



Anomalous effects of helium head pressure carbon dioxide in supercritical fluid chromatography and extraction

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Abstract

Helium head pressure carbon dioxide cylinders are commonly used to facilitate the delivery of liquid CO₂ to supercritical fluid extraction and chromatographic pumps. It is usually tacitly assumed that the helium used to increase the delivery pressure of the CO₂ cylinders is completely insoluble in liquid CO₂ and thus remains isolated in the head space of the delivery cylinder. This assumption is invalid because up to 5 mol% helium can be entrained in the liquid CO₂ delivered from helium head pressure cylinders. Significantly, contamination of liquid CO₂ with even small amounts of helium can cause many unforeseen and usually deleterious effects in supercritical fluid chromatography and extraction schemes. The observed anomalies include decreased density of the fluid phase, irreproducible extraction and retention, ghost peaks, and even phase separation within the column or extraction vessel.

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1. Introduction

One of the technical difficulties encountered in supercritical fluid extraction and chromatography with carbon dioxide is the problem of efficient pumping of this highly compressible fluid. In order to maximize the delivery capacity of either syringe or piston pumps, the fluid in the pump heads must be liquified in order to minimize its compressibility. In practice, this condensation is usually accomplished in one of two ways, viz., by decreased temperature or increased pressure.

Probably the most common optimization method

is cooling the pump head below the boiling temperature of liquid carbon dioxide at the pump pressure. Most commercial SFC pumps are equipped with cooling jackets for the pump; however, an external refrigeration and circulation unit is required. Moreover, if the pressure is subcritical and the temperature of the analytical column, detector or associated plumbing is higher than the temperature of the pump, a liquid to gas or fluid phase transition may occur in these portions of the instrumentation. In common SFC/SFE practice, the pressures are normally supercritical and no liquid phase can exist in the system. Thus, refrigeration is an accepted and efficient pumping optimization strategy.

A simpler and equally efficient method for ensuring the delivery of liquid CO₂ to SFC pumps is to increase the delivery pressure of the storage cylinder.

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ders above the vapor pressure of liquid carbon dioxide at the ambient temperature. Such pressure enhancement is commonly accomplished by the addition of helium to the storage cylinder to produce what is usually called helium head space or helium head pressure CO₂. The vapor pressure of liquid CO₂ at 25 °C is about 63 atm. Addition of helium to the head space of delivery cylinders results in delivery pressures up to 138 atm. At this pressure, liquid CO₂ can be transferred efficiently from a storage cylinder with an eductor tube to a pump without the need for refrigeration. It is usually assumed that helium is completely immiscible with liquid carbon dioxide and thus remains isolated in the head space of the delivery cylinder so that only pure CO₂ is delivered to the pump.

Unfortunately, the assumption regarding the insolubility of helium in liquid CO₂ is not valid. Numerous authors [1–3] have shown that up to 5 mol% helium can be entrained in liquid CO₂ delivered from helium head pressure CO₂ cylinders. Independent vapor–liquid equilibrium measurements with synthetic mixtures of helium and carbon dioxide show that the solubility of helium in liquid CO₂ varies linearly with pressure over a wide range of temperatures [3–5]. The results of some of these investigations are summarized in Fig. 1 where the dotted line marks the pressure normally used for helium head pressure carbon dioxide cylinders. This figure also illustrates some of the very unusual properties of binary mixtures of helium and carbon dioxide. For example, at high pressures, the solubility of helium in liquid CO₂ increases with temperature. So the solubility of helium in liquid CO₂ would be higher in a delivery cylinder or pump at ambient conditions than in an analytical column operated at subambient temperatures *if the systems were at equilibrium*. In addition, the isotherm near the critical temperature [3] illustrates that very high concentrations of helium in liquid CO₂ can be obtained at conditions close to critical. Moreover, this particular binary system displays a very unusual phase behavior called gas–gas immiscibility of the first kind [6]. In such systems, two phases can coexist at temperatures and pressures above the critical temperature and pressure of either pure component, i.e. at conditions where pure CO₂ would be a homogeneous supercritical fluid. In fact, it has

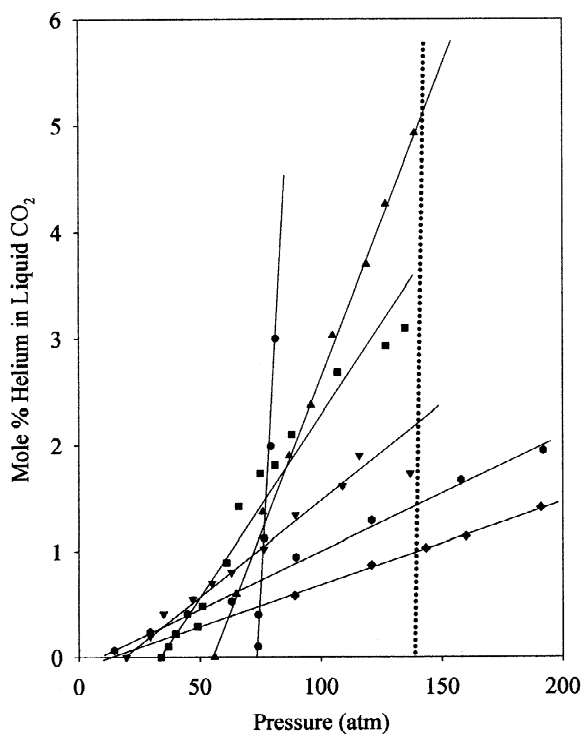


Fig. 1. Solubility isotherms of helium in liquid carbon dioxide: (●) 31 °C [3]; (▲) 20 °C [4]; (■) 0 °C [4]; (▼) -20 °C [4]; (★) -43 °C [5]; (◆) -53 °C [5].

been reported that helium and carbon dioxide will form two phases at pressures up to 8000 atm at a temperature of 80 °C [3,7].

The anomalous phase behavior of binary He–CO₂ mixtures can have unforeseen consequences in any analytical scheme involving the use of helium head pressure carbon dioxide. The primary objective of this investigation is to identify and illustrate the various unanticipated phenomena that can result from the use of helium head pressure carbon dioxide so that practicing chromatographers can recognize and overcome potential problems

2. Discussion

2.1. Density of the mobile phase or extraction fluid

The density of the fluid phase is the key parameter that controls the solvent strength of CO₂-based

chromatographic or solvent extraction systems. Contamination of CO₂ with helium reduces the density of the extraction fluid or mobile phase and results in decreased extraction efficiency [2,8,9] or increased retention time [1,10–12]. The effect of helium concentration on the density of binary CO₂–He mixtures has been measured [2] and calculated using both the Lee–Kestler [13] equation-of-state [12] and the Peng–Robinson [14] equation-of-state [15].

The Peng–Robinson equation-of-state is:

$$P = \frac{RT}{v-b} - \frac{a(T)}{v(v+b) + b(v-b)} \quad (1)$$

where v is the molar volume; b is a size parameter; and $a(T)$ is a temperature-dependent interaction parameter that can be calculated from the critical parameters and a constant, κ , characteristic of each substance:

$$a(T) = 0.45724 \frac{(RT_c)^2}{P_c} \left[1 + \kappa \left(1 - \sqrt{\frac{T}{T_c}} \right) \right]^2 \quad (2)$$

The characteristic parameter κ can be expressed as a polynomial in the acentric factor, ω :

$$\kappa = 0.37464 + 1.54226\omega - 0.26992\omega^2 \quad (3)$$

The acentric factor for CO₂ is 0.0225 [16]. For binary mixtures, the following mixing rules apply:

$$a = \sum_i \sum_j Y_i Y_j a_{ij} \quad (4)$$

$$a_{ij} = (1-k) \sqrt{a_{ii} a_{jj}} \quad b = \sum_i Y_i b_i$$

where Y_i is the mole fraction of component i . The empirical parameter, k , has been determined [15] to be $k = -22.722 + 0.1394T - (1.9887 \times 10^{-4})T^2$ for the helium–CO₂ system over the temperature range from 30 to 100 °C.

Fig. 2 shows the calculated density of pure CO₂ and a binary mixture of 5 mol% helium in CO₂ as a function of pressure at 35 °C. This figure illustrates the dramatic effect a small amount of helium can have on the density of a binary mixture. For example, at 90 atm the reduction in density caused by the presence of helium is almost 50%. It is rather surprising that the presence of even small amounts of helium can diminish the density of binary CO₂–He mixtures so significantly. Such density changes could

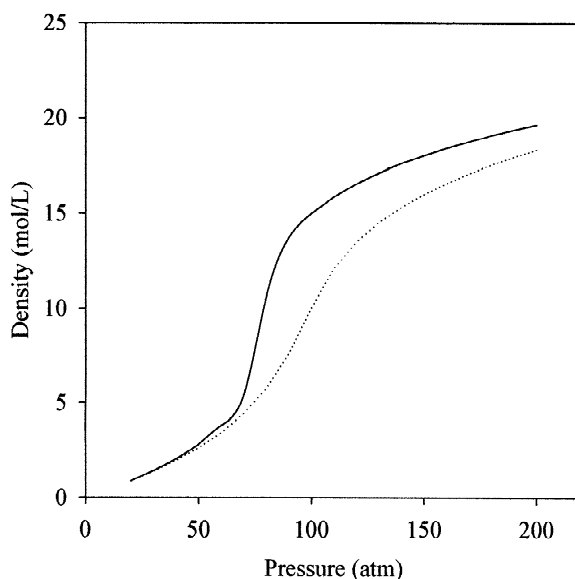


Fig. 2. Calculated density of pure CO₂ (solid line) and a binary mixture of 5 mol% helium in CO₂ (dashed line).

easily account for the 50–70% reductions in extraction efficiencies reported by King [2,9] for the extraction of soybean oils using helium head pressure CO₂. In SFC applications, the reduction in density caused by the presence of helium can result in dramatically increased solute retention.

The reduction in density can also influence chromatographic results in unexpected ways. For example, it has been shown [17–19] that CO₂ will dissolve in polymeric stationary phases as well as adsorb on C₁₈-bonded silica at conditions normally used for supercritical fluid chromatography. This phase distribution process occurs dynamically within the analytical column and can dramatically influence the retention of analytical solutes. Such phase distribution equilibria would also be influenced by density variations in the mobile phase. This phenomenon could in turn exert a secondary influence on the retention of chromatographic solutes in SFC.

Another potential problem is the determination of the mobile phase flow-rate in chromatographic columns. Thermodynamic measurements of high pressure phase distribution equilibria by chromatographic experimental methods require an accurate knowledge of the volumetric flow-rate of the mobile phase. Direct measurement of the flow-rate at high pressure

is usually impossible, and the column flow-rate must be *calculated* from the measured outlet flow-rate at ambient conditions. If the presence of helium in the mobile phase is recognized and the exact composition of the binary fluid is known, an accurate equation-of-state can be used to calculate the volumetric flow-rate at column conditions. However, if the operators are unaware of possible helium contamination, the resulting calculations can be erroneous.

2.2. Mass transport properties

The mass transport properties of CO₂ can also be influenced by the presence of helium. The viscosity and diffusivity of the binary fluids depend upon the composition, and the magnitude and even the direction of the changes are determined by the temperature and pressure of the systems. The mass transport properties influence the flow-rate of the mobile phase through the column along with the peak spreading of eluted solutes.

2.3. Slow equilibration of liquid CO₂–helium mixtures

The diffusion coefficient of helium in liquid carbon dioxide is relatively low [3] ($\sim 10^{-5}$ cm² s⁻¹). Thus, thermodynamic equilibrium of helium with liquid CO₂ in storage cylinders could require several months or even years [3]. The equilibration process is both temperature and pressure dependent, so the storage history and liquid level of a particular cylinder could influence the composition of liquid CO₂ delivered from a helium head pressure CO₂ cylinder. This phenomena is most likely the primary cause for the myriad reported [10–12] instances of irreproducibility observed in SFC experiments with helium head pressure CO₂.

2.4. Concentration pulses observed with binary mobile phases in SFC

When binary mobile phases are used in any type of chromatography, an unusual phenomenon can be observed if an injection alters the concentration of the components of the mobile phase. If one or more of the mobile phase components interacts with the

stationary phase, a concentration pulse can be generated within the column which travels through the column *at a velocity different from that of the injected component*. Thus, additional peaks may appear in chromatograms obtained with a concentration sensitive detector that responds to the mobile phase components. In the case of CO₂–He mixtures, CO₂ will interact with most SFC stationary phases, so a concentration pulse can be generated by the injection of analytical solutes. The concentration pulse will travel at a velocity determined by the mole fraction of helium in the mobile phase and the derivative of the sorption isotherm of CO₂ in or on the stationary phase. The mathematical expression for the retention volume of such a concentration pulse is [20–23]:

$$V_r = V_m + V_s Y_{\text{He}} \left(\frac{\partial q}{\partial c} \right)_c \quad (5)$$

where V_r and V_m are the retention volume of the pulse and the volume of mobile phase in the column, respectively. V_s is the volume of the stationary phase; Y_{He} is the mole fraction of helium; and c and q represent the concentrations of CO₂ in the mobile and stationary phases. The adsorption isotherms of CO₂ at high pressures often display a maximum in the amount adsorbed at the critical pressure and, thus, the concentration derivative can be zero or even negative depending upon the pressure and temperature. In some cases, the concentration pulses can appear at the column dead time. Again, if this phenomenon is not recognized, faulty interpretation of chromatographic results may occur.

2.5. Phase separation of the mobile phase within the analytical column

Finally, another strange phenomenon has been observed in chromatographic columns when helium head pressure CO₂ was used as the mobile phase for subcritical fluid chromatography. The binary CO₂–helium mixture may segregate into two phases within the column. Such phase separation leads to cumbersome three-phase systems in a chromatographic column containing a liquid or solid stationary phase. On the other hand, in an empty column, the CO₂-rich liquid phase can then act as a dynamic stationary phase to produce chromatographic separations [24].

In order to predict the experimental conditions that would result in mobile phase segregation, a complete (P, T, xy) phase diagram is required. The necessary vapor–liquid equilibrium calculations for the CO_2 –He systems can also be carried out with the Peng–Robinson equation of state by calculating the pressure and compositions that produce equal fugacities for both components in both phases. The equation for the fugacity of component 2 is:

$$\ln\left(\frac{f_2}{P_2}\right) = \frac{b_2}{b} \left(\frac{Pv}{RT} - 1\right) - \ln\left(\frac{P}{RT}(v-b)\right) - \frac{0.3536a}{bRT} \left(\frac{2[a_{12} + x_2(a_{22} - a_{12})]}{a}\right) - \frac{b_2}{b} \ln\left(\frac{v + 2.414b}{v - 0.414b}\right) \quad (6)$$

The normal algorithm for calculating vapor liquid equilibrium data for binary mixtures involves the

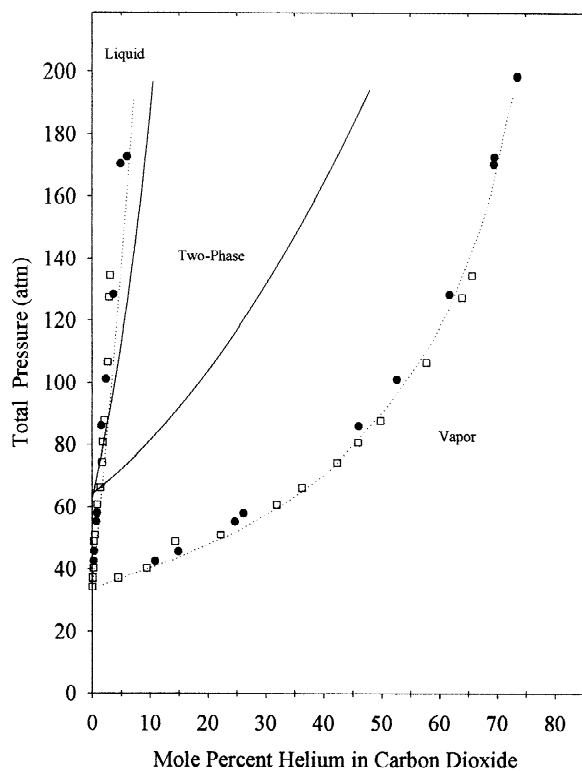


Fig. 3. Phase diagram for helium/carbon dioxide mixtures. Calculated data: solid line 25 °C; dashed line 0 °C. Literature data: (□) 0 °C [4]; (●) 1.7 °C [5].

specification of a temperature and composition of the liquid phase. In an iteration sequence, an initial value of the pressure is specified. The equation-of-state in the form of a third-order polynomial is used to calculate the molar volumes of the liquid and vapor phases. Likewise, an initial estimate of the vapor phase composition is necessary to calculate the fugacities of each component in each phase. If the fugacities are not equal within a specified error, the iteration sequence is repeated with new estimates of the pressure and vapor phase composition.

The results for such calculations at 0 and 25 °C are shown in Fig. 3 along with literature data [4,5] for comparison. Surprisingly, at subcritical temperatures very small concentrations of helium can cause the binary CO_2 –He system to form two phases at conditions where pure CO_2 would be a liquid. Even at room temperature (25 °C), phase separation occurs at helium concentrations as low as 1 mol% at 70 atm and the system will form a single gaseous phase at compositions greater than 4 mol% helium. If such unusual phase behavior is not recognized, formation of two phases in an extraction vessel or chromatographic column operated at ambient temperature could occur with unforeseen consequences.

3. Conclusions

Contamination of a gas or fluid with small amounts (<5 mol%) of an inert gas would normally not be expected to produce truly significant alterations in the physical properties or phase behavior of the gas or fluid. However, in the particular case of carbon dioxide, the influence of low helium contamination is surprising large especially at high pressures and low temperatures. Numerous other authors [1–3,8–12] have previously observed some of the anomalous phenomena discussed above but in a more or less piecemeal fashion. At least three of these authors [1,2,15] have recommended against the continued use of helium head pressure CO_2 cylinders primarily because of the effect of helium on the density of the fluid phase. These warnings are, however, spread throughout the chromatographic literature. The myriad deleterious effects are detailed here in an effort to consolidate all of the findings of the various authors over the years concerning differ-

ent problems observed with helium head pressure CO₂. Despite the repeated warnings, however, many practitioners are unaware of the perils presented by the unusual properties of the binary CO₂–He system that can result from the contamination of SFC-grade CO₂ with even very small amounts of helium. The problem is compounded by the fact that commercial suppliers have not provided adequate warnings of the potential problems posed by the use of helium head pressure CO₂ cylinders for SFE or SFC applications.

Acknowledgements

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