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ORIGINAL ARTICLE

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# The development of a fully automated homemade system for [$^{11}$C] acetate synthesis using an open source PLC

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**ABSTRACT**

Solid phase extraction (SPE) purification method is the efficient and well-known tool for automated [$^{11}$C]acetate synthesis. A fully automated homemade module adopting the SPE method and 'pinch' valves was developed very economically with a universal interface board, a relay card and an open source programmable logic controller. The radiochemical yield of the optimized [$^{11}$C]acetate synthesis by this system was 58.8 ± 2.1% (n=10, decay-corrected) from 15.5 ± 0.19 GBq of [$^{11}$C]$CO_2$ as starting activity, and total synthetic time was 15 minutes. HPLC analysis showed its high radiochemical purity as 97.4 ± 1.1% without possible by-products.
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**Key Word:** [$^{11}$C]acetate, automated synthesis, homemade module, PET imaging

## Introduction

[$^{11}$C]Acetate is one of the well-known PET radiopharmaceuticals in nuclear medicine. It has been widely used for myocardial oxidative metabolism (1), hepatoma (2), prostate cancer (3) as well as brain tumor studies (4). To achieve clinical use, fully automatic [$^{11}$C]acetate synthesis is essential to have stable high radiochemical yields as well as to reduce radiation exposure during preparation.

[$^{11}$C]$CO_2$ bubbling into a Grignard reagent such as methyl magnesium chloride is a conventional way to obtain [$^{11}$C]acetate. Grignard reagent, one of the organometallic chemicals, is powerful for the formation of carbon-carbon bonds. It allows that organic halides add to carbonyl groups such as ketone, aldehyde or ester. But this organometallic chemical leads to a very harsh reaction condition. That is, [$^{11}$C]acetate synthesis looks simple but is accompanied by the harsh reaction condition because of adopting Grignard reagent. For that reason, the efficient purification step must be followed.

There are some of purification methods for the [$^{11}$C] acetate synthesis. In fact, the difference of various synthetic methods for [$^{11}$C]acetate focused on the purification step. Since the half-life of [$^{11}$C]carbon is very short (20.4 min), some factors (e.g. a fast, simple production process, high radiochemical purity, high radiochemical yield) must be considered to get superior results. One of generally used purification methods is distillation (5). Distillation method somewhat takes longer synthetic time and causes impurities with low radiochemical yielding results. Later, a loop method (6) was reported. They made [$^{11}$C]acetate much faster and showed higher yields. However, this

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method is very sensitive to moisture because the inside of the loop has to be coated with Grignard reagent which is extremely moisture sensitive. Thus, anhydrous gas should flow inside the loop up to 30-60 minutes right before synthesis. In 2002, Roeda et al. (7) built a module for [$^{11}$C]acetate based on a solid phase extraction method by Kruijer et al. (8) Corresponding to the loop method, the SPE method provides more stable preparation steps as well as fast, high yielding results. Although they showed high radiochemical yields, their synthetic module was not a fully automatic system. It was not good enough for the routine clinical use. Most recently, Jang et al. (9) reported routine [$^{11}$C]acetate production by self-developed module showing the radiochemical yields and purity as 84.33 ± 8.85% and >98% (n=6, decay-corrected) within 15 min. Meanwhile, some commercial modules for [$^{11}$C]acetate have been industrialized. The representative one is GE TRACERlab FX C series (TRACERlab FX C → FX C Pro → FX2 C). Using them, [$^{11}$C]acetate can be synthesized through bypassing the [$^{11}$C]MeI preparation step to use [$^{11}$C]carbon dioxide directly. A couple of methods using and modifying TRACERlab FX C Pro with SPE purification step have been reported (10, 11). In spite of showing good results, it is inevitable to face damage probability of them. Since the synthesis using organometallic chemicals like Grignard reagent causes piles of solid residues, users must take a risk of the breakdown of the sensitive parts like solenoid valves on the very expensive commercial synthesizers to obtain [$^{11}$C]acetate. Therefore, more simple and economical synthesizer avoiding solenoid valves should fit in with performing the harsh reaction like [$^{11}$C]acetate.

In this study, a simple economical module was developed with the 'pinch' valves for [$^{11}$C]acetate synthesis. It is a fully automated homemade system, and adopts SPE purification method to obtain high radiochemical yields as well as fast, stable production.

## Materials and Methods

### Chemicals and cartridges

3 M methyl magnesium chloride in tetrahydrofuran (THF) was used for Grignard reaction. All solvents and reagents were purchased from Sigma-Aldrich (Seoul, Korea) and used as supplied. [$^{11}$C]$CO_2$ was produced via the $^{14}$N(p,α)$^{11}$C nuclear reaction on a mixture of $N_2$ and 1% $O_2$ from CTI RDS 111 cyclotron (Simens). Disposable ion-exchange cartridges were purchased from GRACE (USA).

### Production of a fully automated homemade system and sequences

Solenoid-operated 'pinch' 3-way, solenoid 2-way and pneumatic valves were positioned on acrylate board as designed in figure 1. All parts were fixed by screws and the vials were connected with silicon tubes for gas/liquid flow. All glass vials and stainless steel needles were disposable. A long needle for [$^{11}$C]$CO_2$ bubbling into Grignard reagent was cleaned up and dried at 120 °C prior to use. To trap the unreacted [$^{11}$C]$CO_2$, all purge lines from the module were connected to an Ascarite II® trap. For the PLC part, general economic interface boards - a K8000 interface board and a K6714-16 relay card (Velleman) were assembled inside the acrylate box. [$^{11}$C]Acetate synthesis sequence as well as a washing protocol to clean up the module after synthesis were programmed using WinPLC, an open source PLC software (http://www.winplc.bvsystems.be/).

### Optimization of reaction condition for the automated [$^{11}$C]acetate synthesis

The optimization tests for automated [$^{11}$C]acetate synthesis were performed to operate our system effectively. Briefly, 10 mL of 1 mM acetic acid, 10 mL

of aq. $NaHCO_3$, 20 mL of distilled water and 2.2 mL of 2 N HCl were added to each disposable vial and placed them on the module. All ion-exchange cartridges (IC-H; cation exchange resin, IC-Ag; $Ag^+$ containing material, and IC-OH; anion exchange resin) were slowly rinsed with 10 mL of distilled water, and also placed on the module. Each 0.1, 0.2, 0.3 or 0.4 mL of 3 M $CH_3MgCl$ was added into 1 mL of THF in a BD Vacutainer® (10 mL, 16 x 100 mm) right before the synthesis and also placed on the system.

After the delivery of starting activity (15.2 ± 0.08 GBq, n=12) from the cyclotron, $[^{11}C]CO_2$ was bubbled into each concentration of Grignard solution in vacutainer at room temperature for 2 minutes with the flow rate of 6 mL/min. To terminate methylation, the reaction mixture was quenched by 1 mM acetic acid, followed by sucking up the mixture and passing it through IC-H, IC-Ag and IC-OH by vacuum. Trapped [$^{11}$C]acetate on IC-OH was washed by 20 mL of distilled water, and eluted with aq. $NaHCO_3$ into the collection vial. The eluted solution added to 2 N HCl in the collection vial was bubbled by $N_2$ gas (99.999%) with vacuum for 2 minutes to remove residual by-product [$^{11}$C]carbonate. Finally, the purified [$^{11}$C]acetate solution passed through a sterile 0.22 μm filter and collected in a 25 mL product vial.

### Automated synthesis of [$^{11}$C]acetate with the homemade module

The starting activity was 15.5 ± 0.19 GBq of $[^{11}C]CO_2$ (n=10), and the optimized concentration of Grignard solution, 0.3 mL of 3 M $CH_3MgCl$ in 1 mL of THF (0.9 mmole) was used for the automated synthesis. The rest of experimental condition was identical with the prior optimizing reaction condition tests.

### Quality control

[$^{11}$C]Acetate was analyzed by a Summit HPLC system (Dionex) with Gabi gamma radioactive detector. Aminex-10 HPX-87H (BIO-RAD) column was used. The mobile phase was 0.008 N $H_2SO_4$ and flowed as 0.6 mL/min under 229 nm of UV and radioactive detectors. Residual organic solvents in the final product were analyzed using gas chromatography (GC). Fused silica capillary column, supelcowax-10 (60 m×0.25 mm × 0.25 μm film thickness, Sigma-Aldrich, USA) was used for GC in the operating temperatures of oven, injector and detector as 75, 250 and 250 °C, respectively. Pyrogen test was carried out using a LAL test kit.

## Results and Discussion

The basic concept of Kruijer et al. (8) was adopted to develop the synthesizer. To reduce production cost, general economic interface boards, a K8000 interface board and a K6714-16 relay card (Velleman) was used. Also an open source freeware, WinPLC was used as programmable logic controller (PLC). On the system body (acrylate board), a general vacuum pump and two pneumatic valves for syringe pumps were used to transfer chemical reagents from site to site. Solenoid-operated 'pinch' 3-way valves chosen for liquid flow. 'Pinch' valves were applied to protect themselves from harsh chemical wastes occurred by Grignard reaction, while solenoid 2-way valves were used only for gas flow. A disposable vacutainer was chosen as reactor to avoid moisture. This automated system is described in figure 1.

A sequence was programed with WinPLC and applied to the developed system. The [$^{11}$C]acetate synthesis was proceeded as figure 2. First, the optimal condition of [$^{11}$C]acetate preparation was searched, and the test with 0.3 mL of 3 M $CH_3MgCl$ plus 1 mL of THF (0.9 mmole of $CH_3MgCl$) showed the best result (Table 1). The lower concentration (0.1 and 0.2 mL of 3 M $CH_3MgCl$ plus 1 mL of THF) showed low labeling yields, whereas the higher concentration (0.4 mL of 3 M $CH_3MgCl$ plus 1 mL of THF) led to cause lots of residual solid wastes

blocking the disposable ion-exchange cartridges such as IC-H, IC-Ag and IC-OH. Bubbling time of [$^{11}$C]$CO_2$ in Grignard reagent was 2 minutes at room temperature. Whether [$^{11}$C]CO2 bubbling was proceeded more than 2 minutes or reaction temperature was lower than room temperature, the labeling yields decreased (data not shown). The lower yielding products contained the known possible by-products such as [$^{11}$C]*t*-butanol, [$^{11}$C] acetone and [$^{11}$C]carbonate (7).

Since residual [$^{11}$C]carbonate was commonly accompanied with [$^{11}$C]acetate preparation, it must have been removed. Kruijer et al. (8) suggested that the final buffer eluting an anion-exchange column to obtain [$^{11}$C]acetate be citrate buffer with air bubbling. Since citrate buffer is slightly acidic (pH=4.7), residual [$^{11}$C]carbonate in acidic condition can be removed by simple air bubbling in the last step. However, the intravenous injection of acidic solution like citrate buffer could make patients painful, $NaHCO_3$ was adopted as eluting buffer complemented by 2 N HCl with a $N_2$ bubbling step. This change led the removal of [$^{11}$C]carbonate residue along with mild pH (pH=7-8) of the final [$^{11}$C]acetate solution for the comfortable i.v. injection to patients. Consequently, the [$^{11}$C]carbonate residue was removed and HPLC analysis showed high radiochemical purity of [$^{11}$C]acetate (Figure 3). There were no pyrogen nor significant residual THF in the final product.

As a result, radiochemical yields and purity were 58.8 ± 2.1% and 97.4 ± 1.1%, from 15.5 ± 0.19 GBq of [$^{11}$C]$CO_2$ as starting activity respectively (n=10, decay-corrected). Also, total synthesis time was fast as 15 minutes. This fully automated homemade system was operated well and occurred no error for clinically routine use. Accordingly,

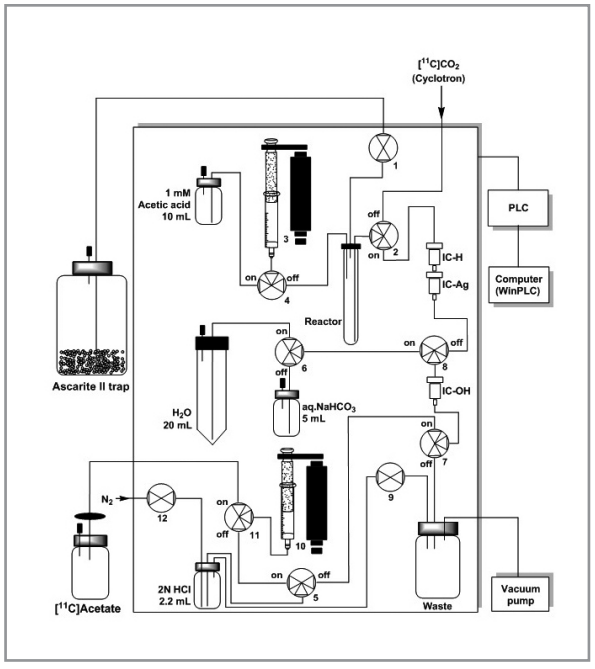


**Figure 1.** Description of the homemade system for [$^{11}$C]acetate synthesis

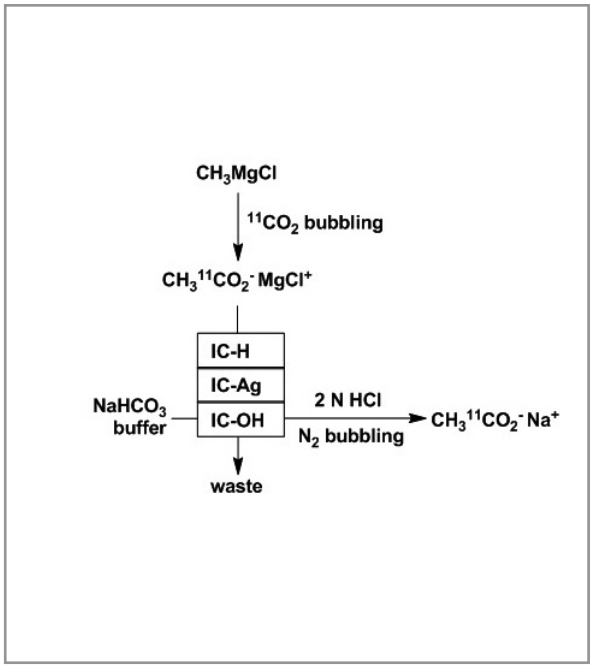


**Figure 2.** The synthetic outline of [$^{11}$C]acetate synthesis

**Table 1.** Optimization of automated [$^{11}$C]acetate synthesis. Each volume of 3 M $CH_3MgCl$ was dissolved in 1 mL of THF and 15.2 ± 0.08 GBq (n=12) of [$^{11}$C]$CO_2$ was used as starting activity. [$^{11}$C]$CO_2$ bubbling was proceeded at room temperature for 2 minutes (n=3 each condition).

| Effect of concentration on automated [$^{11}$C]acetate synthesis (r.t., n=3) | | | | |
|---|---|---|---|---|
| 3 M $CH_3MgCl$ (mL) | 0.1 | 0.1 | 0.1 | 0.1 |
| [$^{11}$C]Acetate (%, decay-corrected) | < 0.84 | 11.2 ± 1.1 | 57.1 ± 5.3 | - |

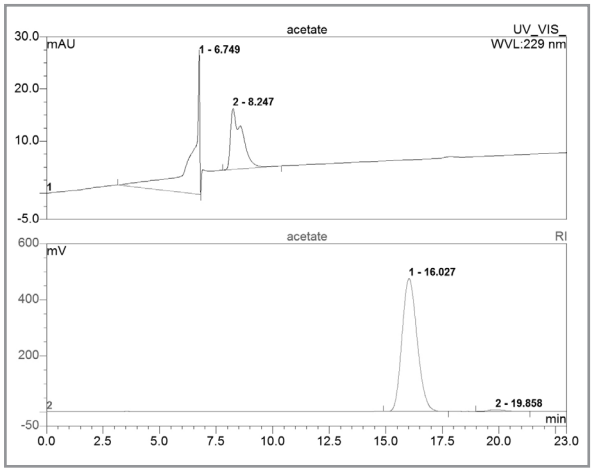


**Figure 3.** HPLC chromatogram of [$^{11}$C]acetate. Aminex-10 HPX-87H (BIO-RAD) column was used, and the mobile phase was 0.008 N $H_2SO_4$ by 0.6 mL/min of flow rate under 229 nm of UV and radioactive detectors. Retention time of 16.0 min is [$^{11}$C]acetate and a minor peak at 19.9 min is residual [$^{11}$C]carbonate. Radiochemical purity was 97.4 ± 1.1% (n=10).

this system have been successfully conducted, and some clinical works were reported (12, 13).

## Conclusion

A fully automated homemade synthesizer was developed for [$^{11}$C]acetate using an open source WinPLC software and two general economic interface boards with 'pinch' valves. This synthesizer performed fast synthesis by SPE method and showed high radiochemical yields with high radiochemical purity for routine clinical use.

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