Acta Chemica Scandinavica, 1997: 51: 418–425
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ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

Supercritical Fluid Synthesis and On-Line Preparative Supercritical Fluid Chromatography of ^{11}C-Labelled Compounds in Supercritical Ammonia

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Jacobson, G. B., Markides, K. E. and Långström, B., 1997. Supercritical Fluid Synthesis and On-Line Preparative Supercritical Fluid Chromatography of ^{11}C-Labelled Compounds in Supercritical Ammonia. – Acta Chem. Scand. 51: 418–425. © Acta Chemica Scandinavica 1997.

An automated supercritical fluid synthesis (SFS) system, designed for reaction and on-line preparative supercritical fluid chromatography (SFC) of ^{11}C-labelled ($t_{1/2}$ = 20.3 min) compounds in supercritical ammonia, is described. The synthesis and on-line SFC purification of [*methyl*-^{11}C]anisole, L-[*methyl*-^{11}C]methionine and 4-methoxyphenyl[^{11}C]guanidine gave a >98% radiochemically pure product within 30 min. [*methyl*-^{11}C]Anisole was obtained from reaction of phenol with [^{11}C]methyl iodide and was used as a model reaction to evaluate the synthetic procedure of the system. 4-Methoxyphenyl[^{11}C]guanidine was produced by reaction of *p*-anisidine with [^{11}C]cyanogen bromide to give the ^{11}C-labelled cyanamide, followed by reaction with ammonia to obtain the ^{11}C-guanidine compound. Both D- and L-[*methyl*-^{11}C]methionine were produced by reaction of [^{11}C]methyl iodide and D- and L-homocysteine thiolactone hydrochloride, respectively. The effect of base concentration on the radiochemical yield and enantiomeric purity of ^{11}C-methionine was investigated, and it was found that 2.5 equiv. of base gave a radiochemical yield >95% with 2–3% racemization. In a typical synthesis and on-line SFC purification of ^{11}C-methionine, a 5 μA h (45 μA, 6.6 min) bombardment to produce [^{11}C]carbon dioxide gave a 3.1 GBq sterile solution of ^{11}C-methionine.

Supercritical fluids have physical properties (e.g., density, viscosity, diffusivity) in between those of a gas and a liquid. The possibility to vary continuously these properties from gas-like to liquid-like by small changes in pressure and temperature makes supercritical fluids attractive as media for chemical reactions, chromatography and extraction.[1–6]

These special properties of supercritical fluids can be an advantage in the development of new techniques for synthesis of compounds labelled with short-lived β^+-emitting radionuclides, such as ^{11}C ($t_{1/2}$ = 20.3 min). The labelled compounds are of interest for *in vivo* studies of biological and physiological processes in the human body, studied by positron emission tomography (PET).[7] In order to obtain the final product in a high radiochemical yield rapid synthetic techniques and purification procedures are required.[8] It is also desirable to reduce the amounts of reactants and solvents to minimize effects related to isotopic dilution and thereby achieve higher specific radioactivity (radioactivity/mol labelled compound) of the products.

Supercritical fluids may also offer an advantage in the purification of short lived radiotracers as rapid separations can be obtained due to their high diffusivity and low viscosity compared with liquids.[9] By performing the SFS with on-line preparative SFC, the total synthesis time could be reduced and no sample loss would occur as the entire reaction mixture is injected onto the column. A further benefit for radiotracer production would be that the products can be trapped directly into a solvent suitable for intravenous injection as many supercritical fluids will disperse as a gas upon depressurization. This would eliminate the time-consuming solvent-separation step which is necessary after conventional LC, and which can result in product loss and reduced specific radioactivity.

We have developed an SFS system for synthesis of ^{11}C-labelled compounds using supercritical ammonia (T_c = 132.5 °C, P_c = 113.1 bar) as a solvent and/or reactant.[10] In the SFS system, highly reproducible reactions in the nanomolar range were performed in 30–500 μl

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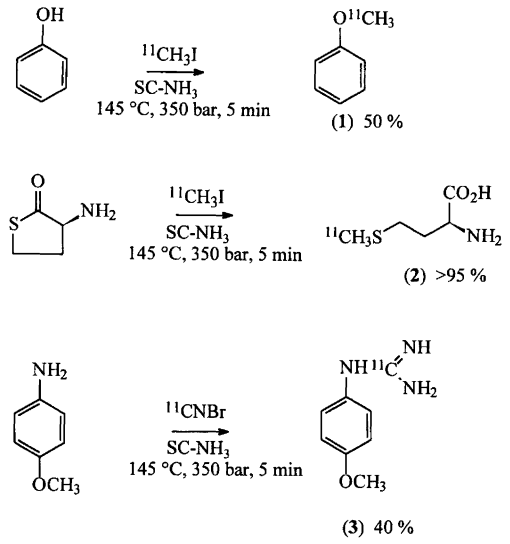

Scheme 1.

reaction cells. In this paper the further development of the SFS system is described, which includes on-line trapping of the ^{11}C-labelled precursor and on-line preparative SFC. The *N*-methylation reaction of phenol with [^{11}C]methyl iodide to produce [*methyl*-^{11}C]anisole (**1**, Scheme 1) was used as a model reaction in the first version of the SFS system, and was chosen in this study to compare the yields obtained with on-line trapping of the ^{11}C-labelled precursor. The total synthesis and purification of L-[*methyl*-^{11}C]methionine (**2**) to a sterile product ready for intravenous injection was performed to evaluate the use of the SFS system for practical applications. L-[*methyl*-^{11}C]Methionine has been used for PET studies in the brain to evaluate, e.g., amino acid transport, amino acid metabolism, or protein synthesis for imaging of tumors,[11] and for that a product with high enantiomeric purity is required. The enantiomeric purity of D- and L-[*methyl*-^{11}C]methionine prepared from D-, and L-homocysteine thiolactone hydrochloride, respectively, was compared with results obtained in conventional liquid reactions.[12a–d] The synthesis and on-line SFC purification of 4-methoxyphenyl[^{11}C]guanidine (**3**) is an example of a two-step reaction utilizing [^{11}C]cyanogen bromide and ammonia as reactants. The SFC purifications were followed by UV and radio detection and the product fractions were collected and analyzed with regard to chemical and radiochemical purity. In our evaluation of the system, radioactivity was used as a measure of mass balance to obtain information about the system efficiency which otherwise would be difficult to measure due to the low concentration of reactants.

Experimental

General. [^{11}C]Carbon dioxide was produced at the Uppsala University PET Centre using a MC 17 cyclotron (Scanditronix AB, Uppsala, Sweden) by irradiation of a target containing 99.95% nitrogen gas (AGA Nitrogen 6.0) and 0.05% oxygen gas (AGA Oxygen 6.0) with a 17 MeV proton beam, producing ^{11}C by the ^{14}N(p,α)^{11}C nuclear reaction. The [^{11}C]carbon dioxide was converted into hydrogen [^{11}C]cyanide using the Scanditronix RNP-17 radionuclide production system according to published procedures.[13]

The [^{11}C]carbon dioxide and hydrogen [^{11}C]cyanide was transported by means of an N_2 gas flow to Synthia, a robotic system for the production of radiopharmaceuticals.[14] In the Synthia system, the [^{11}C]carbon dioxide and hydrogen [^{11}C]cyanide could be either directly transported to the SFS system or first be converted into [^{11}C]methyl iodide[12a,e,f] and [^{11}C]cyanogen bromide,[15] respectively. [^{11}C]Methyl iodide was produced by trapping the [^{11}C]carbon dioxide in 0.5 ml 0.25 M lithium aluminium hydride in tetrahydrofuran. After evaporation of tetrahydrofuran, 1 ml 57% hydrogen iodide was added and the [^{11}C]methyl iodide was transferred in a stream of helium gas to the SFS system. [^{11}C]Cyanogen bromide was produced by passing the hydrogen [^{11}C]cyanide through a bed of pyridinium bromide perbromide at room temperature, and transferred to the SFS system in a stream of N_2 gas.

LC analyses were performed with a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV detector in series with a β^+-flow detector.[16] The following columns were used: (A) Beckman Ultrasphere ODS, 4.6 × 250 mm, 5 μm; (B) Beckman Ultrasil NH_2, 4.6 × 250 mm, 10 μm; and (C) Beckman Ultrasphere Octyl, 4.6 × 250 mm, 5 μm. Mobile phases were (D) 0.05 M ammonium formate, pH 3.5, (E) methanol, (F) 0.01 M potassium dihydrogen phosphate, pH 4.6, (G) acetonitrile–water 50:7 (v/v), (H) 5 mM trifluoroacetic acid (TFA) in water, and (I) 5 mM TFA in acetonitrile. Authentic reference compounds were added and the UV and radio chromatograms were compared.

The SFC purifications were performed on a Hypercarb column (4.6 × 200 mm, 5 μm, Shandon HPLC) isothermally with pressure programming. The compounds were detected at 254 nm with the UV detector.

L-Homocysteine thiolactone hydrochloride was purchased from Fluka, *p*-anisidine and D-homocysteine thiolactone hydrochloride from Sigma, and phenol from Merck.

Supercritical fluid synthesis system (Fig. 1). The SFS system was built based on a modified Gilson SF3 system (Gilson Medical Electronics S.A., Villiers-le-Bel, France), and was designed to perform synthesis in supercritical ammonia, as described earlier.[10] The system consisted of a cryostat refrigerating circulator (F3 CH, HAAKE Mess-Technik GmbH u.Co.), a Gilson model 308 pump, a model 821 pressure regulator, and a model 831 oven. It has now been further modified to include on-line trapping of the ^{11}C-labelled precursor and on-line preparative SFC. Two injection valves, V1 and V2, (6-port

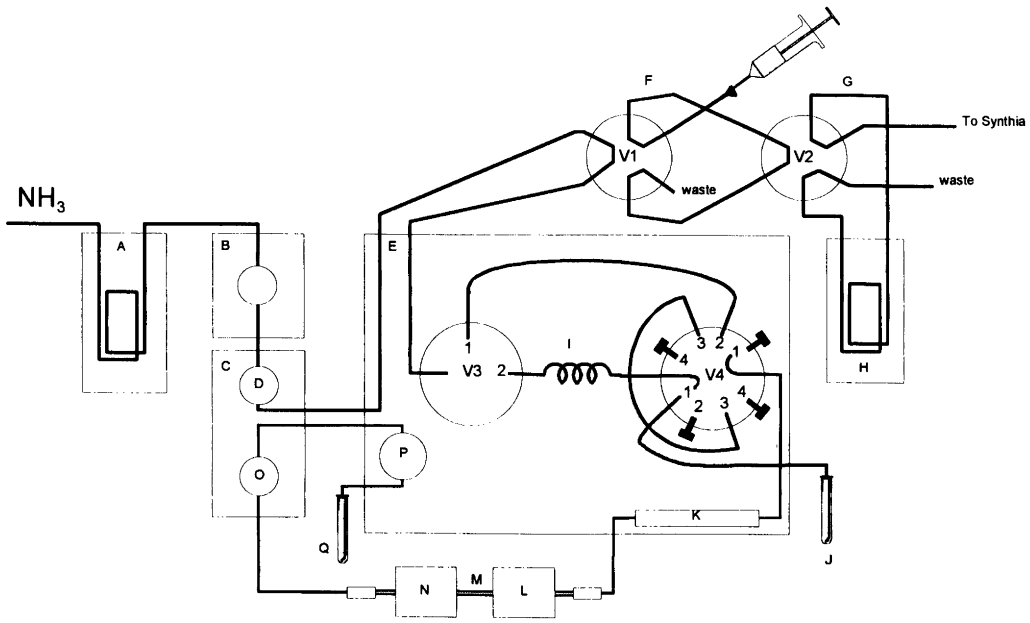

Fig. 1. Schematic of the SFS system: A, cryostat, −10 °C; B, 308 pump; C, 821 pressure regulator; D, pulse dampener; E, 831 oven; F, substrate injection loop (in LOAD position); G, precursor injection loop (in LOAD position); H, cooling bath, −196 or −72 °C; I, reaction cell; J, trapping position after SFS; K, SFC column; L, UV detector; M, fused silica capillary; N, radio detector; O, pressure regulator valve; P, restrictor valve; Q, trapping position after SFC.

two-position valves AC6WHC, Valcon H rotor seals, Valco Instruments Co. Inc., Houston, USA) were located outside the oven, and valves V3 and V4 (three-port two-position valve A3C3WEHCY and 10-port four-position valve A3CST4UWHCY, Valcon E, Valco) were located inside the oven.

The injection loops consisted of 316 grade stainless steel tubing with 0.25 mm i.d. and 1/16″ o.d. The injection loop on V1 (substrate loop, l=40 cm, V=20 µl) was filled with the substrate solution and the loop on V2 (precursor loop, l=80 cm, V=39 µl) was connected to Synthia by Teflon tubing (i.d.=1.5 mm, o.d.=1/8″). A Dewar containing either liquid N_2 (−196 °C) or dry ice–ethanol (−72 °C) was used to cool the injection loop on V2 during trapping of the ^{11}C-labelled precusor. The stainless steel reaction cell (i.d.=0.75 mm, o.d.=1/16″, l=107 cm), connected between valves V3 and V4, had a volume of 473±5 µl. All other tubing in the SFS system was stainless steel with an i.d. of 0.127 mm and 1/16″ o.d.

A UV detector cell utilizing optical fibers was constructed to fit the SFS system. This type of cell has been described in detail elsewhere.[17] Optical fibers of 400 µm o.d. were connected between the UV cell and the detector µPeak Monitor (Pharmacia, Sweden). The UV detector was coupled in series with a radio detector,[16] and the flow from the SFS column was led through both detectors by a piece of fused silica capillary (i.d.=0.198 mm, o.d.=0.356 mm). This allowed an optical window for UV detection to be obtained in the UV cell by burning of a 2–4 mm segment of the polyimide on the fused silica capillary. Also, a higher sensitivity for the radio detector was obtained, compared with using stainless steel tubing, which is otherwise preferred with the use of supercritical ammonia. Both detectors were connected via a Beckman AI 406 interface to a Beckman System Gold Chromatography Software Package for data processing.

The fused silica capillary was connected to the stainless steel tubing by two Valco unions, one before and the other directly after the two detectors. As the polyimide coating on the fused silica was not resistant to ammonia, a special connection had to be made to ensure leak-free conditions. The polyimide was burnt off at both ends of the tubing and glued into a PEEK sleeve (i.d.=508 µm, o.d.=1/16″). Several different glues were tested and the best results were obtained with Loctite 648 (Loctite Sweden AB) which resulted in leak-free connections for at least one week of intensive use. The peek sleeve was fitted into the Valco union with a stainless steel ferrule and nut.

The pressure regulator needed a minimum of 90 bar and 0.5 ml min^{-1} to ensure a linear pressure gradient during the SFC runs. A Rheodyne 7037 pressure relief valve was used to set the pressure to >90 bar when the pressure regulator was fully opened. The valve had an internal volume of 6 µl and was modified with a Gilson factory fitted adjustment screw to enable installation inside the oven.

The SFS system was placed in a lead shielded hood for radiation safety. The valves and cooling bath were operated by remote controlled air actuators. The 308 pump head was placed inside the hood and separated

from its controller keypad to allow the pump and pressure regulator to be programmed from outside the hood.

Experimental procedures for the SFS system.

SFS. The substrate injection loop was filled with the substrate solution as the injection valves V1 and V2 were in load position, V3 in position 2, and V4 in position 1, prior to starting the ^{11}C-synthesis. The ^{11}C-labelled precursor was transported via Synthia to the precursor loop, and trapped at −196 or −72 °C. When the trapping was complete, the cooling bath was removed and valves V1 and V2 were switched to inject position and V4 to position 4. A 0.5 ml min^{-1} flow of ammonia then pushed the reactants into the closed reaction cell, assuring a positive forward flow of ammonia with dissolved reactants. When the desired reaction pressure was reached, valve V3 was switched to position 1 and the flow was reduced to 0.01 ml min^{-1}, as the reaction took place in the closed reaction cell. When the reaction was complete, valves V3 and V4 were switched to positions 2 and 1, respectively, and the entire reaction mixture was trapped in 1–2 ml of a suitable solvent.

SFS with on-line SFC. The substrate loop was filled with the substrate solution as the injection valves V1 and V2 were in the load position, V3 in position 2, and V4 in position 1, prior to starting the ^{11}C-synthesis. The desired ^{11}C-labelled precursor was transported via Synthia to the precursor loop, and trapped at −196 or −72 °C. When the trapping was complete, the cooling bath was removed and valves V1 and V2 were switched to inject position and V4 to position 2. A 0.5 ml min^{-1} flow of ammonia then pushed the reactants into the closed reaction cell, assuring a positive forward flow of ammonia with dissolved reactants. When the desired reaction pressure was reached, valve V3 was switched to position 1 and the column was conditioned as the reaction took place in the closed reaction cell.

The entire reaction mixture was injected onto the column by switching valve V3 to position 2 and V4 to position 3. By isothermal pressure programming from sub- to super-critical conditions, separation of the reaction mixture was attained and the results were monitored by UV and radio detection. The desired fractions were collected in 1–2 ml of a suitable solvent.

[methyl-^{11}C]Anisole. A 2.1 M solution of sodium phenolate was prepared by dissolving 20 mg phenol in 66 µl acetonitrile and 34 µl 5 M aqueous sodium hydroxide (NaOH) solution. The solution was loaded into the 20 µl injection loop (42 µmol phenol, 34 µmol NaOH) on valve V1. [^{11}C]Methyl iodide was trapped at −196 °C in the precursor loop. The reaction was performed at 145 °C; 350 bar for 5 min, as described above, and the reaction mixture was trapped in 2 ml water or injected onto the SFC column. The SFC purifications were performed under the following conditions: temperature, 145 °C, pressure, gradient 0–7 min 82–120 bar; flow, 0.5 ml min^{-1}. The retention time of [*methyl*-^{11}C]anisole was 12.6 min. LC analyses were performed on column A under the following conditions: solvents D–E, gradient 1–7 min 40–85% E, flow 2.0 ml min^{-1}, and wavelength 254 nm. The retention time of [*methyl*-^{11}C]anisol was 7.8 min.

L- and D-[methyl-^{11}C]Methionine. The substrate solution was prepared by dissolving 0.8 mg D- or L-homocysteine thiolactone hydrochloride in 100 µl 0.125 M aqueous NaOH solution. The solution was loaded into the injection loop (1 µmol HCTL, 2.5 µmol NaOH) on valve V1, and [^{11}C]methyl iodide was trapped at −196 °C in the precursor loop. The reaction was performed at 145 °C, 350 bar for 5 min. The reaction mixture was either trapped directly from the reaction cell or injected onto the SFC column. The SFC purification was performed under the following conditions: temperature, 145 °C; pressure, 0–1.5 min 100 bar and a gradient 1.5–6.5 min of 100–200 bar; flow, 0–1.5 min 0.2 ml min^{-1} and from 1.5 min 0.5 ml min^{-1}. This gave a retention time of 6.6 min for [*methyl*-^{11}C]methionine. SFC was also performed with a pressure gradient 2.5–7.5 min of 100–350 bar which gave a retention time of 12.9 min. The product was trapped in 2 ml water. To remove ammonia from the aqueous solution and thereby reduce the pH, the solution was heated (ca. 40 °C) under reduced pressure 2 min. The solution was then diluted with 2 ml 0.1 M phosphate buffer, pH 7.4, and filtered through a 0.22 µm pore sterile filter into a sterile vial. The solution was controlled regarding sterility and pyrogenicity. The reaction mixture and SFC fractions were analyzed by LC on column B under the following conditions: solvents F–G, gradient 1–8 min 95–40% G, flow 2.0 ml min^{-1}, and wavelength 230 nm. The retention time of [*methyl*-^{11}C]methionine was 5.5 min.

To determine the enantiomeric purity of the D- and L-[*methyl*-^{11}C]methionine, they were converted into a diastereomeric derivative by reaction with *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (Marfey's reagent), as described in detail elsewhere.[18] The effect of base concentration on the enantiomeric purity was studied by using 0, 0.125, 0.25, and 1 M NaOH solutions in the [*methyl*-^{11}C]methionine synthesis. LC analyses were performed on column A under the following conditions: solvents D–E, gradient 0–12 min 30–85% E, flow 2.0 ml min^{-1}, and wavelength 340 nm. The retention times for the L-methionine and D-methionine derivatives were 9.6 and 10.7 min, respectively. The optical rotation of D- and L-homocysteine thiolactone hydrochloride was measured at $[\alpha]_D^{26}$ with a Perkin Elmer 241 polarimeter.

4-Methoxyphenyl[^{11}C]guanidine. The substrate solution was prepared by dissolving 2.5 mg *p*-anisidine in 100 µl 1-butanol. The injection loop on valve V1 was loaded with 20 µl (4 µmol) of the *p*-anisidine solution and [^{11}C]cyanogen bromide was trapped at −72 °C in the precursor loop. The reaction was performed at 145 °C, 350 bar for 5 min. The SFC purification was performed

under the following conditions: temperature, 145 °C; pressure, 0–1.5 min 100 bar and a linear gradient from 1.5–6.5 min of 100–200 bar; flow, 0–1.5 min 0.2 ml min^{-1} and from 1.5 min 0.5 ml min^{-1}. This gave a retention time of 10.4 min for 4-methoxyphenyl[^{11}C]guanidine. The collected fractions were analyzed by LC on column C under the following conditions: solvents H–I, gradient 2–4 min 5–15% I and 4–10 min 15–85% I, flow 1.5 ml min^{-1}, and wavelength 230 nm. The retention time of 4-methoxyphenyl[^{11}C]guanidine was 8.3 min.

Results and discussion

General aspects of the system. In the earlier version of the SFS system, the ^{11}C-labelled precursors were first trapped in 150 × 350 µl of solvent, and 5–10 µl of this volume was manually loaded into the injection loop on the SFS system. This way, only a small part of the ^{11}C-precursor batch was used for each synthesis. This was suitable for chemistry development but the possibility to obtain a product with enough radioactivity to perform *in vivo* experiments was limited. In order for the system to be used for production-scale synthesis of radio tracers, an automated trapping procedure of the entire ^{11}C-precursor batch was necessary. This was obtained by integrating the SFS system with Synthia, a robotic system for production of radiopharmaceuticals.[14] In Synthia, [^{11}C]carbon dioxide and hydrogen [^{11}C]cyanide could be delivered directly from the target and processing system, or [^{11}C]methyl iodide and [^{11}C]cyanogen bromide could be obtained after a short synthesis procedure performed by the use of Synthia. The ^{11}C-precursor was transferred from Synthia to the SFS system in a flow of N_2 or He gas and trapped in the precursor loop, cooled with N_2 (l) or dry ice–ethanol.

At the time of injection into the reaction cell, the reaction of the labelled precursor with ammonia sometimes competed with the desired reaction with the substrate solution. In order to increase the yields of the desired products, different injection procedures were investigated for the alkylation reaction of [^{11}C]methyl iodide with phenol and homocysteine thiolactone hydrochloride to produce [*methyl*-^{11}C]anisole and [*methyl*-^{11}C]methionine, respectively. The reaction of the substrate solution with [^{11}C]methyl iodide was performed in the SFS system and the reaction mixture was trapped directly into ca. 2 ml water and analyzed by LC.

In one attempt, the substrate solution loop was placed before the ^{11}C-precursor loop in the flow direction (Fig. 2). The volume of the substrate solution loop was varied in the range 5–20 µl and the ^{11}C-labelled precursor was trapped in a 200 µl loop. The highest radiochemical yields were obtained with the larger substrate solution volume, but the yield of [*methyl*-^{11}C]anisole was still only 28% (Table 1). The volume of the precursor loop was then reduced to 59 µl in an attempt to increase the

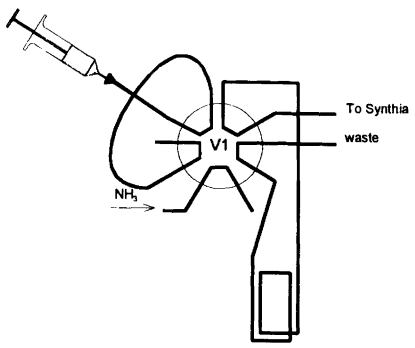

Fig. 2. Injection valve V1 with a 5, 20 or 50 µl substrate loop on the left and a 200 or 59 µl precursor loop, connected to Synthia, on the right.

Table 1. Radiochemical yields[a] as an effect of injection volumes.

Substrate loop/µl	Precursor loop/µl	^{11}C-Anisole (%)	^{11}C-Methionine (%)
5[b]	200	22	22
20[b]	200	27	
50[b]	200	28	
5[b]	59	23	16
20[b]	59	24	52
50[b]	59	40	37
2 × 10[c]	39	50	>95

[a]The radiochemical yields were calculated from the decay-corrected LC chromatograms of the crude product mixtures. [b]The substrate loop and precursor loop were placed on the same injection valve, see Fig. 2. [c]The substrate loop was placed on a separate valve. A total of 20 µl was injected, 10 µl before and 10 µl after the precursor loop, see Fig. 1.

contact between the substrate solvent plug and the precursor. The radiochemical yield of [*methyl*-^{11}C]anisole increased to 40%, but only a 37% yield of L-[*methyl*-^{11}C]methionine was obtained.

The only competing by-product in all reactions with [^{11}C]methyl iodide was [^{11}C]methylamine. At the moment of injection, the gas volume with trapped precursor came in contact not only with the substrate solution, but also with ammonia, which at that time had a pressure of ca. 25 bar throughout the system, causing a back-flow of ammonia into the precursor loop. The rapid diffusion of ammonia against the flow direction could explain the high yields of [^{11}C]methylamine obtained. To avoid this problem, a second injection valve was added to the SFS system (V2, Fig. 1), and the volume of the precursor loop was reduced to 39 µl. By switching the two valves simultaneously, the precursor trapped in the loop came into contact with two 10 µl substrate solution plugs, one on each side. The immediate contact with ammonia was therefore reduced and the yields of [*methyl*-^{11}C]anisole and [*methyl*-^{11}C]methionine increased to 50 and >95%, respectively. This injection procedure was maintained for the rest of the study.

The radiochemical yield of [*methyl*-^{11}C]anisole was

comparable to that obtained in the first published version of the SFS system,[10a] but the fact that ca. 40 times more activity can be obtained by the on-line trapping of the precursor in this new version makes the system more efficient for ^{11}C-labelling synthesis.

Purification of the reaction mixture was obtained by on-line preparative SFC, using a Hypercarb (porous graphite carbon) column, 4.6 × 200 mm, 5 μm. When the reaction was complete, the entire reaction mixture was loaded onto the column using the reaction cell as an injection loop. Separation on the column was obtained by isothermal pressure programming, and UV- and radio-detectors were used to monitor the separations. An SFC chromatogram for the purification of [*methyl*-^{11}C]anisole is shown in Fig. 3A. The radiochemical purity of the product collected at 12.6 min was calculated from the decay corrected chromatograms after LC analysis, and was shown to be >98%. The trace of the radiolabelled product was too low to be visible by UV and only the UV signals from the reactants are seen in the chromatograms.

When ≤30 μl substrate solution was injected into the reaction cell (V=473 μl), the cell contained >97 mol% ammonia. Phase calculations performed on the three-component mixture (ammonia, solvent, substrate), using the Peng–Robinson state equation,[19] concluded that these reactions were performed under supercritical conditions.[10b]

Synthesis of 4-methoxyphenyl[^{11}C]guanidine. The guanidine function is a frequently occurring structural unit in many biologically and pharmaceutically interesting compounds and the synthesis of aliphatic and aromatic ^{11}C-labelled guanidines have been investigated in the SFS system.[10b] In the earlier version of the SFS system, the reaction of 4-methoxyphenyl[^{11}C]guanidine was obtained by trapping [^{11}C]cyanogen bromide in 400 μl *p*-anisidine solution to first obtain the ^{11}C-labelled cyanamide. From this reaction mixture, 20 μl were injected into the SFS system for reaction with ammonia, and a 80% radiochemical yield of the guanidine compound was obtained.

In the new design of the SFS system, the [^{11}C]cyanogen bromide batch could be trapped directly in the precursor loop, and was injected into the reaction cell together with the amine solution. This one-pot reaction gave ca. 40% radiochemical yield of the guanidine. Even though the yields were lower in the one-pot procedure, ca. 40 times higher starting radioactivity could be obtained which gave a 20 times higher degree of radioactivity in the product.

The reaction mixture was purified by on-line SFC and an example of the purification of 4-methoxyphenyl-[^{11}C]guanidine is shown in Fig. 3B. Collecting the fraction at 10.4 min gave a >95% radiochemically pure product. The radiopeaks at 8.2 and 9.4 min were identified as [^{11}C]cyanamide and [^{11}C]guanidine obtained from the reaction of [^{11}C]cyanogen bromide with ammonia.

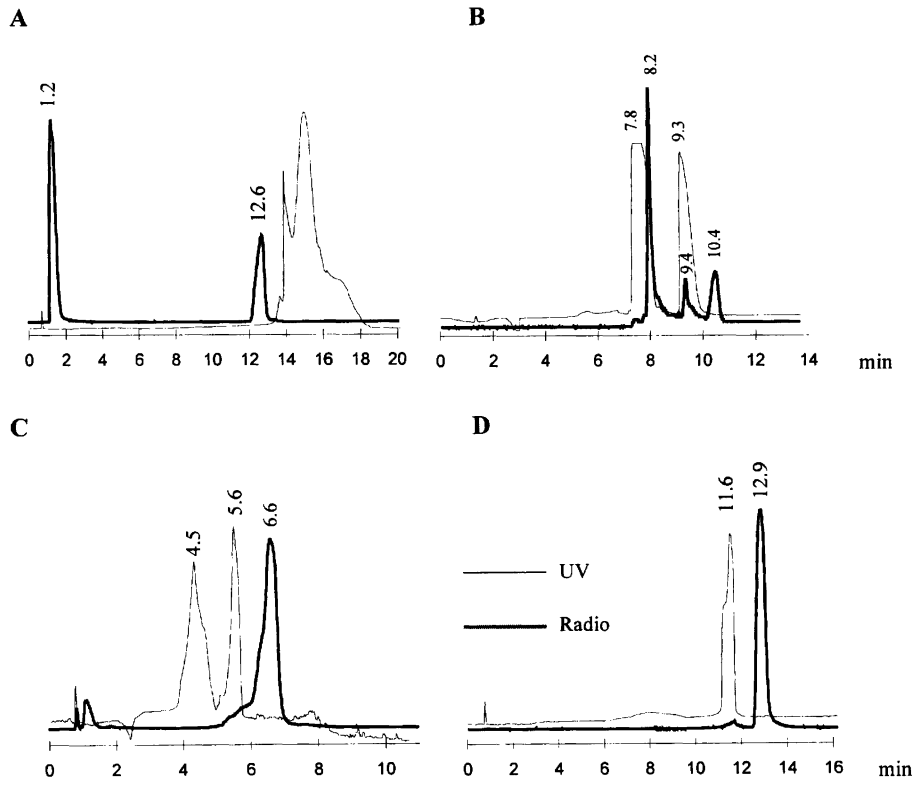

Fig. 3. Preparative SFC (T=145 °C) on a Hypercarb column of: A, [*methyl*-^{11}C]anisole (t_r=12.6 min), 85–120 bar 0–7 min; B, 4-methoxyphenyl[^{11}C]guanidine (t_r=10.4 min), 100–200 bar 1.5–6.5 min; C, [*methyl*-^{11}C]methionine (t_r=6.6 min), 100–200 bar 1.5–6.5 min; D, [*methyl*-^{11}C]methionine (t_r=12.9 min), 100–350 bar 2.5–7.5 min.

Synthesis of ^{11}C-methionine. Several procedures for the synthesis of [*methyl*-^{11}C]methionine have been reported, by reaction of [^{11}C]methyl iodide with L-homocysteine thiolactone hydrochloride[12a–d] or *S*-benzyl-L-homocysteine.[12e,f] For the homocysteine thiolactone reaction, it has been shown that racemization of the starting material increases with increased base concentration, and that the reaction time and reaction temperature have only minor effects.[12c,d] The enantiomeric purity and radiochemical yield of D- and L-[*methyl*-^{11}C]methionine after SFS was investigated using 0.0, 2.5, 5.0 and 20.0 equivalents of NaOH. At least two experiments were performed under each reaction condition, in randomized order. A derivatization reaction of the labelled amino acid with *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (Marfey's reagent[18]) gave a diastereomeric derivative which was easily separated by LC, and the enantiomeric purity was calculated from the decay-corrected peak areas.

2.5 equivalents of NaOH to homocysteine thiolactone were needed to give a radiochemical yield of [*methyl*-^{11}C]methionine at over 95%. With 0.0 or 1.0 equivalents of base only 4–5% radiochemical yield was obtained, and increasing to 5.0 or 20.0 equivalents gave >98% yield. With 2.5 equivalents of base an enantiomeric purity of 94 and 86% ee was obtained for D- and L-[*methyl*-^{11}C]methionine, respectively. Using 20 equivalents of NaOH decreased the enantiomeric purity to 90 and 82% ee for D- and L-[*methyl*-^{11}C]methionine. The low ee for L-[*methyl*-^{11}C]methionine measured even at low base concentrations could be due to small amounts of D-homocysteine thiolactone present as an impurity in the L-homocysteine thiolactone. To verify this, the optical rotation of D- and L-homocysteine thiolactone was measured and compared with the literature value of $[\alpha]_D^{26} \pm 21.5$ (*c* 1.0, in water).[20] For D-homocysteine thiolactone a value of $[\alpha]_D^{26} - 20{\cdot}6$ (*c* 1.0, in water) was obtained and for L-homocysteine thiolactone $[\alpha]_D^{26} + 19.8$ (*c* 1.0, in water), which corresponds to 96 and 88% ee, respectively. The racemization during the SFS was, from these results, calculated to be 2–3% with 2.5 equivalents of base. With starting material of higher enantiomeric purity (>98%), L-[*methyl*-^{11}C]methionine can be produced with high enough enantiomeric purity to be useful in PET studies.

Examples of preparative SFC chromatograms for L-[*methyl*-^{11}C]methionine are shown in Figs. 3C and 3D. In C the retention time of L-[*methyl*-^{11}C]methionine was 6.6 min. A shoulder on the radiopeak at 6.6 min can be observed, although analysis of the collected fraction showed only >98% radiochemically pure L-[*methyl*-^{11}C]methionine when analyzed on two different LC systems. With a later pressure gradient, D, the peak symmetry was improved but the retention time was increased twofold. In D, the effect of increasing the initial isobaric elution at 100 bar by 1 min was that the analytes were retained more strongly on the column and a higher pressure was needed to elute them in a reasonable time. This effect was reproducible even after 4 months use of the Hypercarb column. In a typical synthesis of L-[*methyl*-^{11}C]methionine, a 5 μA h (45 μA, 6.6 min) bombardment gave 3.8 GBq radiochemically pure product. After heating under reduced pressure for 2 min to remove most of the ammonia, dilution with 2 ml 0.1 M phosphate buffer (pH 7.4) and sterile filtration, 3.1 GBq of >98% radiochemically pure L-[*methyl*-^{11}C]methionine with pH 7–8 was obtained within 30 min from the end of the bombardment. The solutions were controlled with regard to sterility and pyrogenicity and were suitable for intravenous injection in humans.

Evaluation of the SFS system. In all the studies, radioactivity was used as a measure of mass balance, i.e., counting the radioactivity in all parts of the system was found to be a fairly good way of controlling the system efficiency. Of the radioactivity batch delivered with the use of Synthia ([^{11}C]methyl iodide or [^{11}C]cyanogen bromide), roughly 80% was trapped in the precursor loop and 20% in the waste connected after the loop. After performing the SFS and collecting the entire reaction mixture, ca. 70% of the radioactivity from Synthia remained, and after preparative SFC ca. 65% was trapped in all the fractions. The trapping efficiency varied somewhat depending on the trapping solvent and the solubility of the products. Of the remaining 10–15% of the radioactivity, ca. 5% was found in the injection loops or on the SFC column, and the rest was probably lost due to insufficient trapping of the analytes.

Conclusion

From this study it can be concluded that on-line SFS–SFC is useful technique for enhanced speed and efficiency in the synthesis and purification of ^{11}C-labelled compounds, and also for small scale synthesis in general. The high compressibility of the supercritical fluid assures effective mixing and transport of the reactants giving highly reproducible results. Supercritical ammonia can be used as both solvent and reactant in the synthesis and also as the mobile phase in preparative SFC.

Acknowledgements. This work was supported financially by the Swedish Research Council for Engineering Science and the Swedish Natural Science Research Council, grant no. K-KU 3463.

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Received August 3, 1996.