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Journal of Chromatography A, 1037 (2004) 393–403
JOURNAL OF CHROMATOGRAPHY A
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Review

Determination of pressure–temperature coordinates of liquid–vapor critical loci by supercritical fluid flow injection analysis

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Abstract

Knowledge of phase behavior as sample is transferred through a chromatograph is necessary for the user to either take advantage of desirable effects, such as peak focusing possibilities, or to avoid disastrous peak broadening. Users staying within the norms of conventional chromatographic techniques may not realize the phase behavior events that might be happening or that might be avoided by virtue of the parameter values they use. However, users working with unconventional conditions or with unconventional fluids, such as near-critical or supercritical fluids, must have an awareness of phase behavior through their chromatograph to ensure success. Complete phase diagrams of binary fluids are rare. However, most chromatographic parameters can be set using only knowledge of the temperature and pressure coordinates of the appropriate critical locus. These coordinates can be quickly determined for Type I binary mixtures using chromatographic equipment and a peak-shape observation technique to perform a simple flow injection experiment. Results and chromatographic applications of this knowledge will be summarized.

Keywords: Reviews; Liquid–vapor equilibria; Supercritical fluid chromatography; Flow injection analysis; Pressure effects; Temperature effects; Mobile-phase composition

Contents

1. Introduction

Let us imagine injecting a small volume of one fluid, *b*, into a stream or another fluid, *a*, flowing through a tube. This is representative of injection, in many forms of chromatography, of a sample solvent into a mobile phase. The mass-transfer behavior of solutes present in *b* will be influenced by the phase behavior of the *a*–*b* mixture. At one extreme, *a* and *b* are miscible, Laminar flow develops, and the solutes broaden due to the differences in flow velocities over the cross-section of the tube. At the other extreme, sharp phase boundaries appear between *a* and *b*. If the boundaries hold together, the resulting segmented flow minimizes broadening of the plug of *b*. While phase separation and segmented flow might be desirable if we want to minimize the broadening of *b* while transferring it with *a* through a tube, if our goal is chromatography then the presence of two

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doi:10.1016/j.chroma.2003.11.058

fluid phases, *a* and *b*, both acting as mobile phases, would greatly complicate the mass transfer. Understanding what phases exist, how they are transported, and how they change during transport is fundamentally important for successful chromatography.

In HPLC we generally dissolve the sample in mobile phase, or in the weaker component of a binary or ternary mobile phase, to avoid phase separation problems. GC can be more complicated: Cold injection techniques deliver liquid sample directly to the column where the liquid is subsequently evaporated by combination of the mobile-phase flow and elevated temperature. Vaporizing injectors evaporate liquid samples prior to delivery to the column inlet, but some injection techniques may purposely or inadvertently condense the sample solvent on the column.

Supercritical fluid chromatography (SFC) and related techniques involving mobile-phase components at temperatures near or above their critical temperature, are even more complicated. It is not always clear or predictable how many phases may be present at a particular time or location in the chromatograph as temperature (*T*), pressure (*P*), and composition (*X*) change both temporally and spatially in the course of the process. Therefore, some knowledge of the phase behavior of the system formed upon injecting sample into mobile phase is necessary to properly set various parameters like pressure and temperature, to take advantage of focusing effects when possible, and to properly transfer separated peaks out of the column and into the detector without introducing broadening or transfer artifacts.

Although virtually any solvent that is chemically stable at the required temperature may be used in SFC and related techniques [1–6], CO_2 is usually a major mobile-phase component. Much information is available on the phase behavior of mixtures containing CO_2 over narrow regions of phase diagrams [7], but complete phase diagrams of binary mixtures of CO_2 and common SFC mobile-phase modifiers and sample solvents are rare. These phase diagrams can be straightforwardly generated using high-pressure view cells or commercial phase analyzers (for example, [8]), but such equipment is not always available to chromatographers. Fortunately, knowledge of the temperature and pressure coordinates of the critical locus, rather than the complete phase diagram, is frequently all that is needed for setting parameters in SFC [9–17]. These coordinates can be estimated quickly using an open-tubular SFC instrument to perform a flow injection experiment [9,14–17]. We will describe the technique, summarize the results to date, and give examples of using this knowledge in setting chromatographic parameters.

2. Phase behavior of Type I binary mixtures

The phase behavior characteristics of pure materials are well known and conveniently displayed by a *P–T* phase diagram, Fig. 1A. The region of this two-dimensional diagram

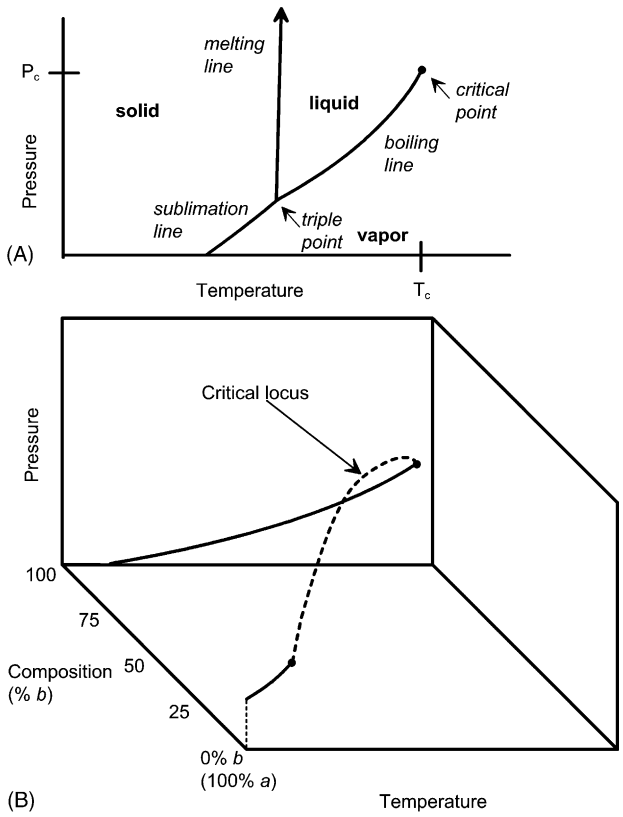

Fig. 1. (A) The phase behavior of a pure, stable fluid can be shown in a two-dimensional phase diagram as in the pressure–temperature diagram shown here. The two-phase liquid–vapor region exists along the boiling line. (B) A binary mixture requires a third dimension since the fluid composition is variable. The boiling lines of the pure components, *a* and *b*, are shown in the limiting planes of the composition axis. (The melting and sublimation lines are omitted from the figure.) Each of these boiling lines ends at its critical point. A locus of mixture critical points (the dashed line) spans the composition dimension, connecting the critical points of the two pure components.

where liquid and vapor can co-exist is a curved line, the boiling line. If we were to conduct a series of observations of the liquid and vapor phases in equilibrium as we move along the boiling line, we would see that the liquid becomes more vapor-like and the vapor becomes more liquid-like as we go in the direction of higher temperature and pressure. Eventually, the properties of the liquid and vapor phases merge at one point, the critical point. This point exists at the end of the boiling line, and the coordinates of the critical point are the critical temperature (T_c) and the critical pressure (P_c). At higher temperatures, there is only one fluid phase. It, like a gas, will expand to uniformly fill its container, but it will also dissolve solutes like a liquid when compressed sufficiently. Unlike liquids, the intermolecular distances and the resulting solvent strength of this fluid are continuously variable from zero to some maximum practical value. Both temperature and pressure affect the fluid density. At constant temperatures above T_c, the fluid density can be easily and continuously adjusted via the pressure.

Type I binary mixtures are formed from two pure fluids that are miscible as liquids [18–20]. Several other mixture types also exist for non-miscible mixtures. However, these mixtures are usually avoided in chromatography because of their inherent miscibility gaps and complicated phase behavior. Therefore, we will mostly limit the scope of this report to Type I mixtures and to more complicated systems that behave like Type I mixtures at temperatures of chromatographic interest.

The complete phase behavior of a Type I binary mixture requires a three-dimensional diagram as shown in Fig. 1B. The boiling lines for the two components, *a* and *b*, exist in the *P–T* planes at the limits of the composition axis. Just as each pure fluid has a unique critical point, every possible mixture of *a* and *b* has a critical point. The locus of *mixture critical points* spans the space of the phase diagram connecting the critical points of pure *a* and pure *b* as shown in Fig. 1B.

For binary mixture systems where the composition is adjustable, the region of liquid–vapor equilibrium is not a boiling line as with a pure liquid, but a volume in the three-dimensional phase diagram, as illustrated in Fig. 2A. At any *P–T–X* coordinate within this two-phase region, liquid and vapor phases separate, and the composition of each phase is given by the intersection of a tie line (running parallel to the *X*-axis through the point *P*, *T*, *X*) with the boundaries of the two-phase volume. The overall ratio of *a* to *b* is defined by the value of the *X* coordinate, but the ratio of the amount of the two phases present is determined by the value of the *X* coordinate and the composition of each phase. A mixture critical point exists at the highest-pressure point on the loops of isotherms drawn anywhere between the critical temperatures of the two pure fluids.

In chromatography and many other techniques, it is often sufficient to use only the *P–T* coordinates of the critical locus, rather than the complete three-dimensional phase diagram, for selecting *P* and *T* values for experiments or processes. If we project the critical locus and the pure-fluid boiling lines from Figs. 1B or 2A into the *P–T* plane, the resulting two-dimensional representation is shown in Fig. 2B. This provides enough information to unambiguously define the *P–T* region where phase separation is not possible at any composition, and the region where phase separation might be possible depending on the overall composition, as shown in the figure.

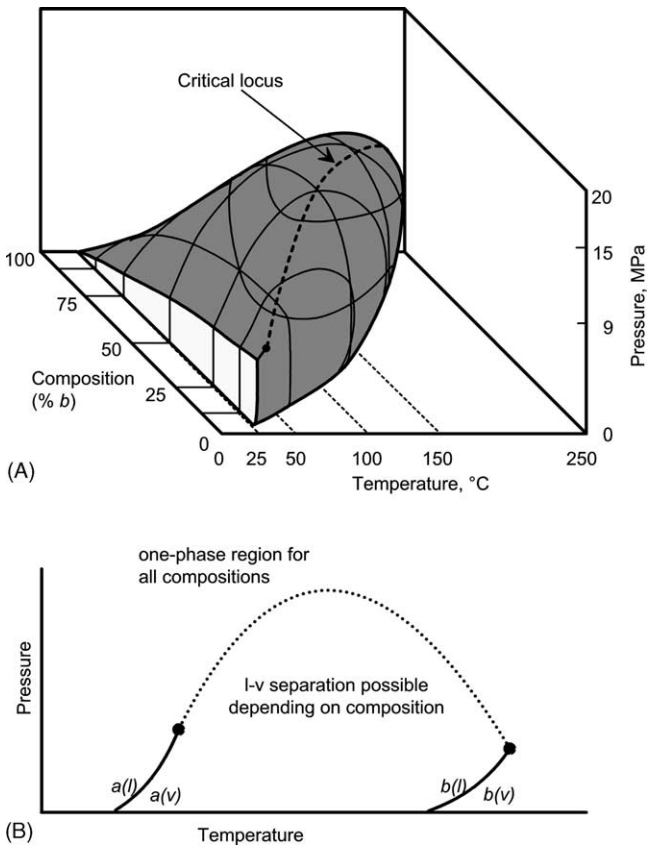

Fig. 2. (A) The two-phase liquid–vapor region for a Type I binary mixture is a volume in the three-dimensional phase diagram as indicated here. The critical locus runs over the top of the two-phase region. The composition of the two phases existing at a *P–T–X* point within the two-phase volume is given where the surface is intersected by a tie line through the point and running parallel with the *X*-axis. (B) Projecting the critical locus of the three-dimensional phase diagram onto the *P–T* plane produces this figure. Only one fluid phase can exist in the *P–T* region outside the critical locus. Inside, liquid–vapor phase separation may be possible depending on the composition. Liquid–vapor phase separation will occur at every point inside the critical locus if all possible composition values are considered.

3. The flow injection peak-shape method

This technique [9] can be implemented using open-tubular SFC instrumentation by replacing the normal SFC column with several meters of fused-silica tube. This tube may be deactivated but is not coated with a stationary phase. The inlet of the tube is connected to the injector (at room temperature), and the outlet is interfaced to the detector. When CO_2 is a component in the binary system being investigated, then it is pumped continuously through the instrument under pressure control at the pump. A flame-ionization detection (FID) system is used because it is blind to the CO_2 but responds to organic liquids injected into the CO_2 stream. A flow restrictor interfaces the outlet of the fused-silica tube to the FID system.

The experiment begins by selecting a test temperature and pressure, both of which are above the critical values for CO_2. Flow of CO_2 is established at the desired pressure, and the detector signal is allowed to stabilize. A small volume of the organic liquid is then injected into the tube using the injector. If this liquid forms a Type I mixture with CO_2, then there will be no phase separation in the room-temperature injector or the room-temperature section of the tube if all this is below the critical temperature of CO_2; thus both components are liquids and are miscible if the mixture is Type I. This is depicted in Fig. 3A. The plug of organic liquid is transported into the heated region of the tube by the flowing CO_2. It is important that the plug volume be large enough that a

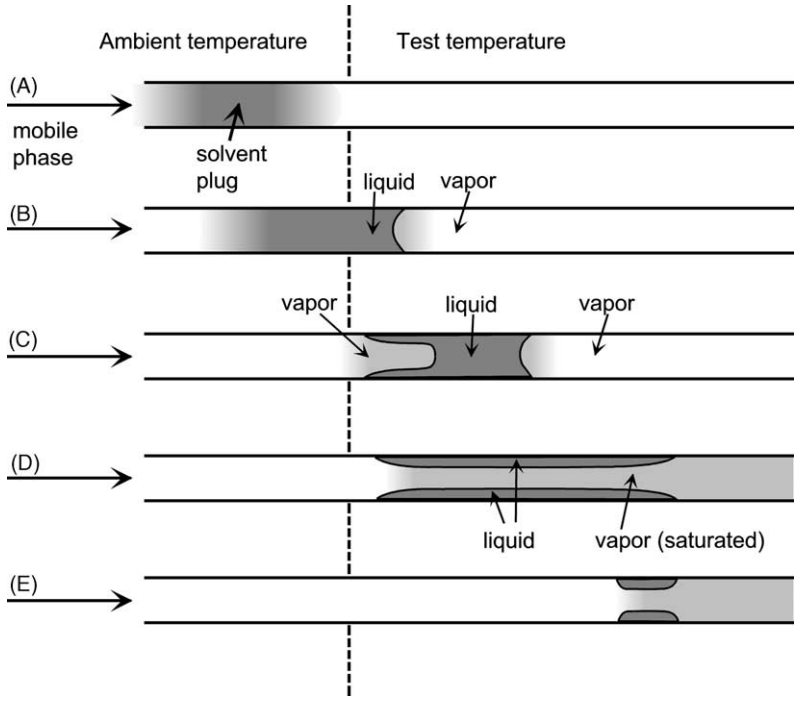

Fig. 3. Schematic depiction of the phase behavior upon the transfer of a plug of liquid injected into a stream of CO_2 at room temperature with an oven downstream set at the test temperature. The pressure-controlling pump and injector are upstream (left in the figure) and the flow restrictor and detector are downstream (right). The pressure and oven test temperature in this example correspond to a point under the critical locus in Fig. 2B. Phase boundaries, when present, are indicated with solid black lines. (A) No phase boundary develops in the room-temperature section of the apparatus where the two components are miscible as liquids. (B) When the injected solvent plug reaches the oven, a leading phase boundary develops. (C) When the trailing end of the solvent plug reaches the oven, a trailing phase boundary develops. Liquid begins dynamically coating the walls as indicated. (D and E) The solvent plug is transported down the tube until it is exhausted by the coating process. The flooded zone may be several meters long in reality. Liquid is removed by evaporation and transport in the vapor phase. The vapor exiting the column is saturated with the component that condensed as long as liquid remains coating the tube.

large fraction of it remains undiluted by CO_2 when the plug reaches the heated zone.

Phase separation may occur at the new temperature, depending on the pressure. If phase separation occurs, then a phase boundary will form between the CO_2 and the leading end of the organic liquid plug. At this point there is only one phase boundary in the system as shown in Fig. 3B. As the trailing end of the organic plug enters the heated zone, a second phase boundary will form. If the organic liquid wets the tube walls, then the phase boundaries would be concave on the CO_2 side if there were no flow. However, the flow likely distorts the leading phase boundary, although its shape is unimportant to the experiment. It is what happens at the back end of the plug that is important: as the trailing end of the plug is transported into the heated portion of the tube by the flowing CO_2, the organic liquid will dynamically coat the walls of the tube, Fig. 3C, in much the same process that a GC column is dynamically coated with a solution of stationary phase during manufacture [21]. This coating process both narrows the inside diameter of the tube available for the trailing CO_2 and depletes the volume of the main part of the plug. Thus, the center of the trailing phase boundary travels faster than the leading phase boundary, eventually catching it and breaking through as the plug is exhausted in the coating process. The coated part of the tube is referred to as the *flooded zone*. If the plug is too large or the tube too short, then the flooded zone will exceed the length of the tube, and the injected liquid will reach the detector. This has to be avoided by experimentally adjusting the injection volume.

The liquid coating is removed by evaporation into “dry” CO_2 delivered from upstream. As the CO_2 flows over the coating, evaporation occurs, saturating the CO_2 with the organic vapor. The saturated CO_2 is then transported through the remaining flooded zone and to the FID system, Figs. 3D and E. Thus, the organic component is removed as vapor, saturating the CO_2-rich phase. This results in the appearance of an FID signal as the organic vapor arrives at the detector. Since the organic vapor concentration is saturated and its concentration is controlled by the phase behavior of the system at the oven temperature and the system pressure, the resulting signal is constant until all of the organic coating has been evaporated and transported to the detector. Thus, the resulting peak in the FID time trace is rectangular shaped if phase separation occurs, and the height of the signal is representative of the mass flow rate of the organic component into the detector. If phase separation does not occur, a liquid coating is not formed on the tube and the organic

component is transported to the detector with ordinary chromatographic broadening as would be associated with a solvent peak. Such a peak is quite tall by comparison, and typically has a distorted Gaussian appearance with no indication of saturation of the mass transport process.

The procedure to map a critical locus involves selecting a temperature, then making injections at various pressures while looking for the pressure where the peaks change from their rectangular appearance to distorted Gaussian. This transition pressure provides an estimate of the mixture critical pressure corresponding to the oven temperature. Repeating at various temperatures produces a set of *P–T* coordinates that, when plotted, provide an estimate the critical locus *P–T* projection.

It is necessary to vary the volume of organic liquid injected to be certain that the disappearance of the rectangular peak shapes is not occurring due to undersized injections combined with dilution and natural broadening in the tube beyond the flooded zone. Additionally, the peak height and width change rapidly when the system pressure is near the transition pressure, thus providing additional discrimination between a mixture critical point and potential artifacts. These may result from the premature exhaustion of a flooded zone if an injection is too small, or from undiluted organic fluid if an injection is too large.

The detector temperature is usually set to 350 °C or higher, well above the critical temperature of the organic component. The intention is to heat the fluid to a temperature well above the critical temperatures of both components while they are still pressurized, and then depressurize near the outlet of the restrictor. Adiabatic cooling will occur when the pressure falls, but if the fluid temperature remains above the critical temperature then a smooth transition to atmospheric-pressure vapor will occur with no chance for phase separation. If a phase separation were to occur in the restrictor, the time would be so short that we would not expect any disturbance in the mass flow rate of either component that would affect the FID signal.

We have completed examination of 23 CO_2–organic solvent systems using this method, and summarize the data in Tables 1 and 2. Several of the most common systems are plotted in Figs. 4 and 5. The first and last points in these plots shown are the critical points for pure CO_2 and for the pure organic component.

Gaps exist in the data in several places for a variety of reasons [14,15]. For pentane and carbon tetrachloride, no problems were encountered, but only a few observations were made. The *n*-hydrocarbons required a larger injection volume than did the more-polar solvents. A larger internal sample loop had to be used, and the slower velocity through this loop may have contributed to peak-shape problems that made results at some temperatures ambiguous. These results were not reported.

Not all the systems reported are Type I, meaning that additional phase behavior may obscure the peak shapes at some temperatures and pressures. CO_2 forms Type II systems with both *n*-heptane and *n*-octane. Decane is even less soluble than these in CO_2. The system CO_2–octanol is distinctly different form the others. Its critical locus does not appear to approach the CO_2 critical point at low temperature. Furthermore, we were not able to get useful results for octanol below 60 °C. This behavior is consistent with a Type III system.

The flow injection peak-shape method has compared well with other methods. Ziegler et al. [14] reported excellent agreement of the peak-shape method results with view-cell results for CO_2–toluene and CO_2–methanol: Ng and Robinson reported four view-cell observations for CO_2–toluene [22]. Interpolations are required for comparison, but the largest deviation with the peak-shape method results is approximately 3%. Brunner reported nine view-cell observations for CO_2–methanol [23,24]. These data essentially agree with the peak-shape results at the level of precision in Table 2 except at the lowest temperatures where the deviation was less than 3%.

Yeo et al. compared view-cell and peak-shape results for CO_2–ethanol and CO_2–1-butanol [25]. The numbers agreed within the uncertainty they reported for the view-cell method. Scurto et al. compared view-cell and peak-shape results for CO_2–chloroform and reported that the methods compare well [26].

4. Applications

4.1. Retention-gap injection in open-tubular supercritical fluid chromatography

If the peak-width contribution of the injection volume is to be less than 5% of the observed peak width, then the injection volume into a 50 μm inside diameter open-tubular SFC column can be no more than about 30 nl for a 10 m long column if no phase separation occurs and if the injection solvent is weak compared to CO_2. However, every organic solvent is stronger than CO_2 for most solutes. Further, if phase separation does occur, then the maximum injection volume may be only a few nanoliters. This volume restriction makes trace analysis very difficult in open-tubular SFC when using mass-sensitive detectors.

Injection volumes reaching 1 μl are possible in open-tubular SFC by using a retention gap [10], that is, an capillary tube of the same diameter as the column but containing no stationary phase. It is installed between a room-temperature injector and the column inlet. Most of the retention gap is required to be in the oven with the column. Sample solvent may flood and dynamically coat the retention gap, as described earlier (Fig. 3), over its entire length without introducing unrecoverable broadening.

The key to making this technique work is in knowing the *P–T* coordinates of the critical locus of the binary system formed by the sample solvent and the CO_2 mobile phase. The pressure and the retention-gap temperature must be selected

Table 1
Estimates of *P–T* coordinates of critical loci for CO_2–solvent mixtures

Temperature (°C)	Pressure (MPa)											
	Acetone [9,14]	Acetonitrile [14]	1-Butanol [14]	Carbon tetrachloride [9]	Chloroform [14]	Cyclohexane [15,16]	Decane [16]	Dioxane [16]	Ethanol [14]	Ethyl acetate [16]	*n*-Heptane [15,16]	*n*-Hexane [14]
31.1	7.39	7.39	7.39	7.39	7.39	7.39	7.38	7.38	7.39	7.38	7.39	7.39
48		9.2										
50	8.9	9.4	9.9		9.1	8.9	10.9		9.6	9.3		8.8
60	9.6	10.5				10.0		10.5		10.1	9.8	9.5
70			13.0		10.6	10.8			12.1	11.0		10.1
71	10.3	11.7					14.5	12.5				
80	10.8	12.7			11.3					11.7		10.7
90	11.3	13.5	15.2		12.0				14.0	12.2	12.2	11.2
91							16.5	14.1				
100	11.7	14.3	16.1	12.8	12.5	13.3		14.9	14.7	12.6		11.6
109	11.9											
110		14.8	16.6		12.9				15.1	12.8		11.7
115							17.9	15.3		12.9		
120	12.0	15.2	17.0		13.2				15.3	13.0	13.3	11.8
125	12.0			13.9		14.4	18.2	15.7				
130	12.0	15.5	17.3		13.4				15.4	13.0		11.7
135						14.5	18.3	16.1			13.3	
140	11.8	15.7	17.4		13.4				15.3	13.0		11.1
145							18.5	16.4				
150	11.4	15.6	17.3	14.2	13.4	14.4	18.5	16.4	15.0	12.9	13.0	10.4
160	11.0	15.5	17.1		13.2				14.6			
161							18.4	16.3		12.5		
170	10.5	15.2	16.7		12.9				14.0			8.6
175				13.7								
180	9.9	14.7	16.2		12.4	13.3	18.2	16.2	13.3		10.9	7.5
186							17.7	15.8				
190	9.1	14.1	15.6						12.5			
200	8.1	13.4	14.9		11.1				11.4			
201										9.9		
210		12.5				11.1			10.3			
220		11.6	13.2		9.6							
230		10.3										
234.2												3.0
235.5	4.8											
240			10.9		7.7							
243									6.4			
250.3										3.9		
260			8.5									
263					5.5							
267.1											2.7	
274.7		4.8										
280.4						4.1						
283.1				4.6								
289.8			4.4									
314								5.2				
344.7							2.1					

Note: 1 MPa = 9.8692 atm, 10 bar, 145.04 psi, and 10.197 kg/cm^2.

to be well inside the critical locus so that phase separation and dynamic coating will occur, and so that *liquid-phase* sample solvent never reaches the analytical column and stationary phase. Phase separation is actually assured at such temperature and pressure because, at any point near the inlet of the retention gap, the composition of the mobile phase will shift from 100% CO_2 to 100% sample solvent when the solvent plug reaches that point, thus completely spanning the unseen composition dimension in a *P–T* projection.

The retention gap must be long enough so that the entire volume of liquid sample is exhausted creating the flooded zone within the retention gap. Thus, the column never sees sample solvent in liquid form. The sample solvent is removed as vapor from the retention gap, and then transported through the column as vapor rather than as a liquid. Although the solutes may be spread out over many meters of flooded retention gap by this process, the solutes can easily be focused into a small initial band at the head of the column by using the solvent effect, or phase-ratio focusing [10]. This technique takes advantage of the large difference in relative retention of solutes on an uncoated tube compared to a column coated with stationary phase, so that broadened solutes eluted from the retention gap under mild conditions are accumulated in a small volume upon reaching the stationary phase.

Table 2
Estimates of *P–T* coordinates of critical loci for CO_2–solvent mixtures

Temperature (°C)	Pressure (MPa)										
	Methanol [14]	Methyl-ethyl ketone [16]	Methyl-*t*-butyl ether [16]	*n*-Octane [14]	1-Octanol [14]	*n*-Pentane [9]	1-Propanol [14]	2-Propanol [14]	Pyridine [15,16]	Tetrahydrofuran [14]	Toluene [9,14]
31.1	7.39	7.38	7.38	7.39	7.39	7.39	7.39	7.39	7.39	7.39	7.39
42						7.9					
44									8.5		
48	9.5										9.1
50	9.8	9.2	8.8	9.4			9.6	9.3	9.1	9.0	
53						8.5					
55									9.7		
60	11.4	10.2	9.6	10.5	16.6				10.3		10.3
70	12.7	10.9	10.1	11.4	17.6		12.4	11.2	11.7	10.5	
71	12.8										11.6
80	13.9	11.8	10.7	12.5	18.7				13.0		12.6
81	14.0										
89											13.4
90	14.8	12.5	11.0	13.3	19.8		14.4	12.7	14.2	11.9	13.5
91									14.3		
96						9.9					
100	15.5	13.1	11.3	13.9	20.8		15.1	13.2		12.5	14.4
110	16.1	13.6	11.3	14.4	21.5		15.6	13.4		12.9	15.2
115			11.2								
120	16.4	13.8	11.3	14.7	22.1		15.9	13.6	17.2	13.1	15.7
130	16.5	13.9	11.1	15.1	22.6		16.0	13.5		13.3	16.2
132						9.3					
135			10.9				16.0				
140	16.4	13.8	10.5	15.2	22.9		16.0	13.3		13.4	16.5
145			10.3							13.4	
150	16.2	13.7	9.9	15.1	23.2		15.8	12.9	19.2	13.4	16.7
160	15.8			14.9	23.3		15.5	12.4		13.2	16.8
161		13.3	9.0								
170	15.3			14.6	23.3		15.0	11.8		13.0	16.7
176		12.8	7.5								
180	14.7				23.2		14.4	11.0	19.6	12.6	16.5
190	13.9				23.0		13.7	10.1		12.1	16.2
196.6						3.4					
200	13.0				22.6		12.9	9.2		11.6	15.7
201		11.0									
210					22.1		11.9	7.9	18.8	10.8	15.2
220					21.6		10.8				14.6
224.1			3.4								
230					20.9					9.2	13.9
235								4.8			
239.6	8.1										
240					20.0		8.4		17.2		13.0
250					18.8						12.1
260											11.1
263.6							5.2				
263.8		4.2									
266.9										5.2	
270									15.0		10.0
280											8.9
290				2.5							7.4
300									11.6		6.3
310											5.1
320.8											4.2
347									5.7		
385.5					2.7						

Note: 1 MPa = 9.8692 atm, 10 bar, 145.04 psi, and 10.197 kg/cm^2.

4.2. Packed-column supercritical fluid chromatography

Injection is not the problem in packed-column SFC that it is in open-tubular SFC. Packed-column SFC is usually done using HPLC-style columns capable of handling injection volumes up to approximately 20 μl. But because of the higher retention of these columns compared to open-tubular columns, packed-column SFC is almost always performed using an organic modifier to increase the strength of the

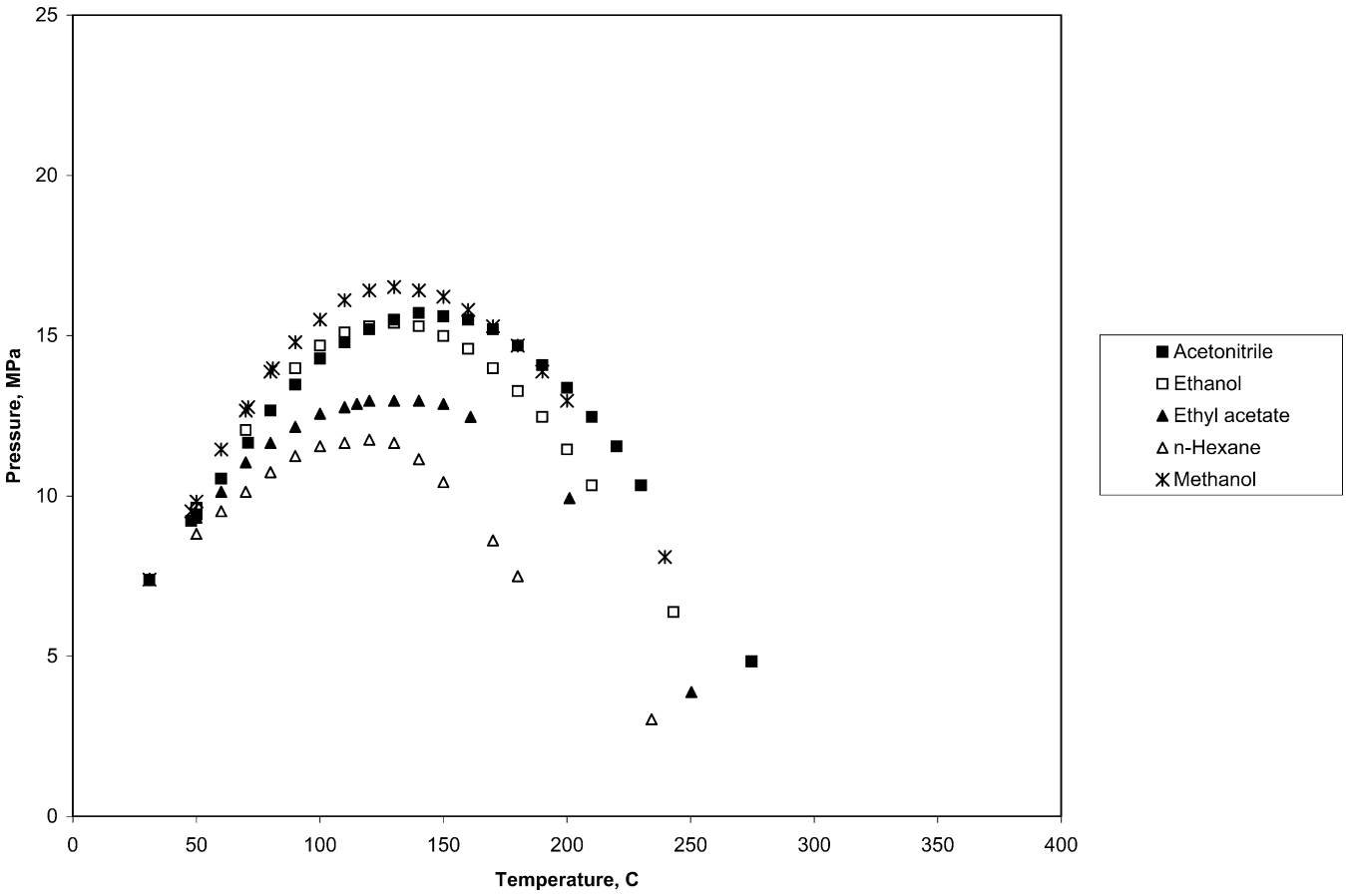

Fig. 4. *P–T* coordinates of critical loci formed from binary mixtures of CO_2 and the solvents indicated, estimated by the flow injection peak-shape method.

mobile phase well beyond that of neat CO_2. Gradient elution at constant temperature and pressure is the most common means of programming packed-column SFC.

Temperature is a powerful selectivity adjustment parameter in SFC, being far more influential than in HPLC. Therefore, temperature is often investigated and adjusted early in developing an SFC method. The concentration of modifier in the mobile phase has the biggest influence on overall retention. So, what is the best pressure to use while investigating the influences of temperature and modifier? The effect of pressure on both solvent strength and selectivity is small in packed-column SFC, particularly if the modifier concentration is more that approximately 20% (v/v). Therefore, it is convenient to set the pressure above the peak pressure value in the critical locus so that both temperature and mobile-phase composition can be varied freely with no possibility of phase separation occurring on the column.

After selecting a temperature, it may be desirable to reduce the pressure to the lowest practical value so that viscosity is minimized, diffusion rates are maximized, and fast mobile-phase velocities can be used with high column efficiency and with little wear on the pump. Once the temperature is chosen, how low can the pressure be set without causing phase separation to occur during a gradient?

It is helpful to simplify the problem by dissolving the sample in the mobile-phase modifier, if possible. If another sample solvent is used, then a ternary system may be formed upon injection, and it may be very difficult to sort out any phase behavior problems. The phase behavior influence of the sample components and mobile-phase additives present in low concentrations can usually be ignored. The operating pressure should be set high enough that phase separation will not be possible at any mobile-phase composition. This allows the sample solvent to go through the column without phase separation, and also ensures that no phase separation will occur in the future if there is a possibility that the gradient parameters might be changed. The minimum operating pressure should be approximately 0.5 MPa higher than the critical pressure corresponding to the chosen temperature for the CO_2–modifier binary system. This pressure can be read directly from the *P–T* projection. The 0.5 MPa cushion allows for drift in the instrument temperature and pressure calibration, and for the small changes that will occur in the phase behavior due to additives and sample components.

Note that the minimum pressure diminishes steeply for nearly all modifiers as the temperature nears the critical temperature for neat CO_2, and that the single-phase region extends far into subambient temperatures as long as the pressure is high enough to keep the CO_2 from evaporating,

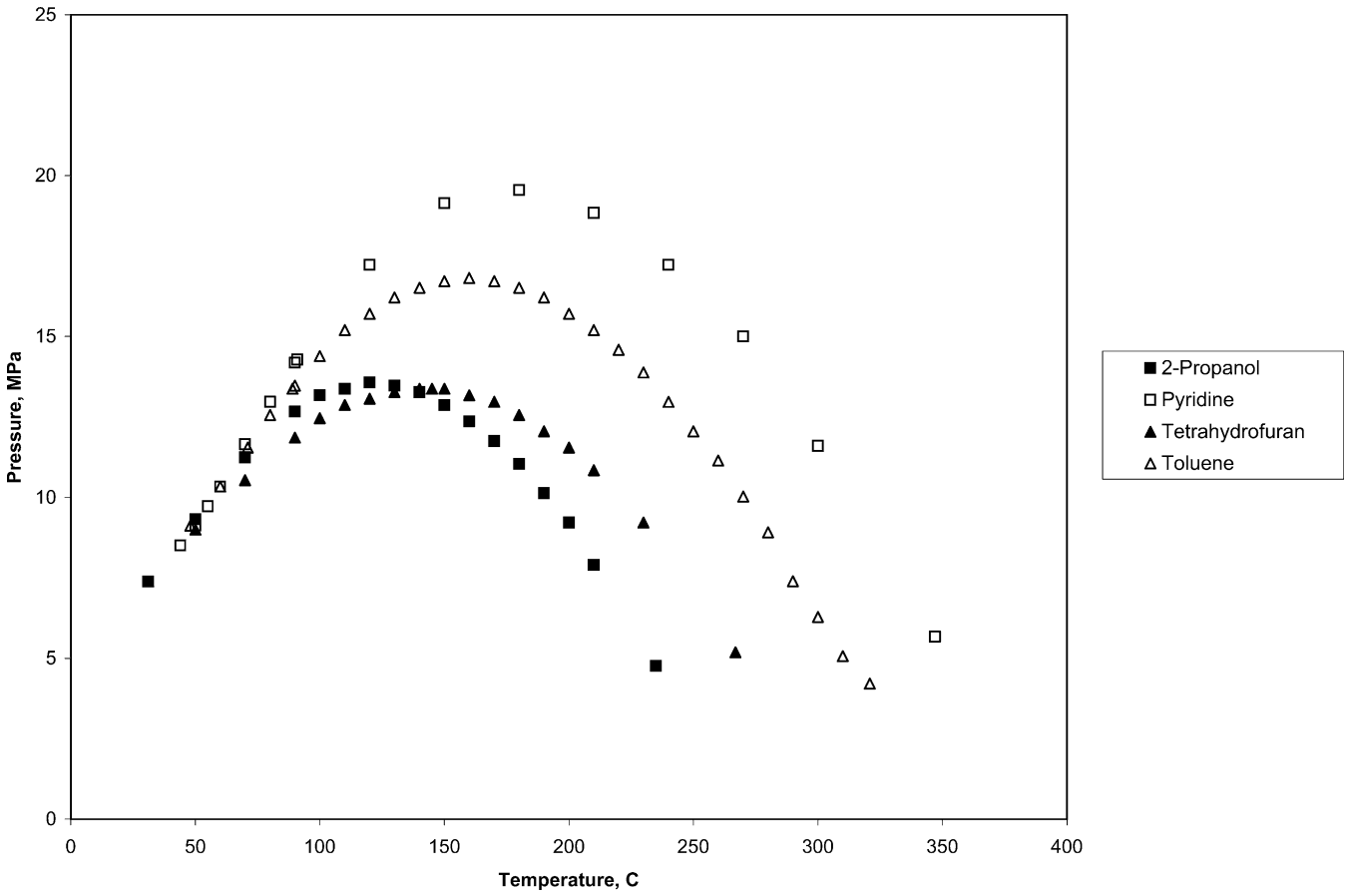

Fig. 5. *P–T* coordinates of critical loci formed from binary mixtures of CO_2 and the solvents indicated, estimated by the flow injection peak-shape method.

and the temperature is high enough to prevent the modifier from freezing. Chromatography performed at temperatures below the critical temperature of CO_2 is called either *subcritical fluid chromatography* if CO_2 is the main mobile-phase component, or *enhanced-fluidity liquid chromatography* if CO_2 is not the main component but is added to the mobile phase to decrease its viscosity [27].

In practice, despite the fact that the phase behavior may only call for small minimum pressures at low temperatures, it is wise to operate the system at slightly higher pressure than the vapor pressure of the CO_2 source. If this source is at ambient temperature, then 7.0 MPa should be the lowest system pressure considered. This is necessary because the vapor pressure of ambient-temperature CO_2 is approximately 6.8 MPa and, if the system is set below this pressure, the CO_2 may simply blow through the check valves in the pump and flow out-of-control into the column.

4.3. Interfacing supercritical fluid chromatographs with low-pressure detectors

Packed-column SFC instruments are normally operated with upstream flow control using two high-pressure pumps, one for CO_2 and the other for modifier. This allows gradients to be blended volumetrically. Pressure is controlled downstream from the column outlet by a pressure regulator or programmable nozzle operated under feedback control. When a UV detector is used, it is placed between the column outlet and the pressure regulator, thus keeping the solutes dissolved in pressurized fluid until detection is finished.

A different arrangement must be used with a low-pressure detector like a mass spectrometer or an evaporative light scattering detector. It is tempting to simply connect the outlet of the pressure regulator directly to the inlet of the low-pressure detector. However, this is not a good idea: the pressure would not be regulated in the connecting tube, and there is a possibility for both phase separation and precipitation of the solutes. The path through the phase diagram with this arrangement is shown in Fig. 6. Even if this works for a particular application involving very volatile solutes, its use can lead to a false expectation of continued success and to eventual failure if problems that are more difficult are addressed in the future, or if the operational parameters are changed. For a research instrument used for a variety of methods, it is wise to take deliberate steps to prevent phase separation and solute precipitation until the mobile phase is vaporized in the low-pressure detector.

One way to avoid this mass-transfer problem is to replace the pressure regulator with a tee that adds make-up flow to the stream. The make-up flow is delivered from a third

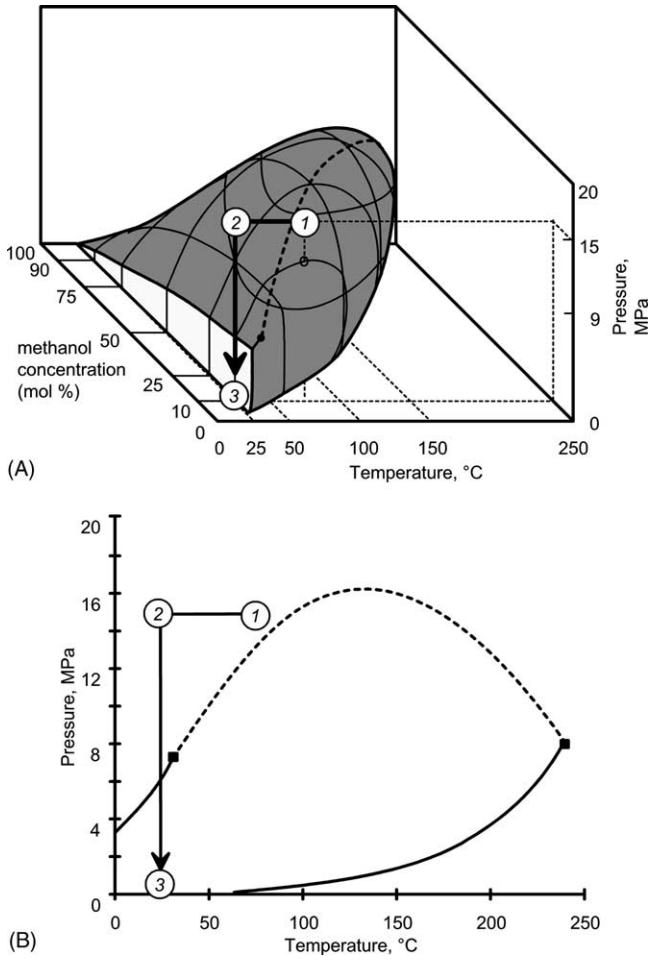

Fig. 6. The path through the CO_2–methanol phase diagram (A) and *P*–*T* projection (B) for direct connection of the pressure regulator outlet to a low-pressure detector using a transfer tube. The column outlet (1) is at the method set points for temperature, pressure, and mobile-phase composition. Temperature diminishes but pressure is maintained to the pressure regulator inlet (2). The transfer tube connecting the pressure regulator outlet with the detector inlet (3) is not pressure controlled, and much of its length may have conditions in the two-phase region, particularly if the transfer tube provides little flow resistance. Low pressure and phase separation in this tube may cause solute precipitation and other mass-transfer problems.

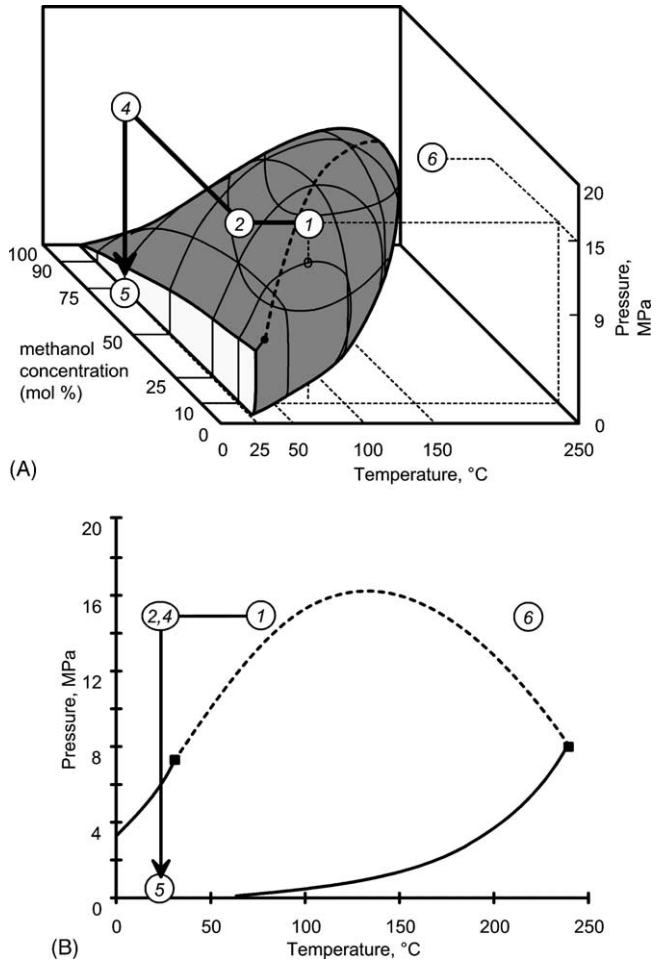

Fig. 7. The path through the phase diagram (A) and the *P*–*T* projection (B) using make-up flow pressure control in place of a pressure regulator. The column outlet (1) is connected to the inlet of a tee (2), placed outside the oven, where pressure-controlled modifier is pumped into the stream. The tee outlet and most if not all of the transfer tube (4) remain at the set pressure. The pressure is finally, abruptly dropped at a restrictor at the detector inlet (5). If the same arrangement is used with the column outlet operated at point (6), the path will go through the two-phase region. Placing the tee in the oven may avoid phase separation from point (6) by making the first move isothermally in the composition dimension and skirting around the two-phase region. The path, 1–2–4–5, is also taken when flow-controlled make-up fluid is added between the column outlet and a pressure regulator although some of the numbers in the diagram now represent different locations than earlier: the column outlet (1) is connected to the tee (2) where make-up fluid is added; the tee is connected to the pressure regulator inlet (4); the transfer tube to the low-pressure detector is the line (4–5). Although the pressure in the transfer tube is not controlled, the pressure usually remains above the transition pressure over enough of the tube length to prevent serious problems.

pump operated under pressure control [12]. It is also usually necessary to add a flow restrictor to the outlet of the transfer tube. The make-up solvent must be the same solvent as the modifier so that the phase behavior will remain predictable. The system pressure is set using the make-up pump. The flow rate of the make-up pump varies, as necessary, to maintain the pressure, and depends on the pressure setting, the flow rate through the column, the flow resistance of the transfer tube and restrictor connecting the tee to the low-pressure detector, and the viscosity of the fluid in the transfer tube. This arrangement keeps the pressure at the system pressure until the last millimeter or so of the restrictor. In some cases, liquid may be sprayed from the outlet of the restrictor. A typical path through the phase diagram is shown in Fig. 7. Note in the figure that essentially all the mass transfer is accomplished at elevated pressure in a region assured of having only one fluid phase.

This arrangement provides trouble-free mass transfer from columns operated at locations in the phase diagram from which the path to the restrictor conditions will not intersect the two-phase region. The solute, although diluted by the make-up fluid, is delivered to the detector without splitting. If the detector can generate a signal dependent only

on the solute mass flux and not on the total flow rate, then this arrangement will be very robust, even if the transfer tube and restrictor resistances change with age. The chromatographic integrity of such an arrangement is excellent since the temporal profile of solute peaks will be unaltered by the make-up flow in a properly assembled system.

If it is desirable or necessary to operate the column from a location in the phase diagram that cannot work with the path shown, such as from point 6, then alternate paths can be devised if the general shape of the two-phase region is known. This shape can be inferred well enough from the appropriate *P–T* projection and the general three-dimensional shape of a Type I two-phase region to plan a suitable path through the phase diagram. In this case from point 6, placing the tee in the oven and isothermally changing the composition first will skirt around the back of the two-phase region, as it is represented in Fig. 7, if enough make-up modifier is added.

Another, more popular arrangement takes advantage of the existing downstream pressure regulator on a typical packed-column SFC instrument, and requires a flow-controlled make-up pump. This pump is teed into the system between the column outlet and the pressure regulator. The purpose is to add enough modifier to increase the viscosity of the fluid, keep the pressure high in the transfer tube, and lower the transition pressure, thus preventing phase separation in the transfer tube connecting the pressure regulator to the detector. Phase separation, if it occurs, will be delayed until very near the transfer tube outlet, and will do little damage to mass-transfer integrity or to peak shapes. This arrangement has the advantage that the pressure can be set using the pressure regulator and the control software provided with the SFC instrument. A potential disadvantage is that the peak must be transferred through the pressure regulator or nozzle, and some broadening may occur. This can be minimized by using a small-volume regulator or nozzle. The normally expected broadening in time caused by the pressure regulator will also be diminished by the make-up flow. Thus, peaks temporally broadened by the pressure regulator with this arrangement can be narrowed by increasing the make-up flow rate.

5. Conclusion

The flow injection peak-shape method is easy to implement and interpret. Although we have applied it only to mixtures of CO_2 and FID-detectable organic solvents, it seems reasonable to expect that the method could be applied to other systems not containing CO_2. The most important requirement is a detector that can see the changes in the concentration of the higher-boiling component as it is delivered as vapor in the lower-boiling component. It is also conceivable that the technique would work if the detector responded sensitively to the lower-boiling component and poorly to the higher-boiling component, although the signals would be negative in this case, and the missing signal would correspond to the dilution of the detectable component by the other component.

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