Acta Chemica Scandinavica, 1994: 48: 428–433
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

Supercritical Fluid Synthesis in the Preparation of β^+-Emitting Labelled Compounds

Gunilla B. Jacobson,[a] Karin E. Markides[b] and Bengt Långström*[,a,c]

[a]Department of Organic Chemistry, Institute of Chemistry, University of Uppsala, S-751 21 Uppsala, Sweden, [b]Department of Analytical Chemistry, Institute of Chemistry, University of Uppsala, S-751 21 Uppsala, Sweden and [c]Uppsala University PET Centre, UAS, S-751 85 Uppsala, Sweden

Jacobson, G. B., Markides, K. E. and Långström, B., 1994. Supercritical Fluid Synthesis in the Preparation of β^+-Emitting Labelled Compounds. – Acta Chem. Scand. 48: 428–433 © Acta Chemica Scandinavica 1994.

A system for synthesis in supercritical fluids has been developed for the microscale synthesis of pharmaceuticals labelled with ^{11}C. Supercritical ammonia was selected as the reaction medium and the following variables were studied in detail: trapping efficiency, cell design, substrate concentration, operation design, and temperature and pressure conditions. Alkylation of phenol by [^{11}C]methyl iodide to yield [*methyl*-^{11}C]anisole was used as a model reaction for evaluation of the system. The results show an increased radiochemical yield in the highly compressible near-critical region.

Compounds labelled with short-lived β^+-emitting radionuclides are of great interest for *in vivo* studies of biological and physiological processes in the human body studied by PET.[1] The commonly used and most interesting radionuclides are ^{15}O, ^{13}N, ^{11}C, and ^{18}F with half-lives of 2.0, 10, 20.3, and 109 min, respectively.

When developing synthetic procedures for these labelled compounds, special considerations must be taken compared with conventional synthesis. Not only the chemical yield but also the total time for synthesis including production of the radionuclides, chemical reaction, separation and purification are important in order to obtain the final product in a high radiochemical yield.[2] It is also desirable to achieve high specific radioactivity to allow the use of tracers at lower concentrations, which reduces perturbations on the system being studied. Typically, the specific radioactivity of the ^{11}C-labelled molecules are 2–6 Ci μmol^{-1}, and for PET investigations in man 5–15 mCi are used, which corresponds to the administration of 1–8 nmol. To reduce isotopic dilution by the stable isotopes, the amounts of reactants and volumes of solvents must be reduced to a minimum. The search for rapid synthetic methods and synthetic techniques in order to reduce such isotopic dilution is an important aim for further development of this tracer technique. If such goals are reached, interesting perspectives of performing *in vivo* studies at levels below the nmol concentration range will be possible.

SFC, supercritical fluid chromatography, and SFE, supercritical fluid extraction, are now well-known separation and sample preparation methods[3–5] that allow density, viscosity, dielectric constant, solubility characteristics, phase behaviour and other physical properties of the mobile phase to be controlled by varying the temperature and pressure within the system.[6–9] The possibility of manipulating the supercritical fluid solvent properties is an interesting parameter to effect the selectivity and rate of chemical reactions.[10–22] It has been stated that, in the near-critical region, the supercritical fluid is highly compressible making solvent–solute and solute–solute clustering effective for local density enhancements.[23–27] This would give increased polarity around the solute as well as considerable negative activation volumes both of which affect the rate constant of the system.[28–39] According to more recent statements (Clifford, personal communication), it is believed that, by judicious control of pressure and temperature, selective solvation of transition states leading to particular products can be achieved. In this way, the yield of some products can be enhanced above that obtained in the liquid phase. Moreover, since depressurization will cause selected supercritical fluids to vaporize at atmospheric pressures, it is possible to eliminate the solvent separation steps: this time-saving aspect is important for synthesis with short-lived radionuclides.

The objective of this study was to use supercritical fluids in order to improve the synthesis of PET-tracers by increasing the possibility of controlling the solvent properties. In this paper we report a supercritical fluid syn-

* To whom correspondence should be addressed.

thesis and a synthesis system. The SFS, supercritical fluid synthesis, system has been developed for on-line small-volume synthesis of ^{11}C-labelled molecules in supercritical fluids. Ammonia was chosen as the reaction medium because of its high polarity, hydrogen-bonding properties, compatibility with basic compounds, ease of vaporization, and critical pressure (P_c = 112.5 atm) and temperature (T_c = 132.5 °C) in a favourable range for instrumentation control and stability of analytes.

A microscale apparatus with automatic control was developed and its instrumental parameters investigated, including the design of cell and trapping device and compatibility of materials with supercritical ammonia. The operational aspects were also studied where temperature, pressure, time, substrate concentration and order of mixing the reactants were found to be important parameters for optimization of the synthesis. Alkylation of phenol using [^{11}C]methyl iodide to yield [*methyl*-^{11}C]anisole was selected as a model reaction for evaluation of synthetic and system parameters.

Results and discussion

The SFS system consisted of a reciprocal pump and a pulse dampener to ensure a pulse-free supercritical ammonia flow, two injection valves for loading the substrate and labelled reactant, and a reaction cell located inside an oven, Fig. 1. In all the experiments, the system was controlled according to the performance procedure described in the Experimental section.

After each reaction, the reaction cell was opened and the product mixture was trapped in a solvent suitable for direct injection on LC. Using a standard anisole solution, different trapping procedures were investigated to optimize the trapping efficiency. By collecting the product mixture for 30 s in 1 ml of solvent, the efficiency of the radiotracer trapped in ethanol, ether, dimethyl sulfoxide

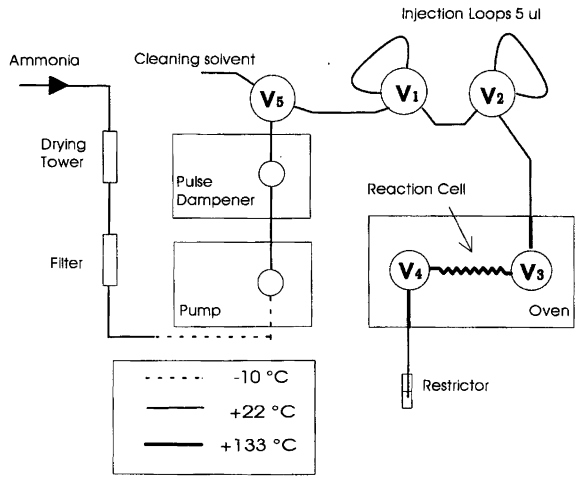

Fig. 1. Schematics of the ammonia supercritical fluid synthesis system.

Table 1. Trapping efficiency in open collection vials as a function of ethanol volume and trapping time.

V/ml	Trapping time/s	Anisole trapped (%)[a]
1	15	59±15
1	30	71±3
1	60	72±2
2	15	84±9
2	30	91±4
2	60	95±4
4	15	95±3
4	30	>95
4	60	>95
6	30	>95

[a] Three experiments were performed for each set of values with the reaction cell 2 at 115 bar, 135 °C and 5 min reaction time. The trapping efficiency was measured in relation to calibrated anisole solutions by LC analysis.

and tetrahydrofuran were compared and shown to be in the range of 70%. For the LC separation, a NH_4HCO_2–MeOH system was used. Ethanol was therefore selected as the solvent for investigating the effect of trapping efficiency with regard to solvent volume and trapping time. The trapping efficiency was improved when the solvent volume increased, Table 1. Trapping times of longer than 15 s and solvent volumes of greater than 2 ml in combination did not improve the trapping efficiency.

Aerosol formation is known to affect the trapping efficiency in open vials.[33–36] Therefore trapping in pressurized vials was compared with trapping in open vials. The vials were capped with a septum; a syringe needle (0.8 mm i.d.) was used as a restriction vent. Trapping in 1, 2 and 4 ml of ethanol was performed for 30 s. Comparable results as for open vials were obtained.

[^{11}C]Methyl iodide was produced from [^{11}C]carbon dioxide by a reaction sequence using lithium aluminum hydride and hydrogen iodide,[37] and trapped in acetonitrile. The stability of [^{11}C]methyl iodide in acetonitrile was analysed and showed a radiochemical purity of 96–

Table 2. Percentage radiochemical yield of [*methyl*-^{11}C]anisole at different pressures and temperatures[a]

	P/bar					
T °C	50	100	115	130	160	200
50	38	39	38	37	38	40
100	43	42	42	46	46	47
115	51	52	54	54	54	54
130	49	55[c]	55[c]	55[b]	54[b]	55
135	51	52[b]	60[c]	55[b]	55[b]	56
147	51	51	53[b]	56[b]	53[b]	54

[a] Three-minute reactions were performed in cell 1 with a 2.12 M sodium phenolate solution. The radiochemical yield was determined chromatographically. Unless otherwise stated, all experimental points were performed twice in randomized order, RSD = 3. [b] Five experiments were performed. [c] Six experiments were performed.

99% for more than 180 min when stored at room temperature.

The reaction products from the [*methyl*-^{11}C]anisole reaction were analysed by LC, giving radiochemical product yields in the range 30–60% depending on reaction conditions, Table 2. A number of experiments were carried out to study whether the substrate concentration, sequence of mixing reactants, pressure, temperature, and reaction cell size had any effect on the radiochemical product yield. The effect of substrate concentration on the radiochemical product yield was studied by injecting different concentrations of sodium phenolate via valve V_2 together with the [^{11}C]methyl iodide solution via valve V_1. Substrate concentrations of above 2 M did not improve the radiochemical yield, Table 3, since the reaction followed pseudo-first-order kinetics with respect to [^{11}C]methyl iodide. The average specific activity for [^{11}C]carbon dioxide was 6 Ci μmol^{-1} at EOB, end of bombardment, and the conversion into [^{11}C]methyl iodide was around 90%, resulting in 20 nmol corrected to EOB. The [^{11}C]methyl iodide was trapped in 200 μl of acetonitrile and 5 μl of this solution contained ca. 0.5 nmol of [^{11}C]methyl iodide at the time of injection, implicating a several thousand times excess of the sodium phenolate.

The sequence of mixing the reactants had a large effect on the radiochemical product yield. By injecting [^{11}C]-methyl iodide through valve V_1, the ammonia flow pushed the [^{11}C]methyl iodide into the substrate solution, giving radiochemical yields of [*methyl*-^{11}C]anisole in the range 50–60%. When the injection sequence was reversed, [^{11}C]methyl iodide reacted with the solvent before being mixed with the substrate, giving a 12–15% radiochemical yield of [*methyl*-^{11}C]anisole. The formation of [^{11}C]-methylamine as a side product was determined by radio GC.

The use of a supercritical fluid as the reaction solvent made it possible to control the physical properties and phase behaviour during the reaction simply by controlling the reaction pressure and temperature. Two to six alkylation reactions on sodium phenolate with [^{11}C]methyl iodide were performed at different temperatures and pressures for each set of values in a randomized order. The addition of reactants effected the supercritical point of the mixture and was therefore different than for pure ammonia. The product yield increased with increasing temperature up to the critical temperature of ammonia (T_c = 132.5 °C), but was less dependent on pressure. The only exception to this was in the near-critical region where a small change in pressure gave larger effects on the radiochemical yield. The highest radiochemical yields were also found in this highly compressible region, Table 2 and Fig. 2.

Four reaction cells of different length and inner diameter were compared to investigate the effect of shape and volume on the reproducibility and radiochemical yield. Repetitive reactions were performed using a sodium phenolate solution and [^{11}C]methyl iodide, Table 4. A decrease in cell volume and an increase in cell length gave increased product yields and reproducibility.

When designing a system to be used for synthesis in supercritical ammonia, special considerations had to be taken when selecting materials for tubing, filters, seals and any other parts that would be in contact with the corrosive ammonia. Only 316 SS steel tubing was used. The tubing was precut and electropolished to reduce dead volumes, surface effects or leaks. An internal diameter of 0.12 mm was selected for all tubing to allow microscale synthesis. The ammonia was first passed through a potassium hydroxide drying tower and two filters to ensure a dry and particle-free fluid. The inlet and outlet check-valve cartridges of PVDF, polyvinyl difluoride, that were used in the pump head appeared to have a limited lifetime of 4–6 weeks. A PTFE-graphite piston seal was successfully used in the pump head. Valcon H rotor material (Valco) was preferred in the valves owing to its resistance to ammonia, while the valves located inside the oven had to be Valcon E to withstand the higher temperature. The Valcon E rotors can survive supercritical ammonia for at least 6 months.

Table 3. Yield of [*methyl*-^{11}C]anisole as a function of sodium phenolate concentration.

Phenolate injected in 5 μl		Radiochemical Yield
μmol	Conc./M	[*methyl*-^{11}C]anisole (%)[a]
1.1±0.2	0.21±0.04	38±3
5.3±0.2	1.06±0.04	55±2
10.6±0.2	2.12±0.05	60±2
21.2±0.2	4.25±0.06	58±2

[a] Two three-minute reactions were performed for each concentration at 115 bar and 135 °C in cell 2. The radiochemical yield was determined chromatographically.

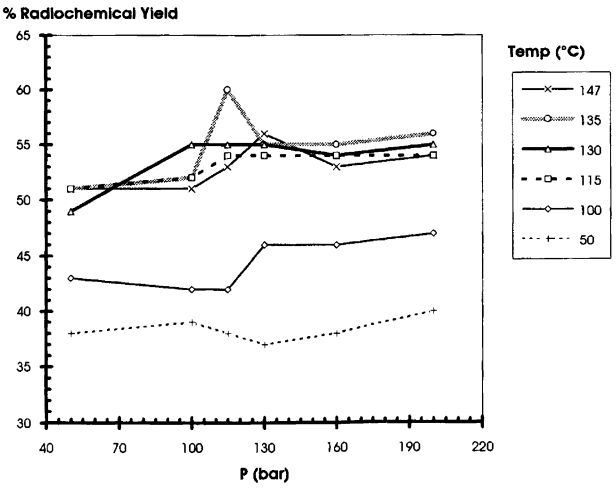

Fig. 2. Radiochemical yield of [*methyl*-^{11}C]anisole from 2–6 experiments, RSD = 3. Values are taken from Table 2.

Table 4. Reproducibility and product yield as a function of cell volume and dimension.

Cell	V/µl	Cell dimension length i.d./mm	[methyl-^{11}C] anisole (%)	RSD[a]
1	318	20×4.50	43–46	3
2	221	500×0.75	52–57	5
3	110	250×0.75	40–53	12
4	30	610×0.25	57–60	2

[a] Five repetitive reactions using a 2.4 M sodium phenolate solution at 140 bar and 145 °C were performed with cells 1 and 4, and four reactions with cells 2 and 3. The radiochemical yield was determined chormatographically.

Conclusions

The SFS system described above can be controlled accurately to perform on-line synthesis with supercritical ammonia and high reproducibility, Table 4. By performing the synthesis in the near-critical region, higher product yields were obtained, and the SFS reaction system was optimized by small changes in pressure and temperature.

Planned developments of the SFS system include on-line micro-preparative supercritical fluid chromatography of the product mixtures. A packed SFC column will be added in series with the reaction cell and on completion of reaction the cell is used as an injection loop for direct injection into the column. SFC is a widely used separation technique, and many procedures for coupled on-line SFE–SFC are described.[38–40]

Experimental

General. Radionuclides were produced at the Uppsala University PET Centre using an MC 17 accelerator (Scanditronix AB, Uppsala, Sweden). A nitrogen gas target was irradiated with a 17 MeV proton beam producing ^{11}C by the ^{14}N(p,α)^{11}C nuclear reaction. The [^{11}C]carbon dioxide produced was trapped in 0.5 ml 0.25 M lithium aluminum hydride in tetrahydrofuran. After evaporation of the tetrahydrofuran, 1 ml 57% hydrogen iodide was added and the [^{11}C]methyl iodide was transferred in a stream of nitrogen gas[37] and trapped in acetonitrile.

Analysis of the product mixtures were performed by LC (Model 1090, Hewlett-Packard, Waldbronn, Germany) equipped with a UV diode array detector in series with a β^+-flow radio detector. A 2.0 × 250 mm Ultrasphere ODS (Beckman Instruments AB, San Ramon, USA) column was used with a mobile phase mixture of 60% 0.05 M NH_4HCO_2, pH = 3.5, and 40% MeOH. The radiochemical yield was calculated by measuring the relative peak areas from the decay corrected radiochromatograms. The chemicals were generally of the following grade: ethanol, 95%; dimethyl sulfoxide, anhydrous, 99 + %; ether, anhydrous, 99 + %. Tetrahydrofuran was distilled from sodium–benzophenone.

Instrumentation. A schematic of the SFS system is shown in Fig. 1. Ammonia gas (grade 4.8, AGA Gas AB, Stockholm, Sweden) was passed through a potassium hydroxide drying tower, a filter for corrosive gases (316 SS, 0.3 µm, F11, AGA Gas AB) and a filter (0.5 µm, 2.5 cm^2, 0.4 ml) included in the thermostatic kit for the 10SC pump head (Gilson Medical Electronics S.A., Villiers-le-Bel, France). A cryostat refrigerating circulator (F3 CH, HAAKE Mess-Technik GmbH u.Co., Karlsruhe, Germany) containing −10 °C ethanol was used to cool the ammonia before it entered the pump (model 308, Gilson Medical Electronics S.A.). The 10SC pump head contained inlet and outlet check valve cartridges of PVDF, polyvinyl difluoride, and a PTFE-graphite piston seal. The pump head was equipped with a thermostatic kit (Gilson Medical Electronics S.A.) connected to the cryostat to keep it cool despite the high pressure used. A pressure regulator (model 821, Gilson) was used to pass ammonia through a pulse dampener to ensure a pulse-free flow and to measure the current pressure. A three-port valve, V_5 (AC3W, Valco Instruments Co. Inc., Houston, USA), was used to allow cleaning solvent to pass through the injection loops and cell between reactions. Two six-port valves, V_1 and V_2, (AC6W, Valco) were used to inject the reactants. Valves V_5, V_1 and V_2 all contained rotor material of Valcon H and could withstand 5000 psi and 75 °C. Both injection loops were made of 316 SS tubing (1/16″, 0.12 mm i.d.) with 5 µl volume. Two three-port valves, V_3 and V_4, (A3C3WEY, Valco) containing rotor material of Valcon E were located inside the oven and could withstand 5000 psi and 150 °C. All five valves were equipped with air actuators and controlled by digital valve interfaces. The aluminum oven was 230 × 90 × 90 mm with a wall thickness of 20 mm. A temperature regulator was used to control the temperature in the interval 20–150 °C (± 2 °C). Different reaction cells were used, all different in volume and length. Cell 1 had a volume of 318 µl (20 mm × 4.5 mm i.d.). It was connected between valves V_3 and V_4 by two 50 mm lengths of 1/16″ 316 SS tubing (50 mm × 0.12 mm i.d.). Cells 2 and 3 were made of 1/16″ 316 SS tubing with a volume of 221 µl (500 mm × 0.75 mm i.d.) and 110 µl (250 mm × 0.75 mm i.d.), respectively. Cell 4 was 1/16″ 316 SS tubing with a volume of 30 µl (610 mm × 0.25 mm i.d.). A pinched restrictor was constructed by crimping the end of a capillary tubing (200 mm × 0.12 mm i.d.). All tubing used from the gas cylinder to the pump head was 1/8″ 316 SS (2.1 mm i.d.), and from the pump head to the restrictor 1/16″ 316 SS (0.12 mm i.d.).

Performance procedure. The desired oven temperature was set and the system was conditioned with ammonia and pressurized to supercritical conditions three to four times. The reactants were loaded into the injection loops on valves V_1 and V_2, while the flow was kept at 0.5 ml min^{-1}. Valve V_4 was closed and V_2 and V_1 were switched to inject position, so that the liquid ammonia pushed the reactants into the reaction cell which was closed at the

outlet side. A positive forward movement of ammonia with dissolved reactants was assured and the desired reaction pressure was reached in the reaction cell. Valve V_3 was then closed and the pump flow was reduced to 0.05 ml min^{-1} to retain pressure between the pump and reaction cell. In practice, the desired reaction pressure was set at the maximum pressure control on the pump ensuring a constant pressure during the reaction. The reaction was carried out for 1–3 min after which valves V_4 and V_3 were opened forcing the products out of the cell and through the restrictor. The pressure then rapidly decreased to 9–10 bar. The restrictor tip was positioned into a solvent to trap the product mixture. After each reaction, valve V_5 was switched to clean the system by flushing 10 ml each of 1% HNO_3 and ethanol through the loops and reaction cell. The system was finally pressurized three times with ammonia to supercritical conditions and conditioned for 1–5 min before performing the next synthesis.

Trapping procedures. 5 µl of a 50:50 v/v mixture of anisole in ethanol were loaded into injection loop V_2. The system was run as described previously with reaction cell 2 (V = 221 µl) and an oven temperature of 135 °C and pressure of 115 bar. The anisole solution was retained in the cell for 30 s and trapped by positioning the restrictor tip 30 mm into the trapping vial. The solvents used were ethanol, dimethyl sulfoxide, ether or tetrahydrofuran. Glass vials (10 ml, 45 mm × 20 mm i.d.) were used as collection vessels either open to atmosphere or capped with a Teflon septum and vented with a syringe needle (0.8 mm i.d.). The trapping efficiency was measured in relation to calibrated anisole solutions by LC analysis.

[methyl-^{11}C]Anisole. A standard solution of sodium phenolate was prepared by dissolving 100 mg (1.06 mmol) phenol in 330 µl acetonitrile and 170 µl (0.85 mmol) 5 M sodium hydroxide, giving 500 µl of a 2.1 M solution. 5 µl of this solution were loaded into injection loop V_2. [^{11}C]Methyl iodide was trapped in 200 µl acetonitrile and 5 µl of this solution were loaded into injection loop V_1. The reaction was run as described previously with the preset temperature, pressure and reaction cell. The products were trapped in 4 ml ethanol over a period of 30 s and analysed by LC.

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.

References

1. Phelps, M., Mazziotta, J. and Schelbert, H., Eds. *Positron Emission Tomography and Autoradiography; Principles and Applications for the Brain and Heart*, Raven Press, New York 1986.
2. Långström, B., Obenius, U., Sjöberg, S. and Bergson, G. *J. Radioanal. Chem. 64* (1981) 273.
3. *Analytical Supercritical Fluid Chromatography and Extraction*, Lee, M. and Markides, K. E., Eds., Chromatography Conference, Provo, USA 1990.
4. Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction, Volume 53, *Journal of Chromatography Library*, Jinno, K., Ed., Elsevier, Amsterdam 1992.
5. McHugh, M. A. and Krukonis, V. *Supercritical Fluid Extraction*, Butterworth, Boston 1986.
6. Kim, S., Wong, J. M. and Johnston, K. P. In: Penninger, J. M. L., Ed., *Supercritical Fluid Technology*, Elsevier, Amsterdam 1985, p. 45.
7. Johnston, K. P. *Am. Chem. Soc. Symp. Ser. 406* (1989) 1.
8. Brennecke, J. F. and Eckert, C. A. *AIChE J. 35* (1989) 1409.
9. Johnston, K. P., Kim, S. and Combes, J. *Am. Chem. Soc. Symp. Ser. 406* (1989) 52.
10. Wu, B. C., Klein, M. T. and Sandler, S. I. *Ind. Eng. Chem. Res. 30* (1991) 822.
11. Paulaitis, M. E. and Alexander, G. C. *Pure Appl. Chem. 59* (1987) 61.
12. Isaacs, N. S. and Keating, N. *J. Chem. Soc., Chem. Commun.* (1992).
13. Aida, T. and Squires, T. G. *Am. Chem. Soc. Symp. Ser. 329* (1986) 58.
14. Brunner, G. *Ion Exch. Solvent Extr. 10* (1988) 105.
15. Eckert, C. A. and Van Alsten, J. G. *Environ. Sci. Technol. 20* (1986) 319.
16. Weder, J. K. P. and Bokor, M. V. *Food Chem. 44* (1992) 287.
17. Johnston, K. P. and Haynes, C. *AIChE J. 33* (1987) 2017.
18. Kim, S. and Johnston, K. P. *Am. Chem. Soc. Symp. Ser. 329* (1986).
19. Subramaniam, B. and McHugh, M. A. *Ind. Eng. Chem. Process. Des. Dev. 25* (1986) 1.
20. Roberts, C. B., Chateaunef, J. E. and Brennecke, J. F. *J. Am. Chem. Soc. 114* (1992) 8455.
21. Kim, S. and Johnston, K. P. *Chem. Eng. Commun. 63* (1988) 49.
22. Hrnjez, B. J., Menta, A. J., Fox, M. A. and Johnston, K. P. *J. Am. Chem. Soc. 111* (1989) 662.
23. Combes, J. R., Johnston, K. P., O'Shea, K. E. and Fox, M. A. *Am. Chem. Soc. Symp. Ser. 488* (1991) 31.
24. Tsugane, H., Yagi, Y., Inomata, H. and Saito, S. *J. Chem. Eng. Jpn. 25* (1992).
25. O'Shea, K. E., Combes, J. R., Fox, M. A. and Johnston, K. P. *Photochem. Photobiol. 54* (1991) 571.
26. Brennecke, J. F., Tomasko, D. L., Peshkin, J. and Eckert, C. A. *Ind. Eng. Chem. Res. 29* (1990) 1682.
27. Morita, A. and Kajimoto, O. *J. Phys. Chem. 94* (1990) 6420.
28. Eckert, C. A., Ziger, D. H., Johnston, K. P. and Ellison, T. K. *J. Phys. Chem. 90* (1986) 2798.
29. Ikushima, Y. and Saito, N. *J. Phys. Chem. 96* (1992) 2293.
30. Bubak, M. and Harder, W. D. *Ber. Bunsenges. Phys. Chem. 81* (1977) 736.
31. Kuei, J. C., Markides, K. E. and Lee, M. L. *HRC & CC 10* (1987) 257.
32. Raynie, D. E., Payne, K. M., Markides, K. E. and Lee, M. L. *J. Chromatogr. In press.*
33. Langenfeld, J. J., Burford, M. D., Hawthorne, S. B. and Miller, D. J. *J. Chromatogr. Sci. 594* (1992) 297.
34. Hawthorne, S. B., Miller, D. J. and Krieger, M. S. *J. Chromatogr. Sci. 27* (1989) 347.
35. Mulcahey, L. J., Hedrick, J. L. and Taylor, L. T. *Anal. Chem. 63* (1991) 2225.
36. Wright, B. W., Wright, C. W., Gale, R. W. and Smith, R. D. *Anal. Chem. 59* (1987) 38.
37. Långström, B., Halldin, C., Antoni, G., Gullberg, P., Malmborg, P., Någren, K., Rimland, A. and Svärd, H. *J. Nucl. Med. 38* (1987) 601.

38. Xie, Q. L., Markides, K. E. and Lee, M. L. *J. Chromatogr. Sci. 27* (1989) 365.
39. Koski, I. J., Jansson, B. A., Markides, K. E. and Lee, M. L. *J. Pharm. Biomed. Anal. 9* (1991) 281.
40. Daimon, H. and Hirata, Y. *Chromatographia 32* (1991) 549.

Received October 4, 1993.