and B.E. Poling. *The Properties of Gases and Liquids,* 4th ed. McGraw-Hill, New York, NY, 1987, p. 47.
17. R.F. Fedors. A method for estimating both the solubility parameters and molar volumes of liquids. *Polym. Eng. Sci.* **14:** 147–54 (1974).
18. J.W. King and J.P. Friedrich. Quantitative correlations between solute molecular structure and solubility in supercritical fluids. *J. Chromatogr.* **517:** 449–58 (1990).
19. C.A. Eckert, B.L. Knutson, and P.G. Debenedetti. Supercritical fluids as solvents for chemical and materials processing. *Nature* **383:** 313–18 (1996).
20. J.F. Kauffman. Spectroscopy of solvent clustering. *Anal. Chem.* **68:** 248A–253A (1996).
21. S. Kim and K.P. Johnston. Clustering in supercritical fluid mixtures. *AIChE J.* **33:** 1603–11 (1987).