Development of an Automated System for the Routine Preparation of Carbon-11 Labeled Radiopharmaceuticals

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An automated system was developed for the routine preparation of carbon-11 (^{11}C) labeled radiopharmaceuticals, which consisted of three major parts including [^{11}C]methylation of the precursor with [^{11}C] iodomethane ([^{11}C]CH_3I), purification of the desired product and formulation of the final ^{11}C labeled radiopharmaceutical. The whole system included seven three-way slider valves, eleven solenoid valves, four pneumatic cylinders, a HPLC (High Performance Liquid Chromatography) system and a rotary evaporator. Using this system, we investigated the radiochemical synthesis of L-[methyl-^{11}C]methionine, which is the most widely used amino acid in tumor PET (Positron Emission Tomography) studies. The overall operation took 30-35 min including the production of [^{11}C]CH_3I (10.5 min) and decay-corrected radiochemical yield was 25%. The automated system we described herein can be widely utilized for the preparation of many ^{11}C labeled radiopharmaceuticals and has been shown to be efficient, reliable and easy to operate.

Introduction

Most ^{11}C labeled radiopharmaceuticals are prepared from reactions of the desmethyl precursors with [^{11}C]CH_3I. These radiopharmaceuticals have been used for diagnosis of various diseases using PET, and their applications are now being extended. For the routine preparation of these radiopharmaceuticals for clinical use, development of an automated system is highly required because ^{11}C has a short half-life (20 min) that requires handling large quantities of the radioisotope and thus laboratory personnels have chances of severe exposure to radiation. Moreover, advantages of the radiosyntheses using an automated system include reliability in the radiochemical yield and simplicity of the radiochemical synthesis.

Automation is defined as a system or method in which many or all of the processes are controlled by self-operating machinery, electronic devices, etc. There are some prerequisites for the automated system: an automated system should be reliable, and the automation should be considered when the frequency of radiosynthesis meets clinical demands because automation requires a lot of time and expense. Radiosyntheses can be divided into four categories.[1] First category includes manual syntheses which are carried out by the chemist's hands. Second is remote syntheses which use valves or manipulators. Third is remote automated syntheses which use a computer to control the operation of valves for a remote process. Last is robotic syntheses which use a mechanical arm to move the reaction vessel, etc. Manual system has advantages in terms of cost and flexibility, but is undesirable in terms of high radiation exposure to personnels and low reliability. As the system gets more automated, going from manual system to robotic system, some advantages would come out, such as low radiation exposure, high reliability in synthesis and possibility of more than one synthesis in a day. At the same time, the costs to develop get higher and more space is required.

Automated systems for the preparation of [^{18}F]FDG (2-[^{18}F]fluoro-2-deoxy-D-glucose), which is the most commonly used PET radiopharmaceutical, are commercially available, however, the systems for [^{11}C]methylation are mostly manufactured by the users. A couple of automated systems are now commercialized, however, the cost is not affordable to many users and the maintenance by the manufacturer is not easily available.

In this study, we developed an automated system which enables the preparation of various ^{11}C labeled radiopharmaceuticals at low cost using pneumatic devices and laboratory equipments, and successfully applied this system to the radiosynthesis of L-[methyl-^{11}C]methionine.

Experimental Section

Materials. L-Homocysteine thiolactone was purchased from Research Biochemicals International (Natick, MA, U. S. A.) and L-methionine from Sigma Chemical Co. (St. Louis, MO, U. S. A.). Ammonium formate was obtained from Aldrich Chemical Co. (Milwaukee, WI, U. S. A.), sterile membrane filters (0.22 μm) from Millipore Corp. (U. S. A.) and HPLC solvents from J. T. Baker (Phillipsburg, NJ, U. S. A.). Pyrogen test of the final compound was carried out using a LAL (Limulus Amebocyte Lysate) kit available from Associates of Cape Cod, Inc. (Woodhole, MA, U. S. A.). Parts used for construction of the automated system are listed in this text where appropriate.

Production of [^{11}C]iodomethane ([^{11}C]CH_3I). Production of [^{11}C]CH_3I is required for the [^{11}C]methylation of the precursor. The $^{14}N(p,\alpha)^{11}C$ nuclear reaction on a

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cyclotron (PETtrace™, General Electric Medical Systems, Uppsala, Sweden) produces ^{11}C as a form of [^{11}C]CO_2 which is then converted to secondary precursors, [^{11}C]CH_3I, [^{11}C]CO, or [^{11}C]HCN, etc. Among theses, [^{11}C]CH_3I is the most useful precursor for many ^{11}C labeled compounds for PET. There are two methods for the production of [^{11}C]CH_3I, such as the conventional and the gas-phase methods. The former is the most commonly used method at most PET centers. [^{11}C]Iodomethane is prepared from the reduction of [^{11}C]CO_2 by $LiAlH_4$ followed by the iodination of the resulting [^{11}C]CH_3OH by HI,[2,3] diphosphorus tetraiodide,[4] or triphenylphosphine diiodide.[5] This method has some disadvantages: $LiAlH_4$ easily absorbs CO_2 from the air, which can lower the specific activity of [^{11}C]CH_3I, and the procedure requires cleaning and drying steps before each synthesis. On the other hand, the gas-phase method produces [^{11}C]CH_3I from the reduction of [^{11}C]CO_2 by H_2 on the Ni catalyst followed by the iodination of the resulting [^{11}C]CH_4 by I_2.[6,7] This method is carried out in the gas phase and thus produces [^{11}C]CH_3I with high radiochemical purity (>99%) and high specific activity. The reaction is carried out in a glass tube packed with ascarite II and iodine, and the tube is repacked every ten runs to avoid ascarite trap from getting clogged.

In this study, [^{11}C]CH_3I was produced by the gas-phase method using a CH_3I MicroLab (General Electric Medical Systems, Uppsala, Sweden). An irradiation of the target at 30 μA for 10 min produced 11.8-12 GBq (320-325 mCi) of [^{11}C]CH_3I with specific activity of greater than 3.7×10^5 GBq/mmol (10,000 Ci/mmol) in our system.

An outline, composition and construction of the system. [^{11}C]Methylation with [^{11}C]CH_3I adopted to the automation is largely classified into two methods in terms of the reaction medium used. One is the method that traps [^{11}C]iodomethane in the reaction solution containing the precursor, and the reaction occurs in the solution.[2,3] The other is on-line method that traps [^{11}C]CH_3I in a small column containing an adsorber and coated substrate, and the reaction occurs in the solid phase.[8,9] We chose to develop the former due to its wide application to the [^{11}C]methylation of most precursors.

The automated system is composed of three major parts, including [^{11}C]methylation part where the precursor is reacted with [^{11}C]CH_3I, purification part of the ^{11}C labeled product and formulation part of the purified product (Figure 1). These three parts are incorporated as one system and placed inside the hot cell (lead shield: 7 cm thick, front side; 5 cm, side walls, bottom and ceiling). The outlet of the CH_3I MicroLab is directly connected to this system through a PEEK tubing (1/16" O.D.).

The first part for [^{11}C]methylation is composed of a 30 cm long mechanical joint type rodless cylinder 8 (MY1B series, SMC, Japan) and a 15 cm long dual rod cylinder 9 (CXS series, SMC, Japan) to move a reaction vessel to the horizontal and vertical directions, respectively. The plate holding the reaction vessel is attached to the vertical cylinder 9 which is also attached to the cylinder 8 (Figure 2). The movement of cylinders are controlled by limit switches, speed controllers (SMC, Japan) and compressed air. The reaction vessel (1.1 mL crimp-top conical vial) is

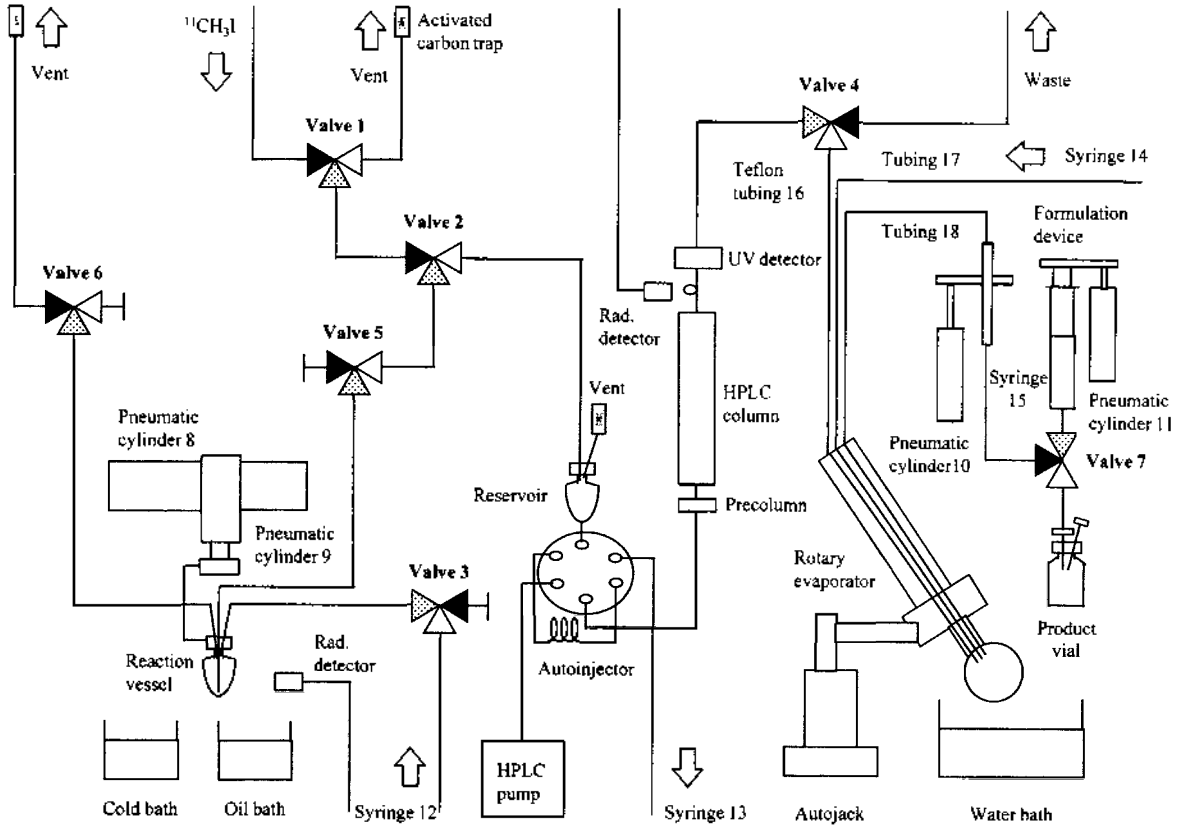


Figure 1. A flow chart of the automated [^{11}C]methylation system. Valves are shown in solid triangles (not actuated), open triangles (actuated) and dotted triangles (common port). The ⊢ or its inverted form attached to valves indicates the blockade of the ports.

thus moved from the cold bath to the oil bath. The cold bath is used for trapping [^{11}C]CH_3I in a reaction vessel containing the precursor and the solvent, and the oil bath for the [^{11}C]methylation. Temperatures of the cold bath and condenser of the rotary evaporator are kept below 0 °C using a refrigerated circulator (Neslab, U. S. A.) and ethanol as the cooling medium (− 5 °C).

The second part for purification of the desired product is composed of a reservoir, an electrically activated 6-port autoinjector (Valco Instruments, U. S. A.), a HPLC system (Thermo Separation Products Co., U. S. A.) and a rotary evaporator (Buchi Instruments, Switzerland). A reservoir is attached to an autoinjector through a teflon adaptor to minimize air bubbles getting into the HPLC column. The HPLC pump is placed outside the hot cell for facile manipulation. The HPLC eluants are monitored simultaneously by a UV detector (214 or 254 nm) for detection of the unlabeled compounds and a NaI(Tl) radiation detector (Bioscan, U. S. A.) for the radioactive compounds. The desired product eluted from a HPLC column is then transferred to a flask of the rotary evaporator movable vertically using an autojack (Eyela, Japan). The HPLC solvents are removed by the rotary evaporator connected to a vacuum pump (Welch Vacuum Technology, U. S. A.). The rotary evaporator is modified such that a three-way glass tube is attached to the top of the condenser. Three teflon tubings (1/16" O.D.) 16, 17 and 18 are inserted to the flask through each way of the three-way tube and the connections are completed *via* teflon adaptors (Surpass Industry, Japan). The teflon tubing 16 is inserted for the transfer of the product from the HPLC column outlet to the flask of the rotary evaporator, the tubing 17 for addition of ethanol from the outside the hot cell to dissolve the compounds which are not completely soluble in saline, and the tubing 18 for transfer of saline to the flask, which is also stretchable to the bottom of the flask to draw out the product solution.

The last part for the formulation of the product (Figure 3) is composed of two pneumatic cylinders 10 and 11 (CGI series, SMC, Japan), a disposable syringe 15, a three-way slider valve 7, three solenoid valves and the teflon tubing 18 stretchable by a stainless steel tube (1/8" O.D.) supported with an adaptor, unions and reducers (Swagelok and Cajon Co., U. S. A.). This part is designed to add saline to the flask containing the product dried in vacuo using a 20 mL disposable syringe 15 containing 9 mL saline, to stretch the teflon tubing 18 to the bottom of the flask to draw out the product in saline, and to transfer the product to a sterile vial through a sterile membrane filter (0.22 μm, Millipore, U. S. A.).

The whole system includes seven 3-way slider valves (Rheodyne Model 53000 and 53010, Alltech, U. S. A.) for the transfer of [^{11}C]CH_3I, the reaction mixture and the product as well as addition of chemicals, and eleven solenoid valves (VZ-1120, SMC, Japan) for operation of pneumatic cylinders and slider valves. Compressed air is supplied to each solenoid valve using an eleven-port manifold (SMC, Japan). All vent tubings are connected to the activated carbon traps that absorb the unreacted radioactive gas. The relative level of radioactivity is monitored using a multi-channel base unit and three ultra-miniature radiation detectors (size: 1×1.5 cm, Bioscan, U. S. A.) which are placed near the reaction vessel, HPLC column outlet, and the flask of the rotary evaporator.

Operation. General procedure for the operation of the system is described below and a flow chart is shown in Figure 1. This procedure can be modified based on the synthetic conditions of radiopharmaceuticals. This system is operated by on-off switches which are mounted on a switch box with a flow chart, and a power supply used is 24 V. The whole process is followed by lighting switches.

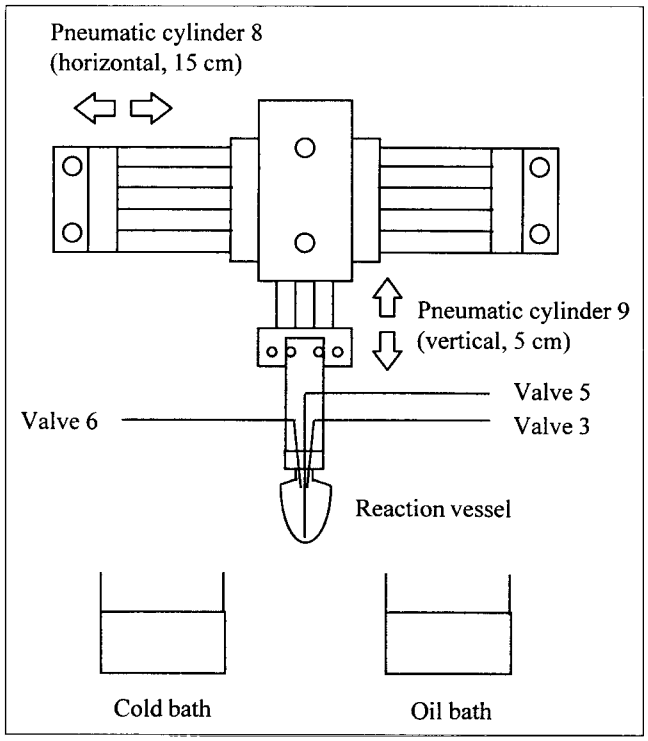


Figure 2. A detailed view of the [^{11}C]methylation part.

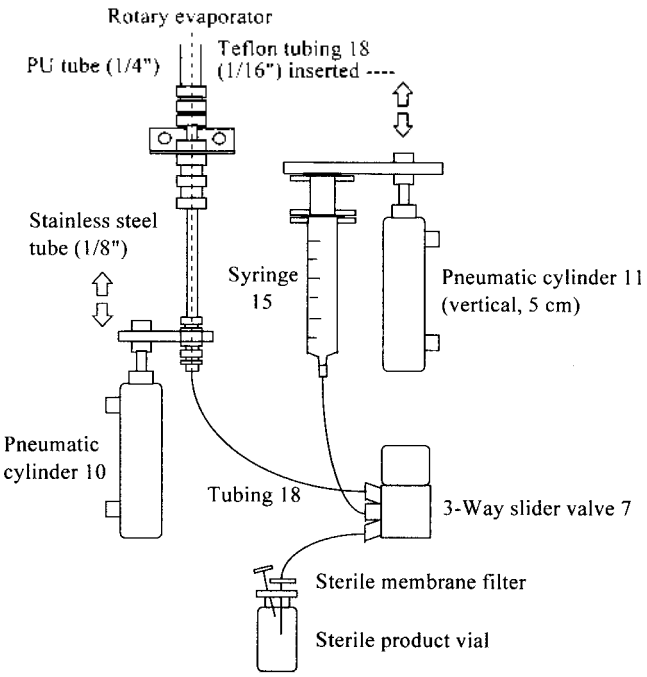


Figure 3. A detailed view of the formulation part.

Step 1. Trapping of [^{11}C]CH_3I

At the end of irradiation (30 μA, 10-20 min), [^{11}C]CO_2 is introduced into the CH_3I MicroLab, from which [^{11}C]CH_3I is released in 10.5 min. [^{11}C]Iodomethane is then trapped in a reaction vessel containing the precursor and the solvent which is dipped in a cold bath (−5 °C). The level of radioactivity in the reaction vessel is monitored by a radiation detector. During this procedure, valve 5 is actuated.

Step 2. [^{11}C]Methylation of the precursor

The reaction vessel dipped in a cold bath is raised and moved by the vertical and horizontal pneumatic cylinders 9 and 8, respectively and lowered to an oil bath set at the appropriate reaction temperature, and then heated for the desired time period. It should be ensured to seal the reaction vessel tightly so that [^{11}C]CH_3I is not allowed to evaporate during the reaction. Valves 1, 2 and 6 are actuated, and valve 5 blocked.

Step 3. Addition of chemicals

At the end of the [^{11}C]methylation, the reaction vessel is raised, and valves 3 and 6 are actuated to put additives to the reaction vessel using syringe 12 through valve 3.

Step 4. Injection onto a HPLC column

The reaction mixture is transferred to a reservoir using air or N_2 pressure into the reaction vessel through valve 3 while valve 5 is actuated. The reaction mixture is then injected onto a HPLC column by pulling out the plunger of the syringe 13 which is connected to the waste port of the autoinjector through a teflon tubing.

Step 5. Running of HPLC

The reaction mixture is purified by generally a reversed phase HPLC system and the eluants are monitored by UV and radiation detectors at the same time. When the desired product is eluted, the fraction is transferred through the actuated valve 4 to a flask of the rotary evaporator equipped with a vacuum pump.

Step 6. Removal of HPLC solvents

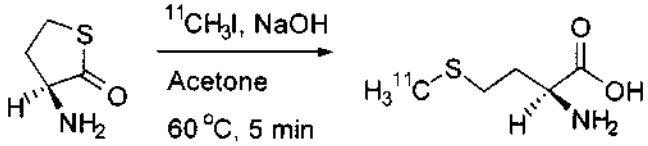
While the appropriate fraction is collected in a flask dipped in a heated water bath, a rotary evaporator and a vacuum pump are operated simultaneously for removal of the solvents.

Step 7. Formulation

Saline (9 mL) loaded in a 20 mL disposable syringe 15 is transferred by the vertical movement of the cylinder 11 to a flask containing the desired product through valve 7. The teflon tubing 18 is then stretched to the bottom of the flask by the cylinder 10 and the solution is withdrawn into the syringe 15 from the flask by the upward stroke of the cylinder 11. The solution is then transferred by the cylinder 11 from the syringe 15 to a sterile product vial through a sterile membrane filter while valve 7 is actuated. The product vial is located in a dose calibrator for ready measurement of the radioactivity.

Step 8. Cleaning-up

The HPLC column is eluted with 95% ethanol at a flow rate of 4 mL/min for 30 min, and the teflon tubing 16 for 5 min with the valve 4 actuated. The teflon tubings located between the reaction vessel and the reservoir are flushed with 95% ethanol several times followed by N_2 through valve 3 while valves 1, 2, 3, and 5 are actuated. The tubing 18 is also flushed by loading 95% ethanol filled in the disposable syringe 15. The reaction vessel and the outlet tubing from the syringe to the sterile product vial are for single use and are not thus cleaned.



Scheme 1. Synthetic pathway of L-[methyl-^{11}C]methionine.

Radiosynthesis of L-[methyl-^{11}C]methionine. The automated system was applied to the preparation of L-[methyl-^{11}C]methionine whose synthetic procedure was based on the literatures with some modifications (Scheme 1).[10,11] To a solution of L-homocysteine thiolactone (1 mg, 8.55 μmol), 150 μL of acetone and 1 N NaOH (30 μL), [^{11}C]CH_3I was trapped at −5 °C. The reaction mixture was heated at 60 °C for 5 min and then neutralized with 150 μL of 0.1 N HCl. The solution was injected onto a semipreparative HPLC column (Alltech Econosil C18, 10× 250 mm) eluted with a 90 : 10 mixture of 0.1 M ammonium formate and methanol at a flow 4 mL/min. The eluant was monitored simultaneously by a UV (214 or 254 nm) detector and NaI(Tl) radiation detector, and the desired product was eluted at 4.5 min. The HPLC solvents were removed *in vacuo*, and the residue was redissolved in 9 mL sterile saline containing sterile sodium bicarbonate solution (50 μL, 8.4% aq.) which was used to adjust the pH to 7.0-7.5. L-[methyl-^{11}C]Methionine was identified by coinjection with unlabeled authentic compound, L-methionine and the radiochemical purity was greater than 99%. Total time was 30-35 min including [^{11}C]CH_3I production (10.5 min) and decay-corrected radiochemical yield was 25% based on [^{11}C]CH_3I. LAL test confirmed the apyrogenicity of the final product solution.

Results and Discussion

An automated system for [^{11}C]methylation was designed and constructed for the routine preparation of many ^{11}C labeled compounds. The [^{11}C]methylation of the precursor with [^{11}C]CH_3I was readily carried out in this system using pneumatic cylinders moving vertically and horizontally. Trapping of [^{11}C]CH_3I was efficient using the circulating cooling medium at around −5 °C: 11.8 GBq (320 mCi) was trapped from around 37 GBq (1000 mCi) of [^{11}C]CO_2. Although lower temperature, which is more costly and troublesome to achieve, would increase the trapping efficiency of [^{11}C]CH_3I, the efficiency we obtained was comparable to the value in the literature. Activated carbon traps were attached to all vent tubings to prevent the unreacted radioactive gas from leaking through the ventilation duct. The oil was used as a heating medium, however, it can be replaced by a heating block or a halogen lamp to avoid the mess of oil. The purification of the desired product was conducted mostly on a reversed phase HPLC system and the removal of HPLC solvents was carried out by a rotary evaporator which was modified to easily add and withdraw the solution to and from the flask. When the desired fraction was eluted, valve 4 was switched on to collect the fraction. Some radiopharmaceuticals are

not fully soluble in saline and in this case, 10% ethanol can be added through the teflon tubing 17 to a flask of the rotary evaporator. At the end of synthesis, the HPLC column and tubings were flushed with 95% ethanol for storage. The reaction vessel, the outlet tubing and the product vial were disposable and thus replaced with new ones for every run.

L-[methyl-^{11}C]Methionine was prepared by the automated system we developed, resulting in average 25% decay-corrected yield over 20 runs. In this preparation, L-homocysteine thiolactone was [^{11}C]methylated with [^{11}C]CH_3I, which method was more readily applicable to this automated system than the other two methods, [^{11}C]methylation of the sulfide ion obtained from L-S-benzyl homocysteine and sodium/NH_3[12] or from reaction of L-homocysteine with Al_2O_3/KF.[13] Although the [^{11}C]methylation of L-homocysteine thiolactone gave better yield in N,N-dimethylformamide (40% decay-corrected yield) than in acetone, acetone was used as the reaction solvent because N,N-dimethylformamide might elute with the product on HPLC due to its polar characteristics. Synthesis of other ^{11}C labeled radiopharmaceuticals using [^{11}C]CH_3I were also carried out by this system and gave the similar results.

We demonstrated that the automated system described herein is easy and simple to operate and also very reliable. Although this system is very flexible to be modified according to various synthetic procedures of ^{11}C labeled compounds, introduction of a PLC (Programmable Logic Controller) to this system is under investigation to gain a better control of the system.

Conclusions

This study shows the design, construction and application of the automated [^{11}C]methylation system which is very easy and simple to operate. Moreover, this automated system can be widely utilized for the preparation of various ^{11}C labeled radiopharmaceuticals which are obtained from [^{11}C]methylation with [^{11}C]CH_3I.

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