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Review Article

Supercritical fluids in medical radioisotope processing and chemistry, Part II: Applications – real and demonstrated

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Summary

In Part I of this review series, an overview was presented on what the basic properties of supercritical fluids are and how they can, and are being used in many of today's industries as solvents for extraction, chromatography and reaction. A good part of this overview detailed the kinds of equipment needed, and techniques on how to use them for optimal performance. Part II of this series will delve into specific applications of supercritical fluid technology as it relates to aspects of medical isotope processing. The reader will note that very few applications of this technology to Nuclear Medicine have been published. Many potential applications cited within the context of this review derive from preliminary studies carried out in the author's laboratory. These examples are presented to spark interest in future developments of this nature.

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

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Introduction

One of the biggest challenges in medical radioisotope processing lies in the ability of radiochemists to design experimental platforms that allow for rapid and efficient separation of isotopes, chemical synthesis, and final radiotracer purification. Emerging technologies are constantly being explored for possible improvements in this overall process. Supercritical fluid technology is but one area whose application could improve future processes for making medical radiotracers.

One of the key features of supercritical states of matter is that the fluids typically will exhibit extremely high compressible behavior by forcing their gaseous or liquid states to exist above some critical pressure or critical temperature defined for that material. Supercritical-phase behavior can exhibit a high level of functionality and controllability in this state influencing both the nano-structures and macro-physical properties of components dissolved in them. As a supercritical fluid, substances retain many of the properties of both gas-like and liquid-like behavior. Thus, they possess strong penetrating and dissolution properties. In addition, they can offer the advantage of being able to change density to a great extent in a continuous manner. For example, the density of carbon dioxide increases almost 13-fold from gas-like (0.5 mol/l) to liquid-like (19 mol/l) behavior in the supercritical region. Water also shows different properties as it moves towards this denser region. This is seen in the drastic change of its dielectric constant reflecting solvent polarizability. Many materials that might not normally be soluble in liquid water are extremely soluble in supercritical water. On this account, use of carbon dioxide or water in the form of a supercritical fluid offers a substitute for conventional organic solvents.

As described in Part I of this series, many substances possess properties that are amenable for their use as supercritical fluids. The intent of Part II of this series is to describe a number of applications, some published and some merely demonstrated in preliminary results from the author's own lab. These examples are meant to show the power and potential of this technology.

Applications – real and demonstrated

PET radiotracer purification using supercritical fluid chromatography

The successful adaptation of supercritical fluid methods to fields of medical isotope processing such as PET depends on the users being able

to demonstrate applications and methods that are better than existing ones. Being better can most often carry with it the qualities of being more efficient, selective, faster or perhaps even less expensive. In PET radiotracer purification reliability is perhaps most important. Additionally, recent concerns over environmental impact and 'green chemistry' brings up another issue which perhaps can become an even stronger impetus for implementing technology of this nature. Without a doubt, carbon dioxide is more environmentally friendly than the organic solvents it can replace in chromatography applications.

Radiochemists working in PET face many challenges while designing and implementing new methodologies for preparing radiotracers that are suitable for human injection. On the one hand, they have to address unique problems of how to sustain efficient chemistry using minute amounts of reactants. On the other, stringent controls must be maintained to minimize chemical and/or radionuclidic impurities during final tracer formulation. Radiotracer chemists typically accomplish this by loading the reaction with an excess of labeling substrate (an organic component to which the labeling precursor bonds), and relying on semi-preparative-scale HPLC to separate the desired radiolabeled product from the starting material, the organic reaction solvents, as well as from reaction by-products. Unfortunately, semi-preparative-scale HPLC in the PET Radiotracer Laboratory is a process that generates a large hazardous waste stream. For example, a single radiotracer synthesis can generate up to 250 ml of an aqueous-organic waste. A logical course of action is to seek an alternate solvent system that would allow radiotracer purification to be carried out in an organic solvent-free environment. This makes for sound environmental and economic sense especially since waste-processing costs will no doubt continue to rise in the future.

To date there are two known applications of supercritical fluid chromatography to medical isotope processing, and both of these are in the PET field.[1,2] The first application was driven out of a desire to combine a supercritical fluid reaction cell with on-line chromatographic purification of the final radiolabeled product using the same supercritical fluid as a mobile phase. This system used supercritical ammonia ($T_C = 132.5°C$, $P_c = 113.1$ bar) for its improved solvating powers of polar analytes over that of carbon dioxide. All purifications were performed on a Hypercarb column (4.6×200 mm, 5 μm, Shandon HPLC) that was operated isothermally, but with pressure programming. The system also utilized a fiber optic UV detector much like that

described in Part I of this review series where 400 μm o.d. fibers served as the high-pressure windows. Fused silica capillary (i.d. = 0.198 mm, o.d. = 0.356 mm) was used to interface the radiation and UV detectors with claims that the radiation detector was more sensitive toward the levels of radioactivity using silica capillary tubing rather than stainless steel tubing for the flow connections. However, it was noted that the polyimide coating that is usually standard on most fused silica tubing was not chemically resistant to ammonia and had to be burned off before use. The authors demonstrated very fast, efficient and extremely reproducible elutions for the chromatographic purification of several carbon-11 labeled compounds including [methyl-^{11}C]anisole, 4-methoxyphenyl[^{11}C]guanidine and [methyl-^{11}C]methionine. One of the key advantages pointed out was the fact that solvent evaporation is eliminated after product collection because the ammonia dissipates as a gas at the outlet thus saving approximately 10 min of process time which improves on specific radioactivity, as well as minimizing product loses from additional manipulations.

The second application was driven not so much out of a desire to seek an environmentally friendly or more efficient process, but more so out of necessity to develop a process that would allow for the purification of a volatile PET radiotracer. This is a unique application in the sense that most, if not all, PET radiotracers are nonvolatile salts or possess sufficiently high boiling points that conventional liquid chromatography followed by evaporation of the mobile phase is practical. However, when attempting to develop a purification scheme for [^{11}C]toluene for PET primate studies as part of a solvent abuse liability investigation, we became keenly aware of the fact that the high volatility of the radiotracer precluded using conventional separation. In addition, a pure aqueous solvent was not appropriate due to the high lipophilicity of this radiotracer, as well as other inhalants under development in our laboratory. The big issue, of course, is how to efficiently isolate something that is volatile from the organic starting materials and solvents that are or can be equally as volatile.

In the specific application, no-carrier-added [^{11}C]toluene was synthesized by the rapid coupling of [^{11}C]CH_3I with tributylphenylstannane mediated by a palladium (0) complex formed from a mixture of *tris*(dibenzylideneacetone) palladium (0) and tri-*O*-tolylphosphine in dimethyl acetamide solvent (DMA).[3,4] An early application utilized a 50% aqueous mixture of DMA mobile phase to affect HPLC purification of the final product.[5] Although final formulation was

diluted to a level containing 10% DMA that was deemed suitable for injection into primates, it was later discovered that the presence of DMA had a strong influence on the pharmacokinetics of the radiotracer.[6] This lead to the more recent application where [^{11}C]toluene was separated from the starting materials using a conventional C_{18} HPLC column and pure supercritical carbon dioxide fluid as the mobile phase operating at 2000 psi and 40°C.[2] Figure 1 shows an overlay of both the UV and radiation detector chromatography traces from the injection of 1 ml volume of a crude reaction mixture containing 5 mCi of [^{11}C]toluene (mixed with 10 μmol of carrier toluene). One can see that toluene, as well as many other small-chained hydrocarbons, is soluble in pure supercritical carbon dioxide fluid. However, toluene has some retention on the column suggesting elution behavior similar to GC. It was also demonstrated in this application that the purified tracer could be quantitatively captured on a solid-support cartridge comprised of Tenax GR material, and later desorbed in a 1.5% cyclodextrin solution that is suitable for intravenous injection or into a breathing tube for direct inhalation. Post-analysis by capillary GC also revealed that the tracer was completely devoid of all starting materials and reaction

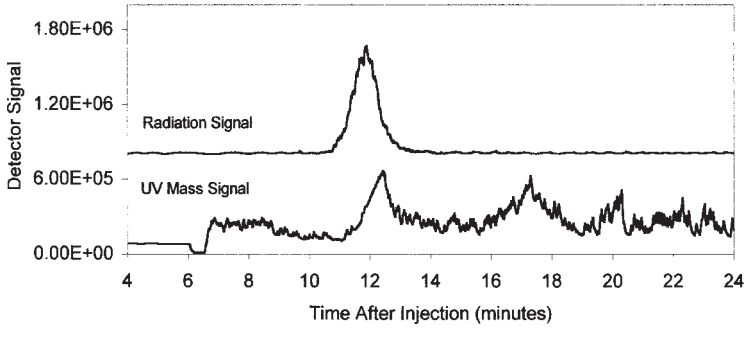


Figure 1. Overlaid elution traces from PIN diode radiation detector and UV mass detector responses from a 5 mCi injection in a 1 ml volume of the [^{11}C]toluene reaction mixture. Note that the sample was 'spiked' with 10 mmol of carrier toluene to provide adequate UV response for system validation. Also note that the radiation detector was interfaced in the fluid flow stream ahead of the mass detector. A single radioactive peak associated with [^{11}C]toluene was detected. (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.)

solvent. This approach can easily be applied to other radiolabeled inhalants or volatile radiotracers with slight refinements to the method.

Expanding this approach to other more conventional nonvolatile PET radiotracers should be possible, as well. Using the same system as described above, and depicted in Part I of this review series, we have been successful at eluting compounds like nicotine and L-deprenyl using pure carbon dioxide fluid on a reversed-phase column. Some of the more polar compounds, for example ritalin, may require refinement of the fluid to include addition of an organic solvent modifier such as methanol or acetonitrile. These refinements would only introduce a small volume of organic waste to the process, but will then require a solvent evaporation step at the end. Another alternative is to slightly humidify the carbon dioxide fluid stream. Supercritical carbon dioxide can be humidified with up to 0.3% water.[7] This can greatly enhance the ability of the fluid to solubilize larger molecular weight and more polar analytes and should perhaps be explored first for efficacy of use before resorting to organic modifiers. The reason for this is the post-chromatography manipulations can be greatly simplified to just rendering the formulation sterile and pyrogen-free without having to remove the small amounts of organic modifier collected along with the product. Several approaches have been described in the literature for humidifying supercritical carbon dioxide. We have been successful at doing this by bubbling the fluid stream through a volume of water maintained at suitable pressure and temperature. In fact, one can use the same extraction vessel design shown described in Part I. Changing temperature of the fluid will control humidification with reasonable precision. A simpler approach is to install a short stainless steel tube filled with silica or alumina in the heated zone of the fluid stream just prior to the sample injection valve. The packing can be primed with water prior to installation, and will provide a sufficient source of water for humidification for several chromatography runs before being depleted.

Supercritical fluid extraction of radioactive metabolites in biological samples

To date, there is only a single known application of supercritical fluid extraction involving medical radioisotopes and this involved PET radiotracer metabolite analysis.[8] This application utilized supercritical ammonia in an attempt to develop a rapid extraction procedure that

allowed subsequent analysis of the radioactive labeled metabolites after *in vivo* injection of *O*-[2-^{11}C]acetyl-L-carnitine and *N*-[^{11}C]methylpiperidyl benzilate in rats. Metabolites were extracted from the kidneys and brains using supercritical ammonia at between 70 and 150°C and a pressure of 400 bar. A 2.2 ml commercial stainless steel extraction vessel (Keystone Scientific, Inc.) was used for this purpose. The rat organs were cut into several small pieces and mixed with purified diatomaceous Earth to fill the extraction volume. However, this material also served as a drying agent to absorb the excess water residing within the tissue samples that often caused plugging of the extractor frits.

For *O*-[2-^{11}C]acetyl-L-carnitine, 66% of the radioactivity was trapped in the collected fractions and 12% remained within the extraction vessel. For the more lipophilic-[^{11}C]methylpiperidyl benzilate, 93% of the radioactivity was collected and less than 1% remained within the vessel. The entire extraction procedure took less than 25 min to carry out which is considerably shorter than conventional liquid solvent extraction.[9] The shorter process time is extremely beneficial in small animal PET studies of this nature where it is quite often necessary to measure radiotracer metabolites and their distribution throughout the animal's organs for proper interpretation of the PET imaging data. Unfortunately, small animal studies of this nature can be restrictive in their scope because the small amount of radioactivity typically injected into the animal, and the short half-life of the radioisotope used generally precludes use of many conventional practices for processing the radioactive biological samples and obtaining useful information. This application clearly demonstrated that the high polarity and solvent strength of supercritical ammonia makes it an attractive media for extraction of polar analytes, with little or no sample pretreatment.

Potential applications of supercritical fluids in the extraction of medical radioisotopes from solid targets

Using supercritical fluids as solvents for extraction of medical radioisotopes produced from solid targets is one area that has yet to be explored but could have an impact. New cyclotron and accelerator technology has intensified interest in solid targets that can produce usable amounts of appropriate medical radioisotopes for diagnostic imaging and radiotherapy using high power density targets. Quite often these target matrices are comprised of costly enriched starting materials often necessitating recovery of the target material. Post-irradiation

recovery of the radioisotope using either dry distillation or dissolution and selective precipitation often renders the target material unusable, or necessitates performing a time-consuming recovery step.[10] The use of supercritical fluids to extract the desired radioisotope from the target material might provide a more efficient, and more cost-effective approach to processing solid targets of this nature. One key advantage here is that the supercritical fluid should not alter the chemical state of the enriched target material thus allowing it to be reused.

Supercritical fluid extraction (SFE) of metal species including organometallic compounds has been the subject of many reports and reviews in recent years.[11–13] Many of these applications were developed as part of radioactive waste remediation projects designed to extract and recover useful amounts of radioactive metals. Most often, pure or modified supercritical carbon dioxide is the solvent of choice for these applications owing to its chemical inertness and commercial availability in pure and modified forms.

It is well known that cationic metal ions are not sufficiently soluble in pure carbon dioxide due to the weak interactions between the positively charged metal ions and the nonpolar carbon dioxide molecule.[14] Thus, extraction and separation of metal ions alone is not a practical consideration here. However, metal ions may be solubilized, extracted and separated by binding them to a suitable organic ligand. One approach of performing SFE of metals is to convert the charged metal ions into neutral metal chelates using suitable organic chelating agents that are dissolved in the supercritical fluid at the source.[15–18] Important considerations here include good solubility and stability of the complexing agents and their metal complexes within the supercritical fluid. One way to assess such properties is through UV-Vis spectroscopy. The high-pressure fiber optic UV cell described for the solvatochromic studies is well suited for this task.[19–21]

Different types of ligands have been used for complexation and extraction. One ligand system which has been extensively studied for SFE of metal species includes the derivatives of dithiocarbamic acid of the general form, R_2NCS_2X, where R is an alkyl group and X is a cation which can be an alkali metal ion.[15,17–19,22] Fluorinated derivatives of this class of chelating agent appear to show greater solubilities over their protonated counterparts.[19] For example, sodium *bis*(trifluoroethyl) dithiocarbamate has a solubility in pure supercritical carbon dioxide fluid at 50°C and 100 bar that is roughly four times that of sodium diethyldithiocarbamate.

β-diketones react with a variety of metal ions to form neutral chelates through interaction of the enolate anions. A number of different metals including Ga, In, Zn, Co, Cu and Li have been investigated as acetylacetone complexes in supercritical carbon dioxide at 60°C, and 290 bar.[23] With the exception of Li, all metals listed showed respectable solubilities ranging from 3 mg/l down to 0.25 mg/l.

Like the dithiocarbamic acid derivatives, several fluorinated β-diketones show enhanced performance due to increased solubilities in supercritical carbon dioxide and have demonstrated applications in the extraction of radioactive lanthanides and actinides in nuclear waste remediation.[11] Several of β-diketone agents tested include trifluoroacetylacetone, hexafluoroacetylacetone, thienyltrifluoroacetone and heptafluorobutanoylpivaroyl-methane.

Organophosphorous reagents, such as tributylphosphate, and phospine oxides, such as tributylphosphine oxide, have a well-established track record for use as extractive ligands for the actinide elements,[24] and more recently for extraction of uranium, thorium and plutonium from solid nuclear waste.[25,26]

Macrocylic polyethers (crown ethers) are yet another class of selective ligands that form stable complexes with metal ions. This complexation is largely based on the ionic radius-cavity size compatibility. Modification of the crown ether structure by attaching negatively charged functional groups will often eliminate the need for adding counteranions that are usually required to facilitate transport of the charged complexes into the organic phase. One type of ionizable crown ether containing triazole groups was shown to selectively extract mercury in supercritical carbon dioxide.[27]

Another class of macrocylic agents that shows promise includes the calixarene-based agents. Calixarenes possess both lipophilic and metal coordinating groups within their structure thus allowing many metals to form highly lipophilic complexes with the deprotonated forms of the caliarene agents.[28]

Designing chelating agents that are highly soluble in carbon dioxide is an area of keen interest in today's scientific community owing to the fact that there is a continuous growing interest in environmental impact and ways to clean up environmental waste. In general, fluorinated compounds exhibit high solubilities in supercritical carbon dioxide, as well as many of the metal complexes derived from these extractants. Molecules which contain functional groups such as fluoroether and fluoroalkyl groups appear to be the most successful in this class.[29]

A number of metal radioisotopes that can be produced on a cyclotron using modest energy particles are listed in Table 1.[10] (This list is not inclusive of all possibilities, but meant to serve as an example based on metal extraction capabilities.) Those targets that are comprised of enriched materials and are listed with an asterisk. The potential also exists that SFE could be applied to solid targets for extraction of nonmetallic radioisotopes. Table 2 presents a partial list of potentially useful radioisotopes that could be explored. Many of the issues and concerns regarding analyte solubility would not exist for this group as carbon dioxide's ability to act as a Lewis acid with these anionic species

Table 1. Metal radioisotopes that are produced using a cyclotron and are candidates for processing via SFE.

Product nuclide	Half life	Nuclear reaction	Nominal energy (MeV)
Mg-28	21 h	^{27}Al(α,3p)	45
V-48	16 d	^{48}Ti(p,n)	11
		^{47}Ti(d,n)[a]	10
Fe-55	2.73 y	^{55}Mn(p,n)	20
Co-55	17.5 h	^{56}Fe(p,2n)	25
Co-57	271 d	^{60}Ni(p,α)	25
		^{55}Mn(^{3}He,n)	40
Cu-61	3.35 h	^{61}Ni(p,n)[a]	12
		^{64}Zn(p,α)[a]	22
Cu-64	12.7 h	^{67}Zn(d,α)[a]	20
		^{66}Zn(d,α)[a]	20
Ge-68	272 d	^{69}Ga(p,2n)[a]	30
As-73	80.3 d	^{74}Ge(p,2n)[a]	11
As-74	17.8 d	^{74}Ge(p,n)[a]	15
Y-88	106.6 d	^{88}Sr(p,n)	11
Zr-89	3.27 d	^{89}Y(p,n)	15
Tc-94m	52 m	^{94}Mo(p,n)	15
Tc-95m	61 d	^{96}Mo(p,2n)[a]	25
Tc-96	4.3 d	^{96}Mo(p,n)[a]	15
Ru-97	2.89 d	^{95}Mo(^{3}He,n)	45
Cd-109	462 d	^{109}Ag(p,n)	20
Ce-139	137.6 d	^{139}La(p,n)	11
Ta-179	1.8 y	^{180}Hf(p,2n)[a]	22
W-178	21.6 d	^{181}Ta(p,4n)	38
Pt-195m	4.02 d	^{192}Os(α,n)	40
Hg-195m	1.67 d	^{197}Au(p,3n)	30
		^{194}Pt(3He,2n)[a]	40
Pb-203	2.2 d	^{203}Tl(p,n)	20
Bi-205	15.3 d	^{207}Pb(p,3n)[a]	30
		^{206}Pb(p,2n)[a]	22
Bi-206	6.2 d	^{207}Pb(p,2n)[a]	22
		^{206}Pb(p,n)[a]	15
Pu-237	45 d	^{237}Np(p,n)[a]	25

[a] Uses isotopically enriched materials.

Table 2. Nonmetallic radioisotopes that are produced using a cyclotron and are candidates for processing via SFE

Product nuclide	Half life	Nuclear reaction	Nominal energy (MeV)
Br-75	97 m	^{76}Se(p,2n)	16
Br-76	16.2 h	^{76}Se(p,n)	25
Br-77	2.37 d	^{78}Se(p,2n)	20
I-120 g	1.35 h	^{120}Te(p,n)	25
I-121	2.12 h	^{122}Te(p,2n)	30
I-123	13.1 h	^{124}Te(p,2n)[a]	20
		^{124}Xe(p,2n)	30
I-124	4.2 d	^{124}Te(p,n)[a]	10
		^{121}Sb(α,n)[a]	30
At-211	7.2 h	^{209}Bi(α,2n)	30

[a] Uses isotopically enriched materials.

could enhance their solubility and facilitate extraction without the addition of suitable chelating agents.

A consideration in applying technology of this nature to any medical radioisotope processing is whether or not the radioisotope is useful in radiotracer development or medical application once it is extracted. This aspect needs to be carefully investigated.

PET radiotracer synthesis using supercritical fluids

Generally, when developing new synthetic procedures for making labeled compounds with short-lived radioisotopes such as ^{15}O, ^{13}N, ^{11}C or ^{18}F, special consideration must be given to sustaining high reaction yields with minute amounts of starting materials in as short a time as possible. These aspects can impact on the quality of the final radio tracer used in PET in terms of its final specific activity. This is especially important in PET receptor imaging studies. Thus minimizing isotopic dilution of the radioisotope is of paramount concern, and is often approached by minimizing the amounts of reactants and volumes of solvents used during the reaction, as well as trying to enhance chemical reactivity in order to shorten reaction time.

The first application of supercritical fluids to PET radiotracer synthesis can be found in the model study using supercritical ammonia to facilitate the methylation of phenol with [^{11}C]methyl iodide to yield [*methyl*-^{11}C]anisole.[30] This work systematically examined a number of operating parameters including cell design, substrate concentration as

well as the influence of fluid temperature and pressure on the radiochemical yield. Radiochemical yields between 40 and 60% were obtained depending on the parameter used. For the most part, higher yields were obtained using smaller volume reaction cells (30 μl) that were longer (610 mm × 0.256 mm i.d.), suggesting perhaps that reactant mixing within the fluid was crucial to efficient reaction. In addition the highest radiochemical yields were obtained when reactions were carried out at or near the critical point ($T_c = 132.5°C$; $P_c = 112.5$ bar). The fluid will be highly compressible in this region of temperature and pressure impacting on its density and reaction rates. Interestingly, studies on the influence of substrate amount on radiochemical yield did not show a linear dependence. Yields remained fairly constant at 55–60% using between 5 and 21 μmol of substrate, but then dropped slightly to 38% when roughly 1 μmol was used. The biggest problem noted was the side reaction between [^{11}C]methyl iodide and ammonia yielding [^{11}C]methylamine which increased with less substrate.

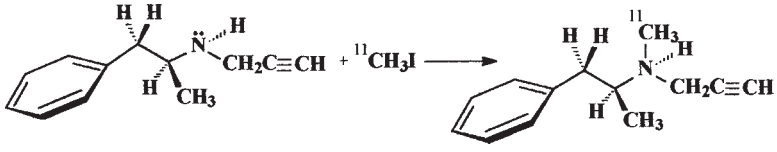
The second application of this technology describes an extension of the above approach to synthesizing a variety of aromatic and aliphatic [^{11}C]guanidines. In the process, the corresponding amines (4-methoxyaniline, 4-methylaniline, aniline, 4-aminobenzamide, 4-aminoacetophone, 4-nitroaniline, benzylamine, n-hexylamine, cyclohexylamine, and piperidine) were converted to their respective [^{11}C]cyanamides by reaction with [^{11}C] cyanogen bromide, and then allowed to react with supercritical ammonia to yield the respective [^{11}C]guanidine.[31]

$$RNH_2 + {}^{11}CNBr \rightarrow RNH\,{}^{11}CN \xrightarrow{sf\,NH_3} RNH\,{}^{11}C(NH)NH_2$$

The nature of the solvents used in the first part of the process, including 1-butanol, acetonitrile and toluene, and their influence on supercritical ammonia phase behavior was investigated using the [^{11}C]guanidine radiochemical yield to probe this behavior. For the most part, 1-butanol provided the highest yields although this trend was not consistent with all systems investigated.

A third application involving mixtures of supercritical carbon dioxide and organic cosolvents as media demonstrated a new strategy for PET radiotracer synthesis that relied on the co-solvent's ability to cluster within such states of matter giving rise to localized regions of high density that can serve as microscopic reactors. In the specific application cited, L-α-methyl-*N*-2-propynyl phenethylamine (nordeprenyl) was methylated with no-carrier-added [^{11}C]methyl iodide to yield [^{11}C]-L-deprenyl using mixtures of supercritical carbon dioxide maintained at a

temperature of 95°C and pressures between 60 and 275 bar, and mixed with 20% acetonitrile.[32]



Reaction under such conditions was shown to produce higher radiochemical yields of product (>92% radiochemical yield based on [^{11}C]methyl iodide) using 40 times less labeling substrate than when reactions were carried out in conventional organic solvents. This observation was attributable to the ability of acetonitrile to cluster, and while doing so to entrain potential reactants within a small reaction volume thus enhancing their chemical reactivity. Unfortunately, the potential of this application was never fully tested to ascertain whether sufficient radiochemical yields could be sustained using even smaller amounts of labeling substrate. The advantage here is that reagents could be reduced to near stoichiometric amounts thus eliminating the need to subject the reaction mixture to a rigorous chromatographic purification that would consume about one half-life of the radioisotope's decay, and reduce the specific activity of the final product by half.

The potential also exists to utilize these clusters to other benefits in radiotracer synthesis. For example, the ability to precisely control positional reactivity on a substrate molecule to an extent that selectively influences where on that molecule the radioactive label attaches, could have significant impact in synthetic methodological design with regard to radiotracer preparation.

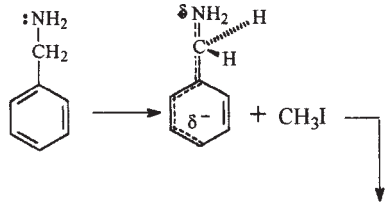
Molecular model calculations carried out in our laboratory suggest that while clusters formed in critical states of matter are only transient in nature, lasting on the order of nanoseconds, they can be highly ordered. Figure 2 depicts a cluster comprised of 48 butanol molecules clustered about a single phenol blue molecule. This is but one of several low energy configurations that we observed in a simple molecular model simulation using ChemSite^{TM} software (Pyramid Learning LLC) designed to conceptualize structures in 3D and provide some rudimentary physical properties. This particular cluster configuration exhibits cubic close packing exerting a net dipole that is near equivalent to the cumulative effect of the individual molecules. The impact this may have on the entrained phenol blue molecule, or any other molecule trapped within, is to cause a dipole interaction resulting in charge

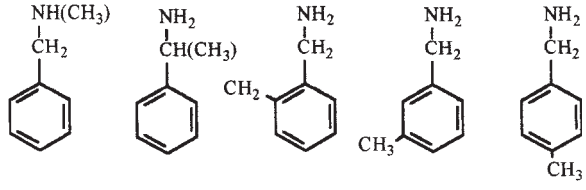
Figure 2. Molecular model simulation of least energy configuration for 48 butanol molecules clustered about a single phenol blue molecule. Dipole alignment in highly ordered cluster exerts a net dipole moment near equivalent to the total moment of the individual molecules

separation. This action can be monitored spectroscopically by relying on spectral shifts in substrate UV absorbance. Similar behavior can be expected when other polar cosolvents are used.

We demonstrated the potential utility of this behavior in a simple reaction model involving chemistry between methyl iodide and benzylamine. When methylation was allowed to occur under conventional conditions in the liquid phase, the only reactive site on the substrate was the amine group yielding *N*-methyl-benzylamine.

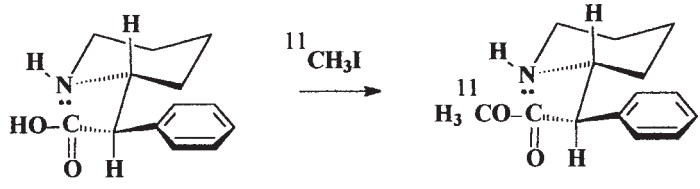
However, when chemistry was allowed to occur in supercritical carbon dioxide fluid doped with 15% acetonitrile and maintained at 50°C, methylation at other sites on the molecule was observed resulting in a distribution *of alpha-, ortho-, meta-* and *para*-methylbenzylamine. In fact, positional reactivity appeared tunable with fluid pressure.



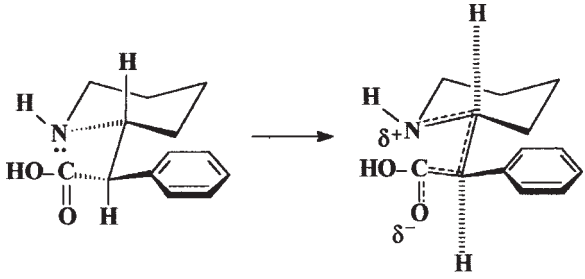


Reaction State					
Liquid	100%	0%	0%	0%	0%
Cluster ($_{137}$ bar)	0%	13%	26%	29%	32%
Cluster ($_{275}$ bar)	0%	0%	10%	6%	84%

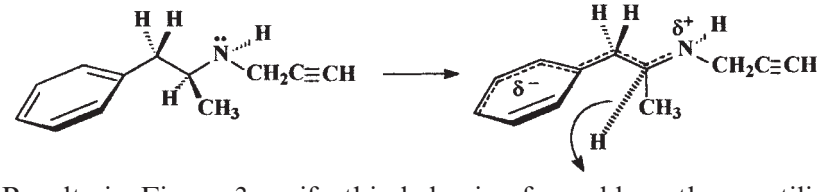
One area where this could be extremely useful is in carbon-11 methylations involving substrate molecules possessing more than one reactive site. Usually under such circumstances it is highly desirable to prevent reaction at one of the two sites. A specific example here is the synthesis of [^{11}C]methylphenidate from reaction of [^{11}C]methyl iodide with ritalinic acid.



Attaching a blocking agent to the nitrogen atom prevents reaction at the more reactive amine group.[33] Once methylation is complete at the less reactive oxygen site, the blocking agent is removed. However, we have demonstrated, at least with unlabeled methyl iodide, that reaction with unblocked ritalinic acid in supercritical carbon dioxide fluid doped with 15% acetonitrile and maintained at 95°C and 150 bar pressure can result in methylation at only the oxygen site yielding the desired methylphenidate product. We attribute this behavior to the dipole induction within the substrate molecule caused by the acetonitrile cluster formed about it in the supercritical state. This induction converts the amino group into a less reactive quaternary ammonium site while enhancing the nucleophilic qualities of the oxygen site.



Considering this, it is interesting to revisit the deprenyl system that was discussed earlier. One might expect to see lower reactivity of the nordeprenyl substrate at or near the critical point for the reaction conditions described due to a similar effect of the acetonitrile cluster on the substrate. For a temperature of 95°C, and a mixture of 20% acetonitrile in carbon dioxide, the critical pressure is reached at about 140 bar. The 92% radiochemical yield (decay corrected to end of bombardment) reported earlier was obtained at 83 bar, substantially less than the critical pressure. Thus, these reactions were actually conducted in a subcritical regime where co-solvent clustering is presumed to be less likely to occur due to a lower density of state for the media.



Results in Figure 3 verify this behavior for cold syntheses utilizing fixed amounts of nordeprenyl and carrier methyl iodide. The deprenyl product yield appears to fall-off exponentially with increasing pressure. The ideal pressure range appears to be in the subcritical regime.

On closer inspection of the charge induction within the nordeprenyl substrate molecule shown in the above schematic, it seems apparent that the C–H bond on the α-position is weakened considerably to an extent that might lend itself to racemization of the starting material or reaction product. A plot of the D(+)-to-L(-) deprenyl product ratio shown in Figure 4, measured by chiral capillary chromatography on a chiraldex column, reveals that the product becomes racemized at the critical pressure for the mixture, roughly 140 bar. However, if pure L-deprenyl is subjected to the same supercritical conditions, no racemization occurs. Therefore, this behavior is most likely due to alteration of the substrate's asymmetry prior to reaction. It is also interesting to note that the stereochemical trend presented in this figure mimics the cluster curve

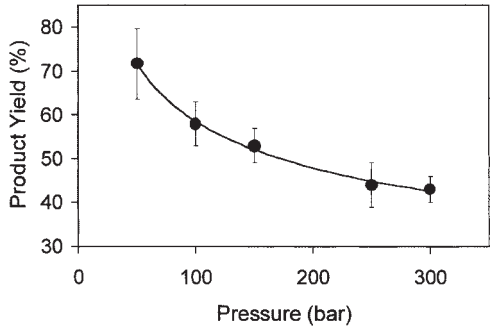


Figure 3. Percent L-deprenyl yield as a function of supercritical fluid pressure. (Reprinted from *Nucl Med & Biol*, RA Ferrieri, I Garcia, JS Fowler, AP Wolf, 'Investigations of acetonitrile solvent cluster formation in supercritical carbon dioxide, and its impact on microscale syntheses of carbon-11 labeled radiotracers for PET,' 26 (1999) 443–454 with permission from Elsevier Science.)

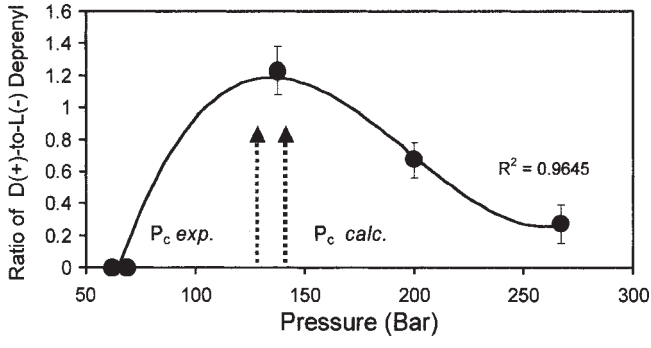


Figure 4. Ratio of D(+)-to-L(-) deprenyl product obtained from the methylation of L-nordeprenyl in supercritical carbon dioxide and 20% acetonitrile maintained at 95°C and pressures from 60 to 275 bar. Dotted lines indicate experimentally and calculated critical pressure for this mixture and condition. This region of pressure is expected to promote co-solvent clustering

for this system that was presented in Part I of this series. Thus, stereochemical alteration directly relates to cluster formation. Unfortunately, we lack a full understanding at this time of the intrinsic relationship between fluid pressure, density of states and cluster nature. Perhaps the cluster is not sufficiently ordered in the subcritical regime to impact on electron distributions within entrained substrates. Clearly, no stereoisomerization was observed in the 50–80 bar pressure range. Thus, investigators must use due caution when attempting to react chiral

substrates. In some instances such as this one, the critical point is not the ideal reaction condition to achieve one's radiolabeling goals.

One final area worth noting is the use of supercritical fluids as solvents for catalyst mediated reactions involving radioisotopes. This area has yet to be explored in radiochemistry. Several examples were cited earlier in this review illustrating the potential use by coupling supercritical fluid technology with organometallic chemistry.

Our personal experience in this area was purely by accident. In one of our recent experiments using supercritical fluid chromatography to purify [^{11}C]toluene for a PET primate study,[2] the outlet restrictor became plugged after the reaction mixture had been loaded onto the column. Taking a brute force approach to remedy the situation, we quickly increased the system pressure from 2000 to 3500 psi. While this action re-established the flow, we observed a new radiolabeled product, identified as [^{11}C]diphenylmethane, which eluted the chromatography system in approximately a 1:1 ratio with [^{11}C]toluene. The synthetic route that was utilized to prepare the [^{11}C]toluene relied on a palladium (0) mediated cross-coupling reaction involving [^{11}C]methyl iodide and tributylphenyl-stannane.[3,4] The Group VIII transition metals, particularly nickel and palladium, are effective in catalyzing the cross-coupling of organometallic reagents with organic halides and related electrophiles.[34] What we did not anticipate was the ability of the supercritical carbon dioxide fluid to promote continued coupling of the [^{11}C]toluene with the solubilized organometallic compounds present in the crude reaction mixture.

This aspect was systematically investigated in unlabeled experiments using carrier amounts of toluene mixed with 13 µl of tributylphenyl-stannane, 0.9 mg of tris(dibenzylideneacetone)dipalladium (0) and 1.2 mg of tri-*O*-toylphosphine in 0.5 ml of dimethylacetamide solvent. This mixture simulated the amounts of material used in an actual radiolabeling synthesis. Cold reactions were carried out for 15 min in a 10 ml volume pressure autoclave maintained at 50°C. Supercritical fluid pressure was maintained at the fluid pump. After reaction, the autoclave was depressurized through a solvent trap containing hexane. The remaining contents of the vessel were then removed and mixed with the same hexane. Aliquots were then analyzed by capillary gas chromatography using a 60 m × 0.25 mm SE-30 column, and flame ionization detection. Results in Figure 5 show the influence of fluid pressure on toluene coupling. The diphenylmethane product yield was based on the µmol of toluene introduced. The results show that the coupling reaction appears optimized at 3000 psi pressure. We have not investigated this

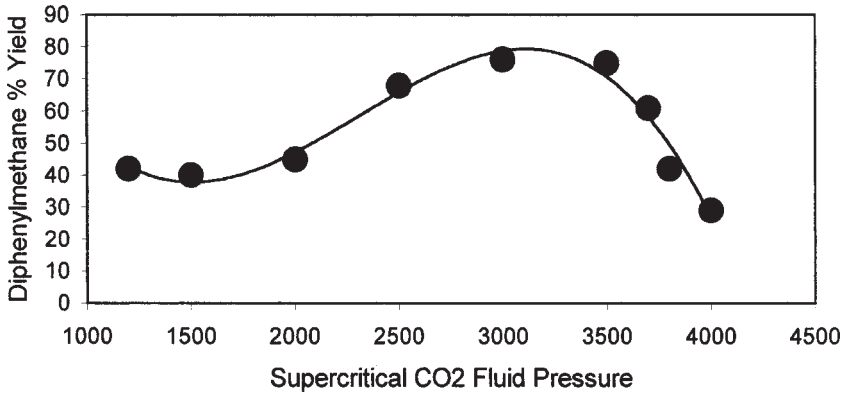


Figure 5. Effect of supercritical CO_2 fluid pressure on diphenylmethane product yield from palladium(0) mediated cross-coupling reaction of toluene with tributylphenylstannane

further to ascertain whether the optimized pressure coincides with critical parameters. This example, however, clearly demonstrates the strength and potential use for supercritical fluids as media for promoting organometallic-based radiosyntheses.

The future for supercritical fluids in medical radioisotope processing

Clearly, there is a future for supercritical fluids in Nuclear Medicine fields involving radioisotope processing. No doubt this future will be driven in part by economics as the cost for processing conventional liquid organic solvents through waste management continues to increase. Radiotracer chemists must therefore look to more eco-friendly solvent systems in their day-to-day operations in order to stabilize and even reduce these operational expenses.

The future role of supercritical fluids in radioisotope research and development will depend on funding agencies recognizing what this technology can offer radiochemistry if properly developed, and their support of fundamental studies that will enable researchers to establish a basic framework of understanding for generalized application.

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References

1. Muller RD, Ferrieri RA, Gerasimov M, Garza V. *Nucl Med Biol* 2002; **29**: 351–357.
2. Jacobson GB, Markides KE, Långström B. *Acta Chim Scand* 1997; **51**: 418–425.
3. Susuki M, Doi H, Bjorkman M, Andersson Y, Långström B, Watanabe Y, Noyori R. *Chem Eur J* 1997; **3**: 2039–2042.
4. Bjorkman M. Palladium-promoted synthesis of compounds labelled with carbon-11'' *Ph.D. Dissertation*, 2000, Uppsala University, (printed in Sweden by Linbergs Grafiska HB).
5. Gerasimov MR, Ferrieri RA, Schiffer WK, Logan J, Gatley SJ, Gifford AN, Alexoff DA, Marstellar DA, Shea C, Carter P, King P, Vitkin S, Dewey SL. Life Sci 2002; **70**: 2811–2828.
6. Gerasimov MR, Logan J, Ferrieri RA, Muller RD, Alexoff D, Dewey SL. *Nucl Med Biol* 2002; **29**: 607–612.
7. Poole CF. *J Biochem Biophys Methods* 2000; **43**: 3–23.
8. Jacobson GB, Moulder R, Lu L, Bergström M, Markides KE, Långström B. *Anal Chem* 1997; **69**: 275–280.
9. Chester TL, Pikston JD, Raynie DE. *Anal Chem* 1994; **66**: 106R–130R.
10. Schlyer DJ. *In Handbook of Radiopharmaceuticals*, Welch MJ, Redvanly CS. (eds). Wiley: New York, 2003: 1–70.
11. Wai CM, Wang S. *J Biochem Biophys Methods* 2000; **43**: 273–293.
12. Ashraf-Khorassani M, Combs MJ, Taylor LJ. *J Chromatogr A* 1997; **774**: 37–49.
13. Wai CM, Wang S. *J Chromatogr A* 1997; **785**: 369–383.
14. M'hamidi JF, Bocquet K, Pommier C. *J Supercrit Fluids* 1991; **4**: 55–59.
15. Wai CM, Lin y, Brauer RD, Wang S, Beckert WF. *Talanta* 1993; **40**: 1325–1330.
16. Lin Y, Wai CM. *Anal Chem* 1994; **66**: 1971–1975.
17. Wai CM. *Anal Sci* 1995; **11**: 165–167.
18. Laintz KE, Wai CM, Yonker CR, Smith RD. *Anal Chem* 1992; **22**: 2875–2878.

19. Laintz KE, Wai CM, Yonker CR, Smith RD. *J Supercrit Fluids* 1991; **4**: 194–198.
20. Carrott MJ, Waller BE, Smart NG, Wai CM. *J Chem Soc, Chem Commun* 1998; **3**: 377–378.
21. Carrott MJ, Wai CM. *Anal Chem* 1998; **70**: 2421–2425.
22. Laintz KE, Yu JJ, Wai CM. *Anal Chem* 1992; **37**: 311–315.
23. Saito N, Ikushima Y, Goto T. *Bull Chem Soc Japan* 1990; **63**: 1532–1533.
24. Ahrland S. *In the Chemistry of the Actinide Elements*, (2nd edn), vol.2, Katz JJ, Seaborg GT, Mass LR (eds.), Chapman & Hall: London, 1986; 1521–1532.
25. Aln A, Miloud M, Hossadi AE, Khaliquzzaman M. *J Radioanal Nucl Chem* 1987; **116**: 271–278.
26. Alcock K, Best GF, Hesford E, McKay HAC. *J Inorg Nucl Chem* 1958; **6**: 328–334.
27. Wang SF, Elshani S, Wai CM. *Anal Chem* 1995; **67**: 919–925.
28. Graham BF, Harrowfield JM, Maucerino M, Ogden MI, Trengove RD. *Proceedings of the 7th International Symposium on Supercritical Fluid Chromatography and Extraction*, Indianapolis, IN, (Abst L-04), March 1996.
29. Yazdi A, Beckman EJ. *Proceedings of the 3rd International Symposium on Supercritical Fluids*, Strasbourg, France, October 1994; 283–288.
30. Jacobson GB, Markides KE, Långström B. *Acta. Chim Scand* 1994; **48**: 428–433.
31. Jacobson GB, Westerberg G, Markides KE, Långström B. *J Am Chem Soc* 1996; **118**: 6868–6872.
32. Ferrieri RA, Garcia I, Fowler JS, Wolf AP. *Nucl Med Biol* 1999; **26**: 443–454.
33. Ding Y-S, Sugano Y, Fowler JS, Salata C. *J Label Compd Radiopharm* 1994; **34**(10): 989–997.
34. Stille JK. *Angew Chem Int Ed Engl* 1986; **25**: 508–524.

