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Review Article

Supercritical fluids in medical radioisotope processing and chemistry, Part I: technology, instrumentation and methodology

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Summary

Although the last two decades has seen a keen interest in using supercritical fluids as media for material processing and chemical reactions owing to their unique physical properties, this technology has been underutilized in medical radioisotope processing and chemistry. The successful application of this technology necessitates not only having a sound knowledge of the physicochemical properties and phenomena occurring within such critical states of matter, but also a clear idea of the tools needed and means for applying them. These topics will be presented under the cover of two parts. Part I will address topics on the general background of supercritical fluid technology, equipment needed, whether for chemistry or separation, and basic techniques to access critical states of matter. Part II will follow in a subsequent volume and is meant to provide a description of applications with cited examples and potential areas for development. The hope is that sufficient interest is stimulated so as to encourage radiochemists to look to alternative technologies of this nature to

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solve their daily laboratory challenges.

Key Words: supercritical fluid; radioisotope extraction; supercritical fluid chromatography; supercritical fluid reactions; PET radiopharmaceutical synthesis

Introduction

Early considerations of supercritical fluid chromatography

While supercritical fluids were first discovered back in the mid-1800s, the first acknowledgement of their potential use in analytical chromatography was not made until 1958.[1] Even so, the scientific community really did not develop an intense interest in this technology until the 1980s when it was realized that critical state chromatography might rival conventional capillary gas chromatography in speed and efficiency, especially when addressing separation challenges of temperature-sensitive substances.[2,3] This interest remained fairly restrictive to this rather specialized area of analytical chemistry for a number of years and was somewhat slow to expand beyond chromatography.

It is interesting to note that while early commercial interests in supercritical fluid technology was deeply rooted in capillary chromatography, it was questionable whether such fluids really could provide any advantages over conventional capillary chromatography techniques. Even so, several commercial supercritical chromatography systems appeared on the market during the 1980s and 1990s in what appeared to be an already waning industry. This rapid commercialization perhaps created a driving force that sustained a basic interest in understanding the properties of supercritical fluids. As a result, the subject has been extensively covered in reviews and textbooks.[1,4]

Recent years have shown a resurgence of interest in supercritical fluid chromatography on both a practical and theoretical level as it applies to packed column separations at both the analytical and preparative scales. Materials that are usually separated by normal-phase high-performance liquid chromatography can most often be separated faster and with higher efficiency using supercritical fluids as the mobile phase.

One of the reasons for this growth is due to the fact that supercritical fluids possess an extremely low compressibility at or near their critical point, which can impact in a good way on composition programming during gradient elution separations.[4]

The relationship between analyte retention and solubility in a supercritical fluid has been addressed from both a thermodynamic aspect based on solubility parameters using the Lee–Kesler equation of state,[5] and a statistical thermodynamic model using the simpler Peng–Robinson equation of state.[6,7] The later approach has been successful at predicting analyte retention at higher mobile phase densities,[7] as well as predicting influences of fluid temperature.[8] However, the reader is cautioned that all of these early theoretical studies are restricted to solubility arguments rather than polarity interactions between the analyte and the stationary phase. While some reasonable correlations may be achieved between theory and practice here, this may not be universally applicable.

More recent systematic investigations have attempted to address normal-phase and reversed-phase behavior particularly in attempting to explain analyte retention characteristics as a function of different functional groups,[9–11] and the relationship between molecular interaction parameters and linear solvation energy.[12–16] While these models are far more complex they do provide a better prediction of observed behavior.

A major resource for chromatography applications is the collection of demonstrated separations from the 1989 Symposium on Supercritical fluid Chromatography applications held in Snow Bird.[17] This collection includes a compilation of 370 examples from almost every area of the chemical industry including pharmaceuticals, food, natural products, bio-molecules, pesticides fuels and polymer additives.

General considerations of supercritical fluids applied to sample extraction

The role of supercritical fluids in sample preparation is more securely established in the today's industries perhaps owing to the fact that it grew out of a more chemical engineering background that was rooted in systematic, realistic approaches that were more goal-oriented rather than knowledge-based. As a result, numerous applications utilizing supercritical fluid extractions can be found in today's chemical, pharmaceutical and food industries.[18] The extraction of caffeine from coffee or tea, and the extraction of nicotine from tobacco are well-known examples in the food industry.[19] Other examples include the extraction of organic pollutants from waste waters,[20–22] the solubilization of dyes in textile processing,[23] as well as the solubilization of polymers in other related industries.[24] A tribute to this success is the fact that, at present, approximately 60 supercritical fluid extraction plants operate worldwide.[25]

General considerations of supercritical fluids applied to chemical reactions

The advantageous physico-chemical properties of supercritical fluids has also lead to an intense interest in their use as solvents for chemical reactions. Classic examples here include pressure influence on stereoselectivity in Diels-Alder reactions,[26–28] polymerizations,[29–36] esterifications,[37,38] as well as various electrochemical reactions.[39–45] Additionally, in recent years we have seen an increased interest in how organometallic compounds behave in supercritical solvents. Many compounds within this class are already being used extensively as reagents in preparation and processing of many inorganic materials, as catalysts in production of a wide variety of chemicals and as chemotherapy drugs. The coupling of supercritical fluid science and technology with organometallic chemistry seems a logical course of action and has already been the topic of a few recent reviews.[46,47] This area is seen by the scientific community has having great potential. Some applications that have already been demonstrated with supercritical fluids include: (i) use of organotransition metal complexes as homogeneous catalysts for reactions;[48,49] (ii) impregnation of polymers with various organometallic complexes from supercritical fluid solutions for property enhancement;[50,51] and (iii) modification of organometallic complexes using supercritical fluids to control size distribution of inorganic powders through rapid expansion of the supercritical solution.[52]

One can only speculate on the reasons why so very little interest in supercritical fluid technology is seen in the medical radiochemistry fields today given such widespread knowledge and understanding of supercritical fluid properties, and demonstrated applications to chromatography, sample extraction and synthesis. Perhaps the major deterrent in the acceptance and use of this technology is the high pressure that is most often necessary to sustain the critical state, and the specialized equipment needed to attain these levels.

Fundamentals on supercritical fluids

What is a supercritical fluid?

A supercritical fluid is a state matter that occurs when a substance is heated and compressed at or beyond some critical point defined by a critical temperature and pressure for that substance. Figure 1 depicts a phase diagram for this behavior. The critical point in the diagram is simply the boiling line defining the phase transition between gas and liquid-like behavior where intermolecular forces and thermal energy become equal. Thus supercritical fluids are not gases or liquids in the strictest sense, but they retain many of the properties of both gas- and liquid-like behavior when in this state. Table 1 presents a generalized comparison of density, diffusion coefficient and dynamic viscosity for gases, liquids and supercritical fluids.

Supercritical fluids possess viscosities that are more gas-like than conventional liquid solvents and diffusion coefficients that are somewhat between the two phases. These combined features allow supercritical fluids to penetrate solid materials more efficiently than conventional liquid solvents providing more efficient sample extraction. In addition, these features allow for more intimate contact of solubilized analytes with stationary phases during chromatographic separations providing higher efficiency of separation in shorter times. Perhaps the most important feature to note about supercritical fluids is their

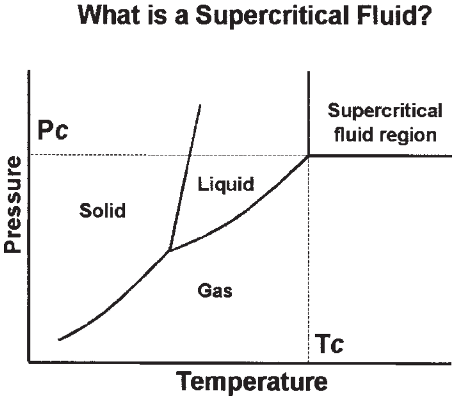

Figure 1. Phase diagram depicting the conditions of temperature and pressure where a substance behaves either as a gas, liquid, solid or supercritical fluid

Table 1. Comparison of general properties of gases, liquids and supercritical fluids

Property	Gas	Liquid	Supercritical fluid
Density [$g\,cm^{-3}$]	10^{-3}	1	0.2–0.8
Diffusion coefficient [$cm^2\,s^{-1}$]	10^{-1}	10^{-6}	10^{-3}
Dynamic viscosity [$g\,cm^{-1}\,s^{-1}$]	10^{-4}	10^{-2}	10^{-4}

pressure-dependent density. At a constant temperature, fluid density can be adjusted from gas-like behavior to liquid-like behavior in a continuous manner. For example, the density of supercritical carbon dioxide can be tuned from 0.5 mol/l (gas-like) to 19 mol/l (liquid-like) thus allowing it to behave in many respects like hexane as a solvent. This quality makes supercritical fluids extremely attractive as tunable process solvents and reaction media.

Types of supercritical fluids and which are actually useful

For the most part carbon dioxide has received the most attention as a supercritical fluid largely because it is environmentally friendly, readily available, inexpensive to purchase and relatively safe to work with. Many other compounds apart from carbon dioxide that are gaseous or liquid at normal temperature and pressure can be rendered in a state of super or hypercriticality. Table 2 presents a useful list of compounds

Table 2. Substances used as Supercritical Fluids[53]

Substance	Critical pressure (psi)	Critical temperature (°C)
Ammonia	1650	133
Argon	705	122
Benzene	715	290
i-Butane	528	135
n-Butane	551	152
Carbon dioxide	1070	31
Carbon disulfide	1108	279
Chlorodifluoromethane	720	96
Chlorotrifluoromethane	561	29
Ethane	718	33
Tetrafluoroethane	571	34
n-Heptane	395	267
n-Hexane	439	234
Hydrogen sulfide	1306	100
Krypton	7961	−64
Methane	673	−82
Methanol	1153	240
Monofluoromethane	824	45
Nitrogen	492	−147
Nitrous oxide	1037	36
neo-Pentane	464	161
n-Pentane	494	197
Propane	617	97
Sulfur hexafluoride	525	46
Sulfur dioxide	1108	158
Trichloromethane	554	29
Trifluoromethane	682	23
Water	3226	375
Xenon	847	17

that have been successfully utilized in supercritical fluid applications over the years. It is by no means comprehensive. In many cases, these compounds require special handling and tend not to be compatible with bench-top work. For example, the aliphatic hydrocarbons require temperatures that are above their boiling and flash points to reach their critical temperature. Thus they can present considerable health and safety risks when used.

Nitrous oxide is perhaps the only widely examined alternative to carbon dioxide. Its critical parameters are nearly the same as those of carbon dioxide. In the early days, there was particular interest in its application for the separation of amines.[54,55] Many researchers were concerned over the possibility that the basic amines could form explosive carbamates in carbon dioxide fluid. Although this behavior of amines has never been observed, nitrous oxide can be troublesome when used with oxidizable analytes, or with organic modifiers added to the fluid.[56,57]

Of the more exotic substances on this list, ammonia deserves special mention only because it was the first supercritical solvent to be utilized in radiotracer synthesis and processing for Positron Emission Tomography (PET).[58–61] Ammonia gas can readily be converted into a supercritical fluid.[62] Past experience, however, has shown it to be such a strong solvent because of its polarity that in many instances it will actually dissolve silica-based chromatography columns it contacts with. It can also be extremely corrosive toward hardware thus special attention must be given to system design.

More recently, there has been considerable interest in halocarbon fluids. Chlorodifluoromethane (Freon-22) has proven to possess excellent solvation properties for chromatography and sample extraction of polar analytes.[63,64] Trifluoromethane also possesses similar properties,[65–67] as does the more ecofriendly tetrafluoroethane (Freon-134a).[68,69] The downside to using these substances as solvents is their limited availability, higher cost and environmental concerns. A recovery system could satisfy the later issue, but leads into more complex equipment.

In practical terms, carbon dioxide is the only substance that has reached widespread acceptance and use. Unfortunately, the polarity of carbon dioxide is effectively the same as liquid hexane, but with one difference in solvent strength residing in the Lewis-acid/Lewis-base properties. Quite often, supercritical fluid applications relying on pure carbon dioxide fall short of success, especially when analytes are extremely polar. However, experience has shown that the addition of small amounts of organic modifiers such as methanol, acetonitrile, tetrahydrofuran and dimethyl sulphoxide will impact on the solvating strength of this fluid. Such mixtures are even available commercially. In packed column chromatography, the addition of a few percent of such modifiers will enable the elution of organic acids and bases.[70] It is believed that such modifiers deactivate the stationary phase surface. Another take on this phenomenon is that the organic modifiers form nanoscopic clusters about the analytes thus providing a buffer against the stationary phase. Such behavior has been reported when using modified supercritical fluids as media for reactions.[71] These nanoscopic clusters can be utilized as tiny reactors. One application of acetonitrile clusters in supercritical carbon dioxide was reported to increase the radiosynthetic yield of [^{11}C]-L-deprenyl,[72] a radiotracer in PET used to measure monoamine oxidase B activity within the brain.[73]

Another approach to modifying carbon dioxide's polarity is through addition of trace amounts of water. While this slight change in composition has little or no effect on the operating critical parameters, the presence of water can impact heavily on solvating power. One thought on this is that water helps promote solvation through hydrogen bonding either with the analyte or with the supercritical carbon dioxide. Supercritical water alone has been found to be too aggressive a solvent quite often causing oxidative breakdown of the analytes. In fact, supercritical water has been effectively used in waste remediation.[74]

Basic Instrumentation

Fluid delivery system

Whether supercritical fluids are used for process or reaction, researchers minimally must invest in a quality high-pressure fluid delivery pump that is capable of delivering reproducibly low flow rates of a compressed fluid that possesses a low-viscosity. Reciprocating pumps common in liquid chromatography (HPLC) tend not to be appropriate. Typically large capacity high-pressure syringe pumps are used for supercritical fluid applications. Several commercial systems are available. The syringe pump manufactured by Isco, Inc. (Lincoln, NE) can be highly recommended for radiochemistry use for two reasons. The unit is modular so that the pump and control box can be located outside of a high radiation area, typically a hot cell, where processes are carried out. The pump capacity can hold up to 500 ml of pressurized fluid (up to 7500 psi) for the larger model up, which from personal experience will allow radiochemistry systems to operate throughout the entire day if necessary. This feature is particularly appealing in the PET field. Because of the nature of the radio isotopes used in PET (for example carbon-11, $t_{1/2} = 20.4$ min; nitrogen-13, $t_{1/2} = 10$ min), typically several radiosynthesis and/or chromatographic separations are carried out throughout the workday. Thus it is highly desirable to have a system operate in almost a continuous mode without refill. These pumps typically can be operated in a pressure-mode, either isobaric or gradient, or constant or gradient flow-mode depending on the application. A single pump station is adequate for most applications including those involving mixing of modifiers as most can be commercially purchased premixed at desired concentrations. Of course, a dual pump station affords the additional advantage of programmable modifier gradients during methods development that can provide big savings of time.

Another useful tip when utilizing pressurized gases like carbon dioxide is to chill the pump head to approximately 5°C or lower.[75,76] Although not an essential piece of equipment, a refrigerated water bath manufactured by Fisher Scientific, Iso-Temp (Pittsburgh, PA) ensures that the pump head is filled to capacity with liquid so that very little head compression is necessary to bring to pump up to operating pressure.

Design considerations for a preparative-scale chromatography module

Application of supercritical fluids to chromatographic separation in radiochemistry most often involves large volume samples (1–2 ml) consisting of the radioactive analyte dissolved in aqueous/organic mixtures. Issues to be considered here include sample introduction, the separation column, appropriate detectors for both mass and radioactivity measurement, and downstream fluid manipulation including diverting valves for compound recovery and pressure restrictors.

Figure 2 shows a schematic of a preparative-scale radio supercritical fluid chromatograph that was recently constructed in our laboratory and used for purifying carbon-11 labeled solvents of abuse.[77] The system is comprised of three components that include the fluid delivery system, chromatography module and downstream a detector/collector module. Considerations for the fluid delivery component have already

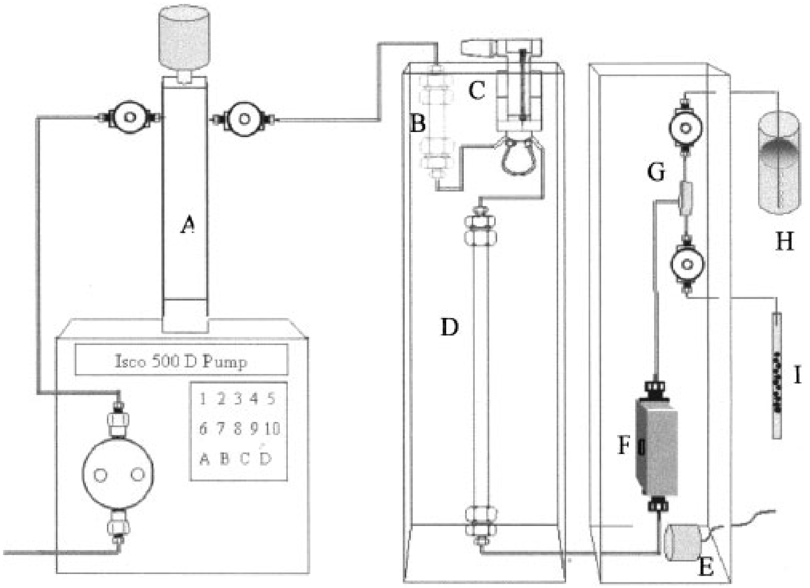

Figure 2. Schematic of supercritical CO_2 fluid radiochromatograph (A: Isco pump module; B: preheat column; C: Rheodyne injection valve; D: chromatography column; E: PIN diode radiation detector; F: Knauer UV absorption cell; G: flow switch valves; H: waste product receptacle; I: solid trap cartridge. (Reprinted from *Nucl. Med. Biol,* RD Muller, RA Ferrieri, M Gerasimov, V Garza, 'Supercritical CO_2 fluid radiochromatography system used to purify [^{11}C]toluene for PET,' 29(2002) 351–357 with permission from Elsevier Science.)

been discussed. The chromatography module in this system was built into an HPLC column heater (Alltech, Inc., Deerfield, IL). The inside compartment is adequate in size to accommodate the preheat exchanger, injection valve and most semi-preparative scale HPLC columns up to 300 mm length. The key advantage to including all of these components in the column heater is that one only has to deal with a single temperature set point and controller for heating these different zones. The heat exchanger is essential in preparative-scale chromatography to ensure that fluid temperature reaches the critical temperature for that substance (or the desired operating temperature above the critical temperature) before it contacts the contents of the sample injection loop. The exchanger can easily be constructed using of several feet of 1/8″ stainless steel tubing tightly coiled into a loop. Our experience has shown that the Rheodyne^{TM} (model 7010) sample loop injectors are extremely robust valves for use with supercritical fluids and can be operated safely up to 5000 psi. Of course, many other manufacturers valves are equally suitable for the task. One thing to note regardless of manufacturer is that it is essential the entire valve body is heated along with the sample loop to prevent sample blockage in the valve rotors channels. The Rheodyne^{TM} valves have been safely tested in this laboratory up to 100°C with no problems. They are also amenable to remote actuation since the chromatography module most likely will reside behind heavy radiation shielding depending on application. The Hamilton Co. (Reno, NV) electric actuator is one of many products of this nature that works very well for this purpose.

Suitable mass detectors for process applications involving supercritical fluids

Several types of mass detectors are suitable for use in supercritical fluid chromatography or other process applications. The attraction here is that one has flexibility to use detectors that are designed for gas chromatography (GC) as well as for liquid chromatography. In the class of gas chromatography detectors, there has been notable success with the flame ionization detector (FID) for mass measurement of volatile and even semi-volatile analytes.[78–80] Such a detector is insensitive to supercritical carbon dioxide fluid, as well as to carbon dioxide fluids modified with water or formic acid. SRI Instruments (Torrance, CA) markets a compact stand-alone unit that is interfaced with a heated sample inlet line. We have successfully used such a detector on the semi-preparative supercritical fluid chromatograph (SFC) by plumbing the interface line into the column effluent stream using a high-pressure HPLC Tee fitting. In radio chromatography where one might desire to recover the radiolabeled peak, it is not entirely practical to use a destructive detector such as this. A solution to the problem is to split the effluent flow to allow 1% or less of the stream to enter the detector. This can be accomplished with the SRI interface by inserting a 10 μm fused silica capillary into the 1/32″ o.d. heated nickel interface tube. The high-pressure seal at the interface Tee can be obtained using an appropriate graphite ferrule. (Several chromatography suppliers sell suitable graphite and graphite-vespel-type ferrules for creating high-pressure seals between standard HPLC components and fused silica capillary tubing.) This setup allows for the majority of the radioactive component to pass to a suitable collection receptacle. We have successfully used this setup for detecting fairly large organic molecules like nicotine. One can see a unique advantage of the FID over conventional HPLC optical detectors in the PET field where quite often a high specific activity radiosynthesis would yield barely nanomolar amounts of mass for detection. If the radiotracer is not strongly chromophoric, one is faced with the challenge of how to obtain a suitable signal-to-noise from the optical detector. The FID is typically 10^3 times more sensitive than optical detectors.

Less widely used in the GC class of detectors is the nitrogen/phosphorous thermionic detector which was successfully used with supercritical carbon dioxide and supercritical nitrous oxide fluids,[81,82] the electron capture detector for measurement of polychlorinated biphenyls,[83] the chemiluminescence detector,[84,85] and the flame photometric detector.[86,87]

Several optical detectors primarily developed for liquid chromatography have found widespread use with SFC including the UV and diode array detectors. Knauer Instruments (Berlin, Germany) markets a useful fiber-optic UV detector that is capable of sustaining up to 300 bar pressure in the 2 mm path length cell. The fiber-optic model offers several key advantages to the radiochemist. Only the optical cell need be maintained at critical temperature thus minimizing the instrumentation footprint that has to reside within the hot cell. This small size also allows one to interface a suitable radiation detector almost at the same juncture of the fluid flow path thus eliminating radiation and mass peak lag times. The utility of light scattering or mass evaporative detectors in SFC have also been explored.[88–91] The disadvantages here are that these detectors tend to be much more costly than conventional optical detectors, as well as somewhat larger.

More exotic detectors for SFC include FT-IR,[92] and MS,[93] which can provide qualitative information about the eluted analyte, but at a higher cost.

The detector/recovery module described in the previous section was designed around the Knauer fiber-optic UV absorption cell and was integrated into a separate heated housing in order to maintain critical temperatures. The outflow stream from the chromatography column can be plumbed into this module or something similar to this design using standard HPLC fittings and 1/16″ stainless steel tubing. Minimizing connection lengths is advisable. Ideally a single heated chamber that houses all components is desirable but sometimes not practical working within small spaces of a shielded hot cell.

Suitable radiation detectors for process applications involving supercritical fluids and radioactive substances

The type and complexity of the radiation detector one chooses for radiochromatography depends on two factors: (i) the nature of the radio isotope involved in the separation process; and (ii) the level of radioactivity that has to be measured. For the most part, medical imaging applications involving radioisotopes are restricted to elements that either decay by direct gamma emission or positron emission that ultimately yield a coincident pair of 511 keV gamma rays. In either case, there exists some flexibility here regarding choice of the type of radiation detector used. Whether it is a scintillation-type detector, diode-type or solid-state detector depends mostly on the desired dynamic range of operation.

Commercial scintillation-type detectors used for this purpose are usually comprised of NaI crystals coupled to a phototube. They tend to provide much more sensitivity than is needed in most applications, and quite often, are prone to high background count rates. This later characteristic can be minimized by either placing a single crystal arrangement inside a heavy lead or steel collimator, or else set up a coincident detector arrangement (for positron emitting radioisotopes) that registers counts associated with only coincident gamma rays in the fluid path. Either way, the radiochemist has two problems to content with for supercritical fluid applications: (i) the size of the radiation detector, whether it is a shielded single crystal or coincident pair, will be rather large considering that the fluid path must be maintained at the critical temperature and (ii) the scintillation crystals tend not to perform with any sense of stability at elevated temperatures.

The PIN diode-type radiation detector offers many more advantages than disadvantages for supercritical fluid chromatography application: (i) the actual detector can be quite small ($\sim 0.25\,\mathrm{cm}^3$), and attached to requisite electronics hardware *via* fairly long leads thus allowing it to be integrated next to the mass detector; (ii) it is fairly tolerant of elevated temperatures (we have tested several commercial detectors up to 60°C and found them to maintain stability in use over time); (iii) it does not require high voltage to operate which provides greater safety to personnel; (iii) it can operate over a fairly broad dynamic range from microcurie levels of radioactivity to millicurie levels; and (iv) it can be relatively inexpensive to purchase or build (albeit the cheapest of the three types mentioned). Several commercial systems are available that are pricey due to excessive packaging that usually includes digital readout with range settings and response times. These are features that are generally unnecessary for chromatography applications. Carroll-Ramsey Associates (Berkeley, CA) markets a very basic and inexpensive dual detector unit that possesses a 10^6 dynamic range with simultaneous analog outputs that can be captured with suitable chromatography acquisition systems. Bioscan, Inc. (Washington, DC) also offers a compact unit with dual channel digital/analog outputs that can be purchased in combination with PIN diode and NaI scintillation detectors.

Other types of detectors that might be worth exploring as gamma sensitive detectors include the small ionization-type detectors (Therados, Inc., Sweden) although they tend to be less sensitive than the PIN diode detectors, or the solid-state-type detectors like CdTe although these types are less stable at elevated temperatures. Both detector types require a modest high voltage to operate.

Radiochromatography applications involving radioisotopes that decay *via* beta particle emission and flow detection of these events typically require intimate contact between the fluid stream carrying the radioactive analyte and some suitable scintillation material.[94] Most commercial radiation flow detectors of this nature utilize plastic scintillators housed within a translucent plastic tube of suitable length to achieve adequate sensitivity toward the level of radioactivity and detection of the corresponding scintillation events through the tube. This works very well for many radio-HPLC applications, but is not suitable for the higher-pressure regime of most supercritical fluids. One suggestion here is to utilize an optical cell, such as the one described above that is capable of withstanding the higher operating pressures, and pumping in an appropriate liquid scintillation mix into the fluid stream. Of course, this increases the complexity of the instrumentation to a new level involving coordination of two syringe pumps. It is worth mentioning here that the control module from the Isco, Inc. syringe pump is capable of controlling more than one pump simultaneously.

Suitable valve hardware for component collection at the outlet of the process application

Basic hardware, and particularly valves, that are involved in redirecting the fluid path after the process application must be of a nature that they can operate reliably at high pressures and elevated temperatures.

Solenoid-type valves are not appropriate for this application nor are stem-type three way valves. If fluid path redirection is a simple setup involving a waste receptacle and desired component collector, then a four-port rotor seal valve such as the more common six-port valves used for sample injection in HPLC is appropriate. Such valves can be operated up to 5000 psi and elevated temperature, as well as can be remotized either electrically or pneumatically. Severally suitable commercial valve kits can be found with any of the larger chromatography supply distributors.

An alternative in this application is the two-way stem valve. Two manufacturers of this type of valve include Valco Instruments, Co., Inc. (Houston, TX) and SSI Equipment Inc. (Burlington, Ontario). The advantage here is that these valves can be obtained in a fairly large bore that can minimize chances for fluid plugs at inopportune moments of operation. Isco Inc. (Lincoln, NE) manufactures a valve kit that operates with encoded motorized controllers on the high-pressure valve-stems. These kits are marketed and intended for use with the company's multi-pump process stations that operate continuously and require redirecting fluid flow at the source to allow the opportunity for pump recharging. We have successfully integrated a single valve kit into the outlet fluid stream in order to redirect flow from a waste receptacle to a product collector. If more collectors are required a second valve kit could be added. Control of these valves occurs directly through the pump control module. Each module is capable of handling up to four valve kits or eight valves. Alternatively, control can be affected through RS-232 *via* a PC computer.

How to maintain adequate back-pressure

In any supercritical fluid process application it is essential that reasonable pressure and density gradients be maintained in the outlet fluid stream in order to sustain the necessary critical pressure that is required to keep analytes adequately solubilized in as much of the system's plumbing as possible. Various passive devices or restrictors have been adopted for maintaining the desired critical state through the fluid pumping rate. Upon exiting such devices, the fluid rapidly expands and depressurizes causing it to revert back to its natural state. In many cases including carbon dioxide, this natural state is gaseous. This adiabatic expansion can cause extreme localized cooling that can give rise to icing, or crystallization resulting in plugging of the flow path.[95,96]

This can be more than a minor annoyance especially when working with fairly large quantities of radioactive material.

Over the years, a variety of approaches have been investigated to maintain control over the pressure gradient without plugging. These included backpressure regulators, variable restrictor valves (Alltech, Inc.) and fixed linear restrictors. The first two choices offer an advantage of variable flow control of the outlet flow stream, but the valves and regulators can be costly. The linear flow restrictors are by far the least expensive and by personal experience the most reliable. Most often, a suitable linear restrictor can be fabricated from a length (typically $< 1\,\text{m}$) of 10–50 um i.d. fused silica capillary tubing. The choice of length and diameter is more or less a trial and error process in order to achieve the desired process pressure and flow rate. A single strand of $50\,\mu\text{m}$ i.d. capillary is most often adequate to allow material to pass even in a preparative-scale process, although we have seen considerable slowing of flow rates when large volumes of water ($\sim 1\,\text{ml}$) are introduced with the sample. This can be corrected by linking several fibers in parallel. This action has little affect on the pressure and density gradients, but does allow greater sample throughput on the outlet stream. An additional advantage to using linear restrictors is that they can be made more impervious to plugging by encasing them in resistively heated metal jackets.[77]

Design considerations for supercritical fluid extraction modules

The development of laboratory supercritical fluid extraction systems closely tracked the development of pressure restrictors for supercritical fluid chromatography. Effectively, these systems are nothing more than a chromatography system with the separation column replaced by a suitable extraction cell. To be suitable, an extraction cell should be constructed of stainless steel. It is highly desirable to minimize and weld seals as these tend to deteriorate over time and can present a radiological control problem for the user. Figure 3 presents a schematic of an extraction cell we built for use in our laboratory. The bottom portion of the cell is machined from 316 stainless steel. Note that the inside cavity is sufficiently large to accommodate a 10 ml volume Reacti-Vial$^{\text{TM}}$. This offers an advantage for loading samples into the chamber. The chamber is heated with four-50 W cartridge heaters that are controlled with thermocouple feedback to an Omega Engineering, Inc. (Stamford, CT) series 9000 proportional controller. The top flange is

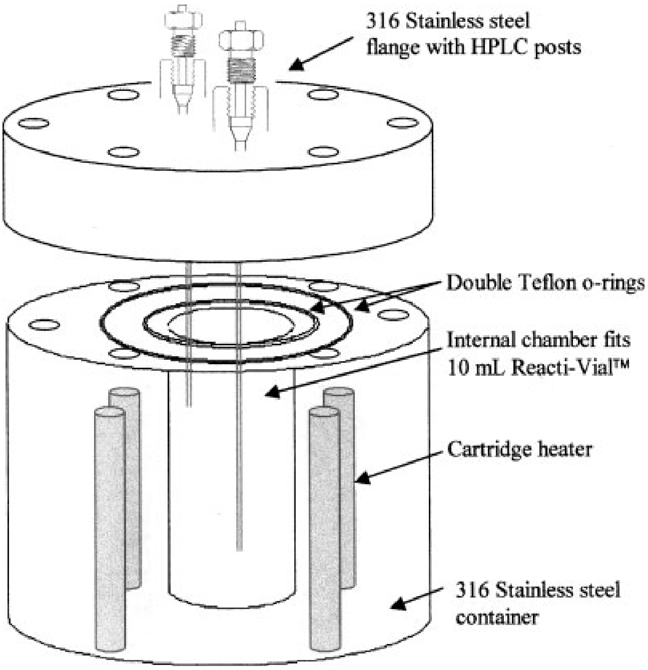

Figure 3. Stainless steel extraction cell for performing supercritical fluid extractions

also machined from 316 stainless steel, and it has two machine ports to accommodate SSI 1/8″ HPLC nuts and ferrules. The inlet and outlet holes through these ports are machined to allow the tubing to slide through to the appropriate depth within the cavity, and then can be locked in place with the HPLC nut and ferrule. The tubing ends are fitted with stainless steel HPLC frits to prevent paniculate from plugging the pressure restrictor. Suitable high-pressure valves can be mounted onto these lines. The top flange is sealed onto the main chamber using a double arrangement (1″ and 1.5″ o.d. × 1/8″ thick) Teflon o-rings. Generally, these must be replaced on every entry into the chamber.

Material collection considerations

The main practical challenges associated with utilizing supercritical fluids for preparative-scale chromatography or process extraction are usually associated with analyte collection. Because of the large volumes of supercritical fluids used in such processes, the extent of fluid expansion at the outlet stream will typically generate large gas flows that will provide uncontrollable release of volatile and nonvolatile analytes thus impacting their recovery. Considerable time and effort has been spent developing ways to quantitatively and reproducibly recover the extract at the end of the process. The trapping method could be as simple as a small volume of solvent,[97] or somewhat more complex involving either adsorption onto solid traps and later thermal desorption,[98] or solidification of the supercritical fluid (if the nature of the material used for this purpose permits this, as is the case with carbon dioxide) with later sublimation to effect recovery of the extract.[98]

Assessing basic properties of supercritical fluids

In order to successfully utilize supercritical fluids, one must be able to accurately predict and/or experimentally measure the solubility of analytes and other added solvents within the fluid. One of the biggest downfalls that can occur is when one assumes a state of criticality with fluid homogeneity when in actuality the system is biphasic. This kind of mistake can be avoided by clearly understanding ways to assess how states of matter behave under certain temperatures and pressures.

Thermodynamic considerations

An empirical description that proceeds from thermodynamic principles can be seen below for the relationship between the temperature-dependent solubility c, and density ρ, (both in g l^{-1}) where a and b are simple constants characteristic of the solvent and solute.[99]

$$c = \rho^{\mathrm{k}}\exp(a/T + b)$$

where k is related to an association number corresponding to the average number of solvent molecules that are associated with a single molecule of a dissolved substance. Typically, k increases nonlinearly with the size of the solute molecule owing to the ability of solvent molecules to cluster about the solute, a phenomenon well known to occur in pressurized liquids and supercritical fluids. This behavior is in part attributable to the high compressibility of the fluid at around the critical loci, and in part because of differences in size and energy between solvent and solute giving rise to strong solvent–solute interactions that yield rather large negative partial molar volumes,[100] and partial molar enthalpies.[101]

Evaluating solvent–solute behavior through geminate radical recombination

Over the years, several practical methods have evolved to investigate solvent–solute behavior in a supercritical state. One of the first methods to develop relied on a photochemical paradigm that is based on the well-established mechanism for α-cleavage of photoexcited triplet ketones yielding geminate radicals.[102,103] Recombination of these radicals can provide a powerful tool to evaluate localized density augmentation caused by solvent clustering. The rationale here is that the denser the cluster cage is the less likely the radical pair will diffuse apart, and the more likely they will recombine to form a distinct measurable product.

We have used this technique in our laboratory to evaluate acetonitrile clustering in supercritical carbon dioxide fluid at 95°C for a number of pressures ranging from 50 to 325 bar.[72] UV photolysis of dibenzylketone at between 250 and 350 nm wavelengths will generate a benzyl radical and the phenylacetyl radical as geminate radicals. The phenylacetyl radical, however, will subsequently undergo rapid unimolecular decarbonylation ($k_{CO} = 9 \times 10^{-6}$ s^{-1} at 20°C) yielding a second benzyl radical.[104,105] This greatly simplifies analysis to measuring ratios of two products, toluene (diffusion/abstraction) and dibenzyl (radical recombination). Figure 4 shows results from some of our earlier work with this approach and demonstrates an optimized cage effect at close to the theoretical critical pressure. While this technique can be highly informative, it does not shed light on whether or not mixtures of solvents are in fact homogeneous or are in some part of vapor–liquid equilibrium.

Measuring liquid–vapor critical loci using peak-shape analysis

Phase separation between the primary supercritical fluid and solvent modifier or injection solvent (note that injection solvents in a preparative-scale application could be of sufficient volume to alter the physico-chemical properties of the fluid) can occur anywhere within a supercritical fluid process application. In a mixture such as this, the phase state depends not only on composition, but also on local temperature and pressure within the system. Phase separation can be

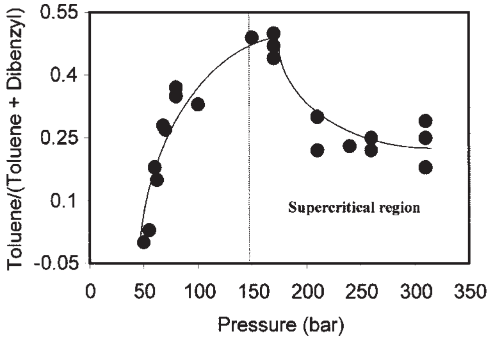

Figure 4. Critical cluster curve for 20% acetonitrile: 80% carbon dioxide at 95°C. Curve generated from geminate radical recombination method. (Reprinted from *Nucl. Med. Biol,* RA Ferrieri, I Garcia, JS Fowler, AP Wolf, "Investigations of acetonitrile solvent cluster formation in supercritical carbon dioxide, and its impact on microscale syntheses of carbon-11 labeled radiotracers for PET," 26 (1999) 443–454 with permission from Elsevier Science.)

either desirable or devastating. Typically, such behavior in supercritical fluid chromatography can lead to peak broadening, peak splitting, irregular peak shapes and irreproducible retention times. All are highly undesirable. However, there are times when controlled phased separation may be put to good use. For example, analyte focusing during direct injection in open tubular chromatography can lead to extremely sharp peaks.[106, 107]

Interestingly, there are no known investigations on the influences of phase behavior in supercritical fluid extraction. In addition, there is a limited amount of phase behavior data even in chromatography applications. This lack of information stems largely from early methodologies to make such measurements, which relied on using high-pressure view cells with either visual inspection or light scattering detection. These kinds of laboratory equipment were not readily available in the laboratory.

In 1993, a simple solvent peak-shape method was described for measuring liquid–vapor critical-mixture curves involving supercritical carbon dioxide and one other solvent combinant.[106] This method is capable of being carried out using standard gas chromatography (GC) equipment. In essence, the source of supercritical carbon dioxide fluid is connected to a length of 10 μm o.d. uncoated fused silica capillary that is housed within the GC column oven for precise temperature control. The outlet of the capillary is connected to a flame ionization detector (FID).

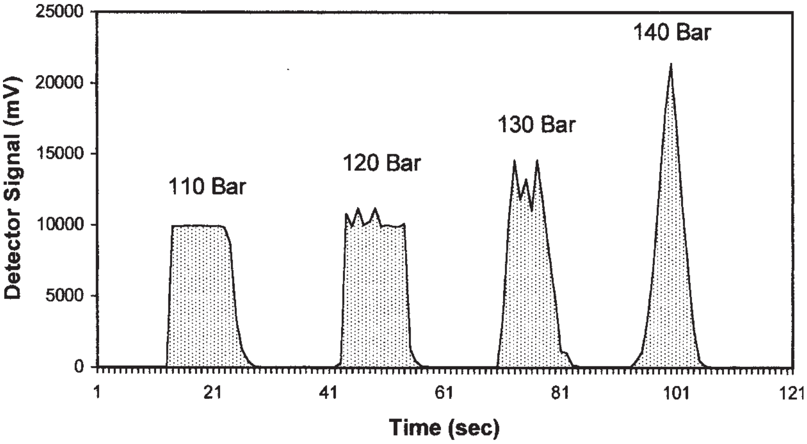

Figure 5. Critical loci measurement of *sc* CO_2-acetonitrile solutions at 95°C using co-solvent peak-shape analysis

A small volume of test solvent is then injected into the fluid stream under controlled temperature and pressure conditions. The nature of the peak shape then reflects the phase behavior between the solvent mixture. For example, if a liquid-phase exists under these conditions then a dynamic film occurs with surface evaporation resulting in a rectangular peak shape. On the other hand, if the binary mixture is homogeneous, then the injected solvent component mixes freely and assumes a Gaussian shape. This behavior can be seen in Figure 5 for acetonitrile–carbon dioxide mixtures at 95°C. At 140 bar the solution behaves in as a single-phase supercritical fluid. One can repeat this exercise for a number of temperatures and ultimately construct a critical-mixture curve for the solvent combination as a function of temperature and pressure. Such a curve can be seen in Figure 6.

While this application may seem specific for supercritical carbon dioxide fluid, there is no reason the same method could not be extended to other fluids. The limitation, of course, is the FIDs sensitivity to the fluid. Likewise, one is not exclusively tied to the FID. As described earlier, there are a host of equally suitable detectors that might be explored for this purpose.

Evaluating solvent properties through solvatochromic behavior

Another way of relating solvent properties is by measuring how the close chemical environment surrounding a suitable chromophoric probe influences the $\sigma \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions in the UV-Vis

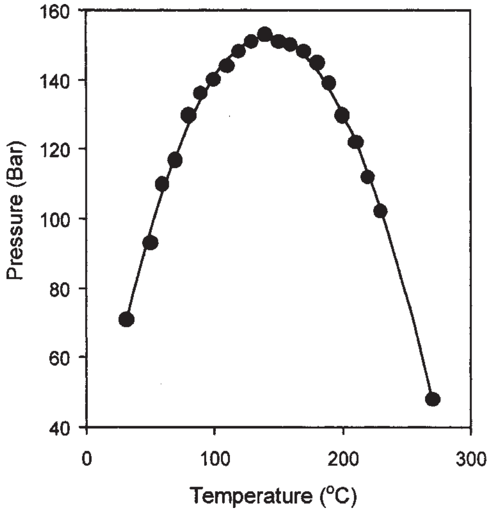

Figure 6. Critical-mixture curve for CO_2-acetonitrile solutions obtained using the peak-shape method. For solutions maintained at 95°C, a pressure of 140 bar will yield a homogeneous supercritical fluid

spectra.[71, 108–111] The rationale here is that measurable shifts in the probe's absorption band can be correlated with the solvent's interaction due to polarity, polarizability and ability to hydrogen bond.

Equipment for carrying out such measurements can be simple. Figure 7 shows a schematic of a suitable optical cell constructed in our laboratory for this purpose. A standard stainless steel HPLC cross can be used as the optical cell yielding a 2 mm pathlength. The optical path can be connected to the light source and spectrometer using 600 μm o.d. fiber-optic leads thus relying on the bare fiber optic end as the high pressure optical window for the cell. Ocean Optics, Inc. (Dunedin, FL) markets fiber optic coupled monochromators that can be configured to desired wavelength range and optical resolution (typically <1 nm resolution is desirable for these measurements). High-pressure coupling to the HPLC cross can be achieved by fitting the fibers into 1/16″ o.d. PEEK tubing and using standard stainless steel HPLC fittings.

We tested 22 cosolvent additives in supercritical carbon dioxide at 50°C and 100 bar pressure using phenol blue as the chromophoric probe. Results from these tests can be seen in Figure 8 where solvatochromic shifts in nm wavelengths were converted to transition energies in kJ/mol and plotted as a function of solvent size. Interestingly, clear class distinctions between cosolvents can be seen

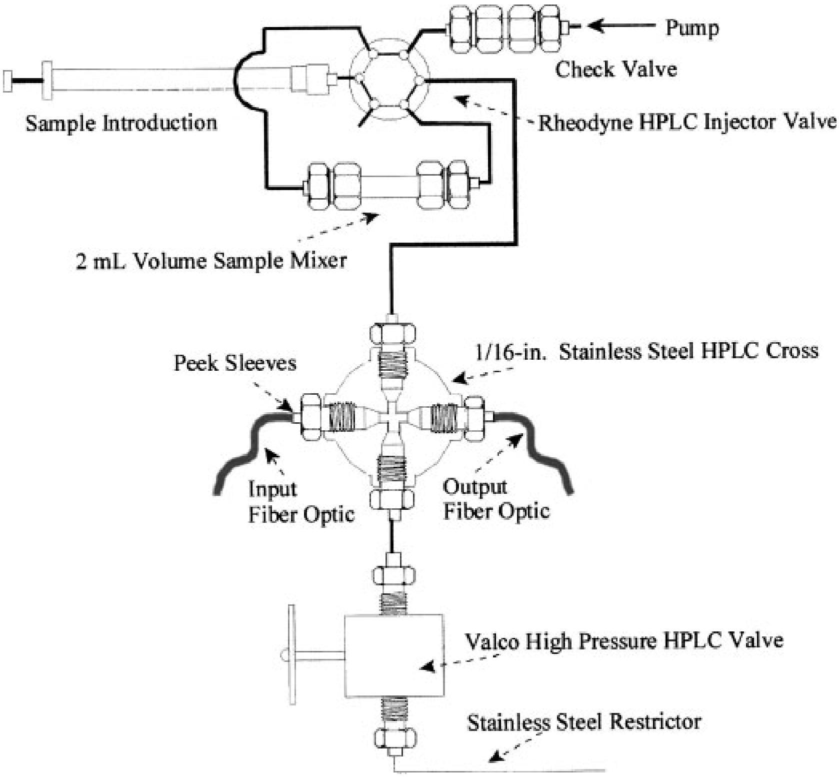

Figure 7. High pressure fiber optic UV-Vis cell and sample apparatus constructed from a commercial 1/16 "HPLC cross and standard HPLC hardware. 600 mm o.d. fiber optics serve as the pressure windows for the cell and can be pressure sealed by encasing within 1/16" o.d. PEEK tubing using standard HPLC nuts and ferrules

under these circumstances that reflect the cosolvent's ability, or lack of, to polarize and hydrogen bond with the solute. In addition, each class of solvent modifiers (including nonpolar hydrocarbons, polar nonhydrogen bonding solvents and alcohols) shows established size related trends that reflect the cosolvents ability to cluster about the solute. For example, methanol produced the largest solvatochromic shift of all the solvents tested. Its compact size and ability to hydrogen bond suggests a highly dense interactive cluster about the solute. This is why methanol is one of the most widely used modifiers in supercritical carbon dioxide. On the other hand, large alcohols behave much the same as a nonpolar hydrocarbon solvent yielding less dense clusters with little or no hydrogen bonding interaction.

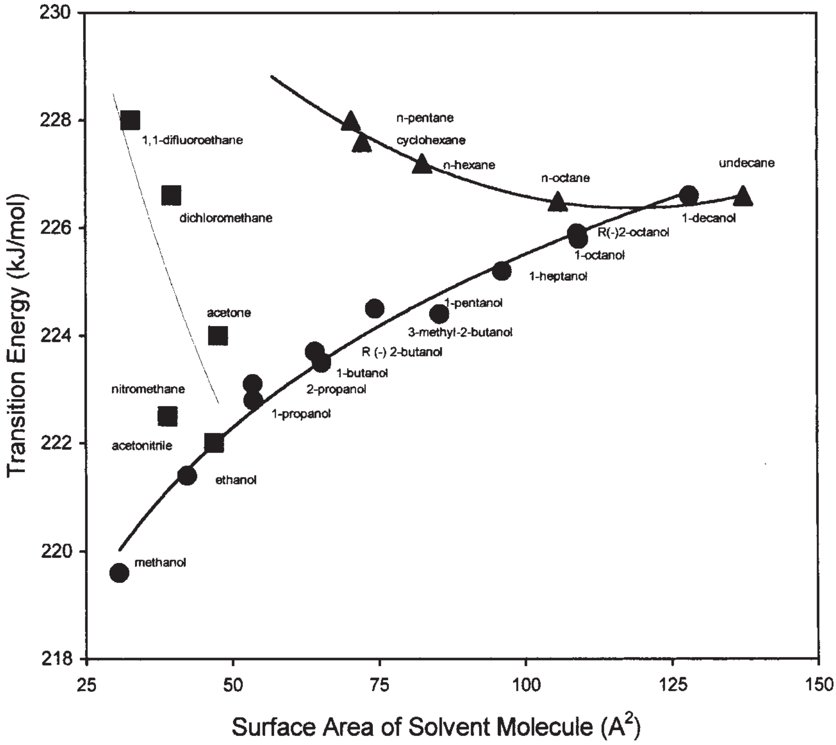

Figure 8. Solvatochromic measurments of various solvents mixed in supercritical carbon dioxide at 50°C and 100 bar. (▲, aliphatic hydrocarbon solvents; ■, polar nonhydrogen bonding solvents; ●, alcohol solvents)

A convincing argument for working at or near the critical loci of the solvent mixture can be seen in Figure 9 where the same 22 cosolvents were retested at 300 bar pressure. (The trend lines in this figure reflect the results from Figure 8.) Note the lack of solvent class distinctions under these circumstances. This is understandable since the solvent additives are less likely to form clusters about the solute when the fluid becomes less compressible at higher pressure.

A further refinement that can aid in interpreting spectroscopic measurements of this nature is to correlate transition energies with an empirical polarization parameter, E_T. This parameter was first introduced in 1990 in a compilation of data relating the molar excitation energy of the charge–transfer band for a highly solvatochromic dye, *N*-phenylpyridine-betaine, with a measure of the strength of the charge interactions of a particular solvent.[112] Replotting the transition energies of Figure 8 against a normalized polarization

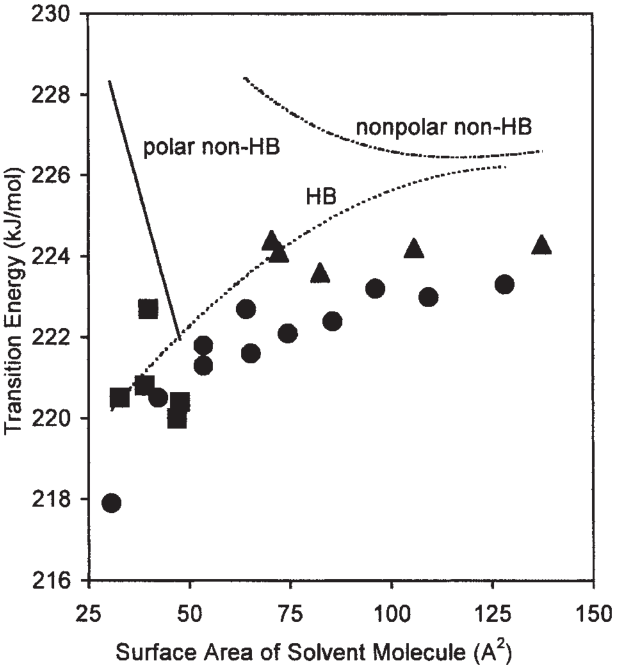

Figure 9. Solvatochromic measurements performed on the same 22 solvents mixed in supercritical carbon dioxide at 50°C and 300 bar. Trend lines depict behavior of these solvent at the lower pressure. (▲, aliphatic hydrocarbon solvents; ■, polar nonhydrogen bonding solvents; ●, alcohol solvents)

parameter, E_T^N, reveals distinct linear correlations in Figure 10 for the three solvent classes suggesting that strong solvent–solute charge–transfer interactions can be elicited within these states of matter. This is an important point that will be revisited later in Part II of this review series.

Concluding Remarks

The intent of Part I of this review was to provide an understanding of the basic properties of supercritical fluids, the equipment necessary to create such states of matter and a basic approach to getting started within the field. Many of the examples described in this part were based on the author's own work, and were presented with an intent to provide additional guidance. Interested readers are encouraged to seek additional information from the many reviews and texts cited within.

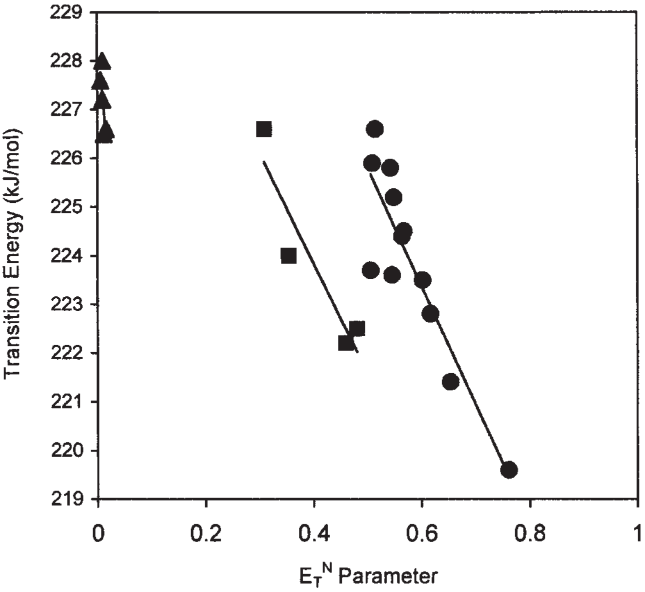

Figure 10. Linear correlation between solvent transition energies and the empirical polarization parameter, E_T^N (▲, aliphatic hydrocarbon solvents; ■, polar nonhydrogen bonding solvents; ●, alcohol solvents)

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