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Unified Chromatography with CO₂-Based Binary Mobile Phases

Exploring chromatographic schemes involving binary mobile phases with CO₂.

Chromatographic methods are usually classified according to the physical state of the mobile phase—for example, GC, supercritical fluid chromatography (SFC), and LC. This admittedly oversimplified classification scheme exists partly because of the similarity of most chromatographic stationary phases, which are usually either polymers or solid adsorbents and are often deactivated by some chemically bonded moiety. Because of the banality of stationary phases, any attempt to unify the various forms of chromatography will naturally center on the mobile phase (1–4). To date, many researchers consider the most promising approach to unification to be the use of CO₂ as the mobile phase because of the “tunable” solvent strength of this fluid.

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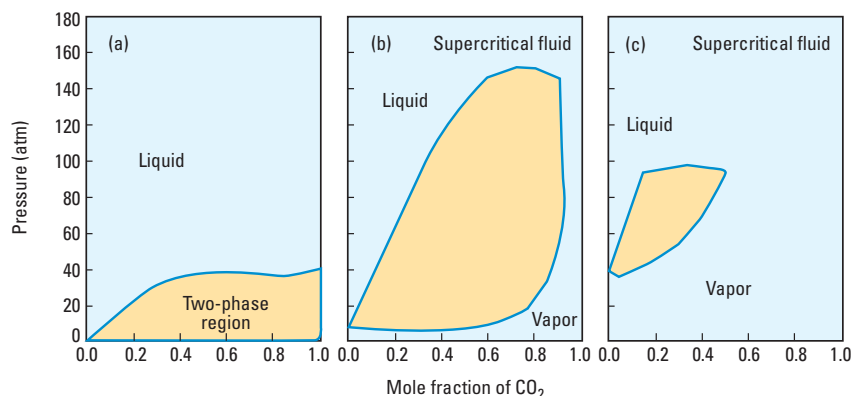


FIGURE 1. Calculated phase diagrams for binary blends of methanol and CO₂ at three temperatures: (a) 0 °C, a temperature below the critical temperatures of both components; (b) 100 °C, a temperature intermediate between the critical temperatures of the two pure components; and (c) 200 °C, a temperature close to but below the critical temperature of methanol.

Unfortunately, neat CO₂ alone is not a good mobile phase because of its poor solvent properties. For this reason, a polar, organic “modifier” is usually mixed with CO₂ to produce a mobile phase with improved solvent properties for polar solutes without significantly altering the physical properties of the mobile phase. Development of practical chromatographic schemes involving binary mobile phases with CO₂ as a component is a promising area of research, and analytical applications of this technology constitute a significant body of chromatographic literature. These systems, although chromatographically useful and interesting, are also very complex, difficult to control experimentally, and often intractable to theoretical modeling and interpretation. For these reasons, a broad discussion of these fascinating systems is both timely and appropriate.

Mobile versus stationary phase

The normally assigned role of the mobile phase in any chromatographic system is simply to transport the solutes through the column. The classical model assumes that all solutes in the mobile phase travel at the same velocity as the bulk mobile phase itself. There are exceptions to this simplistic model, such as size-exclusion and hydrodynamic chromatographies, in which various regions of the mobile phase travel at different velocities. Nevertheless, assuming that all of the solutes spend the same amount of time in the mobile phase, any observed chromatographic resolution of the solutes must be determined solely by the amount of time each solute resides somewhere in the column other than in the mobile phase.

This concept illustrates the normal role of the so-called stationary phase. If molecular diffusion is neglected, the stationary phase provides a place in the column for the solutes to reside without undergoing axial movement. Researchers, however, should

keep in mind that not all the stationary-phase components must be fixed within the column. Also, not all of the mobile phase must move at a uniform velocity. The analytical solutes may interact with mobile-phase components, stationary-phase components, or mobile-phase components dissolved in or adsorbed on the stationary phase (5).

Binary mobile phases and CO₂

Binary mobile phases are used often, particularly in HPLC and SFC. Some very elegant separations have also been achieved with gradient elution and solvent programming techniques. Such multicomponent mobile phases are popular primarily because they can be used to provide continuous control of the solvent or elution strength of the mobile phase; provide continuous, external control of the solvent strength of the stationary phase (i.e., without physically changing the nonvolatile liquid phase or the column itself); deactivate the solid support in packed-column applications; improve the mass transport properties of the mobile phase; and provide a variable-composition fluid phase for thermodynamic investigations of phase-distribution equilibria.

The ability to continuously alter the separating power of a modern chromatographic system represents a major advantage of SFC and HPLC over GC, for which temperature is the only available programming parameter. Although temperature-programmed GC is a powerful and often-used analytical tool, gradient elution HPLC methods and the use of modifiers in SFC have provided an extra dimension for the control of chromatographic selectivity.

The choice of components for binary mobile phases is somewhat restricted primarily because of problems with chromatographic detection systems and the paucity of physical property data and theoretical models for binary systems. However, CO₂ is a particularly useful mobile-phase component because it does not interfere with most chromatographic detectors. It is also well characterized as a pure component, and its binary mixtures are readily modeled with appropriate equations of state. Finally, it is safe, easy to handle, and has minimal environmental impact and mild critical constants.

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The behavior of the binary mobile phase

Phase separation of binary mobile phases is not generally a problem for HPLC systems because completely miscible liquid solvents are the norm. However, if organic modifiers are added to CO₂ or an organic liquid is “enhanced” by adding CO₂ (6–10) to form a binary chromatographic mobile phase, some unique situations can occur. The primary problem is that such

binary mobile phases can, under certain conditions, split into two (gas and liquid) phases. This dramatically complicates the phase distribution equilibria that determine the resolution of analytical solutes in a chromatographic column. Such CO₂-based binary systems are very complex, and their phase behavior can be quite convoluted, as Eckert showed for a series of such systems involving several common SFC modifiers with CO₂ (11, 12). Moreover, significant interactions between the mobile-phase components and common chromatographic stationary phases are usually evident (11–14).

These mobile- and stationary-phase interactions mean that some of the mobile phase becomes part of the stationary phase. Consequently, determining the exact volume and composition of the stationary phase is very difficult. In this case, the stationary phase is created in dynamic equilibrium with the mobile phase, so any change in the properties of the mobile phase will be reflected in the stationary phase, most often in a nonlinear fashion (15). This dynamic stationary phase is fascinating because it exists only inside the column and is very difficult to characterize, unlike the mobile phase, which can be analyzed in detail when it exits the column.

To understand the chromatographic implications of such complex binary systems, a thorough knowledge of their phase behavior is necessary. This information is best conveyed in the form of phase diagrams, even though phase diagrams of multicomponent systems can sometimes be very difficult for the analyst to interpret. A phase diagram for a typical binary SFC-modifier system is shown in Figure 1. This diagram illustrates the influence of composition on the phase behavior of a simple CO₂-methanol system at three temperatures. The isotherms were calculated from the Sanchez-Lacombe model (16, 17).

Figure 1a shows the calculated phase diagram at 0 °C, which is below the critical temperatures of both components. The blue boundary lines—the so-called bubble and dew lines—delineate the orange two-phase region and are continuous throughout the composition range. The system contains liquid, gas, or both phases in dynamic equilibrium, but no supercritical fluid state exists at this temperature. Two phases can coexist over a range of compositions if the pressure is lower than the vapor pressure of liquid CO₂ at 0 °C (~34 atm) but higher than the vapor pressure of methanol (<1 atm). Likewise, at a fixed composition, the bubble-point pressure is higher than the dew-point pres-

sure. Between these two pressures, two phases will coexist in the system. The two phases will have different compositions determined by the pressure.

Unlike the vapor-liquid line of single-component systems, the two-phase region for this binary system can extend over a relatively wide range of pressures and compositions. Such conditions are usually avoided in chromatographic practice and, thus, represent an unpleasant gap in any unification scheme. On the other hand, it is comparatively easy to maintain a stable, two-phase system within a chromatographic column without precise control of the temperature and pressure, if that is the desired mode of operation.

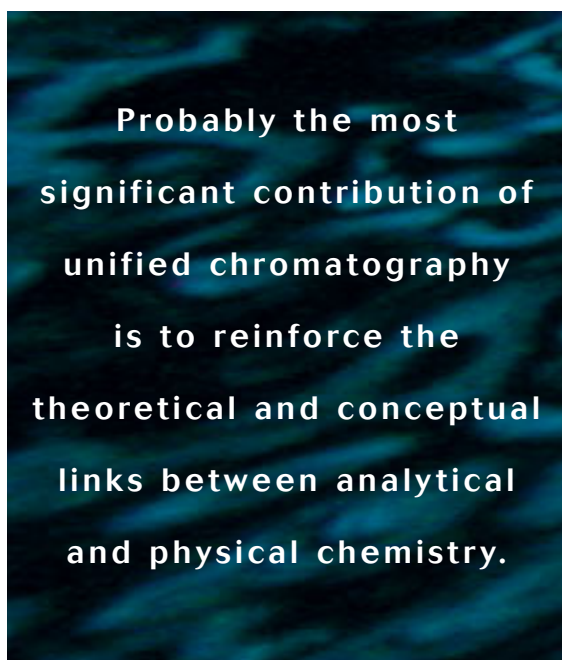
Figure 1b shows the phase behavior for the same system at 100 °C, which is intermediate between the critical temperature

of pure CO₂ (31 °C) and that of pure methanol (240 °C). At this temperature, the two-phase region does not extend over the full composition range. That is, at 100 °C, there is a maximum pressure and a maximum composition where gas and liquid phases cannot coexist. Likewise, for each composition, there is a maximum temperature and pressure above which gas and liquid phases cannot coexist. These maxima can be related to the critical points of the binary systems. But the precise specification of the critical point of a mixture (temperature, pressure, and composition) can be quite complicated and well beyond the scope of this article if the maximum temperature, pressure, and concentration do not exactly coincide, as is often the case. The practicing chromatographer only needs to know the minimum temperature and pressure required

to ensure a single-phase carrier fluid (i.e., to avoid the two-phase region). Surprisingly, the two-phase region extends to relatively high pressures well beyond the range where pure CO₂ would be in a liquid state.

At high concentrations of CO₂, a supercritical fluid is formed. Depending on the pressure, such a fluid can have the properties of a gas—namely, low density and weak molecular interaction. It can also act as an intermediate state or as a liquid, which has high density and strong intermolecular interactions. But the supercritical fluid is not a separate phase with properties of both a liquid and a gas.

Figure 1c shows that at 200 °C, the composition range over which a supercritical fluid can be formed is very large. If the temperature is raised above the critical temperatures of both



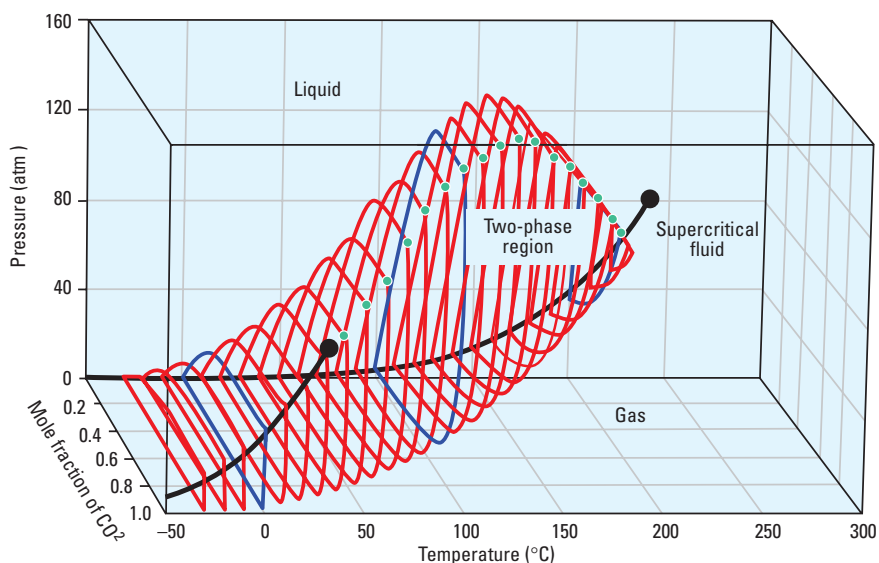


FIGURE 2. 3-D phase diagram for methanol and CO_2 . The blue lines represent isotherms from Figure 1, the black lines and circles represent vapor–liquid lines and critical points for the pure components, and the green circles represent the critical points at each temperature.

methanol and CO_2 , the entire system will form a single, homogeneous fluid phase. This is not true for all binary mixtures, and many different types of phase behavior have been observed (18). One of the strangest phenomena is called gas–gas immiscibility. This anomaly occurs when the critical temperature of a binary mixture is greater at certain compositions than the critical temperature of either pure component. In this case, two phases may form in a binary system at temperatures above the critical temperature of the less volatile component (i.e., at temperatures where both pure components would be gases).

The importance of phase diagrams

To fully exploit the phase behavior of these systems for chromatographic purposes, it is necessary to display the phase diagram in a manner that emphasizes temperature, pressure, and composition. Three-dimensional (3-D) phase diagrams are the best means of displaying the full range of experimental conditions that could result in the formation of two phases within a chromatographic column. This type of full-phase diagram for the CO_2 –methanol system is depicted in Figure 2. In this diagram, the critical points are marked with green circles, and the black lines represent the vapor–liquid lines of the pure components. This figure shows that two-phase systems can be avoided by working at a high pressure, high temperature, or high-mole

fraction of CO_2 . Conversely, the diagram also clearly delineates the exact experimental conditions required to create a two-phase system, if that is desired.

Because of the difficulties in measuring or calculating full-phase diagrams, multicomponent-phase diagrams are often displayed in only two dimensions by neglecting the composition axis, as shown in Figure 3. This 2-D phase diagram is particularly valuable for method development for SFC applications to avoid the inadvertent formation of two phases within a chromatographic column.

fraction of CO_2 -modifier systems.

Most chromatographic applications involve an organic liquid modifier that is miscible with liquid CO_2 and has higher critical pressure and temperature than CO_2 . These systems display what is called type I phase behavior, in which the critical loci of the mixtures exist continuously over the full composition range as shown in Figures 2 and 3. The critical parameters of the mixture may be higher than, lower than, or in between the critical constants of CO_2 and the modifier. Extensive com-

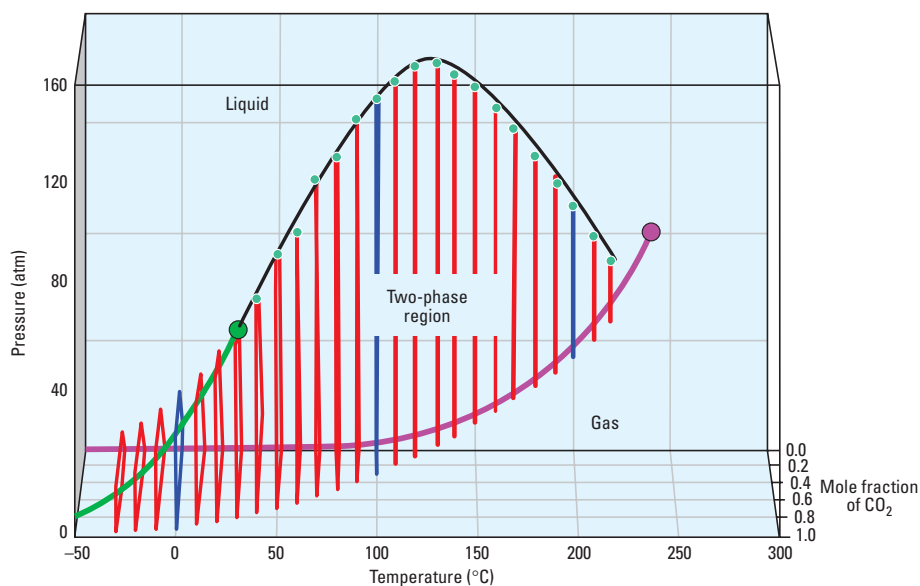


FIGURE 3. 2-D phase diagram showing the critical loci.

pilations of critical data for mixtures of CO₂ and liquid modifiers have been published (18–22). For chromatographic purposes it is often sufficient to know the range of the critical loci without detailed knowledge of the composition. Thus, 2-D plots showing only the critical temperatures and pressures without any compositional detail are sometimes sufficient.

Understanding the binary mobile-phase diagram

Not all binary mobile-phase combinations are so well behaved as the methanol–CO₂ system illustrated in Figures 1–3. For example, mixtures of CO₂ with inert gases display type III phase behavior that is usually observed for mutually immiscible liquids. In this case, the critical loci are discontinuous. Such phase behavior is illustrated in Figure 4 for the helium–CO₂ system at several temperatures. In this example, the isotherms were generated from experimentally measured data (23, 24). The critical points were extrapolated from experimental data reported by Kordikowski et al. (25).

The phase diagram in Figure 4 is discontinuous in composition. That is, the two-phase region extends far above the critical pressure and temperature of either pure component, and there is no measurable critical point over a wide range of composition. The system thus displays gas–gas immiscibility of the first kind (18). In fact, the reported critical points extend as high as 8000 atm and 80 °C (26). Quite surprisingly, very small concentrations of helium force the helium–CO₂ system to break up into two phases at conditions where pure CO₂ would be a single-liquid phase. In fact, about the only way to avoid the formation of two phases in such a system is to operate at temperatures greater than 30 °C or at pressures less than the vapor pressure of pure liquid CO₂, where a single gas phase would be formed. This unusual phase behavior can result in unexpected problems when helium head pressure CO₂ cylinders are used for supercritical-fluid extraction and SFC applications.

On the other hand, such systems that have this unusual phase behavior provide a very simple method for performing gas–liquid chromatography with a volatile stationary phase (27). In this case, the CO₂-rich liquid phase can condense on the walls of an empty capillary column to form a dynamic stationary phase. A helium-rich gas phase can form under conditions where pure CO₂ would be a liquid, and this gas can act as the mobile phase. The two-phase systems are experimentally

stable because small fluctuations in temperature and pressure cause only concomitant changes in the composition of both. Likewise, small variations in the composition of the binary phase delivered by the pump cause only small changes in the relative amounts of the gas and liquid phase in the column. In neither case will fluctuations in the experimental variables cause a change in the number of phases in the column.

In addition to helium–CO₂ mixtures, two phases can be formed from common SFC modifiers and CO₂, such as the methanol–CO₂ systems described in Figures 1–3. For example, a simple chromatogram showing the separation of a series of hydrocarbons in an empty capillary column dynamically coated with liquid methanol and CO₂ is shown in Figure 5. In this experiment, a mass-specific detector was used, and the monitored masses are indicated in the figure. In our experience, this unique

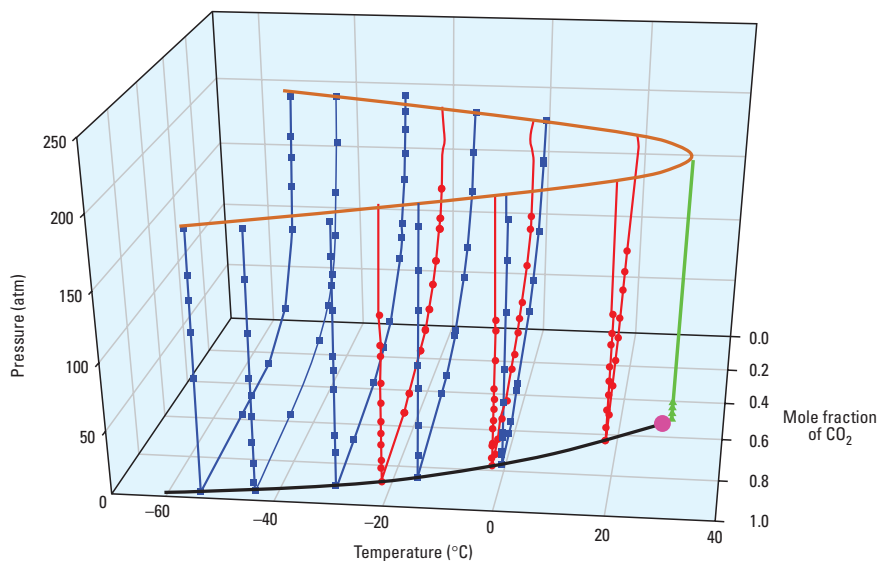


FIGURE 4. Full-phase diagram for helium–CO₂ system: red lines (23) and blue lines (24) are data from the literature; the black line is the vapor–liquid line for pure CO₂; and the green line indicates critical points (25).

type of chromatography is very gentle and avoids all of the problems, including maintenance and cost, commonly associated with classical chromatographic stationary phases.

Unified chromatography and its contributions to analytical chemistry

We have outlined a multicomponent, multiphase variant of gas–liquid chromatography that seems to fill the final major gap remaining in the unification scheme. Rather than avoiding the for-

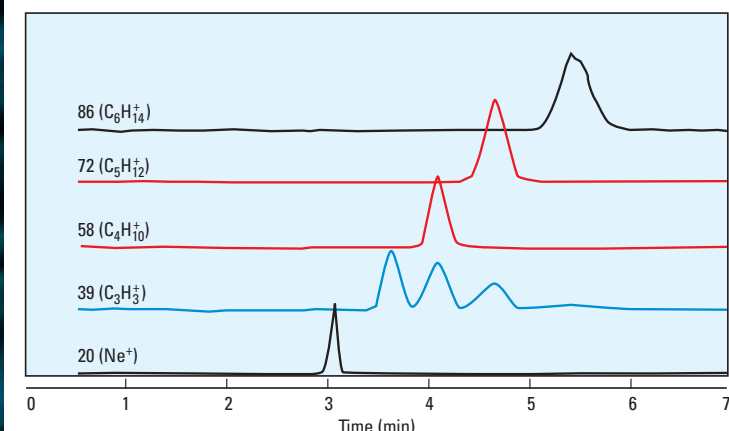


FIGURE 5. Chromatogram illustrating the separation of a series of *n*-alkanes in an empty column dynamically coated with liquid CO₂ and methanol. Column: 500 $\mu\text{m} \times 760$ cm fused-silica-lined stainless steel; mobile phase: methanol-CO₂ (20:80 mole %); stationary phase: none; temperature: 50 °C; pressure: 70 atm.

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mation of two phases within a chromatographic column, this approach makes use of them. In fact, the two-phase region can be explored advantageously to obviate the need for a nonvolatile stationary phase.

Furthermore, it is possible to develop chromatographic applications throughout what is called “phase space.” The continuum can include mobile phases that are gases, liquids, supercritical fluids, and gases in dynamic equilibrium with liquids. Inert gases mixed with CO₂ are particularly useful binary mobile phases for this type of application because they do not interfere with any of the common SFC detectors. However, these are not the only systems that could be used to create dynamic stationary phases.

Probably the most significant contribution of the idealized concept of unified chromatography is to reinforce the vital theoretical and conceptual links between analytical and physical chemistry. Chromatography can provide the necessary experimental results for the development of solution models for complex multicomponent, multiphase systems at high pressure. Conversely, solution models such as those developed by Sanchez and co-workers (16, 17) or Martire and Boehm (28) can be used to model complex chromatographic systems. Unified chromatography using all phases and combinations of phases may provide an excellent experimental method for investigating very complex systems that are not amenable to more classical gravimetric or volumetric methods. This synergism is a unique feature of chromatography and, hopefully, will lead to the development of new and innovative separation methods.

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