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Technical note

A simple Tracerlab module modification for automated on-column [^{11}C]methylation and [^{11}C]carboxylation

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Abstract

A modification of commercial [^{11}C]methylation module which can be implemented for both on-column [^{11}C]methylation and [^{11}C]carboxylation in the same automated system is described. This module configuration was applied to the solid-phase synthesis of *N*-[^{11}C]methyl-choline ([^{11}C]choline) and L-(*S*-methyl-[^{11}C])methionine ([^{11}C]methionine), using [^{11}C]CH_3I as methylating agent, as well as to the synthesis of [^{11}C]acetate by [^{11}C]carboxylation with [^{11}C]CO_2 of methylmagnesium chloride with high and reproducible radiochemical yields in short reaction time, demonstrating to be a fast and reliable tool for the production of these [^{11}C]radiopharmaceuticals for clinical use.

Keywords: On-column [^{11}C]methylation; [^{11}C]carboxylation; [^{11}C] PET radiopharmaceuticals

1. Introduction

Production of [^{11}C]radiopharmaceuticals, such as [^{11}C]choline, [^{11}C]methionine and [^{11}C]acetate, has gained increasing importance in PET diagnostic. At the same time, with the higher throughput of the PET/CT scanners the study of a larger number of patients in a shorter time becomes feasible allowing the use of a single lot of ^{11}C-tracers for several patients.

[^{11}C]choline is a very effective PET radiopharmaceutical for the study of prostate cancer (Hara et al., 1998) and brain tumours (Hara et al., 1997) and [^{11}C]methionine is a useful aminoacidic tracer for the diagnosis of brain tumors (Cook et al., 1999). [^{11}C]acetate is a well known [^{11}C]radiopharmaceutical for the study of myocardial metabolism (Brown et al., 1989) and is also used for the detection of prostate cancer (Oyama et al., 2002). Recently, it has been demonstrated that this radiopharmaceutical seems to have an application in PET imaging of hepatocellular carcinoma (HCC) (Ho et al., 2003). Considering the increasing demand of these tracers we present a modification of a commercial [^{11}C]methylation module to perform both on-column [^{11}C]methylation as well as [^{11}C]carboxylation in the same automated system with the aim of allowing the production of different ^{11}C-tracers in the same day, in order to obtain a simple, fast and reliable tool for production of [^{11}C]radiopharmaceuticals for clinical use.

2. Materials and methods

2.1. Configuration of the [^{11}C]methylation module

The modified configurations of the commercial [^{11}C]methylation module (GE Healthcare Tracerlab C11 methyl, formerly Nuclear Interface [^{11}C]methylation module) are depicted in Fig. 1 ([^{11}C]methylation) and Fig. 2 ([^{11}C]carboxylation). The original reactors were replaced with reaction vessels connected to valves with disposable needles as described elsewhere (Matarrese et al., 2003). [^{11}C]CO_2 was produced by the classical nuclear reaction $^{14}N(p,\alpha)^{11}C$ on a mixture of nitrogen (N60 purity grade) and 0.5–1% oxygen in a PETtrace cyclotron (G.E.

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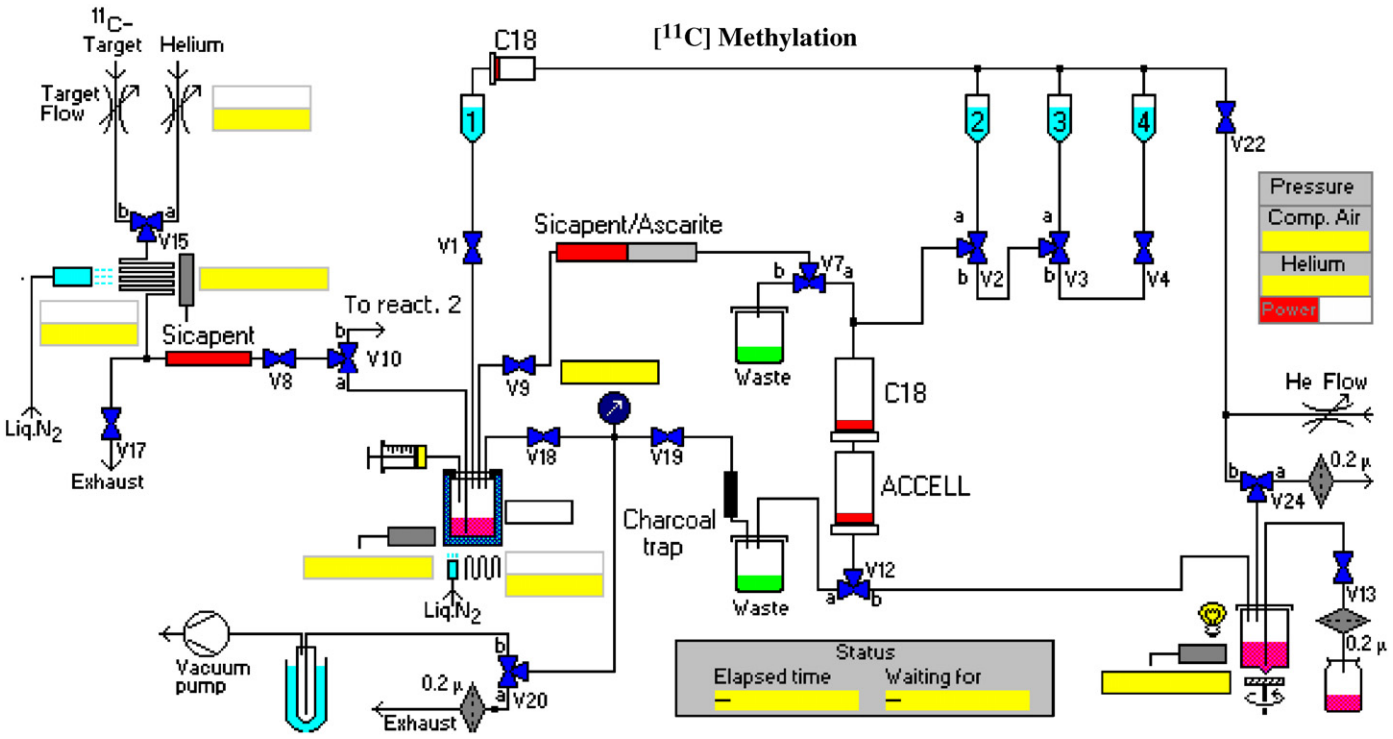

Fig. 1. Schematic diagram of the configuration for on-column [^{11}C]methylation (reactor 1).

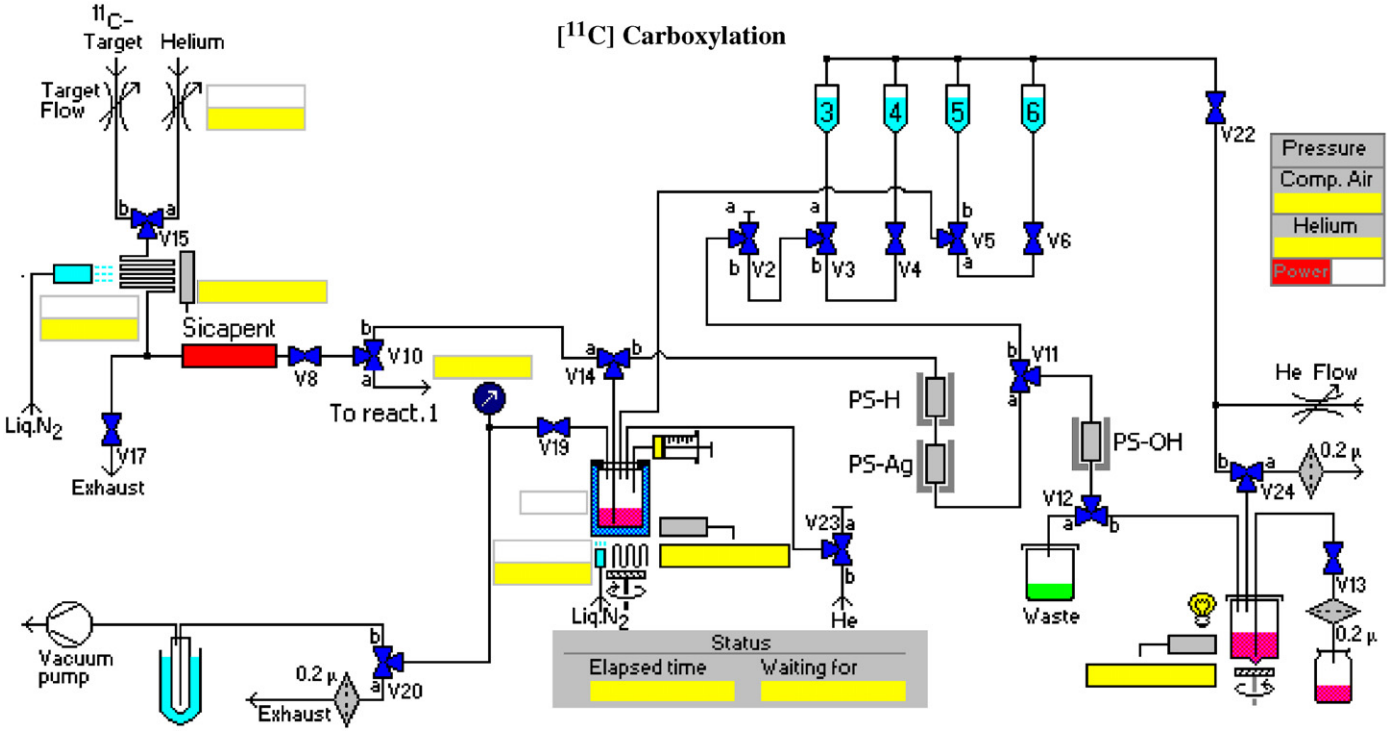

Fig. 2. Schematic diagram of the configuration for [^{11}C]carboxylation (reactor 2).

Healthcare, 16.5 MeV protons) and trapped in a coil cooled at −160 °C with liquid nitrogen. After coil warming, [^{11}C]CO_2 was delivered under helium flow through a dehydrating agent P_2O_5 (Sicapent, Merck) and, via valves V8 and V10, in the reactor 1 or reactor 2 of the module: switching V10 in a position allows the delivery of [^{11}C]CO_2 in the reactor 1 for [^{11}C]CH_3I production while V10 in b position delivers [^{11}C]CO_2 in the reactor 2 for [^{11}C]carboxylation of Grignard reagent. The module configuration shown in Fig. 1 was applied to the synthesis of both

[^{11}C]choline and [^{11}C]methionine whereas Fig. 2 shows the module configuration for the synthesis of [^{11}C]acetate.

2.2. Reagents for the synthesis and QC of [^{11}C]choline and [^{11}C]methionine

$LiAlH_4$ 1 M in tetrahydrofuran, further diluted to a concentration of 0.2 M in a glove box under argon atmosphere, 2-naphthalene sulphonic acid (⩾90%), L-methionine (⩾99%), L-homocysteine thiolactone hydrochloride (⩾99%) were purchased from Fluka. HI (57%) was purchased from Riedel-de Haën, 2-dimethylaminoethanol (99.5%), L-homocysteine (95%) and Ascarite were obtained from Aldrich. The Sep-Pak light C18, plus tC18 env. and Accell plus CM columns were obtained from Waters. Absolute ethanol, H_3PO_4 (85%), NaOH (99.9%), $NaH_2PO_4 \cdot 2H_2O$ (extra pure 98–105%, pharmaceutical grade) and Sicapent (P_2O_5 dehydrating agent) were purchased from Merck.

2.3. Reagents for the synthesis and QC of [^{11}C]acetate

Methylmagnesium chloride 3 M in tetrahydrofuran was purchased from Fluka and diluted to a concentration of 0.1 M in a glove box under argon atmosphere. Glacial acetic acid (99–100%) was obtained from J.T. Baker Chemicals, citric acid and sodium citrate were purchased from Merck. The Chromafix columns PS–H+, PS–AG+ and PS–OH were obtained from Macherey-Nagel.

Before use PS–H+, PS–AG+ columns were rinsed with 10 ml of sterile water, whereas PS–OH columns were activated with 10 ml of NaOH 1 N followed by 10 ml of sterile water.

NaOH 1 M in water was obtained from Merck (Titrisol) and diluted to 0.1 M with distilled water.

2.4. Synthesis of [^{11}C]choline

[^{11}C]choline was synthesised according to the solid-phase [^{11}C]methylation of the precursor, 2-dimethylaminoethanol (DMAE) on a C18 column using [^{11}C]CH_3I as methylating agent (Pascali et al., 2000).

[^{11}C]CO_2 produced by the cyclotron (1.8 Ci, 35 μA/25 min irradiation) was bubbled under helium flow at 20 ml/min in a solution of $LiAlH_4$ (0.2 ml, 0.2 M in THF) at −40 °C within 2 min. After elimination of the solvent at 75 °C under vacuum and cooling at 55 °C, 0.7 ml of HI 57% were added and the reactor was warmed at 140 °C. [^{11}C]CH_3I was distilled under stream of helium (20 ml/min), purified by a column of P_2O_5 (Sicapent, Merck) and NaOH (Ascarite, Aldrich), and transferred within 2 min into a Sep-Pak C18 column where 60 μl (591 μmol) of 2-DMAE was previously loaded. After a washing step with 10 ml of absolute ethanol, to remove the excess of precursor, and with 10 ml of sterile water, to remove the residual ethanol, [^{11}C]choline retained on a cation-exchange resin Sep-Pak Accell Plus CM was eluted with 4 ml of saline and collected in a vial with 4 ml of saline in a final volume of 8 ml. The solution of [^{11}C]choline was sterilised by 0.22 μm filter and was ready for clinical use.

2.5. Synthesis of [^{11}C]methionine

[^{11}C]methionine was synthesised according to the solid-phase [^{11}C]methylation of the precursor L-homocysteine thiolactone on a C18 column, using [^{11}C]CH_3I as methylating agent (Pascali et al., 1999).

[^{11}C]CH_3I produced and purified as described for the synthesis of [^{11}C]choline was transferred within 2 min under stream of helium (20 ml/min) into a Sep-Pak Plus tC18 env. previously loaded with 210 μl of a solution of L-homocysteine thiolactone hydrochloride 7.7 mg (50 μmol) dissolved in 500 μl of NaOH 0.5 M in ethanol/water 50/50.

[^{11}C]methionine was eluted with 2.5 ml of $NaH_2PO_4 \cdot 2H_2O$ 0.05 M buffer and collected in a vial containing 3.3 ml of the same phosphate buffer and 4.2 ml of saline. The final volume was 10 ml.

The solution was bubbled with helium for 2 min to remove the unreacted [^{11}C]CH_3I and sterilised by a 0.22 μm filter.

2.6. Synthesis of [^{11}C]acetate

The synthesis was a [^{11}C]carboxylation of a Grignard reagent (methylmagnesium chloride or bromide) with [^{11}C]CO_2, followed by hydrolysis, ion-exchange purification and elution with an isotonic buffer solution (Kruijer et al., 1995; Roeda et al., 2002).

[^{11}C]CO_2 produced by cyclotron (1.8 Ci, 35 μA/25 min irradiation) was bubbled under stream of helium (10 ml/min) in a solution of methylmagnesium chloride (0.1 M in THF, 1 ml) at room temperature within 3 min. The solution was then hydrolysed with 4 ml of acetic acid 1 mM and pulled, under helium flow, through the columns PS–H+, PS–AG+ and through the anion-exchange column PS–OH. The reactor was rinsed with others 4 ml of acetic acid 1 mM and the [^{11}C]acetate, retained on PS–OH, was washed with 10 ml of sterile water and eluted with 10 ml of isotonic citrate buffer. The final solution was bubbled with helium for 2 min to remove the unreacted [^{11}C]CO_2 and was sterilised with 0.22 μm filter. [^{11}C]acetate was finally ready for clinical use.

2.7. Quality control

pH were measured by standard pH meter.

2.7.1. [^{11}C]choline

The radiochemical purity was determined by RP–HPLC with μBondapackC18 column (3.9 × 300 mm, Waters) using 2-naphthalene sulphonic acid 3 mM + H_3PO_4 1 mM as mobile phase, at the flow of 1 ml/min. The [^{11}C]choline was monitored by NaI radiodetector (retention time 7.0 min).

2.7.2. [^{11}C]methionine

The chemical and radiochemical purity were determined by RP–HPLC with Nucleosil 100-3 C18 column (3×250 mm, Macherey-Nagel) using $NaH_2PO_4 \cdot 2H_2O$ 0.05 M with 2% ethanol at the flow of 0.3 ml/min. The elution of [^{11}C]methionine was monitored by UV detector (220 nm) and NaI radiodetector (RTs: radiolabelled impurity 4.2 min, L-homocysteine thiolactone 4.3 min and L-homocysteine 4.5 min, [^{11}C]methionine 5.5 min).

2.7.3. [^{11}C]acetate

Radiochemical purity was determined with strong anion-exchange column CarboPac PA10 column (4×250 mm, Dionex) with NaOH 0.1 M as mobile phase at the flow of 1 ml/min. The [^{11}C]acetate was monitored with UV detector (220 nm) and NaI radiodetector (retention time 6.6 min).

The residual organic solvents were measured by GC on a capillary column RTX-200 ($30\,m \times 0.3$ mm, Restek). Pyrogen contents were measured by LAL test, using a gel-clotting method.

3. Results and discussion

The radiochemical yields of [^{11}C]choline and [^{11}C]methionine with on-column [^{11}C]methylation were, respectively, $79 \pm 8\%$ EOB decay corrected (results of 145 syntheses, mean $\pm$ SD) and $70 \pm 5\%$ EOB decay corrected (results of 35 syntheses, mean $\pm$ SD), with average production consistently higher than 600 mCi available for patients administration. The pH of the injectable solution of these [^{11}C]radiopharmaceuticals was 6.3 ± 0.1; the radiochemical purity was greater than 99% for [^{11}C]choline and greater than 98% [^{11}C]methionine.

The residual amounts of L-homocysteine thiolactone and L-homocysteine were lower than maximum concentration allowed according to [^{11}C]methionine monograph reported in the European Pharmacopoeia.

The concentration of residual organic solvents was 12 ± 4 mg/ml for ethanol, while acetone and acetonitrile was below 0.01 mg/ml. The total synthesis time was 16 min for [^{11}C]choline and 12 min for [^{11}C]methionine, including the trapping of [^{11}C]CO_2.

The radiochemical yield of [^{11}C]acetate was $74 \pm 8\%$ EOB decay corrected (results of 28 syntheses, mean $\pm$ SD) with average production higher than 650 mCi. The pH was 5 ± 0.2 and the radiochemical purity was greater than 97%. The residual concentration of THF was below 0.1 mg/ml. The total synthesis time for [^{11}C]acetate was 10 min including the trapping of [^{11}C]CO_2. All the lots tested of these [^{11}C]radiopharmaceuticals were demonstrated to be sterile and pyrogen free. This new configuration obtained by a modification of a commercial [^{11}C]methylation module allows to perform both on-column [^{11}C]methylation as well as [^{11}C]carboxylation in the same automated system. [^{11}C]CO_2 produced can be delivered to reactor 1 or 2 simply by turning a three-way valve and the passage from on-column [^{11}C]methylation to [^{11}C]carboxylation is fast and simple. The high and reproducible yields of synthesis and, therefore, the activity available for patients administration demonstrate that this proposed procedure on this easily modified module is a reliable tool for routine clinical practice.

Replacement of the original reactors with those suggested by Matarrese et al. allows an extremely simple change of the reacting vials as well as the use of sterile needles every synthesis.

This configuration also allows the implementation of an easy automated procedure for the cleaning of the module, which is mandatory for a routine daily use of the module itself.

4. Conclusion

We have described a modification of a commercial [^{11}C]methylation module to perform both on-column [^{11}C]methylation and [^{11}C]carboxylation in the same automated system. We have applied this new configuration to our routine production of [^{11}C]choline, [^{11}C]methionine and [^{11}C]acetate with high and reproducible radiochemical yield in short time of synthesis. These results and the high activity (>600 mCi starting from 1.8 Ci of [^{11}C]CO_2 produced) available for patients administration demonstrate that this modified module is a fast and a reliable tool for the production of these [^{11}C]radiopharmaceuticals for clinical use.

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