Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 195-203 (2000)

[^{11}C]Methylation on a C_{18} Sep-Pak cartridge: a convenient way to produce [*N*-methyl-^{11}C]choline*

Claudio Pascali[1], Anna Bogni[1], Ren Iwata[2], Mara Cambiè[1], Emilio Bombardieri[#,1].

[1]National Cancer Institute, Nuclear Medicine Division, V. Venezian,1, Milan 20133, Italy. [2]CYRIC, Tohoku University, Aramaki, Aoba-ku, Sendai 980-8578, Japan

SUMMARY

[*N*-Methyl-^{11}C]choline has been simply and efficiently labelled at room temperature by solid supported [^{11}C]methylation of 2-dimethylaminoethanol on a commercial C_{18} Sep-Pak Light cartridge. The labelled product was retained on a cation exchange resin (Sep-Pak Plus Accell Plus CM) and washed with ethanol and water to remove any excess precursor. Then [*N*-methyl-^{11}C]choline was desorbed from the cartridge by elution with saline, passed through a 0.2 μm sterile filter and collected in a sterile vial. Overall, the total synthesis time was 12 min from the end of bombardment (EOB). The radiochemical yield from [^{11}C]CO_2, decay corrected (EOB) was 87% and the radiochemical purity was greater than 99%.

Very conveniently, the automated apparatus employed is a very simple modification of the system routinely used for the synthesis of L-[*S*-methyl-^{11}C]methionine.

Key words: [^{11}C]choline, PET, solid supported methylation

INTRODUCTION

Tumor cells are in general characterized by the active incorporation of choline for production of phosphatidylcholine, a cell membrane constituent, to assist their rapid duplication (1). This has been confirmed by ^{31}P-magnetic resonance spectroscopy.

*A preliminary account of this work was presented at the 13th International Symposium on Radiopharmaceutical Chemistry, 27 June – 1 July 1999, St. Louis, USA.

#Author for correspondence. Phone: +39-02-2390220, Fax: +39-02-2367874, E-mail: bombardieri @istitutotumori.mi.it

CCC 0362–4803/2000/020195–09$17.50

Received 19 August 1999
Revised 20 September 1999
Accepted 22 September 1999

Because of this, ^{14}C-labelled choline has been proposed as an indicator of growth activity *in vitro*. (2) Recently, human PET studies with ^{11}C-labelled choline have shown the effectiveness of this radiotracer for the imaging of brain tumors (3,4), prostate cancer (5) and cervical cancer (6). These positive results have led us to introduce this radiotracer into our clinical programme.

Since the early reports on the synthesis of [*N*-methyl-^{11}C]choline (7,8) only minor changes have been made to its preparation, mainly aimed at improving the efficiency and speeding up the purification step. (3,9,10,11) The common feature of all of these methods is the conventional distillation of [^{11}C]methyl iodide into a vessel containing the precursor for methylation. This procedure has the main drawback that it requires cooling during the transfer of [^{11}C]methyl iodide, and heating during the subsequent reaction. Moreover, the need to reduce the relatively large volume of 2-dimethylaminoethanol used (up to 500 μL) and to remove the unreacted [^{11}C]methyl iodide forced the authors to perform a total evaporation followed by redissolution in water. All these factors are detrimental to automation and may be a source of variability in the performance of the system. Also a recent procedure based on methylation using [^{11}C]$CH_3OSO_2CF_3$ and having the precursor adsorbed on quartz wool did not bring any notable improvement. While the radiochemical yield was not reported, the reaction time was still long (20 min). (12)

Following our own experience with on-column [^{11}C]methylation (13) we recently reported (14) on the synthesis of L-[*S*-methyl-^{11}C]methionine using a commercial C_{18} Sep-Pak cartridge as a solid-phase support material for the reagents. This approach considerably simplified the setup, shortened the process time and allowed operation at room temperature.

Following this successful application, we decided to apply the same method and apparatus to the synthesis of [*N*-methyl-^{11}C]choline.

EXPERIMENTAL

Materials and methods

Reagents and solvents were used as purchased without further purification, unless specified. Choline chloride (99%), 2-dimethylaminoethanol (99%; further purified by

distillation), 2-naphthalene sulfonic acid (70%), acetonitrile (99.9%), phosphoric acid (98%) and ^{13}C-enriched methyl iodide (99 atom %) were purchased from Aldrich. Absolute ethanol and sodium dihydrogenphosphate were purchased from Carlo Erba, 10 mL sterile vials from Nycomed Amersham Sorin and Millex-GS 0.22 μm filter units from Millipore. All cartridges (Sep-Pak C_{18} , tC_{18} and Accell Plus CM), both of the Light and Plus size, were obtained from Waters. [^{11}C]Carbon dioxide and [^{11}C]methyl iodide were produced as previously described. (14)

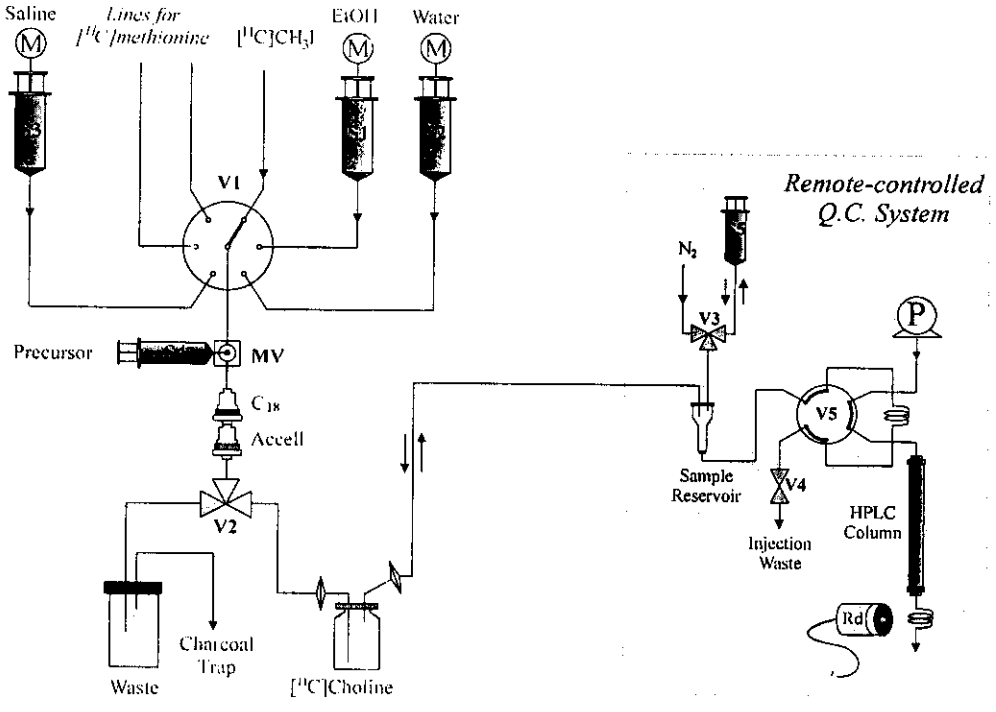

Figure 1. Diagram of the automated system for the production of [*N*-methyl-^{11}C]choline. Also depicted is the remote-controlled apparatus for quality control by HPLC. **MV**: 3-way manual valve. **V1**: pneumatically-operated six-position rotary valve (Rheodyne 5012P). **V2-V3**: 3-way slider valve (Rheodyne 5301) with pneumatic actuator (Rheodyne 5300). **V4**: 2-way solenoid valve (Series 9, General Valve). **V5**: pneumatically-operated 6-way PEEK injector valve (Rheodyne 9010P). **S1-3**: motorized syringe. **S4-S5**: manually operated syringe. **P**: HPLC pump. **Rd**: radiodetector NaI(Tl).

Synthesis and quality control

Figure 1 shows a schematic diagram of the automated system for the [*N*-methyl-^{11}C]choline synthesis. This system was directly linked to a remotely-controlled apparatus for the withdrawal and injection of an aliquot of final solution into an HPLC system for quality control.

2-Dimethylaminoethanol (60 μL, 591 μmol) was loaded on to the C_{18} cartridge by syringe S4 prior to the start of synthesis. [^{11}C]Methyl iodide, produced *via* the classical $LiAlH_4$/HI method, was distilled across the loaded cartridge using a nitrogen flow (20 mL/min, 130 s) and the fraction coming through was trapped in a charcoal trap. Ethanol (10 mL) and pyrogen-free sterile water (10 mL) were then passed through the two joined cartridges in order to wash out any unreacted precursor (collected in the waste) and [^{11}C]CH_3I. The product, now immobilized on the Accell Plus CM Sep-Pak, was desorbed from the cartridge by elution with sterile saline (5 mL), passed through a 0.22 μm sterile filter and collected in a vented sterile vial. The final solution had a pH of 7 and proved to be sterile and pyrogen-free.

Table 1. Activity distribution for the solid-phase synthesis of [*N*-methyl-^{11}C]choline.

	% of activity d.c. at EOB*	Minutes from EOB
Trapping of [^{11}C]CO_2 in liquid nitrogen	100	1.5
[^{11}C]CH_3I at the end of distillation	91	8.1
[^{11}C]Choline solution	87	12.1
C_{18} Sep-Pak Light	1.1	
Accell Plus CM	0.1	
Waste	1.7	
Charcoal trap	0.5	
Sterile filter	0.6	

*Data represent the average of 30 experiments.

The total synthesis time from EOB, including the 2.5 min required for concentrating the target charge in a liquid nitrogen refrigerated loop and then releasing it to the hot-cell, was 12 min. The radiochemical yield from [^{11}C]CO_2, decay corrected at EOB, was 87% (Table 1), which corresponds to a conversion rate of 97% from [^{11}C]CH_3I.

The radiochemical purity of the formulated product was evaluated by analytical HPLC on a YMC-Pack PolymerC_{18} column (4.6x250 mm; 6 μm). The mobile phase was 1mM naphthalene-2-sulfonic acid + 0.05M H_3PO_4 and the flow rate was 1 mL/min. The eluent was monitored by a refractive index detector (HP 1047A, Hewlett-Packard) which was located upstream from a flow radio-detector (Radiomatic Flo-One A515 TR, Canberra-Packard). However, during routine preparations of large activity batches, a sample was withdrawn and injected into an HPLC column for quality control by means of a remotely-controlled setup, as previously reported. (14) In such a case only a radiodetector was used (NaI(Tl) / PMT, type FC-002 Flow-Count, Bioscan) and for convenience the mobile phase was 0.05M NaH_2PO_4. Radiochemical purity was found to be always greater than 99.5%.

Gas chromatographic analyses were conducted on a Hewlett-Packard instrument (mod. 5890 series II) using a flame ionization detector and a splitless injection technique in order to assess the residual amount of precursor. The analyses employed a SGE BP20 capillary column (25 m x 0.33 mm i.d.) with oven temperature increased from 70°C to 220°C at a 10°C/min rate. With the above described reaction conditions the residual precursor was, on average, 28 μg/mL.

Table 2. Decay corrected radiochemical yield of [*N*-methyl-^{11}C]choline *vs.* [^{11}C]CH_3I distillation flow.

[^{11}C]CH_3I distillation (mL/min x sec)	Precursor loaded (μL)	Radiochemical yield from [^{11}C]CH_3I (%)*
15 x 170	40	96.7
20 x 130	40	95.8
10 x 255	60	97.5
15 x 170	60	97.3
20 x 130	60	96.6
25 x 105	60	93.5
25 x 105	80	96.0

*Data represent the average of at least 3 experiments.

The validity of the synthesis was further confirmed by parallel preparation of [*N*-methyl-^{13}C]choline from [^{13}C]CH_3I (2 μL) and examination of the product by ^{1}H-decoupled Fourier transform ^{13}C-NMR spectroscopy (Bruker AC 300 MHz). The only peak recorded had the same chemical shift (δ_{TSP} = 56.8 ppm) as the *N*-CH_3 carbons in the corresponding spectrum of reference choline chloride.

RESULTS AND DISCUSSION

The radiosynthesis of [*N*-methyl-^{11}C]choline through a solid supported [^{11}C]methylation on a commercial C_{18} Sep-Pak Light cartridge has been successfully carried out in remarkably short time (12 min) and very good radiochemical yield (87%, decay corrected at EOB). Furthermore, this approach has considerably simplified the whole procedure and thus its automation.

A summary of the relative activities measured in the system components is reported in Table 1. Several experiments were done in order to determine the optimum conditions with respect to the [^{11}C]CH_3I flow rate, volume of precursor loaded and washing conditions. The major constraint was the need to limit the amount of 2-dimethylaminoethanol in the final solution since this compound can compete with choline for brain uptake. (9) Thus, we tried to keep the volume of precursor loaded

Table 3. Residual 2-dimethylaminoethanol in the final solution *vs.* rinsing conditions. Cartridges used: C_{18} Sep-Pak Light and Accell Plus CM Sep-Pak.

Precursor loaded (μL)	Rinsing solvent(s)	Rinsing volume(s) (mL)	Residual precursor (μg/mL)
100	H_2O	20	238
80	H_2O	20	177
60	H_2O	15	150
40	H_2O	20	133
60	EtOH/H_2O 1:9	15	123
60	EtOH/H_2O 1:9	20	89
40	EtOH/H_2O 1:9	20	78
60	EtOH/H_2O 1:1	20	29
40	EtOH/H_2O 1:1	15	28
80	EtOH	15	48
60	EtOH	20	41
60	EtOH	15	46
40	EtOH	15	35
40	EtOH	10	42
100	EtOH + H_2O	5 + 10	86
60	EtOH + H_2O	5 + 10	38
60	EtOH + H_2O	10 + 10	28
40	EtOH + H_2O	10 + 10	25
40	EtOH + H_2O	3 + 5	86

as low as possible without decreasing the [^{11}C]methylation efficiency (see Table 2). Obviously, larger amounts would allow us to achieve the same high radiochemical yield in a shorter time thanks to a higher flow rate of [^{11}C]CH_3I.

The residual amount of 2-dimethylaminoethanol determined in the final 5 mL solution varied greatly according to the volume loaded on the cartridge and the conditions used for the rinsing step which precedes the elution of the product with saline. Some of the conditions tested are shown in Table 3. Even if not optimized, these data give a fair idea of the trend. The procedure involving the EtOH/H_2O 1:1 solution was abandoned because of a back pressure problem which suggested a moderate elution rate if any leak was to be avoided.

A few comments can be made on the cartridges used. Ideally, the "Light" type is preferred because of the smaller volume which allows a shorter time for its elution. However, unlike in a previous report (10), the Accell Plus CM Sep-Pak Light was unable to adequately retain [*N*-methyl-^{11}C]choline, which was found in considerable amount (>20%) in the washing. Conditioning of the cartridge before its installation in the apparatus, as suggested in the user manual, gave no better result and was therefore abandoned. Experiments were also performed using a tC_{18} Sep-Pak Light cartridge in place of the normal C_{18} type because of their larger carbon load (17% *vs.* 12%). However, this solution also gave lower radiochemical yields.

Since some liquid is always retained on a cartridge no matter how much flow is passed through, we decided also, as a test, to remove the C_{18} Sep-Pak and directly load the precursor (60 μL) on the Accell cartridge. The observed radiochemical yield from [^{11}C]CH_3I (90%) was slightly worse when compared to our standard procedure (97.5%). On the other hand, this method afforded a better removal of the starting material, with only 22 μg/mL found in the final solution. Thus, this procedure can be a useful alternative whenever the clinical investigation calls for very low levels of 2-dimethylaminoethanol.

According to the reported HPLC method (10) for the analysis of [*N*-methyl-^{11}C]choline, it should be possible to determine the mass of both choline and 2-dimethylaminoethanol by a refractometer. However, in our hands, this method failed to afford the expected result, since both compounds had a very low response to the

refractometer (Hewlett-Packard HP 1047A; sensitivity $1.5x10^{-7}$ RIU). The lack of a mass signal prevented us from assessing the specific activity of [*N*-methyl-^{11}C]choline, as well as determining the residual 2-dimethylaminoethanol by HPLC. However, the levels of precursor were evaluated by gas chromatography.

Furthermore, in order to confirm the identity of the radioactive product by comparison of its retention time with that of authentic choline chloride (4.35 min) we injected an amount of standard (1 mg/mL) several hundred-fold higher than the predicted carrier accompanying [*N*-methyl-^{11}C]choline[#]. A 5 μL injection of a 1-4 mg/mL solution of choline chloride was sufficient to give a discernible peak having the same retention time as the desired radioactive product. Concentrations greater than 4 mg/mL gave a shorter retention time and thus lead to an erroneous interpretation of the radiochromatogram.

CONCLUSION

Sterile and pyrogen-free isotonic solutions of [*N*-methyl-^{11}C]choline have been produced in very high radiochemical yield in 12 min from EOB (18 min if we include the remote-controlled quality control by HPLC). The use of a solid-phase support for the reaction has allowed us to shorten the reaction time and considerably simplify both the procedure and its automation. Furthermore, the system has proved to be easily adaptable to the preparation of other radiopharmaceuticals since it is currently used also for the synthesis of L-[*S*-methyl-^{11}C]methionine.

Acknowledgements - This work was partially supported by a grant-in-aid for scientific research from the Associazione Italiana per la Ricerca sul Cancro and from the "Progetto Finalizzato" of the Italian Ministery of Health. The authors are grateful to Ing. V. De Sanctis for running the cyclotron operations.

REFERENCES

1. Aiken NR, Gillies RJ. *Anticancer Res.* **16:** 1393-1397 (1966).

[#] This assumption is based on the known specific activity of our [^{11}C]CH_3I.

2. Waki A., Yoshimoto M., Kato H., Yonekura Y., Fujibayashi Y. *J. Labelled Compd. Radiopharm.* **42:** S690-692 (1999).
3. Hara T., Kosaka N., Shinoura N., and Kondo T. *J. Nucl. Med.* **38:** 842-847 (1997).
4. Shinoura N., Nishijima M., Hara T., Haisa T., Yamamoto H., Fujii K., Mitsui I., Kosaka N., Kondo T., Hara T. *Radiology* **202:** 497-503 (1997).
5. Hara T., Kosaka N., and Kishi H. *J. Nucl. Med.* **39:** 990-995 (1998).
6. Que T.H., Boonstra H., vd Zee A., Vaalburg W., Hollema H., Pruim J. *J. Nucl. Med.* **40:** S255P (1999).
7. Friedland R.P., Mathis C.A., Budinger T.F., Moyer B.R., Rosen M. *J. Nucl. Med.* **24:** 812-815 (1983).
8. Diksic M., Yamamoto Y.L., Feindel W. . *J. Labelled Compd. Radiopharm.***21:** 815-820 (1984)
9. Rosen M.A., Jones R.M., Yano Y., and Budinger T.F. *J. Nucl. Med.* **26:** 1424-1428 (1985).
10. Hara T., and Yuasa M. *Appl. Radiat. Isot.* **50:** 531-533 (1999).
11. Mulholland G.K., Zheng Q-H., Mock B.H., Vavrek M.T. *J. Labelled Compd. Radiopharm.* **42:** S459-461 (1999).
12. Lehikoinen P., Bergman J., Hallsten U., Kokkomaki E., Eskola O., Solin O., Viljanen T. *J. Labelled Compd. Radiopharm.* **42:** S480-482 (1999).
13. Iwata R., Pascali C., Yuasa M., Yanai K., Takahashi T., Ido T. *Appl. Radiat. Isot.* **43:** 1083-1088 (1992).
14. Pascali C., Bogni A., Iwata R., Decise D., Crippa F., Bombardieri E. *J. Labelled Compd. Radiopharm.* **42:** 715-724 (1999).