REMOTE SYSTEM FOR PRODUCTION OF CARBON-11 LABELED PALMITIC ACID

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ABSTRACT

Carbon-11-labeled palmitic acid is produced routinely at Washington University School of Medicine for studies of myocardial metabolism. To accomodate the needs of 8 - 15 preparations per week and the large amounts of radioactivity (>20 mCi/study) required, a remotely-controlled system for the preparation of [1-^{11}C]palmitic acid has been constructed. The system is small (0.04 m^3), completely portable and contained totally within a small lead-shielded hood. Large quantities of C-11 palmitic acid (up to 675 mCi) have been prepared, with minimum radiation exposure to the operator. This system has been in full operation for two years, and has been used for more than 200 syntheses with a failure rate of less than 5%.

INTRODUCTION

The Cardiovascular Division of the Washington University School of Medicine requires multiple preparations per week of [1-^{11}C]palmitic acid as a diagnostic agent for myocardial metabolism in patients (1) and animal models (2). Although [1-^{11}C]palmitic acid is easily synthesized by the reaction of carbon-11 carbon dioxide with pentadecyl magnesium bromide, the actual preparation for in vivo studies entails several additional steps of isolation, binding of the labeled fatty acid to human serum albumin, and sterilization via Millipore filtration. The preparation of ^{11}C-palmitic acid has for many years been done manually (3), but the radiation dose to the chemist is appreciable. Thus, the need for repetitive preparations of large amounts of [1-^{11}C]palmitic acid has prompted the development of a remote system for synthesis of this radiopharmaceutical. Previous descriptions of "remote" palmitic acid systems are either incomplete (4), neglect to describe the shielding necessary (4,5), or are unnecessarily complex and involving a multiple vessel synthesis (5). We describe here the construction and operation of a totally remote system, utilizing a single reaction vessel,

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which is capable of delivering large amounts of sterile, pyrogen-free and radiochemically pure [1-^{11}C]palmitic acid, with minimum radiation exposure to the operator.

EXPERIMENTAL

Remote System: Construction. The remote system is housed totally within a shielded hood 102 cm wide, 67 cm deep and 67 cm high (Fig. 1). Sides, front, and top are constructed of 5 cm thick lead; the front has a lead glass window 30.3 cm wide, 45.5 cm long and 10 cm thick. Access into the hood is afforded by two sliding doors in the rear (each 1.26 cm thick lead) and 15 cm X 15 cm openings (with 5 cm thick lead doors) in each side. Permanently installed in the hood is a stainless steel radiator trap for $^{11}CO_2$ collection; the trap is

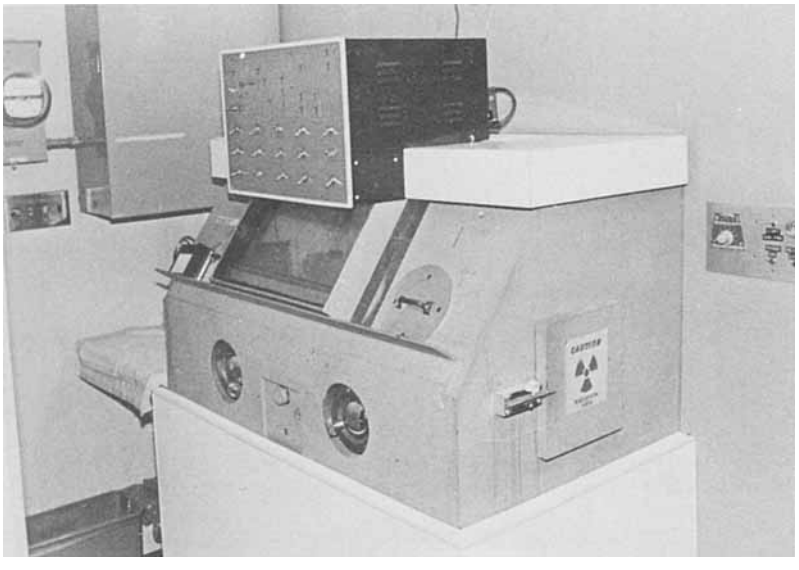

Figure 1: Shielded hood with control panel for the synthesis of [1-^{11}C]palmitic acid.

cooled using a dewar filled with liquid nitrogen (dewar raised or lowered by a pneumatically powered platform). The trap is heated by resistance.

The actual synthetic equipment is all portable and easily moved in and out of the hood. The letters used in the following description refer to the schematic shown in Figure 2: the actual apparatus is shown in Figure 3. The reaction vessel (B; 13.5 cm long X 3.5 cm wide) is equipped with two side-arms and the lower section is in the shape of a standard test tube, with the 1 ml volume point clearly marked. Agitation of the vessel is done using a standard laboratory vortex mixer (C). Through one side-arm of the vessel (B) is inserted a 1 mm ID teflon tube (L1), through which reagents are added from outside the hood. Through the second side-arm is inserted a 1 mm ID teflon tube (L2) which extends to the bottom of vessel B. (Teflon tubing is used in conjunction with the reaction vessel, as the disposable polyethylene tubing used elsewhere cannot be used with diethyl ether). The other end of this teflon tube enters a teflon block (D) (4 x 5.5 cm, carefully bored as indicated in Fig. 2), which is the common path for connecting the reaction

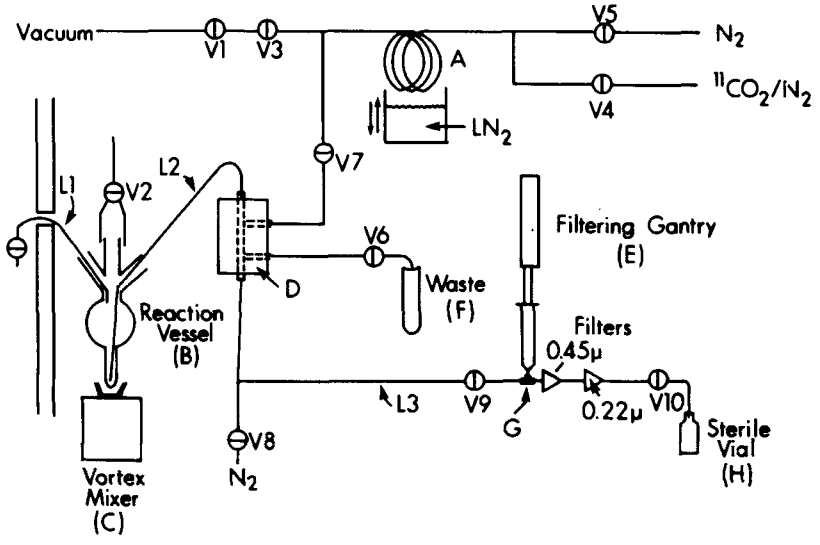

Figure 2: Schematic representation of reaction vessel, valves and filtering gantry used for synthesis of [1-^{11}C]palmitic acid.

vessel (B) to the waste receptable (F) and the filtering gantry (E).

The filtering of the final product solution through 0.45 and 0.22 micron Millipore filters is done using a gantry (E) consisting of two air cylinders (E-1, E-2) (capable of 40-45 psi pressure) attached to the plungers of 12 ml disposable plastic syringes, and two air driven pinch valves (V9, V10). Sterile disposable polyethylene tubing (L3) and three-way valve (G) are used in the fluid path from the teflon block (D) through the filtering gantry. The final filtered solution is finally delivered into a sterile vial (H). The dimensions of this filtering gantry are 24 cm wide, 40.5 cm high and 20 cm deep. The entire synthetic apparatus (vessel, mixer and filtering gantry) occupy a volume of approximately 0.04 cubic meters (1.5 cubic feet).

The switches controlling all remote operations (mixer, valves, pneumatic

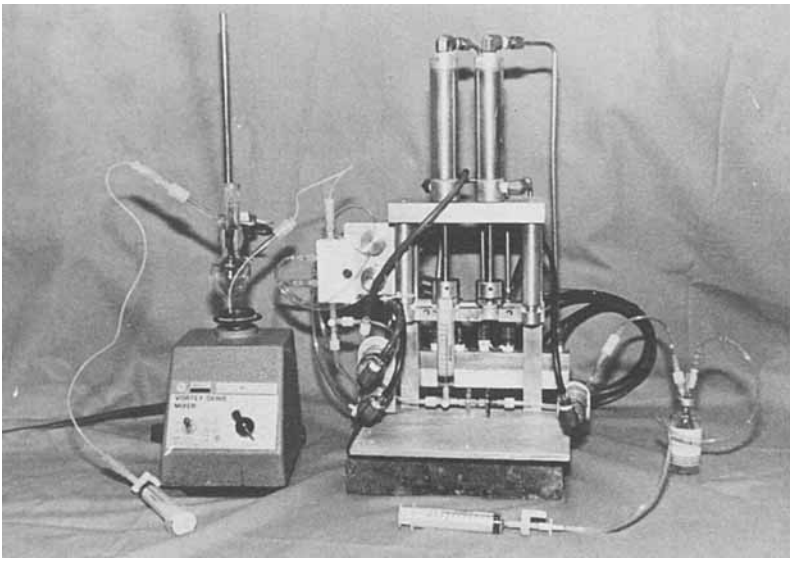

Figure 3: Reaction vessel, vortex mixer and filtering gantry system used for the totally remote synthesis of [1-^{11}C]palmitic acid.

pistons) are mounted on a single control panel at the top of the hood, and the function of each clearly marked as shown in Figure 3.

Production of $^{11}CO_2$. The $^{11}CO_2$ is produced via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by proton bombardment (15 MeV, 30 μA) of a 2 atm gas target (2% O_2/98% N_2) using the Washington University Medical School Cyclotron Corp. CS-15 cyclotron. Irradiations of 10-30 minutes give yields of 400-1200 mCi of $^{11}CO_2$ at the end of bombardment.

Synthesis of [1-^{11}C]Palmitic Acid. At the end of bombardment, the $^{11}CO_2$ is collected by opening valve 4 and evacuation of the target gas through the cooled radiator trap (A) (valves 1 and 3 open, all other valves closed). When the target has been emptied, valves 1 and 3 are closed, the trap pressurized to 16 psi with helium (flow through target), then valve 4 closed.

A slow flow of nitrogen (3 ml/min) through the trap and reaction vessel is established by opening valves 5,7 and 2 (vent). Then 3 ml of 0.1 M pentadecyl magnesium bromide (3) in diethyl ether is introduced into the vessel B via line L1. The dewar is lowered, the radiator trap (A) warmed and the $^{11}CO_2/N_2$ bubbled through the Grignard reagent solution for 3-5 min. The nitrogen flow is then stopped by closing valve 7.

Excess Grignard reagent is destroyed by addition (via line L1) of 3 ml of 1 N hydrochloric acid. The vessel is shaken using the vortex mixer (C), and the layers allowed to separate. The lower aqueous layer is drawn off by closing valve 2 (vent), opening valve 7 briefly to slightly pressurize the vessel, then opening valve 6; the water is pushed through the teflon line (L2) and teflon block (D) and into the waste receptacle (F). The meniscus is observed visually. The remaining ether layer is washed twice with 2 ml of 0.9% saline solution, and each time the lower aqueous layer discarded into the waste receptacle (F). Valve 6 is then closed.

The ether solution is then diluted with 1 ml of 95% ethyl alcohol (added via line L1). The diethyl ether is evaporated by establishing a 40 ml/min flow of nitrogen through the solution, done by opening valves 8 and 2.

Evaporation is continued until only 1 ml of liquid is left, then valve 8 closed.

The ethyl alcohol solution is then diluted with 8.2 ml of a 3.5% solution of human serum albumin in 0.9% saline (kept ready at 40-42° C during the synthesis), mixed well using the vortex mixer, then left to stand for 2 min to effect binding of the fatty acid to the albumin.

As the final step, the albumin-fatty acid solution is Millipore filtered. The vessel is slightly pressurized (close valve 2, open valve 8 briefly), then the solution pulled into a 12 ml syringe by opening valve 9 and raising the piston of the air cylinder (E1). Valve 9 is closed, valve 10 opened, and the syringe barrel pushed down, forcing the solution through 0.45 and 0.22 micron Millipore filters and into the sterile vial (H). The gantry (E) was originally constructed with separate syringes and pistons for each Millipore filter, but we have since found this unnecessary.

Quality Control. The preparations of [1-^{11}C]palmitic acid have been routinely analyzed for radiochemical and chemical purity, sterility and apyrogenicity. Radiochemical and chemical purity are determined on random preparation of [1-^{11}C]palmitic acid, using HPLC (Spectra-Physics Model 3700) and the following column and conditions: Waters fatty acid analysis column, 10 μ, 3.9 mm ID x 30 cm long; mobile phase THF: CH_3CN: H_2O 35:30:40 (v/v), 2 ml/min, refractive index and NaI (Tl) detectors. On every tenth batch apyrogenicity is determined using standard U.S.P. XX procedures, and sterility by checking microbial growth under both aerobic and anaerobic conditions.

RESULTS AND DISCUSSION

Carbon-11-labeled palmitic acid is prepared by the carboxylation (with $^{11}CO_2$) of pentadecyl magnesium bromide. The overall synthesis involves the chemical reaction, isolation and purification, solubilization by binding to human serum albumin and filtration.

Use of the remote system described herein allows for the preparation of large amounts of [1-^{11}C]palmitic acid; our maximum yield has been 675 mCi at one time. This system has been used for more than 200 preparations of [1-^{11}C]palmitic acid in the past two years, with a failure rate of less than 5%. The average radiochemical yield (corrected for decay) has been 65%, with the radiochemical purity consistently >99%. Synthesis times are 10-12 minutes from the end of bombardment, and the specific activity of the final product is 700-1500 Ci/mmol (determined by HPLC).

There are several important features of this shielded synthesis hood and portable synthesis system. Construction is simple, consisting of readily available equipment (vortex mixer, disposable tubing and syringes) or easily manufactured (teflon block, glass reaction vessel, filtering gantry). Although the pinch valves used here were home-built, similar small pinch valves are commercially available.* The entire apparatus can be easily and quickly removed from the hot box; as we have had two identical systems constructed, this allows for rapid repetitive synthesis, limited only by the time needed for decay of residual activity in the vessel, lines and filters: the distribution of radioactivity within the hood is shown in Table 1. The entire system can be assembled in less than 10 minutes, making possible [1-^{11}C]palmitic acid preparations for emergency clinical situations. Finally, the shielded remote system accomplishes the most necessary function of reducing the radiation exposure to the operator; exposure per preparation is less than 1 millirem. The hood used in this work is much larger than needed: a small (approx. 30 cm x 30 cm x 50 cm) hood would suffice, reducing shielding costs and space requirements.

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Table 1. Distribution of Carbon-11 Activity Within Shielded Synthesis Hood After [1-^{11}C]Palmitic Acid Synthesis

Location	%
Vessel + L2	13.5 ± 2.8
Waste	1.45 ± 0.7
Teflon block + L3	4.8 ± 0.3
Filters	14.9 ± 3.1
Product Solution	65.5 ± 0.8

Although designed for ^{11}C-palmitic acid synthesis, the hood and remote control functions (electrical outlets, valves and gantry arrangement) are easily adapted to the preparation of other carbon-11 compounds: we have used it in syntheses of [1-^{11}C]pyruvic acid (6) and a variety of carbon-11 labeled alcohols and ethers (7).

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