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October 5, 1998

Jane Axelrad
Food and Drug Administration
5600 Fishers Lane
Mail Code HFD5
Rockville, MD 20857

Dear Ms Axelrad:

Enclosed you will find examples of SOPs describing the synthesis and quality control operations for [F-18]fluorodeoxyglucose, [F-18] sodium fluoride, 6-[F-18]fluoro-L-DOPA, [O-15]water and [N-13]ammonia, five of the PET radiopharmaceuticals in current clinical use. The information originates in two institutions, but is typical of the procedures now in place for their preparation at different sites. Please let me know if you have any questions on these.

Also, on a related issue, and as a follow-up of our recent telephone conversation, please let me know how you would like to proceed with the USP monographs.

Sincerely

Jorge R. Barrio

Encls.

JRB/iaa

98D-0372

C 3

Procedure: **Preparation of Fludeoxyglucose F-18 for Injection, USP**

I. OVERVIEW

This procedure details the preparation of [^{18}F]FDG, using the GE PETtrace FDG MicroLab®. The [^{18}O]water target is bombarded with protons in the cyclotron. The resulting [^{18}F]water is shunted, via 0.030" PEEK (polyetheretherketone) tubing, to the MicroLab® where the actual synthesis process occurs. The resulting [^{18}F]FDG is collected in a sterile vial, checked for quality control, then delivered to the PET facility for patient use.

II. EQUIPMENT

1. CS-15 or JSW-168BC cyclotron
2. [^{18}O] water
3. GE PETtrace FDG MicroLab®, (Model # P5005AG)
4. GE cassette, pre-packaged with 6 Eppendorf syringes (5 mL) and 4 reagent vials
5. Acetonitrile, 99+%
6. Mannose triflate (1,3,4,6-tetra-O-acetyl-2-O-trifluoromethane sulfonyl manno-pyranose), 99+%, kept frozen and dry when not in use
7. Sterile 100% ethyl alcohol (single use vial)
8. Sterile Water for Injection (SWI), USP (single use vial)
9. Sterile hydrochloric acid, 1.0 N and 0.4 N concentrations
10. Sterile sodium phosphate buffer solution (134 mM NaOH / 180 mM Na_2HPO_4) in a single use vial, kept refrigerated when not in use

11. Sterile 8.4% sodium bicarbonate solution, USP

12. Sterile, pyrogen-free 18 and 21 gauge needles (1.5 inch)

13. Sterile, pyrogen-free 1, 3, 6 and 12 mL syringes

14. Gas tight glass Hamilton syringe, 25 µL

15. Millipore Sep-Pak™ C-18 Plus extraction cartridge

16. Millipore Sep-Pak™ Alum-N Plus extraction cartridge

17. Millex-GS™ 0.22 µm sterile, pyrogen-free, vented and non-vented filters (25 mm diameter)

18. Millex-GV4™ 0.22 µm sterile, pyrogen-free, non-vented filter (4 mm diameter)

19. 70% Isopropyl alcohol pads

20. Sterile 20-24 inch collection tubing

21. Sterile, "Y" shaped flush tubing

22. Customized remote injection device, with tubing and 3-way stopcock

23. Sterile, 10 mL and 30 mL glass vials

24. Millex®/Sterivex™ Integrity Tester (Millipore SLTE ST0 00)

25. Milli-Q water

III. PROCEDURE

A. Pre-Run Checks

1. Turn on the light located in the top right corner of the MicroLab® hot cell.

2. Check that the valve to the compressed nitrogen tank is open, and the pressure regulator is reading 70-80 psi.

3. Check that the printer is on line, and has an adequate paper supply.

4. Press the TEST button on the Operator Console, located behind the hot cell. The Operator Console should read "Ready for New Cassette".

5. Remove the old cassette, if any, from the MicroLab®.

   a) Remove and discard the needle/filter assemblies from the outlet line beneath the hot cell.
   b) Pull out the old cassette, including the outlet line which has been fed through the opening in the bottom of the hot cell.
   c) Push in the front block of the MicroLab® until it moves (it will then pop back out).

B. Preparation of the [^{18}O] Water Collection Vial

1. Replace the old collection vial whenever a new lot of [^{18}O] water is used for [^{18}F] production. The material can be distilled and reused at a later time. (If you are using the same lot skip to section C).

2. Disconnect the outlet line, on the left rear side of the MicroLab®, from the [^{18}O] water collection vial. Give the old collection vial to the cyclotron operator for storage.

3. Label a new sterile, 30 mL empty vial with the following information:

   a) Lot # of the current batch of [^{18}O] water
   b) Manufacturer's name
   c) Current date

4. Insert a sterile 18 gauge needle through the septum of the vial.

5. Attach another sterile 18 gauge needle to the [^{18}O] water outlet line from the GE MicroLab®; insert this needle into the collection vial.

6. Place the collection vial into a small lead vial shield and push it into a corner of the hot cell.

C. Activation of the Solid-Phase Extraction Cartridges

1. Activate a Sep-Pak™ C18 Plus cartridge by passing 10 mL of 100% ethyl alcohol through the cartridge using a sterile, disposable syringe.

2. Next, pass 11 mL of SWI through the C18 Plus cartridge.

3. Firmly attach the top (female luer) of the activated C18 Plus cartridge to the bottom (male luer) of the Sep-Pak™ Alum-N Plus cartridge.

4. Firmly attach the bottom of the C18 Plus cartridge to the female luer of the 20 inch extension tubing.

D. Filling the Reagent Vials

1. Remove the four reagent vials from the GE package.

2. The following reagents are prepared by S. Schwarz, R.Ph. Full preparation details can be found in the Reagent Formulary binder.

   a) Sodium phosphate buffer solution
   b) 100% ethyl alcohol
   c) HCl, 1.0 N and 0.4 N concentrations

3. Fill the vials, in the following order, to the inscribed fill lines with:

   a) Vial 4: $\geq$ 2.5 mL of 1 N HCl
   b) Vial 3: $\geq$ 11 mL of the sodium phosphate buffer solution
   c) Vial 2: $\geq$ 6 mL of acetonitrile
   d) Vial 1: 30 $\pm$ 5 mg of mannose triflate

   Note: Triflate supplied by RBI comes in a ready to use pre-measured vial which can be snapped directly into place on the cassette.

4. Using a sterile, pyrogen-free needle and syringe, aseptically withdraw the remaining SWI from the vial. Discard all but 9 mL, which is returned to the SWI vial.

5. Add 1 mL of the 8.4% sodium bicarbonate solution to the remaining 9 mL of sterile water in the SWI vial. Shake the vial to mix.

E. Installation of the GE Cassette

1. Confirm that the "Ready for New Cassette" light is visible on the Operator Console.

2. Remove the cassette from its foil package and inspect for any visible

signs of damage. (There should be several methanol pads in the foil package along with the cassette).

3. Attach Vials 1-4 to the appropriately numbered positions on the cassette.

4. Attach the top (female luer) of the Alum-N Plus extraction cartridge to Position #5 on the right side of the cassette.

5. Attach the six Eppendorf syringes to the six positions on the top of the cassette. Make sure they are firmly attached.

6. Slide the cassette into the MicroLab®, pulling it forward until it is seated.

7. Thread the collection tubing through the opening in the bottom of the hot cell, checking that the line is not twisted or pinched.

8. Simultaneously press in both buttons located on each side near the top of the MicroLab®, until the block snaps shut.

9. Check that each syringe is straight and well seated in the box by pushing on each one firmly.

10. Use the Hamilton syringe to inject 10 µL of the diluted sodium bicarbonate solution from the SWI vial into the target water reservoir on top of the cassette (between syringes #2 and #3). Do not push the needle below the neck of the reservoir into the narrow tubing.

11. Put a sterile, pyrogen-free 21 gauge needle on the target inlet PEEK tubing line. Bend the needle slightly, ~45-60° angle, and insert in the target water reservoir.

12. Close and lock the hot cell door.

F. Preparation of the [^{18}F]FDG Collection Vial

1. Obtain two pre-printed sample labels (see example below), which will be filled in with the following information:

   a) Lot #
   b) Time and date of assay
   c) Assay (mCi/mL)
   d) Total product volume delivered for patient use (mL)

e) Expiration time and date (6 hours after assay)
f) MicroLab® operator's initials

CAUTION
RADIOACTIVE MATERIAL

Cyclotron Facility
Fludeoxyglucose F-18 Injection, USP

Lot:_____
Time:_____ Date:_____
Assay:_____ mCi/mL
Volume:_____ mL
Expiration:_____

2. Label a sterile, 10 mL collection vial with the date/lot #, and preparer's initials.

3. Wipe the septum of the collection vial with an isopropyl alcohol pad.

4. Aseptically add 0.4-0.5 mL of 0.4 N HCl to the vial by using a sterile, pyrogen-free syringe and a needle attached to a 0.22 µm unvented (4 mm diameter) filter.

5. Place the collection vial into a lead vial shield. Put the top of the shield above the vial and screw the nuts on tightly.

6. Replace the blue absorbent pad in the lead drawer located below the hot cell. Put the lead shielded collection vial in the drawer.

7. Swab the collection vial, and the top of the lead shield, with an isopropyl alcohol pad.

8. Aseptically attach a sterile, 0.22 µm vented filter to a capped 18 gauge sterile, pyrogen-free needle. Prepare another needle with a 0.22 µm non-vented (25 mm diameter) filter.

9. Aseptically insert the vented filter/needle assembly into the vial. The needle tip must be above the future liquid phase in the vial.

10. Aseptically insert the non-vented filter/needle assembly into the vial. The second needle tip must also be above the future liquid phase in the vial.

11. Aseptically attach the outlet line from the extraction cartridges, at position #5, to the vented filter/needle assembly.

12. Place the lead shielded collection vial as close as possible to the hole in the bottom of the hot cell, without placing excessive stress on the

collection line.

13. Close the drawer containing the lead shielded collection vial.

G. Direct Injection of [^{18}F] Activity into the MicroLab®

1. Check the Operator Console and confirm that the MicroLab® "Ready for Target Water" light is on. This should take ~6 minutes from the time the cassette is installed.

2. Notify the cyclotron operator that the GE cassette is ready for target elution.

3. When ready, the activity will be automatically shunted, via PEEK tubing, from the cyclotron target to the MicroLab®.

H. Manual Injection of [^{18}F] Activity into the MicroLab®

1. If some of the [^{18}F]water from the cyclotron target is being diverted to another project, the remaining activity must be injected into the MicroLab®, usually by the cyclotron operator, using the remote injection device.

2. Collect the activity for FDG in a sterile vial, placed inside the specially marked lead shield. Carry the activity to the MicroLab®.

3. Attach the customized remote injection device, with 3-way valve, to the top of the lead shielded vial. (The air and water lines, with syringes and stopcock, are kept in the hot cell).

4. Attach a sterile 21 gauge needle to the outlet tubing. Bend the needle slightly, then place it in the target water reservoir on top of the cassette (between syringes #2 and #3). Do not push the needle beyond the neck of the reservoir.

5. Close and lock the door of the hot cell. Check that the air and water lines hang unhindered outside of the hot cell.

6. Transfer the activity from the vial to the target water reservoir using the 3-way valve attached to the remote device. (Water pressure is used to remotely control the device).

a) Take the syringe filled with deionized water and push in the plunger until you see the valve on the remote device switch positions.
b) Turn the 3-way stopcock on the water line, shutting off the flow from the water syringe to the device. This locks the remote valve in the transfer position.
c) Withdraw the target water from the vial by pulling back on the plunger of the air syringe.
d) Watch the target water fill the 1 cc syringe attached to the device inside the hot cell. (Do not pull too quickly or the activity may be pulled right out of the hot cell).
e) When the desired amount of target water has been withdrawn, turn the 3-way stopcock back to the water syringe position. (Internal pressure will switch the valve on the device in the hot cell).
f) Using the air syringe, push the target water out of the internal syringe and into the target water reservoir.
g) To capture all of the activity, refill the air syringe then push more air through the device.
h) Repeat the process until the desired amount of activity has been transferred into the target water reservoir.

I. Preparation for 3 or More Patients

1. To produce enough activity for $\geq$3 patients, (or for late afternoon delivery), it will be necessary to flush the extraction cartridges and collection tubing with Sterile Water for Injection (SWI).

2. Connect the shortest leg of the "Y" tubing to Position #5 on the right side of the cassette.

3. Connect the leg with the short tubing to the top (female luer outlet) of the Alum-N Plus extraction cartridge.

4. Connect the leg with the long tubing to a sterile, pyrogen-free syringe.

5. Close and lock the hot cell door, allowing the long tubing and syringe to hang unhindered outside the hot cell.

6. Close off the tubing, using the attached blue pinch clip, as it exits the hot cell.

7. At the end of the synthesis process, when all of the [^{18}F]FDG has entered the collection vial, unpinch the long tubing using the blue clip, then push

air through the "Y" assembly with the attached syringe.

8. Next, fill the syringe with 1-1.5 mL SWI, and push this through the "Y" assembly. This will flush any residual [^{18}F]FDG from the extraction cartridges and add it to the collection vial.

J. Initiation of Radiopharmaceutical Synthesis

1. Check the Operator Console and confirm that the MicroLab® "Ready for Process" light is on. This will appear if at least 10 mCi of activity has entered the MicroLab®.

2. Wait for direct delivery to be completed, then press the ACTIVATE key on the Operator Console.

3. If the "Ready for Process" light did not come on, despite the delivery of a sufficient level of radioactivity, synthesis can be manually initiated:

   a) Simultaneously press the ACTIVATE and TEST buttons.
   b) Press the ACTIVATE button a second time.

4. The automatic synthesis process takes 50 minutes. During this time the MicroLab® operator should:

   a) Fill out the batch record and BET/Sterility Request Form for this sample (see attached examples).
   b) Attach a completed, pre-printed label to the first page of the batch record.
   c) Clean the preparation area for future use.

IV. PRE-RELEASE TESTING

A. Measuring Product Concentration and Volume

1. Disconnect the needles from the septum of the sample vial.

2. Remove the shielded collection vial from the lead drawer.

3. Carry the lead shielded collection vial to the dose calibrator. Unbolt and remove the top of the vial shield.

4. Using tongs, remove the sample vial from the lead shield.

5. Place the vial into the well of the dose calibrator. Record the time and the activity on the batch record, under end of synthesis or EOS activity.

6. Using tongs, replace the vial in the lead shield and move to the QC drawing bench area.

7. Place the shielded vial in the rotating holder, locking it in place.

8. Label a sterile, pyrogen-free, 3 mL syringe with the date/lot # and [^{18}F]FDG preparer's initials:

9. Rotate the shielded vial holder until the septum of the vial is facing downwards.

10. Using a plexiglass syringe shield, withdraw 0.8-1 mL of sample from the vial using the labeled QC syringe.

11. Measure the activity level in the syringe using the dose calibrator.

12. The product concentration, or assay, is recorded in mCi/mL.

13. The volume of product being delivered to the PET facility is calculated by dividing the total EOS activity in the vial (mCi) by the concentration (mCi/mL).

B. Determining Product pH, Color and Appearance

1. Before sending the QC sample syringe for TLC analysis, place one drop of sample on a pH indicator strip. Record the pH on the batch record.

2. Look carefully at the sample while it is in the syringe or vial. Record the color and appearance of the sample on the batch record. The acceptance criteria for patient administration is:

a) pH:	from 4.5 - 8.5
b) Solution clarity:	clear
c) Solution color:	from colorless to dark yellow

C. Sending the Sample for TLC Analysis

1. Remove the needle from the 3 mL QC syringe and replace with a sterile, pyrogen-free cap.

2. Place the syringe in the pneumatic line sender, taping it into the sender with masking tape.

3. Notify the individual performing the Quality Control that the sample is ready for testing.

4. Send the syringe to the QC laboratory via pneumatic line.

5. Confirm that the sample was received.

D. TLC Analysis

1. The radiochemical purity must be > 90%. (See the Quality Control Procedure for full details).

2. The QC operator will notify the MicroLab® operator whether the radiopharmaceutical is acceptable or unacceptable for patient use.

E. Bubble Point Test

1. While the MicroLab® operator is waiting for QC results, the integrity of the vented 0.22 µm (25 mm) sterilizing filter's membrane can be tested using the Integrity Tester incorporated into the hot cell.

2. Remove the needle and collection tubing from the sterilizing filter in the shielded drawer beneath the hot cell.

3. Flush the filter with 5-10 mL of Milli-Q water.

4. Place the filter firmly into the bracket mounted in the back of the shielded drawer. Check that the outlet tubing is attached to the bottom of the bracket.

5. Attach the compressed air (or nitrogen) tubing to the filter's inlet.

6. Look through the glass window in the hot cell door until you can see the

transparent, water-filled test tube and outlet tubing.

7. Gradually increase the gas pressure until you can see a stream of bubbles coming out of the outlet tube. The minimum acceptable Bubble Point pressure for this type of filter is **50 psi**.

8. Indicate on the batch record whether the filter has passed/failed the test.

V. PRODUCT RELEASE

Under normal circumstances the QC operator will notify the MicroLab® operator that the batch is acceptable for patient use. The MicroLab® operator will take the shielded vial (and batch label) to the PET Facility, where Nuclear Medicine personnel will withdraw and administer the patient doses. (The lead vial shield must be returned to the Cyclotron Facility for reuse).

For patients scheduled for PET scanning in the East Building, double the required level of activity is withdrawn from the patient vial, placed in a labeled, 10 mL sterile, pyrogen-free syringe, and sent to the East Building via pneumatic tube. (Note: Use the special pneumatic line sender prepared by the cyclotron operator).

If any abnormal circumstances occur during production immediately notify radiopharmacist They will be responsible for final product release.

VI. POST-RELEASE TESTING

A. BET and Sterility Testing

1. A one hour bacterial endotoxin test is performed on every batch of [^{18}F]FDG released for patient use.

2. Every tenth batch of [^{18}F]FDG is tested for sterility by incubating the sample for 14 days then visually checking for growth. (See the appropriate Quality Control Procedures for full details).

B. Patient Tracking

The identity of every patient (by ID #) who receives a dose from a batch of [^{18}F]FDG is listed on the batch record. If a sample is subsequently found to

be contaminated, the appropriate patient can be identified and contacted to determine adverse reaction to the dose.

VI. SPECIAL HANDLING

A. General Use of the Equipment/Facility

Due to the presence of radioactive material, use of the GE PETtrace FDG MicroLab® should only be done by authorized, trained personnel under the supervision of R.Ph.

B. Personal Protection

1. All personnel must wear lab coats, eye protection, and disposable protective shoe covers over their street shoes, upon entering the cyclotron facility.

2. Disposable latex gloves and sleeve protectors must be worn during radiopharmaceutical chemical preparation.

3. All personnel must wear TLD radiation badges and rings.

4. Before leaving the cyclotron facility, personnel must remove disposable gloves and shoe covers, and check themselves for contamination using the hand/foot survey meter.

5. In case of personal contamination:

   a) Immediately wipe activity from exposed skin.
   b) Wash using soap, water and a soft brush; recheck.
   c) If there is still residual radiation, use Rad-Con foam decontaminant until all radiation has been removed/reduced to a nontransferable level.
   d) Notify the Radiation Safety Officer, at 8425.

6. Contaminated garments should be left in the cyclotron facility to decay to background levels. This requires at least ten half-lives.

7. Upon returning to the department, all personnel must check themselves for contamination by using the hand/foot monitor in Room 4424.

CAUTION
RADIOACTIVE MATERIAL

Cyclotron Facility
Fludeoxyglucose F-18 Injection, USP

Lot: _____
Time: _____ Date: _____
Assay: _____ mCi/mL
Volume: _____ mL
Expiration: _____

RADIOPHARMACEUTICAL COMPOUNDING RECORD
FLUDEOXYGLUCOSE F 18 INJECTION

Date: _____ Batch / ID #: _____ Prepared By: _____

I. REAGENTS

Chemical	Manufacturer	Lot #	Expiration	Quantity	√ New Vials	Initials
[O-18] Water						
Absolute Ethanol, USP						
Sterile Water for Inj., USP (to flush C-18 / to dilute buffer)						
1 N HCl						
0.4 N HCl						
Acetonitrile						
8.4% Sodium Bicarbonate Inj., USP (in SWI / in cassette)						
134 mM NaOH in 180 mM Na_2HPO_4						
1,3,4,6-Tetra-O-acetyl-2-O-Trifluoro-methane sulfonyl mannopyranose						

II. EQUIPMENT

Equipment	Manufacturer	Lot #	Expiration	Initials
Disposable Cassette				
Vented 0.22 µm Sterile Filter				
Unvented 0.22 µm Sterile Filter				
GV_4 Sterile Filter (4 mm diam.)				
Sterile 18 Gauge Needles				
10 mL Sterile Vial				
Alumina N Plus Cartridge				
C-18 Plus Cartridge				
20-24" Extension Tubing				
"Y" Flush Tubing				

Comments/Observations: _____

_____

_____

III. CYCLOTRON BOMBARDMENT

Operator: _____ Cyclotron: _____ Full Delivery (Y/N): _____

Integrated Beam Current: _____ Time: _____ (hrs) Avg. Beam Current: _____ (µA)

Comments: _____

_____

IV. PRE-RELEASE TESTING

Assay: _____ mCi in _____ mL = _____ mCi/mL at _____

SOS Activity:_____(mCi) EOS Activity: _____(mCi) % Yield: _____

Total Vol:_____(mL) pH: _____ Color / Appearance:_____

Delivered: _____mL to _____ Delivered: _____mL to _____

Radiochemical Purity (% Total Counts): _____ RF: _____ Bubble Test: ( ) Pass ( ) Fail

Checked By (R. Ph.)_____ Date: _____

V. POST-RELEASE TESTING

Bacterial Endotoxin Test: ( ) Passed ( ) Failed ( ) Not Run _____

Sterility: ( ) Passed ( ) Failed ( ) Not Run

(Note: See attached report for details.)

VI. PATIENT TRACKING

Patient ID #: _____ Amount Received: _____mCi

_____ _____

_____ _____

_____ _____

_____ _____

Division of Radiological Sciences

BET/Sterility Request Form

Product: $^{18}F^{-}$fludeoxyglucose **Batch #:** _____

Prepared By: _____ **Date:** _____

Total Batch Volume: _____ **Sample pH:** _____

( ) Check here if the preparation procedure has been altered, requiring Inhibition and Enhancement Testing for USP and RDRC approval.

Comments: _____

_____

_____

Sample Handling: All FDG samples must be properly labeled, put in a lead shield and stored in the freezer until ready for testing.

- -

Q.C. Lab Log in Date / I.D. _____

Procedure: **Preparation of ^{15}O-H_2O Using the CS-15 Cyclotron**

I. OVERVIEW

This procedure details the production of ^{15}O-labeled water, using the CS-15 cyclotron. The target gas pre-mix, 0.1% O_2 in 99.9% N_2, is bombarded with deuterons for 3-15 minutes at a beam current of 10-20 μA. The target gas is initially circulated through a closed loop from the target to the furnace, then back to the target. It is then circulated through a wider loop consisting of the target, furnace, filter, bubbling saline bag, filter, and target where it is again bombarded. The sample is finally collected in a sterile syringe which is automatically shunted, via pneumatic line, to a dose calibrator located at the PET imaging center.

II. EQUIPMENT

1. CS-15 cyclotron
2. Aluminum target, 125 mL volume, covered in front by a double foil assembly using 0.00025 inch (0.25 mil) Havar foils
3. Stainless steel 375-475 °C electric furnace
4. 0.1% O_2 in 99.9% N_2 gas pre-mix (UHP grade N_2)
5. Wide bore ionization chamber (to contain the bubbling bag)
6. Sterile, disposable plastic bubbling bag and tubing (Medex 6057-1)
7. Disposable latex gloves
8. Sterile 19 gauge, 1.5 inch needles
9. Sterile 20 mL plastic syringe with rubber septum
10. Sterile Acrodisc 0.45 μm micropore filter

11. Sterile, pyrogen-free, 0.9% sodium chloride solution (U.S.P. saline)

12. Sterile 12 mL plastic luer-lock syringe

13. Bellows pump

14. Keithley 616 Digital Electrometer radiation detector

15. Picker Transistorized Radiation Lab Monitor

16. Sample label

III. PROCEDURE

A. Power-up Stage

To make it easier to locate various controls on the Cyclotron Control Panel, the sections of the panel will be described from left to right as: sections A, B, C and D.

1. Turn on the O_2-N_2 pre-mixed gas cylinder and check that the pressure is set at 50 psi.

2. Go over to the gas manifold area and find the water furnace temperature gauge. Under the gauge, turn the rotary switch to the **H_2O** position.

3. Section B - <u>Deflector Control</u> and <u>Main Magnet Control</u>
   Flip **UP** the ^{15}O toggle, then press the ON/OFF and EVACUATE buttons until they light up. This turns the furnace on. Allow a minimum of 20 minutes for the furnace to heat up.

4. Check that the **H_2O System** toggle switch, in the ^{15}O hood, is in the **CC** position (for the CS-15 cyclotron).

5. Section A - <u>Control Power Distribution</u>
   a) Turn all of the toggle switches in this row **ON** (up).

   b) Check the vacuum gauge above the toggles. There should be some level of activity, but not at the far right of the meter.

   c) To the right of the meter, check that the sensitivity knob is at **10^{-5}**.

6. Section A - General On-Off
   Turn all of the toggles **ON** (up), in the following order:

Water	(first)
Mag. P.S.	
Osc. Fil.	
I.S. Gas	
I.S. Arc	
Anode P.S.	
Deflector P.S.	(last)

7. Section B - Current Indicator & Integrator
   Turn **ON** the power button below the meter.

8. Section C - Frequency Meter
   Turn this knob to **AUTO**.

9. Section B - Harmonic Coil Control
   Flip up the **ITT** toggle valve.

10. Section B - Main Magnet Control
    Turn the coarse adjust **ON**; for deuterons the meter should read 175 A. If there is no reading, zero the meter, wait a minute then try again.

11. Section A - Ion Source Control
    a) To strike an arc, hold down the Arc Current Adjust **increase** button until the Arc Current meter reaches maximum.

    b) When the Arc Voltage reaches ~1000-1500 V, tap the Ion Source Pressure **prime** button. Tap it again at ~2000 V.

    c) Use the Arc Current Adjust **decrease** button to reach an Arc Current meter reading of **0.3-0.4 A**.

12. Section B - Deflector Control
    Use the Deflector Voltage Adjust dial to get **28,000 V** on the meter.

13. Section C - R. F. Voltmeter
    a) Set the Dee Voltage dial for **350**.

    b) Check that the DVR Loop is **OPEN** (^{15}O).

14. Section C - Position Control
    Use the Probe IN and OUT buttons to position the probe at **~1200**.

15. Section B - Beam Current
    Set the Input at **#1** and the Range at **30 μA**.

16. Section B - Main Magnet Control
    a) Set the Fine adjust knob at **500**.

    b) Use the Fine and Coarse knobs to adjust the magnet and "peak" the beam.

    c) Lock the Coarse knob down.

17. Sections B & C - Main Magnet Control and R.F Voltmeter
    Use the Coarse Adjust and Dee Voltage knobs to bring the beam current up slowly, checking the Beam Current meter to get **~20 μA**.

    (Note: you peak the beam using the coarse adjust, and set the beam using the Dee voltage.)

18. Section D - Beam Steering P.S.
    a) Turn **ON** the power to the following panels:

Vertical (VERT)	Line - ON
Horizontal (HORZ)	Line - ON
Wobbler	Power - ON
Switching Magnet	Power - ON

    b) Starting with the vertical control, use the Coarse and/or Fine knobs to adjust the current to **12 A** on the meter.

    c) With the horizontal control, use the Coarse and/or Fine knobs to adjust the current to **12 A** on the meter.

    d) On the wobbler, use the Amplitude Adjust knob to set the meter at **60 V RMS** (root mean square).

    e) On the switching magnet, set the polarity to REVERSE; set the Current Control knob to **~15**.

19. Section C - Position Control

Retract the probe by using the Probe IN and OUT buttons. When fully retracted the indicator light will come on.

20. Section B - Beam Current
    Change the Input setting to **#3**.

21. Sections B & C - Deflector Control, Main Magnet Control, Harmonic Coil Control and R. F. Voltmeter

    You may lose ~40% of the beam current once the probe has been retracted, since more energy is now hitting the deflector. Therefore, you must peak the beam several times to reach an optimum level.

    a) Adjust the beam current by using the following knobs, aiming for a beam current of **~25 μA** with the Dee Voltage knob **<400**.

       Deflector Voltage Adjust knob
       Fine Adjust knob
       X, and Y knobs

    b) Increase the Dee Voltage level slightly (turn the knob slowly) and repeak the beam using the four knobs listed above. Repeat the process several times as needed.

B. Target Fill Stage

1. Turn **ON** the Keithley detector by flipping up the toggle switch. This will allow you to determine the level of activity (mCi) of the sample.

2. Open the O_2-N_2 pre-mixed gas tank; the pressure on gauge should read **~45** psi.

3. Install daily a new set of sterile, disposable Medex tubing ("bubbling bag"), with the 5 inch extension tube, in the ^{15}O hood.

4. Open the pinch valve at the back of the hood and insert the extension tubing.

5. Install Acrodisc 0.45 μm filters at the gas inlet and outlet ports.

6. Fill out the preprinted sample label, initial it, and stick it on the outside

of the syringe. The expiration time is 30 minutes post-assembly of the syringe. Any sample arriving at a PET center without an attached label is not to be used for patient testing. (See label example).

CAUTION
RADIOACTIVE MATERIAL

Cyclotron Facility
Water O 15 Injection

Date: _____
< 150 mCi (Measure before use)
Expiration: _____

7. Put a sterile needle and syringe in-line with the tubing, then snap in place.

8. To load the bubbling bag with 10 mL of the sterile saline solution, first withdraw the saline from the vial using a 12 mL sterile syringe and needle. Remove the needle, then connect the syringe to the bubbling bag and inject the saline.

9. Turn the stopcock until it is closed to the syringe line, but open to the bubble bag line. Close the door to the hood to minimize radiation exposure.

10. Go over to the gas manifold area and find the water furnace temperature gauge. Flip the EVACUATE toggle switch until the indicator light comes **ON**. Select target **#2** (water). Look at the digital display to check that #2 has been selected.

11. When the target pressure gauge reads zero turn **OFF** the EVACUATE toggle. Open the **PM** needle valve, wait for the pressure gauge to reach **20** psi then close the needle valve.

12. Section B - Harmonic Coil Control
    Under the Ion Target Tube Controls flip the ^{15}**O** toggle. This opens the vacuum gate sends water to cool the target and air to cool the foil.

13. Section B - Current Indicator & Integrator
    Reset the beam counter to zero using the red button.

14. Section D - Beam Shutter Colimator Control
    Flip **ON** (up) the **O**^{15} (B) toggle.

C. Focusing the Beam

Sections B & D - Current Indicator & Integrator and Beam Steering P.S.

1. Find the Direct Current Integrator meter and the Beam Current meter at the top of panel B.

2. To maximize the beam current on the target you must focus the beam as narrowly as possible. In other words, you must maximize the levels of direct integrated current while minimizing the levels of beam current.

3. To maximize the current, adjust the VERTICAL and HORIZONTAL quads using the COARSE and FINE current knobs. Aim for a direct current of **10-20 μA**.

4. Keep adjusting the beam until the integrated beam current counter registers the required level for this particular sample.

D. Sending the Sample

1. Select the location to which the ^{15}O-H_2O sample will be automatically shunted using the rabbit line control panel. (Timers are automatically set to open/close valves.

2. Section B - Main Magnet Control
   Press the BUBBLE button **IN** (light on). This sends the gas through the saline bubbling bag. When the Keithley detector reads the required level of activity for the sample, call the PET center to confirm delivery, then press the BUBBLE button **IN** again (light off), which turns off the gas flow to the saline bubbling bag.

3. Section B - Deflector Control
   Press the **SEND** button.

4. Section D - Beam Shutter Colimator
   Flip **OFF** (down) the ^{15}**O** (B) toggle.

5. Section B - Harmonic Coil Control
   Turn **OFF** the ^{15}**O** toggle.

6. Section B - Main Magnet Control

Press the EVACUATE button **IN** (light on).

7. Fill out the run card for this batch. (See attached example).

E. Complete Cyclotron Shut-down Stage

1. Section C - Position Control, put the probe back **IN**.

2. Section B - Beam Current, set Input at **#1**.

3. Section C - R. F. Voltmeter
   Turn the Dee Voltage down and the Frequency Meter **OFF**.

4. Section A - Ion Source Control
   Reduce the Arc Current by holding the decrease button down until the limit light comes on.

5. Section B - Deflector and Main Magnet Controls
   Turn the Deflector Voltage Adjust, Main Magnet and Coarse Adjust knobs to **ZERO**.

6. Section A - General On-Off
   Working from right to left, turn all of the toggles **OFF** (down), except for the water toggle.

7. Section A - Control Power Distribution
   Turn all of the toggles in this row **OFF** (down).

8. Section D - Beam Steering P.S.
   Turn down the power to the Vertical & Horizontal quads and Wobbler panels, then turn power **OFF**. Turn power to the Switching Magnet **OFF** then turn power down.

9. Section A - General On-Off
   Turn **OFF** the water toggle.

10. Section B - Harmonic Coil Control
    Flip down the **ITT** toggle valve; turn **OFF** the ^{15}O toggle; push the ON/OFF and EVACUATE buttons (lights off).

11. Turn the gas tanks **OFF**.

12. In the ^{15}O hood, turn **OFF** the H_2O switch and the light.

F. Partial Shut-down Stage

If another sample is scheduled for preparation within one hour, the entire system does not need to be shut down.

1. Section C - Position Control, put the probe back **IN**.
2. Section B - Beam Current, set Input at **#1**.
3. Section D - Beam Steering P.S., turn **OFF** the 4 main panels.
4. Section C - R. F. Voltmeter, use the Dee Voltage knob to turn the Beam Current to **~10 μA**.

G. Restart after Partial Shut-down

1. Increase the Dee Voltage.
2. Turn **ON** the power to the Beam Steering P.S. panels.
3. Continue the process from step III. A. 18.

IV. COMMUNICATION

Due to the short half-life (~2 minutes) of ^{15}O, there must be close communication between the cyclotron controller and the PET imaging center. The following steps must be taken before a radiopharmaceutical sample is prepared:

1. PET center will contact controller to initiate process.
2. Controller will power-up cyclotron.
3. PET center will call again to confirm sample demand.
4. Controller will fill target.
5. Controller will call PET center to reconfirm readiness, then send sample.

V. SAMPLE ANALYSIS

Every 2 weeks, or more frequently if the need arises, a sample is tested for sterility and pyrogenicity according to USP XXIII standards. Every 6 months, a sample is tested for Radionuclidic Purity by decay analysis. (For full details see the appropriate Quality Control Procedures).

VI. SPECIAL HANDLING

A. General Use of the Facility

Due to the presence of radioactive material, use of the cyclotron should only be done by authorized personnel, trained in Radiation Safety, under the supervision of Dr. . There should be two persons in or near the cyclotron control room at all times, to insure a rapid response to any injuries or emergencies. Access to the facility should be limited to authorized personnel only.

B. Personal Protection

1. All personnel must wear lab coats, eye protection, and disposable protective shoe covers over their street shoes upon entering the cyclotron facility.

2. Disposable latex gloves and sleeve protectors must be worn during radiopharmaceutical chemical preparation.

3. All personnel must wear TLD radiation badges and rings.

4. Before leaving the cyclotron facility personnel must remove disposable gloves and shoe covers, and check themselves for contamination using the hand/foot monitor by the door.

5. In case of personal contamination:

   a) Immediately wipe activity from exposed skin.
   b) Wash using soap, water and a soft brush; recheck.
   c) If there is still residual radiation use the Rad-Con foam decontaminant until all radiation has been removed or reduced to a non-transferable level.
   d) Notify the Radiation Safety Officer, at -8425.

6. Contaminated garments should be left in the facility to decay to background levels. This requires at least ten half-lives.

7. Upon returning to the department, all personnel must check themselves for contamination using the hand/foot monitor in Room 4424.

Rad. Sci. - 1 rev. 6/91

CC MEDICAL CYCLOTRON

No. 1833 | Date 1/5/96

a D P He_3	
Beam	Ua
EFNCY	%
B+	KV
DI AL	A
BIAS	KV
RF	MhZ
DEE	KV
ANODE	KV
CATH	KV
MAG	A
F	C
X	Y
ARC	KV
ARC	MA
GAS	MM
DEF	KV
VAC	

Investigator KL	OPR 5	Investigator WJP	OPR 5	Investigator Cyc	OPR 9
Recipient QC		Recipient 10400 B		Recipient E.Build 05 Test	
Units	Isotope ^{15}O	Units	Isotope ^{15}O	Units	Isotope ^{15}O
RFF 45.8	ION F 53.9	RFF 49.0	ION F 57.1	RFF 50.3	ION F 58.2
S 44.0	S 52.1	S 46.2	S 54.3	S 49.4	S 57.5
T 1.8	T 1.8	T 2.8	T 2.8	T .9	T .7
CO 11	W (39)	OO 1	W (41)	CO	W (43) OO 111

Investigator SB	OPR 5	Investigator LL	OPR 8	Investigator WJP	OPR 10
Recipient SP3D		Recipient 104 BC		Recipient 104	T. G. 4
Units	Isotope ^{15}O	Units	Isotope ^{15}O	Units	Isotope ^{15}
RFF 46.2	ION F 54.3	RFF 49.4	ION F 57.5	RFF [illegible]	ION F [illegible]
S 45.8	S 53.9	S 49.0	S 57.1	S 50.[illegible]	S [illegible]
T .4	T .4	T .4	T .4	T 1.5	T [illegible]
CO 1	W 1 (40)	OO	W 1 (42)	CO	W 111 (44) OO

Procedure: **Preparation of ^{15}O-H_2O Using the JSW-BC168 Cyclotron**

I. OVERVIEW

This procedure details the production of ^{15}O-labeled water, using the JSW-BC168 cyclotron. The target gas pre-mix, 0.1% O_2 in 99.9% N_2, is bombarded with deuterons for 3-10 minutes at a beam current of 10-40 μA. The gas is then circulated through a closed loop from the target to the furnace, then back to the target where it is bombarded again. It is then circulated through a wider loop which consists of the target, furnace, filter, bubbling saline bag, filter, and target where it is again bombarded. The sample is finally collected in a sterile syringe which is automatically shunted; via pneumatic line, to a dose calibrator located at the PET imaging center. Total bombardment

II. EQUIPMENT

1. JSW-BC168 cyclotron
2. Aluminum target, 250 mL volume, covered in front by a double foil assembly using 25 micron Havar foils
3. Stainless steel 375-475 °C electric furnace
4. 0.1% O_2 in 99.9% N_2 gas pre-mix (UHP grade N_2)
5. Wide bore ionization chamber (to contain the bubbling bag)
6. Sterile, disposable plastic bubbling bag and tubing (Medex 6057-1)
7. Disposable latex gloves
8. Sterile 19 gauge, 1.5 inch needles
9. Sterile 20 mL plastic syringe with rubber septum

10. Sterile Acrodisc 0.45 μm micropore filter

11. Sterile, pyrogen-free, 0.9% sodium chloride solution (U.S.P. saline)

12. Sterile 12 mL plastic luer-lock syringe

13. Bellows pump

14. Keithley 616 Digital Electrometer radiation detector

15. Picker Transistorized Radiation Lab Monitor

16. Sample label

III. PROCEDURE

A. Power-up Stage

1. Turn on the O_2-N_2 pre-mixed gas cylinder and check that the pressure is set at 60 psi.

2. Turn **ON** the following electrical control panels in the order listed:
   ARIS G-1 controller (both circuit breakers)
   Interface/CPU
   Control panel (power, main key, drive unit and cooling pump)
   Main Coil P.S.
   RF High Volt P.S. (power and standby)
   Trim Coil/Aux.Coil/PQM P.S.
   Ion Source/Deflector P.S.

B. Computer Control Stage

1. Using the mouse, select the following parameters on the ARIS computer status screen, then select [Process].

radioisotope	furnace	target
$^{15}O_2$ on		auto off
		TB6 Aux.

2. Check the status screen to make sure the $^{15}O_2$ target has been selected

(the TB6 box should say Aux.).

3. On the JSW cyclotron control panel press in the blue **H_2O Furn. On** button, then press in the red **Evacuate** button, until they light up.

4. Allow a minimum of 20 minutes for the furnace to heat up.

5. Check that the **H_2O System** toggle switch, in the ^{15}O hood, is in the **JSW** position (for the JSW cyclotron).

6. On the cyclotron control panel, press **Deuterons**. Check the cyclotron status screen to make sure deuterons have been selected; look for D 8 MeV.

7. If deuterons have not been selected, press the **Reset** button on the Interface/CPU unit.

8. To flush the ion source gas line press the **Manual Mode** button, then increase the gas flow to ~5 sccm.

9. Wait 5-10 minutes for flushing to end, then decrease the gas flow to 0 sccm. Press the **Data Fetch** button on the control panel.

10 On the cyclotron computer status screen select [Menu], [End], [Main] then [Execute]. This activates the drive units which are on pre-set parameters.

11. Next, turn **ON** the Keithley detector by flipping up the toggle switch. This will allow you to determine the level of activity (mCi) of the sample.

12. Press the cyclotron standby **ON** button, on the control panel, after you see the computer prompt. The control panel lights should be red. If the program is not running properly the control lights will be green.

    (Check the Control Panel's Cyclotron Interlock warning lights for an indication of the problem, then take whatever appropriate corrective actions are necessary.)

13. An audible buzzer will sound, indicating that the cyclotron vault must be evacuated immediately, and the door must be shut.

C. Gas/Syringe Fill Stage

1. Near the gas manifold, flip the **VAC. PUMP** toggle switch (light off).

2. Open the O_2-N_2 pre-mixed gas valve until the pressure on the rough gauge reads **3** psi.

3. Install daily a new set of sterile, disposable Medex tubing ("bubbling bag"), with the 5 inch extension, in the ^{15}O hood.

4. Open the pinch valve at the back of the hood and insert the extension tubing.

5. Install Acrodisc 0.45 μm filters at the gas inlet and outlet ports.

6. Fill out the preprinted sample label, initial it, and stick it on the outside of the syringe carrier tube. The expiration time is 30 minutes post-assembly of syringe. Any sample arriving at a PET center without an attached label is not to be used for patient testing. (See example label below).

Cyclotron Facility
Water O 15 Injection

Date: _____
< 150 mCi (Measure before use)
Expiration: _____

7. Put a sterile needle and syringe in-line with the tubing, then snap in place.

8. To load the bubbling bag with 10 mL of the sterile saline solution, first withdraw the saline from the vial using a 12 mL sterile syringe and needle. Remove the needle, then connect the syringe to the bubbling bag and inject the saline.

9. Turn the stopcock until it is closed to the syringe line, but open to the bubble bag line. Close the door to the hood to minimize radiation exposure.

D. Fill Target Stage

1. Using the CS-15 cyclotron control panel, flip up the ^{15}O toggle switch,

then press the green **On/Off** button (light on).

2. Select the location to which the ^{15}O-H_2O sample will be automatically shunted using the rabbit line control panel. (Timers are automatically set to open/close valves.)

3. On the cyclotron control panel, find the manual operation button and press **Manual Mode**.

4. Press the **Bellows Pump** button in.

5. Use the small knob to increase the Filament Current until the ARC Voltage is reduced to <50. The cyclotron status screen should show I_A between 0.04-0.05.

6. Check the RF Mode **CW** button. It should be red. If not, press it.

7. Turn the Beam Current up to ~40 μA, using the ARC Current knob.

8. Use the following 3 small knobs to peak the beam current, staying at ≤40 μA: Aux. Coil Current, Dee Voltage and Deflector Voltage.

9. Decrease the Filament Current, balancing it with the ARC Current, to obtain ~40 μA. These fine adjustments must be performed for about 10-15 minutes, until the system has stabilized.

10. When the Beam Current Integrator reaches 40 μA.min, press in the **Bubble** button. This sends the gas through the saline bubble bag.

11. Check the Keithley detector. In most cases you will want ~80 mCi of activity to be delivered. When the detector indicates you are ~40 mCi above that, call the PET center to confirm delivery.

12. Turn the **Bubble** button off then press the **Send** button.

13. Turn the ARC Current down.

14. Turn the Bellows Pump **OFF**.

15. Turn the Evacuate button **ON** (light on).

16. Fill out the run card for this batch. (See attached example).

E. Complete Cyclotron Shut-down Stage

1. On the cyclotron control panel, press the Operate **Off (Reset)** and Standby **Off (Reset)** buttons.

2. Turn the RF High Volt P.S. **standby** switch **OFF**.

3. Wait 10 minutes, then turn **OFF** the electrical control panels in the opposite order listed on page 2, section III. A. 2.

4. Turn the CS-15 control panel **OFF**.

5. Turn off the **H_2O Furn.** and **Evacuate** buttons.

6. Turn the gas tanks **OFF**.

F. Partial Shut-down Stage

If another sample is scheduled for preparation within one hour, the entire system does not need to be shut down. Press the Operate **Off (Reset)** button. This will take some of the strain off of the filament.

G. Restart after Partial Shut-down

1. On the cyclotron status screen select [Resume].

2. Continue the process from step III. C. 9.

IV. COMMUNICATION

Due to the short half-life (~2 minutes) of ^{15}O, there must be close communication between the cyclotron controller and the PET imaging center. The following steps must be taken before a radiopharmaceutical sample is prepared:

1. PET center will contact controller to initiate process.
2. Controller will power-up cyclotron.
3. PET center will call again to confirm sample demand.
4. Controller will fill target.
5. Controller will call PET center to reconfirm readiness, then send sample.

decontaminant until all radiation has been removed or reduced to a non-transferable level.

d) Notify the Radiation Safety Officer, , at 3425.

6. Contaminated garments should be left in the facility to decay to background levels. This requires at least ten half-lives.

7. Upon returning to the department, all personnel must check themselves for contamination using the hand/foot monitor in Room 4424.

JSW MEDICAL CYCLOTRON

No. 436

Date 12/1/95

Rad. Sci. -2 rev. 6/91

Beam I 36 (Pb)	Investigator WJP OPR 12-1	Investigator EYAL OPR 4	Investigator KL OPR 4
Aux Coil 2.71	Recipient 104 P	Recipient Chen	Recipient Chen QC
Dee Volt 37.9	Units Isotope 15O	Units 1500 Isotope 18F tr	Units Isotope 15O
Def Volt 36.9	F	F 10.9	F 13.1
Fil I 237.3	S 9.9	S 10.1	S 12.3
Arc I 196 V 148.8	T .1	T .8	T .8
Gas 1.01	CO W OO 1 (1105)	CO W OO (1107)	CO W (4) OO (1109) 10μA 3min, 10min 40μA 5min, 10min
ION 18.9 Ø 1.05 Z			
POS 1.44 X 4.43 Y			
Plug .83 U .65 L	Investigator JSP OPR 12-1	Investigator WJP x2 OPR 4	Investigator JSP OPR 5
Def .05 EN 4.1 Ex	Recipient EBP	Recipient 104 P x2	Recipient EBP
Sep .05 EN 4.07 Ex	Units Isotope 15O	Units Isotope 13O	Units 3420 Isotope SPECIP
M.C. [illegible] EN [illegible] Ex	F 10.1	F 12.3	F 14.6
C.T.C. 0 A 0 B	S	S 10.9	S 13.1
H.T.C. 17 A 27 B	T .1	T 1.4	T 1.5
PQM 14	CO W OO 1 (1106)	CO W OO TNL 1 1 1 (1108)	CO W OO (1110)

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2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

(FDG)

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SYNTHESIS OF

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

(FDG)

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

The synthesis of 2-[F-18]fluoro-2-deoxy-D-glucose (FDG) follows the procedure reported in the literature [Hamacher et al., J. Nucl. Mec. 27, 235-238 (1986). A copy of the paper is enclosed]. The synthesis is routinely carried out using an automated module as reported in the literature [Padgett el al., Appl. Radiat. Isot. 40, 433-445 (1989). A copy of the paper is enclosed].

Description of the production of [F-18]fluoride ion

No carrier added [F-18]fluoride ion is made by the $^{18}O(p,n)^{18}F$ nuclear reaction conducted in a silver target body. The target is filled with 330 µL of [O-18]H_2O and irradiated with 11 MeV protons. After bombardment, the radioactive water is transferred through 0.8 mm ID polyethylene tubing to the FDG synthesis module.

Description of the synthesis of FDG

No carrier added $K^{18}F$ was prepared by the addition of K_2CO_3 (1 mg) and Kryptofix 222 (10 mg) to the proton irradiated [O-18] water. The water was evaporated at 115°C with a stream of dry nitrogen and the last traces of moisture were removed by azeotropic distillation with acetonitrile (3 x 1 mL). A solution of 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose (10 mg) in acetonitrile (1.5 mL) was added to the dry $K^{18}F$/Kryptofix complex and the reaction mixture was heated for 15 min at 85°C. The fluorinated product solution was through a silica Sep-Pak cartridge (Waters Associates) and eluted with ether (8 mL). The eluent was evaporated with a stream of nitrogen in a glass vessel kept at 115°C. One mL of 1.0 N hydrochloric acid was added to the residue and heated at 115°C for 15 min. The hydrolyzed

mixture was then transferred onto the top of a column of ion-retardation resin (Bio-Rad, AG11A8, 50-100 mesh) pre-equilibrated with sterile water. The hydrolysis vessel was rinsed with water (18 mL) onto the column and the FDG eluted from the column was passed through a tandem alumina and C-18 Sep-Pak cartridges (Waters Associates) which had been washed with absolute ethanol (3 mL) followed by sterile water (10 mL). The resulting solution was finally sterilized by passing through a Millipore filter (0.22 μM) into a sterile 30 mL multi-injection vial containing 1 mL of sterile NaCl (180 mg/mL).

The step by step procedure for the setting up of the FDG automated synthesis module and the production of FDG are described in the following section.

2 - [F-18]Fluoro - 2 - deoxy - D - glucose (FDG) Synthesis

Introduction

This is the procedure for the automated synthesis of 2-[F-18]fluoro-2-deoxy-D-glucose (FDG) using the RDS-112 Cyclotron System. The synthesis consits of six steps; target unloading, dehydration of the fluoride ion, fluorination of the precursor molecule, Kryptofix/fluoride ion removal, hydrolysis, final purification and sterlization of the final product. After the Chemical Process Control Unit (CPCU) has been prepared, with clean glassware and fresh reagents, the target bombardment and the entire chemical synthesis is controlled without operator intervention by the Radiochemical Delivery System (RDS-112).

Materials

1. one 0.22um vented sterilizing filter (Millipore #SLGSV255F)
2. one C-18 Sep-Pak cartridge (Millipore #51910)
3. one Alumina Sep-Pak cartridge (Millipore #51810)
4. one Silica Sep-Pak cartridge (Millipore #51900)
5. two glass CPCU Module synthesis tubes
6. one 30 ml sterile vial
7. several glass syringes
8. several hypodermic needles
9. one 1 x 15 cm LC column (Biorad #737-1030)
10. alcohol swabs

Chemicals

1. Kryptofix® (K_{222}) (1 mg) (4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8,8,8]-hexacosane) (Aldrich #29,111-0) / K_2CO_3 (10 mg) in 1 ml of water
2. 10mg mannose triflate compound (1,3,4,6-Tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannosepyranose) (Aldrich #31,025-5)

3. 8 ml acetonitrile (CH_3CN)
4. 8 ml ethyl ether (anhydrous)
5. 20 ml vial of sterile water for injection
6. 1ml 180 mg/ml NaCl solution (USP)
7. 5 ml ethanol
8. 0.3 ml [O-18]H_2O
9. AG 11A8 Resin (Biorad #142-7834)
10. 1.0ml 1N HCl solution

RDS Cyclotron Automatic Operation

1. The cyclotron system is assumed to be in the standby condition, ready to generate beam. If it is not ready, refer to the cyclotron operation documents for further information.

Automatic FDG Production

1. Select **Produce Labeled Product** from the **RDS Function Selection Menu**. Press **ENTER** to continue.

2. From the **Produce Labeled Product Menu**, for **Target Zone 4** select either **[F-18] FDG CPCU 1 or [F-18] FDG CPCU 2**, depending on which CPCU is to be used for the synthesis. Press **ENTER** to continue.

3. On the **Target Zone 4 Setup Page**; enter the beam current, normally 20μA, and the bombardment time, normally 60 min.

4. Press **ENTER** to begin the automated production sequence; target loading, bombardment and chemistry.

CPCU Synthesis Module Set-up (Ref: fig. 1)

NOTE When facing the CPCU, the Fluorination Vessel (Vessel #1) is on the left, and the Hydrolysis Vessel(Vessel #2) is on the right . The reagent vessels left to right are: Kryptofix, Acetonitrile,Triflate, Ether, HCl, and Sterile Water.

CAUTION: Use separate syringes for each of the reagents. Insure that there is no contamination between the Kryptofix solution and the Triflate solution, as this will poison the reaction. .The syringes must be rinsed with methanol and dried between each use.

1. At the terminal select **Execute a Command File**. When the Command File Menu appears select **INITCPCU**. This will initialize the CPCU; turn on the oil baths, fan, nitrogen gas supply, move the elevators to the initial position and check the operation of the oil bath positioning mechanism. This must be done at least 20 minutes prior to EOB to allow the oil baths time to come to their proper temperatures.

NOTE: Be sure to have the correct directory selected for the CPCU in use ; SYNYH1 for CPCU #1 or SYNYH2 for CPCU #2

2. Add to the Kryptofix Vial 1.0 ml of the Kryptofix solution.

3. Add to the Acetonitrile Vial 1.5 ml of acetonitrile

4. Insert a spinal needle through the septum of the Mannose Triflate vial, containing 10 mg of the compound. Place the vial in the clip holder and insert the pressure line needle through the septum. Add 1.5 ml of acetonitrile to the Mannose Triflate and dissolve.

5. Add 8.0 ml of ether to the ether vial.

6. Add 1.0 ml of 1N HCl to the HCl Vial

7. Place a 20 ml vial of sterile water into the clamp, and insert the short pressure needle and long fluid out needle into the vial.

8. Remove two clean glass CPCU Synthesis Tubes from the oven, allow to cool. Insert the tubes into the holders at the top of the Synthesis Module. Place the appropriate silicone stopper with the liquid transfer lines into the recess on the top of the CPCU. Insure the tubing is inside the synthesis tubes. Clamp the stoppers in place, taking care not to pinch any of the liquid transfer lines with the clamp.

9. Connect a Silica Sep-Pak between the the Fluorination Vessel and Hydrolysis Vessel using the male luer connections at the top of the module. Connect the long end of the Sep-Pak to the Fluorination Vessel line and the short end to the Hydrolysis Vessel line.

10. Take the 1 x 15cm chromatography column and place about 12cm of AG11A8 resin in the column. Drain the water to just above the top of the resin.

11. Wash a C-18 Sep-Pak cartridge with 5 ml of ethanol and insert a double male luer adapter into long end of the Sep-Pak. Attach the short end of the Alumina Sep-Pak to the other end of the luer adapter. Wash the entire assembly with 10 ml of sterile water. Insert the bottom of the resin column into the long end of the Alumna Sep-Pak. Insert one end of the output delivery line into the short end of the C-18 Sep-Pak. Attach the column assembly to the FDG Module by the clips provided and insert the silicone stopper from the hydrolysis vessel into the top of the resin column. The flow path is out of the hydrolysis vessel through the resin column, alumina, C-18 and into the sterile vial.

CAUTION: Insure the silicone stopper is tight in the top of the resin column, the liquid transfer is by pressure. A loose stopper could pop out causing the loss of the product

12. Take a 30ml sterile vial and swab the top with an alcohol wipe. Insert two sterile hypodermic needles through the septum and attach a 0.22 μm vented sterilizing filter to one of the needles. Withdraw 1.0 ml of the 180 mg/ml saline solution and inject into the vial through the sterilizing filter. Affix a filled out label on the sterile vial. Attach the output delivery line to the sterilizing filter and place in a lead pig.

13. Push the CPCU into the USS cabinet and close the door. Be sure none of the cabling or tubing becomes tangled or pinched.

Chemistry Process

1. At the End of Bombardment the program will start unloading the target into the selected CPCU. The unloading process takes about 4 min.

2. When the computer tells the operator the target is unloaded ALWAYS check to insure the radioactive material is in the CPCU before telling the computer to continue. At the computer, from the Maintainence Page **TARG1** turn **OFF R70**, the low pressure supply valve to the target. Observe the target pressure read out, if the water bolus has cleared the delivery line the pressure will bleed down rapidly. If not turn **ON R70** for a few moments then turn it off again and observe the pressure reading. Check for complete unloading by opening the USS door part way and using a survey meter to check for presence of radioactivity.

3. When the synthesis is complete remove the output line and filter from the vial using tongs leaving the vent needle in place. Assay the vial for activity, pull a sample for HPLC analysis using the vent needle and a 1cc syringe, ship vial to the clinic for use. Inform the clinic of the amount of activity delivered.

Glassware Preparation

1. Wash the glassware in hot soapy water, rinse thoroughly with tap water and then deionized water, and rinse with methanol.

2. Dry in the oven at 130°C.

Solutions Preparation

Kryptofix - Carbonate Solution

1. 250 mg Kryptofix (K_{222}): 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8,8,8]-hexacosane: Aldrich #29,111-0
2. 25 mg Potassium carbonate (K_2CO_3)
3. 1 ml Deionized Water

4. 25 ml Acetonitrile (CH_3CN)

Dissolve the Kryptofix and potassium carbonate in the deionized water, then add the acetonitrile. Cap the vial with a teflon septum.

Saline Solution(180 mg/ml)

1. 9 gm NaCl (USP)
2. 50 ml sterile water

Dissolve the NaCl in the sterile water.

CPCU Glassware Removal

1. Remove the stopper assemblies from their clamps.

2. At the computer console select Command File **VESOUT**; corresponding to the CPCU in operation, and follow the instructions on the screen.

Computer Command Files

1. SYNTH1 (2): Unloads target to CPCU and produces the labeled FDG product.
2. INITCPCU: Prepares the CPCU for chemistry, turns on oil baths etc.
3. INITVES1: Moves vessel #1 (fluorination) to it's initial position.
4. INITVES2: Moves vessel #2 (hydrolysis) to it's initial position.
5. DRY1: Dries the fluoride ion from the target.
6. BATHLEFT: Moves the oil baths to the left position.
7. BATHRIGHT: Moves the oil baths to the right position.
8. BATHCENTER: Moves the oil baths to the center position.
9. CYCLBATH: Moves oil baths back and forth for testing.
10. ADDVIAL1: Adds the kryptofix solution to the fluorination vessel.
11. ADDVIAL2: Adds the acetonitrile to the fluorination vessel.
12. ADDVIAL3A: Adds the triflate solution to the fluorination vessel.
13. ADDVIAL4: ADDVIAL4A: Adds the ether to the fluorination vessel.
14. ADD VIAL5: Adds the 1N HCl to the hydrolysis vessel.
15. ADDVIAL6: Adds the water to the hydrolysis vessel.
16. TOPVES1: Moves vessel #1 (fluorination) to it's top position.
17. TOPVES2: Moves vessel #2 (hydrolysis) to it's top position.
18. HEATVES1A: Lowers vessel #1 into oil bath for evaporation step.
19. HEATVES1B: Lowers vessel #1 into oil bath for fluorination step.
20. HEATVES2A: HEATVES2B, HEATVES2C: lowers vessel #2 into oil bath in successive steps for ether evaporation.
21. HEATVES2D: Lowers vessel #2 into oil bath for hydrolysis.
22. EVAPVES1: EVAPVES1B: Evaporates the contents of vessel #1

23. EVAPVES2: Evaporates the contents of vessel #2
24. TRANSFER1: Transfers ether from vessel #1 to vessel #2 and evaporates the ether form the vessel #2.
25. TRANSFER2: Adds water to vessel #2 and transfer it through the resin column and into sterile vial.
26. TRANSVES1: Transfers the contents of vessel #1 to Vessel #2.
27 TRANVES2A: TRANVES2B, TRANVES2C: Transfers the contents of vessel #2 through the resin column into the sterile vial.
28. VESOUT: Pushes the reaction tube out the top of the chemistry module for easy removal and replacement.
29. MIXVES1: Bubbles nitrogen to mix the fluorination solution.
30. MIXVES2: Bubbles nitrogen through the hydrolysis solution.
31. REFLUX2: Hydrolysis reaction step
32. OFFCPCU: Resets the CPCU, shuts all open valves.
33. F18DELIV1 (2): Unloads the target to CPCU 1 or 2.
34. CD(XXXX): Changes directory of user command files, ie. CDSYNTH2 changes the directory to the SYNTH2 set of commands. Whrer XXXX is the directory name PROD, TEST, SYNTH1, or SYNTH2
35. HOLDVES1: Holds vessel #1 against the stopper assembly for liquid transfer.
35. HOLDVES2: Holds vessel #2 against the stopper assembly for liquid transfer.
36. REACT1: Adds Triflate, heats,mixes and cools reaction.
37. COOLVES1: Raises vessel #1 and turns on fan to cool reaction.

NOTE: Various subroutines are called by the FDG synthesis programs to conduct the chemical synthesis. Therefore in the event of a malfunction, causing a halt to the synthesis, it may be finished by calling the appropriate subroutine in the proper order. Refer to the synthesis in the CPCU manual.

The chemical synthesis subroutines can only be halted by pressing **Control C**. When the program halts all control functions remain in their present state either on or off. In order to reset the CPCU, close any open valves turn off the oil bath and stop the elevator motors, select the , when the new menu appears select **OFFCPCU** and press **ENTER** in order to stop all CPCU operations.

$H_2{}^{18}O$ Syringe Filling Procedure

1. Open the target vent valve, using the switch on the TSU valve board. Allow the target to vent for about 60 seconds

CAUTION: Failure to vent the target before disconnecting the fill line could cause the [O-18]water in the line to be blown out.

2. Disconnect the fill line from the syringe.

3. Remove the syringe from the syringe pump

4. Place a fresh hypodermic needle on the syringe and with draw 7 ml of [O-18] water from the vial.

5. Remove the hypodermic needle. Slowly push in the syringe plunger and push out any air bubbles in the syringe.

NOTE: Any air bubbles in the syringe can cause inaccurate water dispensing.

6. Place the syringe in the syringe pump clamp. Turn the syringe pump power switch **OFF**. At the computer turn **ON R63**. Turn the syringe pump power switch **ON**, and allow the ram to push the syringe plunger until a water drop begins to form at the luer fitting, turn the syringe pump power switch **OFF**. At the computer turn **OFF R63**. Reattach the fill line to the luer fitting. Turn the syringe pump power switch **ON** to the ml/min position.

7. Close the target vent valve.

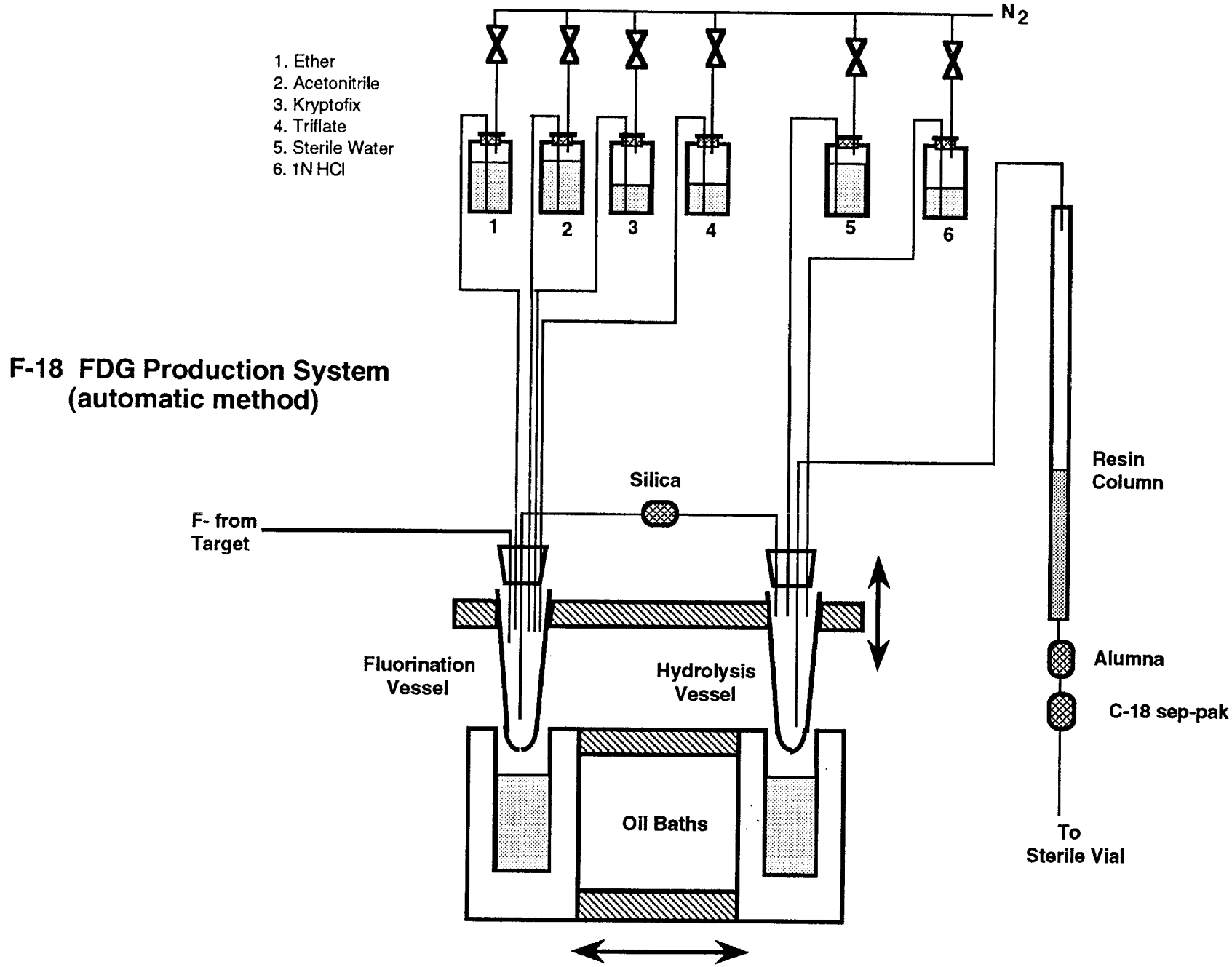
CHEMICAL PROCESS CONTROL UNITS RELAY NUMBERS

CPCU #1	CPCU #2	OPERATION
R 00	R 144	Oil bath moves right
R 01	R 145	Oil bath moves left
R 02	R 146	Oil bath moves right
R 03	R 147	Oil bath moves left
R 04	R 148	Spare, high pressure valve
R 05	R 149	Spare, high pressure valve
R 06	R 150	Spare, low pressure valve
R 07	R 151	Spare, low pressure valve
R 08	R 152	Spare, low pressure valve
R 09	R 153	Bubbler to Vessel #2
R 10	R 154	Vial #1 pressure (Kryptofix)
R 11	R 155	Vial #3 pressure (Triflate)
R 12	R 156	Vial #2 pressure (Acetonitrile)
R 13	R 157	Vial #5 pressure (HCl)
R 14	R 158	Nitrogen supply to valves R40, R 41 CPCU#1, and R 184, R185 CPCU#2
R 15	R 159	Vial #6 pressure (Water)
R 24	R 168	Oil bath #1 controller

R 25	R 169	Oil bath #2 controller
R 26	R 170	Air flow heater
R 27	R 171	Fan
R 28	R 172	Vessel #1 up
R 29	R 173	Vessel #1 down
R 30	R 174	Vessel #2 up
R 31	R 175	Vessel #2 down
R 32	R 176	Limit switch - Vessel #1 top
R 33	R 177	Limit switch - Vessel #1 bottom
R 34	R 178	Limit switch - Vessel #2 top
R 35	R 179	Limit switch - Vessel #2 bottom
R 36	R 180	Limit switch - Oil baths right
R 37	R 181	Limit switch - Oil baths center
R 39	R 182	Limit switch - Oil baths left
R 40	R 184	Bubbler vessel #1
R 41	R 185	Vial #4 pressure (Ether)
R 42	R 186	Pressure test manifold valve (normally open)
R 43	R 187	Pinch valve - target delivery line
R 44	R 188	Vessel #1 hold current
R 45	R 189	Spare relay
R 46	R 190	Vessel #2 hold current
R 47	R 191	Spare relay

TARGET CONTROL VALVES

VALVE NO.	OPERATION
R 63	Target syringe pump power
R 67	Target pinch valve
R 68	Target vent valve
R 69	Target relief valve
R 70	Target low pressure valve
R 71	Target high pressure valve


F-18 FDG Production System
(automatic method)
1. Ether
2. Acetonitrile
3. Kryptofix
4. Triflate
5. Sterile Water
6. 1N HCl
N_2
1
2
3
4
5
6
Silica
F- from Target
Fluorination Vessel
Hydrolysis Vessel
Oil Baths
Resin Column
Alumna
C-18 sep-pak
To Sterile Vial

QUALITY CONTROL PROCEDURES

FOR

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Radiochemical Quality Control for [F-18] FDG

Introduction

The following protocol is used to check the purity of a sample of [F-18] FDG, utilizing a radioactivity detector.

Materials

1) Freshly made vial of [F-18] FDG
2) CH_3CN ; 18 M ohm H_2O
3) Econosphere NH_2 5U column (Alltech)
4) HPLC injector with 20ul load loop
5) Rainin Rabbit-HP Pump
6) Beckman Model 170 Radioisotope Detector
7) Macintosh Computer running Rainin Dynamax HPLC controller and data collection system
8) 1ml syringe and an injection needle.

[F-18] FDG sample preparation

For FDG synthesis procedure refer to the RDS F-18 Fluorodeoxyglucose Production Protocol. With a 1ml syringe withdraw ~ 0.2 ml of the FDG sample and inject it into a plastic syringe case. Add ~ 0.2 - 0.3 ml of deionized water to the sample to dilute the concentration. The sample is now ready for injection.

Preparation of Acetonitrile Mobile Phase

Measure out 850 ml of CH_3CN and 150 ml of 18 M ohm H_2O for every 1L of the HPLC solvent. Filter the solution through a 0.45 micron nylon filter to remove any particulate matter and then vacuum degas it. Note: give the HPLC system sufficient time to equilibrate with the solvent system (usually 30 min. at aflow rate of 1 ml / min).

HPLC System Preparation and Shutdown

The HPLC system is stored in the same mobile phase that is used for sample analysis and therefore does not require equilibration time. The system is immediately ready for use at any time.

Computer Program Settings

1)Click twice on the hard drive symbol on the Mac screen. At this point a menu will appear.

2)Double click on the "Dynamax" folder

3)Choose "DA" folder and double click

4)Go to "File" on top of the screen and pull it down. Select "Open Method". Select "FDG Q.C." folder from the pop-up window.

5)Go to "Control" on top of the screen and pull it down. Select "Run Control "

6)Select "Ramp to Start" and click on it. The system will take 30 seconds to come up to pressure and the computer will set the flow rate to 2.0 ml / min. In the mean time follow the Sample Injection procedure outlined below.

Sample Injection

With a 1ml injection syringe draw about 0.3 ml of sample. Make sure to remove all of the air bubbles from the syringe by tapping it gently on the sides.

Turn the injector to the "LOAD" position and insert the needle. Gently inject the contents of the syringe.

7)Select "Start Run" and click on it.

8)Printer Ready ! "OK" window will pop up. Check if printer is on and click on "OK". (If printer is off, turn it on and click on the window)

9)The following two tasks have to be performed simultaneously:

a) Turn the injector handle from "Load" to "Inject" position.

b) Click on the "Inject" icon

Everything from now on is performed automatically.

Data analysis and discussion

The FDG should elute as a single peak at roughly the 2.8 minute mark. Impurities show up as one or two small peaks at or around the two minute mark. They usually account for no more than 2 % of the final FDG product and are believed to be partially hydrolyzed Fluorodeoxyglucose derivative.

RADIOCHEMICAL QUALITY CONTROL

RADIOANALYTICAL HPLC OF

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Column: Alltech Econosphere NH_2 column (250 x 4.6 mm)

Eluent: 85% CH_3CN + 15% H_2O

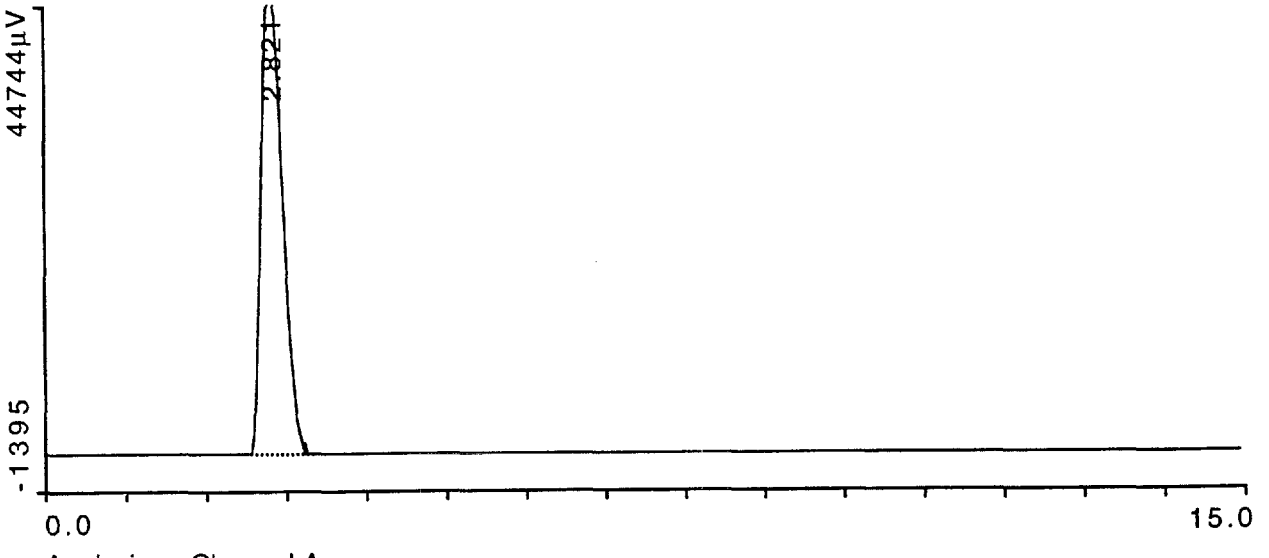
Flow rate: 2.0 mL/min

Chart speed: 1 cm/min

Radioactivity detection

Processing File:
Method: FDG Q.C.
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(µV)	Area(µV-sec)	Area%
1	2.821	N	43937	736428	100.000
Total Area				736428	100.000

Chemical Quality Control for [F-18] FDG

Introduction

The following protocol is used to determine the chemical purity of a sample of Fluorodeoxyglucose (FDG).

Materials

1) Dionex Carbopak PA1 (4x250mm) column

2) Dionex Carbopak PA1 Guard column

3) Dionex Gradient Pump

4) Dionex Pulse Electrochemical Detector

5) A1-450 data collecton program

FDG sample preparation

Follow the synthesis procedure outlined in RDS-112 Florodeoxyglucose Production Manual to obtain a unit of FDG. Upon completion of synthesis, draw 0.4 - 0.5 ml of liquid from the FDG collection vial into a 1ml syringe.

Preparation of NaOH Mobile Phase

100 mM NaOH mobile phase is used for specific activity measurement. It is prepared by mixing 1.9894 liters of 18 Mohm water with .0106 liter of 50%(v/v) NaOH solution for a total volume of 2 liters. The solution is filtered through a .2um filter to remove any particulate matter and then vacuum degassed.

HPLC System Preparation and Shutdown

Three hours before analysis perform the following tasks (refer to the appropriate manuals if in doubt):

1) Turn the He gas bottle on.

2) Loosen the cap on the 100mM NaOH reservoir.

3) Turn the System swith "ON".

4) Flip the switch from "PRESSURE" to "SPARGE". Let sparge for 15-30 min.

5) Tighten cap on the 100mM NaOH reservoir.

6) Flip the switch from "SPARGE" to "PRESSURE" and adjust the black pressure knob until the scale reads 7-10 psi.

7) Swith the Gradient pump (GP) to "LOCAL". Set program to #2; flow rate to 1.0ml/min; gradient to 100% 100mM NaOH. At this point press "START" and let the column equilibrate for 2-3 hours upon which time push the "STOP" button to stop the buffer flow.

8) Set the Pulse Electrochemical Detector (PED) to "INTEGRATED AMPEROMETRY"; anode range to 100uC and program to #9. (This detector uses a gold cell as a detection device).

9)When ready to run the samples turn both the GP and the PED to "REMOTE". The "REMOTE" mode delegates the control of the two units to the computer.

10) Upon completion of sample analysis turn GP and the PED back to "LOCAL"

11) FlipSYSTEM switch to"OFF" and close the He bottle.

Desired Computer Program Settings

1) On the computer terminal double click on the A1-450 MENU icon .

2) Double click on the "RUN" icon.

3) At the top of the screen, under LOAD menu choose Method. Select " fdg.met" file and double click on it. Press OK in the method menu screen and wait until the program loads intself into the system.

4) At the top of the screen, under LOAD menu choose Schedule. Select "fdg.sch" file and double click on it. Once loaded, select the desired sample number to start from. Choose option "UPON RECEIVING SIGNAL AT INTERFACE" instead of "AUTOMATIC". Click "OK"

5) At this point make sure that the sample is in the injector ready to be processed.

6) At the top of the screen, under RUN menu choose START.

7) Everyting from now on is done automatically.

Due to the complexity of preparation of the Method and Schedule files the procedure will not be describved here, instead preformulated files "fdg.met" and "fdg.sch" will be used. Please refer to the DIAMEX software manual for detailed instructions regarding formulation of the above mentioned files.

Sample Injection

With a 1ml syringe draw about 0.2 - 0.3ml of sample. Make sure to remove all of the air bubbles from the syringe by tapping it gently on the sides.

Make sure the injector is in the"LOAD" position and insert the needle. The injector is pneumatic and should return to the "LOAD" position after each run is completed. Gently inject the contents of the syringe.At this point the sample is in the injector ready to be processed.

Execute the "RUN" - "START" command on the computer to commence the sample analysis.

For detailed description of computer procedures refer to the paragraph above.

Data Analysis and Discussion

The chromatogram showed a tiny peak around 10 min for cold 2 - fluoro - 2 - deoxy - D - glucose (FDG). The size of the peak is consistent with the high specific activity of the final product. A relatively large peak at ~ 5 min due to D - glucose was also observed. D - Glucose is formed as a side product during the synthesis of FDG.

CHEMICAL QUALITY CONTROL

ANALYTICAL HPLC (PULSE AMPEROMETRIC DETECTION) OF

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Column: Dionex Carbopak PA1 column (250 x 4 mm)

Eluent: 100 mM NaOH in water

Flow rate: 1.0 mL/min

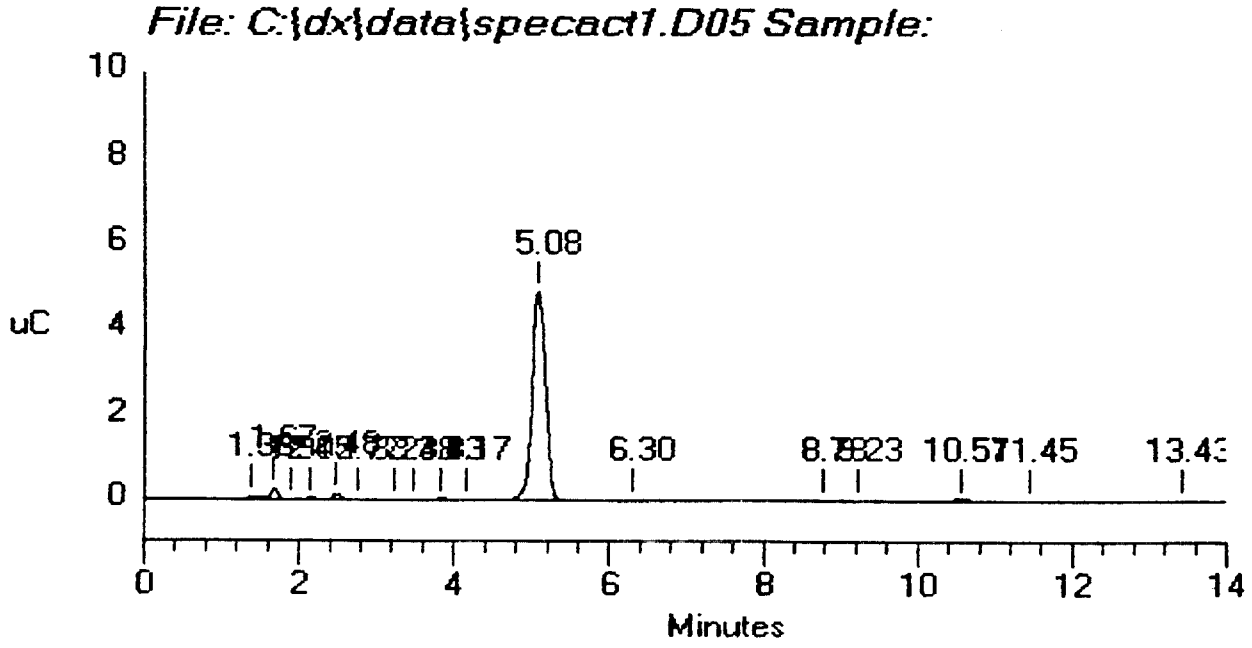
Chart speed: 1 cm/min

Detector: Dionex Pulse Electrochemical

```
==============================================================================
¦ Sample Name: 4                                  Date: Thu Sep 05 11:57:04 1996¦
¦ Data File  : C:\dx\data\specact1.D05                                         ¦
¦ Method     : C:\dx\method\fdg.met                                             ¦
¦ ACI Address: 1        System : 1        Inject#:   5  Detector: PED-IAMP    ¦
==============================================================================
```

REPORT	VOLUME	DILUTION	POINTS	RATE	START	STOP	AREA REJ
External	1	1	4200	5Hz	0.00	14.00	20000

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
15	10.57	fdg	0.003	25921	547360	1	-1.71
		Totals	0.003	25921	547360		



CHEMICAL QUALITY CONTROL

ANALYTICAL HPLC (PULSE AMPEROMETRIC DETECTION) OF

2-FLUORO-2-DEOXY-D-GLUCOSE STANDARD

Column: Dionex Carbopak PA1 column (250 x 4 mm)

Eluent: 100 mM NaOH in water

Flow rate: 1.0 mL/min

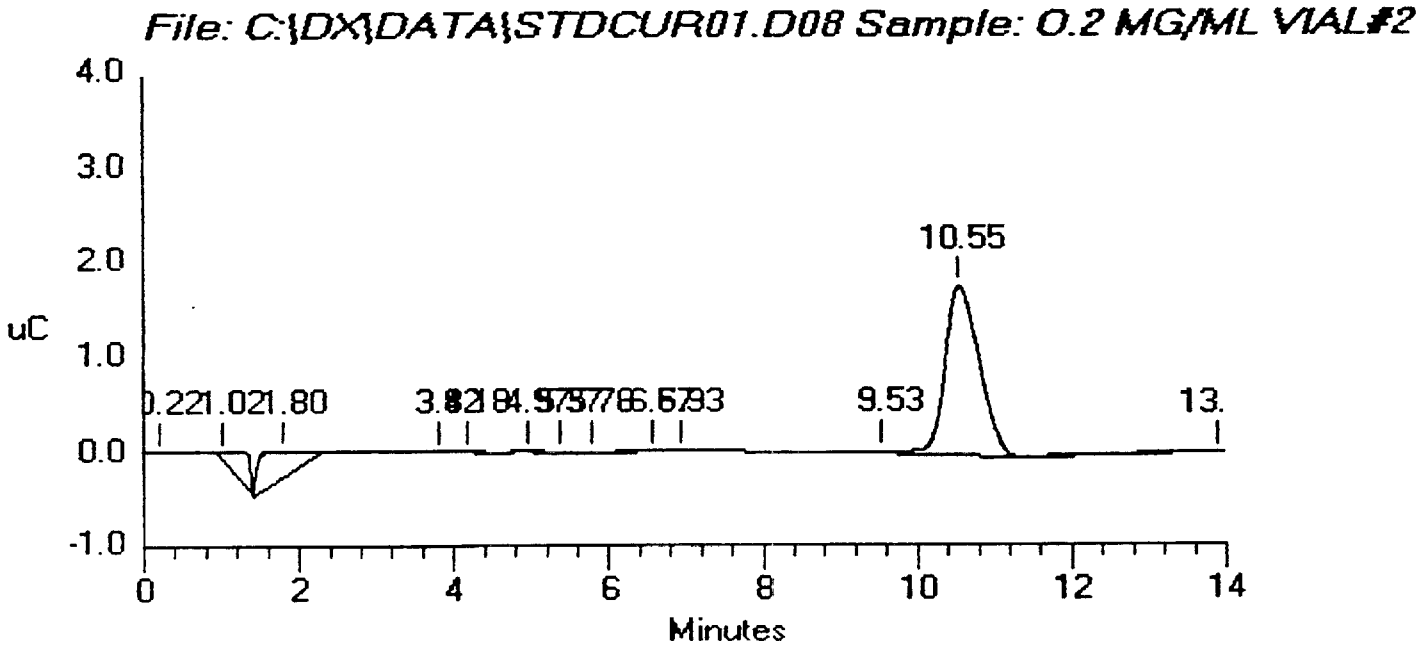
Chart speed: 1 cm/min

Detector: Dionex Pulse Electrochemical

```
==============================================================================
| Sample Name: 0.2 MG/ML VIAL#2                Date: Thu Aug 15 13:25:44 1996|
| Data File  : C:\DX\DATA\STDCUR01.D08                                       |
| Method     : C:\DX\METHOD\FDG.MET                                          |
| ACI Address: 1        System : 1        Inject#:   8  Detector: PED-IAMP  |
==============================================================================
```

REPORT	VOLUME	DILUTION	POINTS	RATE	START	STOP	AREA REJ
External	1	1	4200	5Hz	0.00	14.00	1000

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
12	10.55	fdg	0.298	1773093	54169662	2	1.44
		Totals	0.298	1773093	54169662		



RADIONUCLIDIC QUALITY CONTROL OF 2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE (FDG)

Introduction

The following protocol is used to determine the radionuclidic purity of a sample of 2-[F-18]fluoro-2-deoxy-D-glucose.

Materials

1) ORTEC Gamma-Ray Spectrometer
2) Gamma Vision Software
3) Capintec Radioisotope Calibrator Model CRC-15R

2-[F-18]Fluoro-2-deoxy-D-glucose Sample Preparation

For [F-18]fluoro deoxy glucose synthesis procedure refer to the Production Protocol described earlier.

Analysis and Discussion

The γ-ray spectrometer was first calibrated with a NBS standard radioactive source for energy and efficiency. A 1 μL capillary tube was dipped into a sample of FDG and the capillary tube was placed in a 5 mL glass beaker and kept in the counting chamber of the γ-ray spectrometer. The γ-ray spectrum showed only 511 keV peak attesting to the F-18 radionuclidic purity in the sample as 100%.

A 0.1 mL sample of FDG taken in a glass test tube was placed in the radioisotope calibrator chamber. The decrease of radioactivity with time was measured and from which the half-life of the isotope was determined to be 109.8 min consistent with the half-life of F-18 isotope.

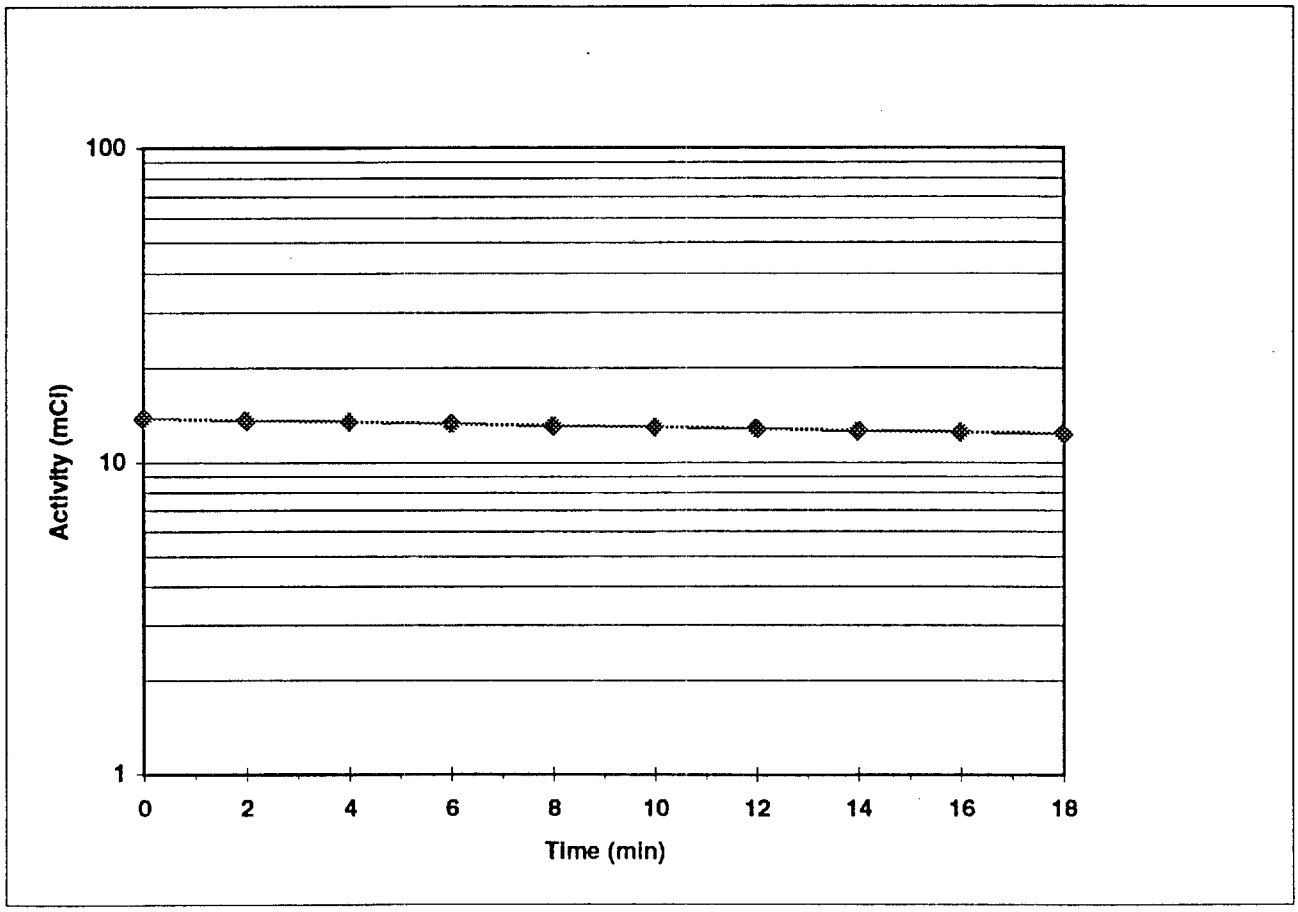
DECAY CURVE ANALYSIS FOR

[F-18] HALF LIFE

Time (min)	Observed Activity (mCi)	Calculated Half-Life (min)	Corrected Activity (mCi)
0	13.74		
2	13.57	111.3	13.74
4	13.40	110.6	13.74
6	13.23	109.9	13.74
8	13.06	109.2	13.74
10	12.90	109.9	13.74
12	12.74	110.1	13.74
14	12.58	110.0	13.74
16	12.42	109.8	13.74
18	12.26	109.5	13.74

Average Half-life **110.0** min

% Error From 109.8 min **0.21%**



PROCEDURE FOR TESTING STERILITY OF 2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Materials

1. Incubator set at 34°C
2. Thioglycollate medium, enriched with vitamin K1 and Hemin, 8 ml (Baxter Scientific)
3. 0.5 ml of sterile water (as negative standard), bacteria contaminated solution (as saliva for positive standard) and test specimens

Procedure

1. To use optimally: before use, boil thioglycollate tubes for 10 min to rid media of oxygen (have tubes slightly open to prevent any cracking of tubes)
2. Allow tubes to cool to room temperature
3. Add approximately 0.5 ml of specimen to thioglycollate tubes
4. Close tube leaving them slightly loose and incubate
5. Examine cultures for 7 days for evidence of growth
6. Report results as "No growth" or negative or positive if there is any growth

Reference

1. Saule, B.M. (ed.), 1983. The APIC Curriculum for Infection Control Practice Association for Practitioners in Infection Control.

2. Widmann, F.K. (ed.), 1985 AABB Technical Manual, Ninth edition, Arlington, VA, American Association of Blood Banks; pp 325-343.

PROCEDURE FOR TESTING PYROGENICITY OF 2[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Materials

1. Limulus amebocyte lysate (LAL) lymphylized
2. E-coli endotoxin 0.55: B5 10 mg lymphylized Kit Cat # N 284 from Whittaker Bioproducts, Inc. 8830 Biggs Ford Road, Walkersville, MD 21793-0127
3. Pyrogen free water-commercially available sterile water for injection (USP) is usually satisfactory
4. Pipettes: 1, 5, 10 ml and 100 µl - pyrogen free
5. 10 x 75 ml glass reaction tubes, pyrogen free - available from Whittaker Bioproducts, Inc. or may be rendered pyrogen free by heating at 180°C for 4 hr

Procedure

1. Set up pyrogen free reaction tubes (10 x 75 m) accordingly for standards and unknown samples in triplicate
2. Reconstitute control standard endotoxin (CSE) with required amount of pyrogen free water as stated on vial. Reconstituted CSE maybe stored at 2-8°C for up to 4 weeks
   a. Vortex well and dilute to concentrations of 1 EU/ml
   b. Make series of dilution of 0.5, 0.25, 0.12, 0.06 and 0.03 EU/ml for standards
   c. Use pyrogen free water as negative control
3. Reconstitute the lysate with pyrogen free water according to vial instructions. Swirl gently but thoroughly for at least 30 sec
4. Carefully transfer 100 µl of water, standards and test samples into appropriate reaction tubes
   (NOTE: Test specimens should be in the pH range of 6.0-7.5. If necessary pH adjust with pyrogen free acid or base. Do not adjust unbuffered solutions)
5. Add 100 µl of reconstituted LAL to each reaction tube, immediately mix and place in a 37°C water bath. After exactly 1 hr of incubation, remove tube from both and examine for gelation
6. Record results as either positive (gelation) or negative (no gelation)

Initial Quality Control - for lysate sensitivity

Endpoint dilution is determined as the last dilution of endotoxin which still yields a positive result

Assay Results
CSE DILUTIONS (EU/ml)

Replicates	0.5	0.25	0.12	0.6	0.3	H_2O	Endpt.
1	+	+	+	-	-	-	0.12
2	+	+	+	-	-	-	0.12
3	+	+	+	+	-	-	0.06
4	+	+	+	-	-	-	0.12

Lysate sensitivity is calculated by determining geometric mean of the end point. Each end point is converted to log 10. Individual log 10 values are averaged and the lysate sensitivity is taken as the antilog 10 of this average log value.

Calculation of Geometric Mean Endpoint

Endpoint EU/ml	Log 10 Endpoint
0.12	-0.921
0.12	-0.921
0.06	-1.222
0.12	-0.921

Mean = -0.996
Antilog 10 mean = 0.10 EU/ml
Data of endotoxin is unknown
Lysate sensitivity = 0.10 EU/ml

Determination of Endotoxin in Unknown Sample
Lysate Sensitivity = 0.10 EU/ml

Sample Dilution

Replicate	1/2	1/4	1/8	1/16	1/32	1/64
1	+	+	+	-	-	-
2	+	+	+	+	-	-

Endpoint dilution	Log 10 endpoint
1/8 (0.125)	-0.903
1/16 (0.0625)	-1.204
	Mean -1.054

Antilog 10 mean = 0.088 = 1/11.3

Endotoxin Conc. = lysate sensitivity x endpoint dilution
= 0.10 x 11.3 = 1.13 EU/ml

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UCLA RADIOPHARMACY DELIVERY SERVICE

PRODUCT: 2-[F-18]Fluoro-2-Deoxy-D-Glucose DATE: 6-3-96

LOT NO.: 6-3-96-1

FORMULATION: Fluorine-18 Fluorodeoxy - D - Glucose

PURPOSE: PET Research/Clinic

CONTAINER: 30 ml. sterile glass vial

CLOSURES: Rubber stopper
Aluminum seal

Test	Sampling Requirements (mL)	Limits	Results
pH	0.1	3.5-8.5	7.0
Appearance	stock	clear, colorless	√
Bacterial Endotoxin	0.5	175 EU/v	negative
Sterility	1.0	sterile	no growth

LITERATURE REFERENCES

FOR

2-[F-18]FLUORO-2-DEOXY-D-GLUCOSE

Efficient Stereospecific Synthesis of No-Carrier-Added 2-[^{18}F]-Fluoro-2-Deoxy-D-Glucose Using Aminopolyether Supported Nucleophilic Substitution

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An aminopolyether mediated synthesis of fluorine-18 (^{18}F) 2-fluoro-2-deoxy-D-glucose (FDG) has been developed. The nucleophilic fluorination with accelerator-produced [^{18}F]fluoride works at the no-carrier-added level and gives epimerically pure 2-^{18}FDG with an uncorrected radiochemical yield of a maximum 50% in a synthesis time of ~ 50 min from EOB.

J Nucl Med 27:235–238, 1986

In conjunction with positron emission tomography (PET), 2-[^{18}F]fluoro-2-deoxy-D-glucose (2-FDG) is presently the most important radiopharmaceutical and is used to measure regional cerebral glucose metabolism (*1*). The broad application of this radiolabeled carbohydrate leads to a variety of alternative syntheses with the aim of providing higher radiochemical yields and increasing the stereoselectivity of the fluorination reaction.

The synthesis routes of 2-FDG include electrophilic fluorinations with ^{18}F-F_2 (*2–4*), Xe$^{18}F_2$ (*5–7*), or acetylhypofluorite (*4,8–10*) as fluorinating agents and nucleophilic reactions with anhydrous [^{18}F]fluoride (*10,11*). Although the electrophilic reaction of acetylhypofluorite with tri-*O*-acetyl-D-glucal (*9*) is the most commonly used method to produce 2-FDG for medical research, the synthesis exhibits only limited stereospecificity (*12*) similar to the reaction of F_2 with D-glucal. Any electrophilic process starting from ^{18}F-F_2 leads to a loss of 50% in addition to losses by lacking stereospecificity of the available fluorine-18 (^{18}F). Furthermore, the product has only low specific activity in the order of a few Ci/mmol.

The synthetic procedure starting from [^{18}F]fluoride has several advantages. In contrast to the production of carrier-added ^{18}F by the ^{20}Ne(d,α)^{18}F reaction in the presence of $^{19}F_2$, the [^{18}F]fluoride can be obtained with very high specific activity (no-carrier-added), e.g., from the nuclear reaction of ^{18}O(p,n)^{18}F using an oxygen-18 (^{18}O) enriched water target (*13*). This reaction can be carried out with a small 10 MeV proton accelerator.

Three successful nucleophilic syntheses of 2-^{18}FDG are published. One based upon the replacement of the triflate group of methyl 4.6-*O*-benzylidene-3-*O*-methyl-2-*O*-trifluormethanesulfonyl-β-D-mannopyranoside by $^{18}F^-$ (*10*) and the other on the reaction of [^{18}F]fluoride with methyl 4.6-*O*-benzylidene-2.3-*O*-sulfuryl-β-D-mannopyranoside (*11*). The substitution of the triflate-group proceeds with a yield of about 30%, but the difficulty in removing the methyl group from the 3-*O*-position reduced the overall yield significantly (~10%). The method developed by Tewson (*11*) leads to an excellent incorporation of $^{18}F^-$ into the cyclic sulfuryl-compound (>90%), but the hydrolysis of the glycoside resulted in a considerable reduction of the radiochemical yield to about 40% or less. A paper has recently been published describing the reaction of anhydrous no-carrier-added KH[^{18}F]F_2 with 1.2-anhydro-3.4:5.6-di-*O*-isopropylidene-1-C-nitro-D-mannitol (*14*). Hydrolysis of the ^{18}F-labeled derivative with trifluoroacetic acid afforded 2-^{18}FDG in a radiochemical yield of 10%.

The goal of this study was to use (a) the tetraacetylated D-mannose, i.e., 1,3,4,6-tetra-*O*-acetyl-2-trifluormethanesulfonyl-β-D-mannopyranose (*1*, Fig. 1) as a precursor, and (b) the aminopolyether potassium complex [K/2.2.2]$^+$$^{18}F^-$ as a phase-transfer catalyst. This

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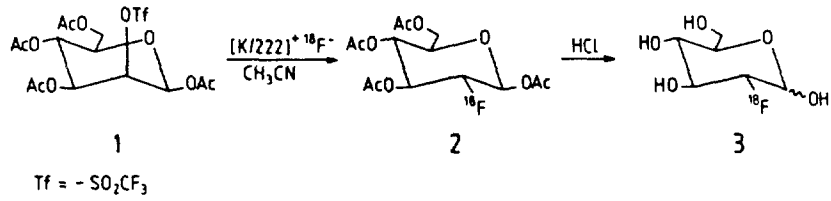

FIGURE 1
Reaction scheme—syntheses of 2-fluoro-2-deoxy-D-glucose

complex has recently been shown to allow a mild and efficient nucleophilic fluorination at a no-carrier-added level (*15–17*). The resulting increase of nucleophilicity greatly facilitates the fluorination procedure. By using the tetraacetylated precursor which can be selectively prepared (*19*), the removal of the protecting groups can be carried out rapidly under mild conditions and hence higher yields of 2-FDG can be obtained.

MATERIALS AND METHODS

Accelerator Production of [^{18}F]Fluoride

Fluoride-18 was produced* by the $^{20}Ne(d,\alpha)^{18}F$ reaction using a Ne (15% H_2) target (*18*) to produce no-carrier-added ^{18}F-HF, which was removed from the target wall after bombardment by rinsing with triply distilled water. The conversion of no-carrier-added ^{18}F activity to a reproducible and reactive fluoride labeling system using the bicyclic aminopolyether Kryptofix 222† was carried out similar to the method reported previously (*15–17*).

Cold Syntheses

The synthesis and characterization of the precursor 1.3.4.6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-*β*-D-mannopyranose have been published elsewhere (*19*).

For the purpose of confirming the structure of the synthetic product by ^{19}F nuclear magnetic resonance (NMR) spectroscopy, it was necessary to carry out an inactive synthesis to obtain macroscopic amounts of unlabeled 2-fluoro-2-deoxy-D-glucose. The reaction scheme is shown in Fig. 1. The experimental details for the synthesis are as follows: 1.3.4.6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-*β*-D-mannopyranose (0.48 g; 1 mmol) and 0.38 g (1 mmol) of the cryptand Kryptofix 222 were dissolved in 10 ml of dry acetonitrile and heated under reflux for 15 min in the presence of 41 mg (0.7 mmol) KF and 25 mg (0.15 mmol) K_2CO_3. The residue was filtered off and the solution concentrated on a rotary evaporator to dryness (bath-temperature <50°C). To remove the phase-transfer catalyst and inorganic components the viscous residue was extracted three times with 5 ml of water and then heated under reflux in the presence of 10 ml of 1*M* hydrochloric acid for 20 min. The acid solution was deionized using ion retardation resin AG11A8 (50–100 mesh)‡, concentrated to dryness and dissolved in D_2O to measure the ^{19}F-NMR spectrum.

Sugar Analysis

The crude reaction mixture of the 2-FDG synthesis was analyzed by anion exchange chromatography (AEC) of the sugar borate complexes. The AEC was performed with an automated sugar analyzer Biotronik ZA 5100§ as described previously (*20*).

Thin layer chromatography (TLC) was performed on silica gel 60† with the solvent system acetonitrile/water (95:5). The spray reagent orcinol-sulfuric acid was used for detection. The radiochemical purity was ascertained by TLC on monosodium phosphate impregnated silica plates (*12*). This modified TLC method allows the separation of FDG and FDM by developing the plates several times with CH_3CN/H_2O, (95:5) as eluent. Radio high performance liquid chromatography (HPLC) on Lichrosorb-NH_2 (*10*) (column 250 × 4¶, eluent CH_3CN/H_2O, 95:5, flow: 1 ml/min) of the no-carrier-added product was compared with authentic samples of 2-FDG/2-FDM.

Reactive [^{18}F]Fluoride Labeling System

In a cylindrical reaction vessel of pyrolytic carbon (Sigradur-G, 18 × 70 mm)** the aqueous solution of no-carrier-added ^{18}F (0.5–1 mCi for the test runs and 20–50 mCi for a production run) was added to a solution of 4.6 mg (0.03 mmol) potassium carbonate and 26 mg (0.06 mmol) Kryptofix 222 in acetonitril-H_2O (86:14) (v/v). At an oil bath temperature of about 105°C the solution was purged with helium (~50 ml/min) and concentrated to dryness. After the solvent was removed (~3 min) the drying process was extended for about 3 min to remove traces of remaining water.

Synthesis of 2-^{18}FDG

A solution of 20 mg (0.04 mmol) 1.3.4.6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-*β*-D-mannopyranose in 1 ml anhydrous acetonitrile was added to the dry residue of [^{18}F] fluoride containing aminopolyether (APE)-complexed potassium carbonate. This mixture was heated under reflux for about 5 min. The solution was concentrated to ~0.4 ml, transferred into a syringe with about 5 ml distilled water, and passed through a C18 SEP-PAK cartridge†† which had been previously washed with 2 ml of THF and 5 ml of water. Residual

aminopolyether was desorbed completely by washing the C18 SEP-PAK cartridge with 5 ml of hydrochloric acid (0.1 mol/1). The acetylated carbohydrates were subsequently eluted from the cartridge with 2 ml THF and the solution evaporated to dryness in the Sigradur reaction vessel. Two milliliters of 1*M* hydrochloric acid was added and heated under reflux for 15 min (bath temperature: 130°C). The hydrolysate was decolorized by passing through the same C18 SEP-PAK cartridge as used before. The carbon vessel was rinsed with 1 ml of water and used to elute residual ^{18}FDG from the cartridge. For deionizing the hydrolysate, the acid solution was transferred to a column packed with AG11A8 retardation resin and neutral aluminium oxide 90† (*4*). The resulting neutral eluent was adjusted to an isotonic solution and finally sterilized by passage through a Millipore filter (0.22 μm).

RESULTS AND DISCUSSION

Fluorination of 1.3.4.6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-β-D-mannopyranose using APE-complexed potassium fluoride on a macroscopic scale yielded peracetylated 2-fluoro-2-deoxy-D-glucose (*2*, Fig. 1), in ~ 50%. Based on the ^{19}F-NMR data of the crude synthetic product after acid hydrolysis, it is obvious that the nucleophilic substitution gave pure 2-^{19}FDG without any formation of the epimer 2-fluoro-2-deoxy-D-mannose.

The chemical shifts observed were in agreement with data available from the literature for 2-FDG (*4,21*). The δ-values found for 2-FDG were 32.44 ppm (α-anomer) and 32.26 ppm (β-anomer) with a ^{1}H-^{19}F coupling constant of $J_{H\text{-}2,F}$ = 50 Hz and $J_{H\text{-}3,F}$ = 15 Hz, respectively. TLC of the hydrolized crude reaction product showed two major products, with Rf 0.37 identical to 2-FDG and a spot with Rf 0.05 which was due to aldohexoses. Ion exchange chromatography of the sugar borate complexes demonstrated that besides 2-FDG only glucose could be detected in the acid hydrolysate of the crude reaction mixture.

The displacement of the triflate group using the aminopolyether-complexed potassium salt of no-carrier-added [^{18}F]fluoride in the presence of potassium carbonate occurred within less than 5 min (80° C). Although some decomposition was observed during refluxing of acetonitrile, the no-carrier-added [^{18}F]fluoride was incorporated with formation of acetylated 2-^{18}FDG to about 95%. Labeling results of no-carrier-added substitution reaction are reproducible since the amount of [^{18}F]fluoride remaining on the wall of the glassy carbon reaction vessel is <3%. The acetylated 2-^{18}FDG and the excess of triflate were separated from the water-soluble components using a C18 SEP-PAK cartridge that adsorbed the lipophilic substances while the hydrophilic aminopolyether (Kryptofix 222) and inorganic salts were eluted completely by water-acetonitrile (90:10) and subsequently washed with 0.1*M* hydrochloric acid. After desorption of the labeled acetylated carbohydrate from the cartridge, the final step was to remove the acetyl groups to give 2-^{18}FDG.

In contrast to the etherified and glycosylated sugar-derivatives (*10,11*) the acetyl groups could be removed easily using acid hydrolysis conditions as described for the corresponding glucal derivative (*9*).

The light-yellow acid hydrolysate containing the unprotected 2-^{18}FDG could be decolorized by filtration through the same C18-cartridge used before. The 2-^{18}FDG solution was neutralized by an ion retardation resin and traces of fluoride were adsorbed on aluminium oxide. After this purification step the uncorrected yield of 2-[^{18}F]fluoro-2-deoxy-D-glucose was 44±4% (n = 7) and the total time for the preparation was 45 to 50 min. Within experimental error no change in radiochemical yield was observed for runs ranging from 1 to 50 mCi of no-carrier-added [^{18}F]fluoride. TLC (MeCN:H_2O/95:5) of the FDG solution has shown that ~ 99% of the [^{18}F]fluorine was present as 2-^{18}FDG (Rf 0.37) whereas only 0.5 to 1% of the $^{18}F^-$ activity was located at the starting point. The TLC on monosodium phosphate impregnated silica plates as described by van Rijn et al. (*12*) makes it feasible to separate the epimeric sugars FDG and FDM. Using this modified TLC method, only one radioactive component with a Rf-value equivalent to that of FDG appeared to be present.

Additionally, the isocratic HPLC (Lichrosorb-NH_2 column) gave the same retention time for the radiochemical product and the authentic 2-FDG sample (HPLC retention time 3.9 min). As in the case of the TLC, radiochemical impurities were not detected.

The nucleophilic ^{18}F fluorination was also performed satisfactorily in anhydrous THF but the reaction time of 25 to 30 min was significantly longer than in the dipolar solvent acetonitrile (5 min).

CONCLUSION

The advantage of the synthetic method presented here is the high yield (max. 55% uncorrected) of no-carrier-added 2-^{18}FDG based on the phase-transfer mediated substitution of triflate by [^{18}F]fluoride. The stereochemical specificity of the nucleophilic displacement combined with a rapid hydrolysis of the acetylated sugar derivative makes it possible to synthesize epimerically pure 2-^{18}FDG with high specific activity. The synthesis of 2-^{18}FDG was carried out successfully with larger quantities of [^{18}F]fluoride suitable for clinical use. In addition, the precursor 1.3.4.6 tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-β-D-mannopyranose can be easily prepared in a two step reaction starting with D-mannose.

FOOTNOTES

* Jülich (Compact cyclotron CV-28), Jülich, FRG.
† E. Merck AG, Darmstadt, FRG.
‡ BIO-RAD, Richmond, CA.
§ Biotronik, Munich, FRG.
¶ Merck, Darmstadt, FRG.
** SIGRI, Meitingen, FRG.
†† Waters Chromatography Div., Millipor, MA.

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Computer-controlled Radiochemical Synthesis: A Chemistry Process Control Unit for the Automated Production of Radiochemicals

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A computer-controlled general purpose chemistry process control unit (CPCU) suitable for the automated production of radiochemicals has been developed. This valve-and-tubing synthesis system can be user programmed to accommodate a variety of chemical processes. In a practical demonstration of its utility, the CPCU has been configured and programmed to synthesize 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-[^{18}F]FDG) using aqueous [^{18}F]fluoride ion. Using this instrument, the yield of 2-[^{18}F]FDG from [^{18}F]fluoride ion is 54.9% ($\pm$11.2%, n = 125) corrected to EOB, after a synthesis time of 50–55 min. The average total activity produced (for runs of 5–10 μA) is 28.1 mCi/μA ($\pm$5.03 mCi/μA). Thus, the amount of 2-[^{18}F]FDG produced from a 10 μA for 1 h bombardment was 154.3 mCi ($\pm$27.4 mCi). The unit has been similarly configured and programmed to synthesize 2-deoxy-2-[^{18}F]fluoro-D-mannose (48% EOB), 3-(2′-[^{18}F]fluoroethyl)spiperone (29% EOB), and [^{18}F]fluoroacetate (66% EOB) from aqueous [^{18}F]-fluoride ion, and 2-[^{18}F]FDG from gaseous acetyl hypo[^{18}F]fluorite (20% EOB).

Introduction

Successful development and operation of commercial enterprises and university- or hospital-based research centers that utilize various radiochemicals requires the routine availability of these labeled compounds. The key to this availability has been either to support the large professional and technical staff required for daily and/or on-demand production of the various agents labeled with in-house cyclotron-produced radioisotopes, or to establish access to an external source, such as an off-site cyclotron facility or commercial distribution center, for the requisite radiochemical precursors (which may or may not undergo further chemical processing). With the aim of dramatically reducing the personnel requirements associated with these efforts, an instrument for automated remote radiochemical syntheses has been developed (Schmidt *et al.*, 1987a,b; Padgett *et al.*, 1987; Schmidt *et al.*, 1987c). This unit allows routine fully automated in-house preparation of labeled compounds without the dependence on numerous highly trained research personnel. Indeed, after menu-oriented product selection, somplete radiochemical synthesis, including product purification, may be carried out without further operator intervention.

While the value of developing totally automated complex radiochemical syntheses has been appreciated for many years, and although this goal has been approached by several reports of remote semi-automated chemical synthesis systems (Barrio *et al.*, 1980, 1981; Fowler *et al.*, 1981; Padgett *et al.*, 1982a,b; Berger *et al.*, 1979; Langstrom *et al.*, 1982), it has only been recently that this idea has reached fruition for on-line gas processing (Welch and Kilbourn, 1985; Strijckmans *et al.*, 1985), and more complex radiochemicals (Iwata *et al.*, 1984; *Davis et al.*, 1982; Brodack *et al.*, 1987, 1986; Alexoff *et al.*, 1986). In addition to allowing a research or commercial production program requiring these labeled compounds to operate with reduced highly trained personnel capability means lowered radiation exposure to the technical staff and the reliability that comes with computer-control of complex processes.

We report herein the development of a fully automated computer-controlled general purpose chemistry process control unit (CPCU) (CTI Cyclotron Systems, Inc., Berkeley, California, U.S.A.). In order to demonstrate its potential, this unit has been configured and programmed to synthesize a variety of radiochemicals labeled with the cyclotron-produced radionuclide fluorine-18 ($t_{1/2}$ = 109.7 min) using both aqueous [^{18}F]fluoride ion (nucleophilic fluorination) and gaseous acetyl hypo[^{18}F]fluorite (electrophilic fluorination) as the labeled chemical precursors. For example, 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-[^{18}F]FDG) has been synthesized from aqueous [^{18}F]fluoride ion generated by the bombardment of an expendable [^{18}O]water target. The pure 2-[^{18}F]FDG is obtained in good yields (average 54.9%, $\pm$11.2%, corrected to EOB, n = 125) after a synthesis time of 50–55 min. The CPCU has also been programmed to prepare 2-deoxy-2-[^{18}F]fluoro-D-mannose, 3-(2′-[^{18}F]fluoroethyl)spiperone, and [^{18}F]fluoroacetate from aqueous [^{18}F]fluoride ion, and, to demonstrate functional and chemical compatibility with gaseous radiolabeled precursors, [^{18}F]FDG from gaseous acetyl hypo[^{18}F]fluorite.

Materials and Methods

(A) Synthesis of Positron-emitter Labeled Radiochemicals Using [^{18}F]Fluoride Ion

1. Production of [^{18}F]fluoride ion

No-carrier-added [^{18}F]fluoride ion was produced via the ^{18}O(p,n)^{18}F nuclear reaction by proton bombardment of 98–99% enriched [^{18}O]water (Mound Facility, Miamisburg, Ohio; Isotec, Inc., Dayton, Ohio). The target holder used was constructed of copper and electroless plated with nickel. A vacuum window of 0.025 mm aluminum and a target window of 0.013 mm Havar reduce the measured extracted proton energy of 11 MeV to 10.7 MeV entering the target water. A water cavity of 10 mm dia and 2.0 mm depth was used, resulting in centerline thickness of 2.15 mm due to outward deflection of the window with the target pressurized to 15 psig. A 0.2 mL reflux chamber is provided above the minimum loaded water level and extra target water is added to provide make-up for radiolysis and reflux redistribution.

The target was remotely loaded using a syringe pump through a fill port that accessed the target chamber via the top of the target. A second port at the top of the target was used for venting during loading, irradiation and

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pressurization for irradiated water transfer. During irradiation, the target body is water cooled (typically 12–15°C) and the aluminum and Havar foils are cooled with recirculated helium gas (flow rate = 100 L/min).

After irradiation, the [^{18}O]water containing the [^{18}F]-fluoride ion is transferred to the CPCU from a valved port at the bottom of the target with 0.1–0.5 psig nitrogen gas pressure through as much as 25 m of 0.86 mm i.d. polyethylene tubing. Typically, 90% of the initial target charge of [^{18}O]water is recovered after irradiation.

2. Synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose

The synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-[^{18}F]-FDG) was adapted from the method of Hamacher *et al.*, 1986). The [^{18}O]water [^{18}F]fluoride ion (0.3 mL) was delivered into a cylindrical Pyrex vessel (17 × 112 mm), the "fluorination vessel" (VES1, Fig. 3), containing a solution of the aminopolyether 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix 222) (10.0 mg, 0.027 mmol) and potassium carbonate (1.0 mg, 0.007 mmol) in acetonitrile–water (96:4, 1.0 mL). Nitrogen gas flow (30 mL/min) was introduced through Teflon tubing (1/16 in. o.d.) extending to the bottom of the vessel and the vessel was immersed in an oil bath heated to 110–115°C for 3 min. During this time, a flow of heated air (125°C) was directed across the top of the vessel. At the end of this period, the vessel was removed from the bath and acetonitrile (1.0 mL) was added to the residue. The vessel was reimmersed in the 110–115°C oil bath for another 3 min. This addition, evaporation sequence was repeated two additional times. To the dry [^{18}F]fluoride ion/K_2CO_3/K222 residue was added acetonitrile (0.5 mL), followed by a solution of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-beta-D-mannopyranose (mannose triflate, 10.0 mg, 0.02 mmol) in acetonitrile (1.0 mL). The reaction mixture was mixed by bubbling nitrogen gas through the solution as before and then was placed in an oil bath maintained at 80–85°C for 5 min. The vessel was then removed from the oil bath, and after cooling briefly (cool air bath, 30 s), ethyl ether (4.0 mL) was added. The resulting ether/acetonitrile solution was passed through a silica gel cartridge (Waters Associates Sep-Pak silica cartridge, No. 51900) into a second Pyrex vessel, the "hydrolysis vessel" (VES2). The solvents were evaporated with nitrogen flow and heat (110–115°C). A second portion of ether (4.0 mL) was added, transferred through the silica gel cartridge and evaporated as before.

In order to obtain a sample of the fluorinated intermediate for ^{1}H- and ^{19}F-NMR analysis, a synthesis was carried out starting with the mannose triflate (100 mg), potassium fluoride (1.0 g), and K222 (6.0 g) in acetonitrile for 10 min at 80°C. The desired intermediate, 2-deoxy-2-[^{19}F]fluoro-1,3,4,6-tetra-*O*-acetyl-beta-D-glucopyranose, was obtained in 5% yield (as determined by gas chromatography), and gave the expected spectra (^{19}F-NMR ($CDCl_3$/$CFCl_3$) 201.6 ppm (lit. (Adamson *et al.*, 1970) 201.4 ppm); ^{1}H-NMR ($CDCl_3$/TMS) δ 2.05, 2.08, 2.09, 2.18 (4s, 3H, CH_3), 3.86 (ddd, 1H, J_{5-6a} = 4.0 Hz, J_{5-6b} = 2.1 Hz, J_{4-5} = 9.7 Hz, H_5), 4.1 (dd, 1H, J_{6a-6b} = 12.6 Hz, H_{6a}), 4.3 (dd, 1H, H_{6b}), 4.45 (ddd, 1H, J_{2-1} = 8.0 Hz, J_{2-3} = 8.7 Hz, J_{2-F} = 51 Hz, H_2), 5.07 (dd, 1H, J_{4-3} = 9.4 Hz, H_4), 5.38 (ddd, 1H, J_{3-F} = 14.2 Hz, H_3), 5.79, 1H, J_{1-f} = 3.0 Hz, J_{1-2} = 8.0 Hz, H_1).

To the residue of the intermediate 2-deoxy-2-[^{18}F]-fluoro-1,3,4,6-tetra-*O*-acetyl-beta-D-glucopyranose (radioTLC: R_f = 0.51, silica gel 60, ether) was added hydrochloric acid (1 M, 1.0 mL). The acid solution was mixed by bubbling nitrogen through the mixture as before and then was placed in an oil bath at 110–115°C. After heating for 15 min, the aqueous solution was transferred to the top of a column (11.0 × 0.7 cm i.d.) packed with ion-retardation resin (Bio-Rad Laboratories AG 11 A8). The output of this column is connected in succession to an alumina cartridge (Waters Associates Sep-Pak alumina N cartridge) and a C-18 cartridge (Waters Associates Sep-Pak C-18 cartridge). The product is eluted by the addition and subsequent transfer of three portions of water (5.0 mL) to the hydrolysis vessel, and is collected in a serum vial. Radio thin-layer chromatography demonstrated that the product 2-[^{18}F]FDG had the same retention factor as the literature value (Hamacher *et al.*, 1986) (R_f = 0.35, silica gel 60, acetonitrile/water, 95:5), and was found to be >99% pure. When carrier KF is added to the initial reaction mixture, the final product also produced a ^{19}F-NMR spectrum consistant with the literature (Bida *et al.*, 1984) (D_2O/hexafluorobenzene (external)) +32.73 ppm (alpha-anomer), +32.60 ppm (beta-anomer)). The yield of 2-[^{18}F]FDG from [^{18}F]fluoride ion is 54.9% (±11.2%, n = 125) corrected to EOB, after a synthesis time of 50–55 min. The average total activity produced (for runs of 5–10 μA) is 28.1 mCi/μA (±5.03 mCi/μA). Thus, the amount of 2-[^{18}F]FDG produced from a 10 μA 1 h bombardment was 154.3 mCi (±27.4 mCi). Chemical stability of the 2-[^{18}F]FDG product was determined by radioTLC analysis every 6 h for 24 h; no decomposition products were observed.

3. Synthesis of 2-deoxy-2-[^{18}F]fluoro-D-mannose

The synthesis of 2-deoxy-2-[^{18}F]fluoro-D-mannose (2-[^{18}F]FDM) (Luxen *et al.*, 1986a) was very similar to that described above for 2-[^{18}F]FDG except for the following changes.

The dry [^{18}F]fluoride ion/K_2CO_3/K222 residue, prepared as described above, was reacted with a solution of the protected glucose triflate, methyl 3-*O*-benzyl-4,6-*O*-benzylidine-2-*O*-trifluoromethanesulfonyl-beta-D-glucopyranoside (10 mg, 26.7 μmol) in acetonitrile (1.0 mL) for 30 min. After transfer of the [^{18}F]fluorinated intermediate through the silica gel cartridge and evaporation of the ether, the hydrolysis was carried out using 6 M hydrochloric acid (1.0 mL) for 30 min at 120°C. Subsequent purification (Luxen *et al.*, 1986a) (i.e. larger ion-retardation resin column to neutralize the acid) gave the desired 2-[^{18}F]FDM which was >99% pure by radioTLC (R_f = 0.35, silica gel 60, acetonitrile/water, 95:5). Starting with 8.4 mCi of [^{18}F]fluoride ion, 2.15 mCi of 2-[^{18}F]FDM was obtained after a synthesis time of 100 min.

4. Synthesis of 3-(2′-[^{18}F]fluorethyl)spiperone

For the synthesis of 3-(2′-[^{18}F]fluoroethyl)spiperone ([^{18}F]-FESP) (Satyamurthy *et al.*, 1986) the CPCU plumbing was configured as follows to allow carrying out the published procedure: the silica gel cartridge was replaced by a C-18 cartridge and the output from this column was connected to the common port of a 3-way Teflon valve located on the back of the CPCU. The normally open port of the valve was directed to a waste vial, and the normally closed port was connected to the product vial. The reservoir position taken by ether for the sugar syntheses is replaced by water, and an additional reservoir of methanol was added. Thus only VES1 of the CPCU (*vide supra*) was used (for preparation of the dry [^{18}F]fluoride ion and the subsequent fluorination reaction) and the product was removed for purification by preparative HPLC.

To the dry [^{18}F]fluoride ion was added a solution of 3-(2′-bromoethyl)spiperone (2 mg, 4 μmol) in acetonitrile (1.0 mL), and the resulting mixture was heated for 20 min at 110°C. After removal of VES1 from the oil bath, water (4.0 mL) was added, and the aqueous mixture transferred through the C-18 cartridge with the solvents collecting in the waste vial. Additional water was added (2 × 4.0 mL) and collected in the same way. The valve was then activated, methanol (2.0 mL) was added to VES1, and the solvent was transferred through the C-18 cartridge eluting the 3-(2′-[^{18}F]fluoroethyl)spiperone into the product vial. RadioTLC analysis (silica gel 60, chloroform/methanol 90:10) of the product solution showed two peaks corresponding to [^{18}F]fluoride ion (R_f = 0.0, 6.45%) and

(a)

(b)

Fig. 1. Photo

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orrected to EOB.
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A) is 28.1 mCi/μA
f 2-[^{18}F]FDG pro-
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hyl)spiperone ([^{18}F]-
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as added. Thus only
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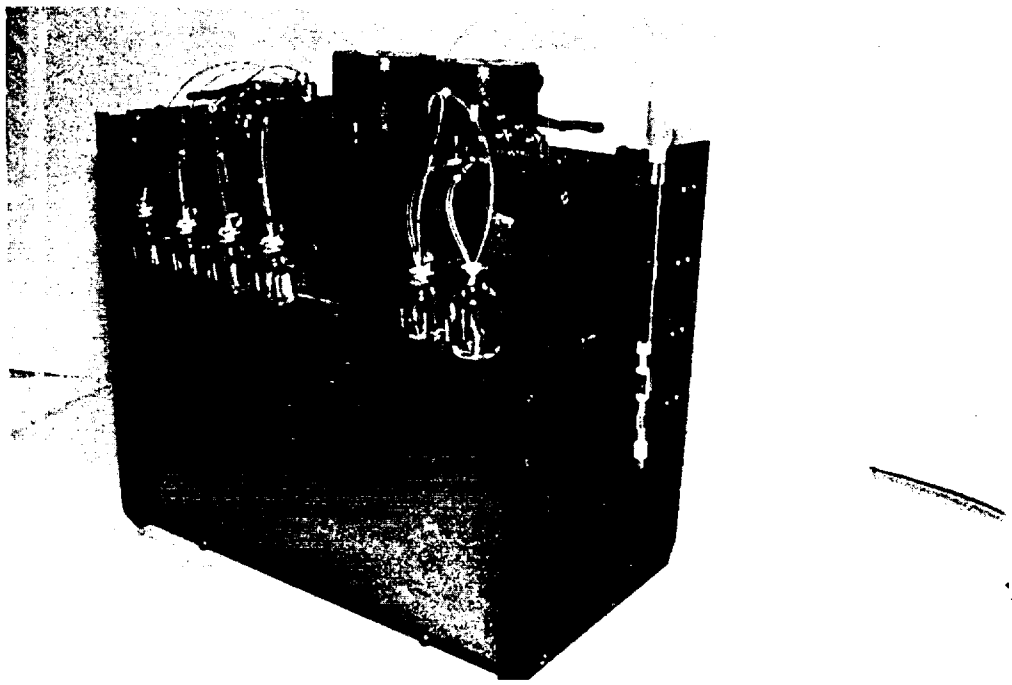
(a)



(b)

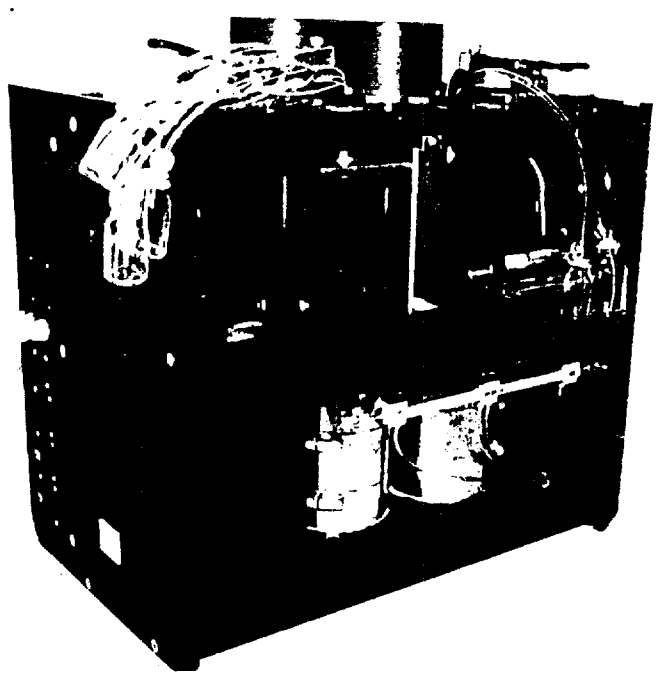


Fig. 1. Photographs of CPCU. (a) Set-up for synthesis of 2-[^{18}F]FDG. (b) With front cover removed showing interior components.

[^{18}F]FESP (R_f = 0.40, 93.55%). Starting with 2.7 mCi of [^{18}F]fluoride ion, 0.605 mCi of product solution was obtained after a synthesis time of 57 min.

5. Synthesis of [^{18}F]fluoroacetate

The synthesis of [^{18}F]fluoroacetate ([^{18}F]FA) (Sykes *et al.*, 1986) was carried out using the same unit operations and CPCU configuration as used for the synthesis of the labeled sugars with the following changes.

To the [^{18}F]fluoride ion/K_2CO_3/K222 residue, prepared as described above, was added benzyl bromoacetate (2 mg, 8.7 μmol) in acetonitrile (1.0 mL), and the mixture was heated as before for 5 min. After transfer to VES2, to the residue containing benzyl [^{18}F]fluoroacetate was added 3 N sodium hydroxide (1.0 mL), and the hydrolysis was achieved by heating at 110°C for 5 min. The basic solution was then transferred directly to the product vial by the successive additions of water (3 × 5 mL), and the solution was brought to pH 7 by the addition of 3 N hydrochloric acid (1.0 mL). Analysis of the product solution by radio-HPLC (Bio-Rad Laboratories HPX-87H column, 300 × 7.8 mm, with Brownlee Labs Polypore H guard column, 4.6 × 30 mm, 0.01 M sulfuric acid, flow rate = 0.4 mL/min) showed one peak (R_t = 27 min) corresponding to [^{18}F]FA. Starting with 4.8 mCi of [^{18}F]fluoride ion, 2.42 mCi of [^{18}F]fluoroacetate was obtained after a synthesis time of 42 min.

(B) Synthesis of Positron-emitter Labeled Radiochemicals Using Gaseous Acetyl Hypo[^{18}F]fluorite

1. Synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose

The synthesis of 2-[^{18}F]FDG by electrophilic [^{18}F]fluorination was carried out essentially as described in the literature (Bida *et al.*, 1984). The CPCU plumbing was modified to allow the bubbling of the gaseous acetyl hypo[^{18}F]fluorite ([^{18}F]AcOF) to occur with the VES1 opening sealed by the silicon stopper; the exiting gas stream passed through a soda lime cartridge to trap unreacted [^{18}F]AcOF. The exit of the trap was connected to a Teflon 2-way valve. Only one solvent reservoir was used. The "fluorination vessel" used for the fluoride ion-based syntheses was replaced by a longer Pyrex vessel (17 × 130 mm). The [^{18}F]AcOF was then bubbled (flow rate = 150 mL/min) through a solution of 3,4,6-tri-*O*-acetyl-D-glucal (45 mg, 165 μmol) in Freon-11 ($CFCl_3$) (15 mL) at 25°C. After the target emptying was completed, the soda lime trap valve was closed, and VES1 was lowered into the oil bath to evaporate the remaining $CFCl_3$. Ether was then added and the transfers and subsequent steps were exactly the same as for the [^{18}F]fluoride ion-based synthesis of 2-[^{18}F]FDG described above in Section (A)2. The radiochemical yield was calculated by measuring the activity distribution. The product 2-[^{18}F]FDG solution, obtained after a synthesis time of 60 min, contained 19.7% of the activity delivered to the CPCU, and radioTLC analysis confirmed a single product. The sodium acetate trihydrate cartridge contained 62.5%, and the soda lime trap contained 5.5%, of the activity delivered.

(C) The Chemistry Process Control Unit

1. Chemistry process hardware

The chemistry process control unit (CPCU) hardware is pictured in Fig. 1. The complete CPCU consists of three different types of components: the chemistry process control module (CPCM), or the CPCU hardware, the CPCU control chassis, and the CPCU operating system and control software.

The chemistry process control unit hardware physically occupies a rectangular box measuring 45 cm wide × 45 cm high × 28 cm deep made of aluminum plates with a removable front panel of stainless steel. An opening through each end allows the passage of air through the unit. The air inlet opening is fitted with a coiled band heater which when turned on heats the incoming air. The air outlet opening is equipped with a fan. With the front panel in place and the fan on, air for heating or cooling the reaction vessels is pulled into the enclosure through the inlet opening only. Inside the unit are two glass reaction vessels (VES1 and VES2) and two oil baths, each with a controller. The vessel positions are vertically adjustable allowing for passing the incoming air flow (heated or ambient) either over the opening or around the outside of the two vessels. The oil baths are on a horizontal movable carrier operated by gas cylinders which allows the oil baths to be in either of two positions under the vessels. There is also an intermediate position whereby the vessels may be ejected from the holders allowing their retrieval out the top of the unit after removal of the silicone stoppers. The mechanical schematic of the CPCU is shown in Fig. 2.

Each of the two vessels can be independently positioned vertically by activation of a stepping motor which turns a ball screw attached to each vessel holder. We have loosely defined four basic positions for the vessels. At the lower position, the vessel is fully immersed in the oil bath; this is for maximum heating such as in the final solvent evaporation. At the uppermost position the vessel opening is sealed against a silicone stopper; this is for the low pressure transfer of the contents of the vessel to the next vessel or to the columns for purification. With the vessel opening sealed, pressurization of the vessel forces the contents of the vessel to be transferred via a Teflon tube which extends through the stopper to the bottom of the vessel. Two intermediate vessel positions are defined; the higher position for addition of solvents/reagents to the vessel, and the lower position has the vessel partially immersed in the oil bath with the upper portion of the vessel in the (cool) air flow stream; this is for refluxing a solvent during a reaction.

Optical limit switches corresponding to the two extreme vessel positions and the three oil bath carrier positions allow remote position sensing of the vessels and the oil baths by the computer. In addition, the oil bath temperatures are read (and adjusted if necessary) by the computer. The two silicone stoppers used to seal the vessels extend through the top plate of the unit and hold the Teflon tubes that connect the individual reservoirs to the two vessels and the two vessels to each other. These small-bore tubes extend from the bottom of each bottle (or vessel) through the silicone stopper and are used to transfer the solvent/reagent solutions to the vessels and the reaction mixture from one vessel to the next and to the purification columns. These reservoirs (various sized serum bottles) containing each solvent/reagent solution are mounted on the front panel.

Each reservoir bottle is also connected to a three-way valve such that when the valve is off, the interior of the bottle is connected to the inside of the unit, thus insuring pressure equalization between the vessels and the reservoirs and avoiding unwanted transfers due to a pressure differential. When activated, the valve delivers 2–5 psi of nitrogen pressure to the reservoir bottle forcing the liquid contents of the bottle out the exit tube to the vessel or delivering this pressure to the vessel and forcing the reaction mixture either to the next vessel or to the purification columns. These three-way valves are attached to a manifold and mounted on the back of the unit. The purification columns may be mounted on the end panel and the final product is delivered to a shielded container located outside the unit.

All transfers and evaporations are carried out by the timed opening and closing of the appropriate gas pressure valves and the turning on and off of the stepping motors and air flow heater. We have found that these operations are very reproducible and the added complexity of temperature and/or pressure feedback loops does not offer any advantage over the simple timed approach; in fact, the more complex the control system, the more likely that there will be component failure.

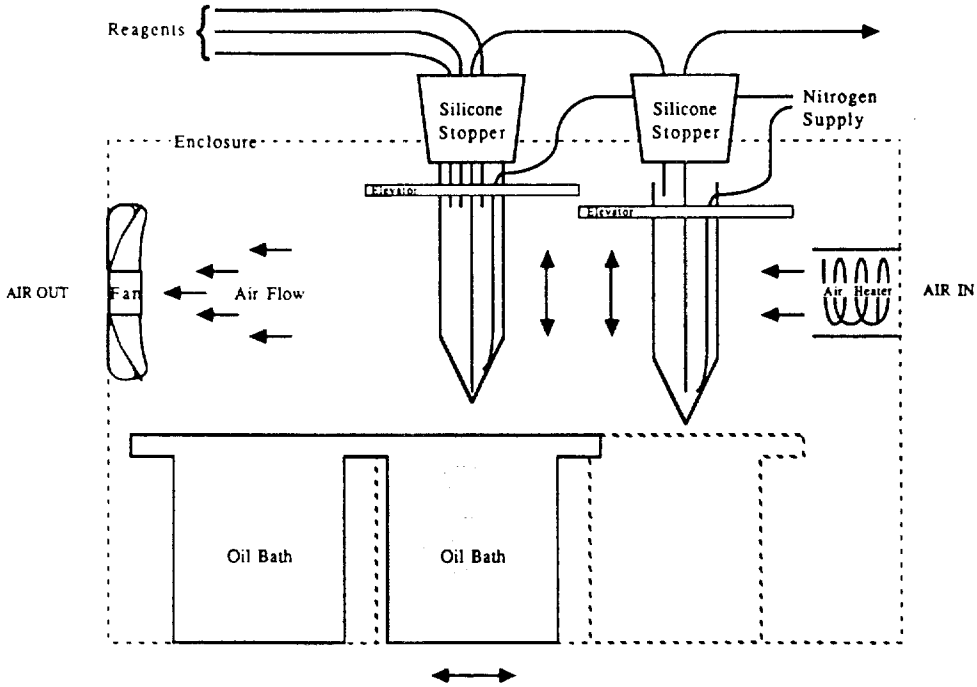


Fig. 2. Mechanical schematic of CPCU showing air flow path and oil bath and vessel movements.

A list of CPCU hardware components is provided in Appendix 1.

2. Operation of process hardware and function of control software during synthesis of 2-[^{18}F]FDG

Preparatory to creating the command file program (*vide infra*) to accomplish an automated synthesis, for example, 2-[^{18}F]FDG from [^{18}F]fluoride ion, the complete synthetic process was broken down into appropriate unit operations (Padgett *et al.*, 1982a). The synthetic process for the synthesis of 2-[^{18}F]FDG proceeds in five basic steps: (a) drying of [^{18}F]fluoride ion, (b) nucleophilic [^{18}F]fluorination of the mannose triflate, (c) isolation of tetraacetyl 2-[^{18}F]FDG, (d) hydrolysis of the acetyl protecting groups, and, (e) purification and delivery of the final product, 2-[^{18}F]FDG. This synthetic process is carried out by the CPCU as shown schematically in Fig. 3. These unit operations or processing tasks are divided into two groups which can be labeled major and minor; the major tasks are not repeated but contain minor tasks which may or may not be repeated within this synthesis. With few exceptions, when each minor operation or task has finished, the status of the system is always the same. Thus, when each operation starts, the status is the same: all valves are off and the vessels are at the same position. This allows these unit operations to be interchanged and inserted as desired without continuity problems.

The total synthetic procedure containing the individual operations is labeled SYNTH1. The unit operations that comprise the SYNTH1 synthesis control program are described in part below and summarized in Table 1.

Prior to an actual SYNTH1 synthesis, the system status is determined by the system diagnostic operation DIAG which checks each function such as oil bath carrier movement, oil bath temperature and temperature readout, vessel positioning, etc., for readiness. Failure to pass these tests prevents the synthesis from commencing.

Drying of the [^{18}F]fluoride ion. This major task is labeled DRY1. This is where the synthesis starts; nonrepeating minor operations are the addition of the potassium carbonate K222/CH_3CN solution, and the delivery of the [^{18}O]water/[^{18}F]fluoride ion. Repetitious operations are the positioning of the oil baths, addition of an aliquot of acetonitrile and its subsequent evaporation. These minor operations are achieved by the subroutines BATHLEFT, ADDVES1, F18UNLOAD, ADDVIAL2, and EVAPVES1. It is these subroutines that actually carry out the timed opening and closing of the valves and turning on and off the stepping motors and air heating that constitute the operation of the unit.

The actual text of the SYNTH1 command file is reproduced in Fig. 4.

3. Description of CPCU control system

The control system for the CPCU is designed around the IBM PC AT computer and the STD Bus. Figure 5 shows the relationship of the control system components.

The three primary components of the CPCU control system are:

(a) The IBM PC AT computer system. All elements of our control system are controlled by a single computer. One obvious advantage of this is that an entire radiochemical process, including purification, may be carried out by a single operator at a single terminal.

(b) The STD bus subsystem. The STD, or standard, bus is a widely accepted bus structure and many manufacturers offer a variety of I/O cards for this system. An 8-bit parallel link between the computer and the bus carries out two functions:

—Reads limit and position switches, temperatures, pressures, etc.;

—Transfers on/off commands to the valves, switches, and motors.

(c) The software. The computer runs the RTX286 real-time, multi-tasking, multi-user operating system. A program written in Fortran-286 interprets user commands and performs hardware control.

A complete list of control system components appears in Appendix 2.

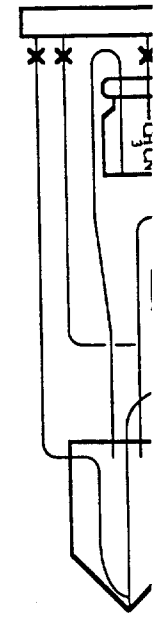


Fig. 3. Plumb

There are three level
system:

(i) Routine operati
User interaction is via
final radiochemical pr
(ii) Research and d
vidual command files
files might be for a cor
simple or unique task
(iii) Fully manual.
mands are entered usi
starts here.

Unit operations major task description
1. DRY1: [^{18}F]Fluoride K222/K_2CO_3 to prepare reaction.
2. REACT1: [^{18}F]Fluor with mannose triflate.
3. TRANSFER1: Tran solution of protected 2- followed by evaporatio
4. REFLUX2: Acid hy groups to give 2-[^{18}F]F
5. TRANSFER2: Puri product.

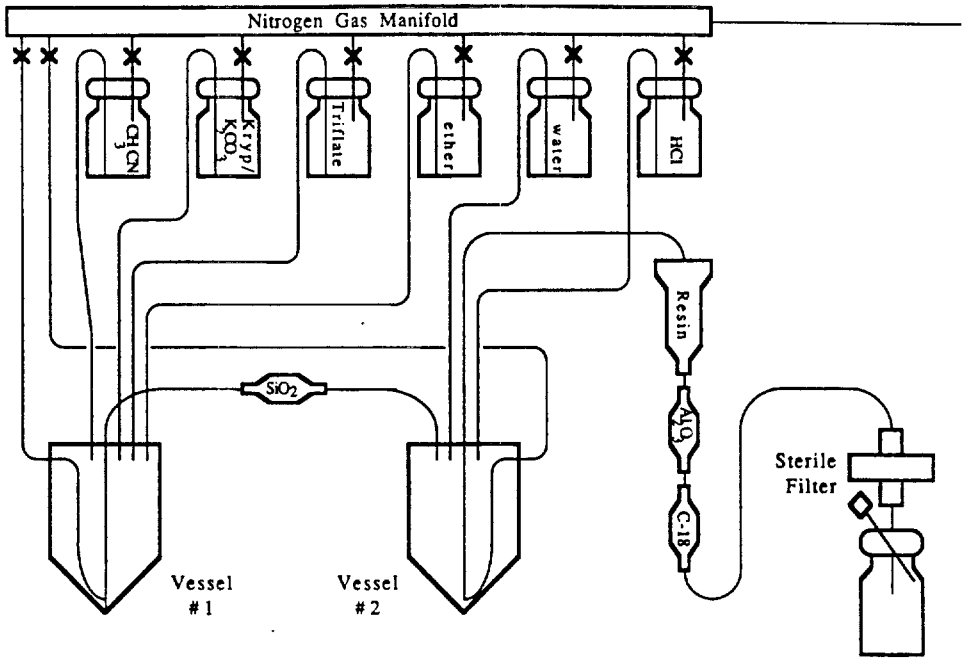


Fig. 3. Plumbing schematic of CPCU showing relationship of solvent/reagent reservoirs to the vessels and purification columns.

There are three levels of user interaction with this control system:

(i) Routine operation. This mode is totally automatic. User interaction is via menu selection oriented toward the final radiochemical product.

(ii) Research and development. This mode is where individual command files are specified via menu selection. These files might be for a complex, multi-step process, or for a very simple or unique task.

(iii) Fully manual. This mode is where individual commands are entered using the keyboard. Process development starts here.

The syntax of the command language to control the CPCU is simple and straightforward. No knowledge of a programming language is necessary in order to write the desired command file programs. For example, to move the oil baths to the left position requires opening the two valves allowing gas pressure to act on the air cylinders controlling the left motion of the oil baths for 10 s. This action is carried out with the command sequence:

```
on r01
on r03
wait 10
off r01
off r03
```

Table 1. Summary of 2-[^{18}F]FDG synthesis control program

Unit operations major task description	Associated minor tasks command file names	Minor task descriptions and time required
1. DRY1: [^{18}F]Fluoride ion dried in the presence of K222/K_2CO_3 to prepare for nucleophilic fluorination reaction.	INITVES1 INITVES2 BATHLEFT ADDVIAL1 F18UNLOAD ADDVIAL2 EVAPVES1	Vessels and baths positioned. K222/K_2CO_3 solution added; [^{18}F]fluoride added. 3 × 1 mL CH_3CN added and evaporated at 110–115°C. 15 min.
2. REACT1: [^{18}F]Fluoride ion displacement reaction with mannose triflate.	BATHRIGHT ADDVIAL3 HEATVES1 MIXVES1	Baths repositioned. Mannose triflate added to [^{18}F]fluoride ion K_2CO_3 K222. Reaction mixture heated at 80–85°C. 6 min.
3. TRANSFER1: Transfer of acetonitrile/ether solution of protected 2-[^{18}F]FDG from VES1 to VES2 followed by evaporation of solvents.	COOLVES1 ADDVIAL4 TRANSVES1 EVAPVES2	Ether (2 × 4 mL) added to VES1 and solution transferred through silica gel cartridge into VES2; ether solvent evaporated. 12 min.
4. REFLUX2: Acid hydrolysis of hydroxyl-protecting groups to give 2-[^{18}F]FDG.	ADDVIAL5 HEATVES2 MIXVES2 COOLVES2	Aqueous HCl (1M, 1 mL) added to residue of tetraacetyl 2-[^{18}F]FDG; mixture heated at 110–115°C. 16 min.
5. TRANSFER2: Purification and delivery of product.	TRANSVES2A ADDVIAL6 TRANSVES2B	Solution transferred to resin, alumina, and C-18 columns and VES2 rinsed with water (3 × 5 mL) to elute and deliver 2-[^{18}F]FDG. 7 min.

```
;START OF "SYNTH1.CMD" [SYNTHESIS OF 2-[F-18]FDG]
text 20,0,THE SYNTHESIS OF 2-[F-18]FDG IN PROGRESS...
BEAMOFF
@INITCPCU
@DRY1
@REACT1
@TRANSFER1
@REFLUX2
@TRANSFER2
@OFFCPCU
text 20,0,THE SYNTHESIS OF 2-[F-18]FDG IS COMPLETED
text 21,0,Press "ENTER" to terminate synthesis command file
waitc <D>
;END OF "SYNTH1.CMD"
```

Fig. 4. Hard copy of the command file used for the synthesis of 2-[^{18}F]FDG.

where r01 and r03 control the relays connected to the appropriate valves. In the research environment, these commands may initially be entered individually to test the actual hardware connections. More typically, this sequence is entered into a command file with a text editor, and the command file is executed as a single command. In addition, a command file may call another command file, to a total nesting level of six. This allows the researcher to modularize and use structured techniques.

Since there are optical limit switches to indicate when this movement is completed, inclusion of this input instead of a timed sequence allows the successful movement of the baths to be a prerequesite for continuation of the synthesis. In addition, each command may specify the name of yet another command file to be executed if an error occurs. In the previous example, if we want to have the activation of the appropriate limit switch allow synthesis continuation, and if we wish to specify the name of a file OFFCPCU to be executed on an error condition, the command sequence becomes:

```
on r01
on r03
waits r38, off, 30, @OFFCPCU
off r01
off r03
```

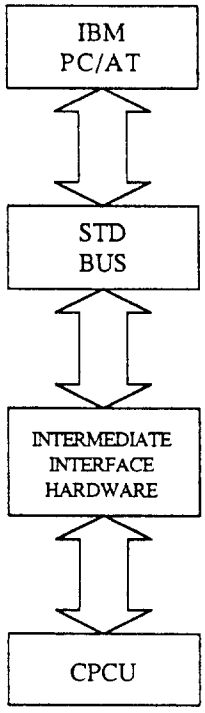


Fig. 5. CPCU control system components.

where r38 is the optical limit switch relay that must be turned off within 30 s by the completed bath movement. If the bath movement does not occur, as signaled by the continued activation of the limit switch, then the program OFFCPCU, which contains commands to turn off all relays of the CPCU and to signal that the chemistry process has been interrupted, is performed.

Results and Discussion

The computer-controlled chemistry process control unit (CPCU), used in these automated syntheses of various fluorine-18 labeled radiochemicals from aqueous [^{18}F]-fluoride ion and gaseous [^{18}F]AcOF, is a logical continuation of the design philosophy that produced unit operations-based remote, semi-automated synthesis systems for the routine production of 2-[^{18}F]FDG from [^{18}F]fluorine or acetyl hypo[^{18}F]fluorite, [1-^{11}C]2-deoxy-D-glucose, [1-^{11}C]-palmitic acid (or other acids produced via Grignard reactions with [^{11}C]CO_2), and carbon-11 and nitrogen-13 amino acids (Barrio *et al.*, 1980, 1981; Fowler *et al.*, 1981; Padgett *et al.*, 1982a,b; Baumgartner *et al.*, 1981; Barrio *et al.*, 1982a,b, 1983). Fluorine-18 in two different chemical forms was chosen as the radionuclide used in this demonstration of the CPCU for several reasons including the two different types of chemistry available (nucleophilic substitution using aqueous fluoride ion and electrophilic fluorination using gaseous acetyl hypofluorite), and its increasing popularity as a tool, in conjunction with the application of the tracer kinetic method, for studying the basic chemical and functional processes in biochemistry, physiology, pharmacology, etc. (Fowler and Wolf, 1986). In addition, its 110-min half-life permits relatively long synthesis procedures and commercial distribution center-based dissemination.

At the onset of the design of this instrument, it was decided that our goal was to construct an automated chemistry process control unit that exhibits the following characteristics:

—simple, both of construction and of use, i.e. user friendly to set-up, use and maintain;
—flexible, that is, adaptable to a variety of different radiochemical procedures using gaseous or liquid labeled precursors;
—reliable and dependable to insure the highest quality product with a minimum of routine maintenance;
—compact in size to allow for easier shielding of the processing unit; and,
—inexpensive to build and maintain.

To best meet these design goals, we made the following basic decisions: we chose to stay with the traditional valve-and-tubing style system rather than the newer robot radial-array approach (Brodack *et al.*, 1987) because it offered the most synthetic flexibility, was easily compatible with an existing control system, and would not occupy large areas which would require shielding. While robots clearly have the edge

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when the task is to physically move a piece of hardware (a vial, etc.) from one place to another, many of their attributes seemed to be inapplicable to the problem at hand. We also chose to achieve all transfers of liquids (solvents, reagents) by gentle gas pressure instead of vacuum since this would reduce the number of valves and associated plumbing required and a source of gas pressure is easier to obtain and maintain than a source of vacuum. In addition, we decided to use heated oil baths for our thermal energy instead of a metal heater block or hot air bath because of the ability to closely control the temperatures using microprocessor-based computer-compatible controllers and the superior heart transfer characteristics of an oil bath.

In order to maintain simplicity in the hardware/software interface, our approach here was to avoid feedback monitoring of active processes such as solvent evaporation and temperature control. Rather, we felt that by controlling the way processes are carried out, simple timing with a slight margin of safety would allow the same result to be achieved without the considerable added complexity and expense that active feedback loops would entail. Of course, with our control system this capability can be introduced in the future. We have incorporated various passive feedback capabilities such as optical limit switches to allow remote position sensing of the oil bath carrier and the two vessels, and the use of microprocessor-based temperature controllers which permit communication with the computer. With the information these feedbacks provide, coupled with correct system set-up, the quality of the process and the product is assured.

For each radiochemical product desired, the CPCU is configured to allow the required chemical steps to be carried out in the proper sequence. The configuration consists of connecting the reagent/solvent reservoir vials both to the corresponding nitrogen supply valve on the valve manifold board and to the appropriate reaction vessel, and setting the oil bath temperatures. In addition, chromatographic purification cartridges and columns are specified. For each synthesis, each vessel has a silicone stopper through which the Teflon tubing passes to connect the vessels and the vials and purification column(s). Different synthetic routes may require different configurations, and different numbers of tubes through the silicone stoppers. For example, for reactions starting with a gaseous precursor, such as [^{11}C]carbon dioxide or acetyl hypo[^{18}F]fluorite, the introduction of the labeled gas is carried out by bubbling this precursor through the reaction mixture with the vessel opening sealed against the stopper. The stopper, in addition to the plumbing for the addition and transfer of liquids, is also fitted with a vent tube which is connected to a soda lime trap. Thus, the activity is either trapped in the reagent solution or in the soda lime; none is released.

In order to best demonstrate the potential usefulness of the CPCU, we chose to initially focus on configuring and programming the instrument to prepare 2-[^{18}F]FDG. Thus, using the unit operations approach, the system was organized according to the processes to be carried out; drying of the fluoride ion followed by nucleophilic fluorination of the mannose trifflate in the first vessel, purification during transfer to the second vessel, hydrolysis of the acetylated 2-[^{18}F]FDG in the second vessel, and final purification during transfer of the 2-[^{18}F]FDG to the product delivery vial.

The unit operations approach as applied here is a powerful tool to help organize the total process and to integrate the hardware and the software. However, it is not intended to generate software operations that will be repeatedly used as in all chemical procedures that may be adapted to the CPCU. Using our operating system and the command file architecture, these routines are so easy to compose that the exact program one desires can be generated either by starting with an existing program which is then modified as needed, or by creating a new one. For example, in our 2-[^{18}F]FDG synthesis program called SYNTH1, the command files TRANSVES2A, TRANSVES2B, and TRANSVES2C are identical except for the wait time allotted to achieve the transfer (since in each case the amount of liquid transferred is different), because the same basic operation is taking place, i.e. the transfer of a liquid from VES2 to the purification columns. Also the command files HEATVES1/HEATVES2, MIXVES1/MIXVES2, and COOLVES1/COOLVES2 are identidal except for the changes necessary to make them vessel specific (i.e. a different valve turns on the VES1 bubbler than turns on the VES2 bubbler, etc.). In addition, this approach allows for synthesis specific comments and notation to be included.

It was intended from the very beginning of the development of the CPCU concept that all operations would be controlled by a single computer and operating system, and this integrated system would be capable of being run by a single operator. The RTX286 operating system, a real-time, multi-tasking, multi-user operating system, in conjunction with an STD bus and an 8-bit parallel link, offered the capability of controlling a wide range and virtually unlimited number of functions, such as reading limit and position switches, temperatures, pressures, and sending on/off commands to valves, motors, switches, fans, heaters, etc. Thus, from a control standpoint, the task was to decide what functions would be best used to achieve the desired result, the automated preparation of radiolabeled chemical compounds. Control of these functions, or operations, then followed in a straightforward manner.

In order to illustrate how the chemical process control unit functions, the following describes the CPCU-based synthesis of 2-[^{18}F]FDG using the synthetic method of Hamacher *et al.*, 1986) which has been modified to be compatible with our system.

Prior to commencing the synthesis, the CPCU is first subjected to the operation DIAG which checks each mechanical function of the process hardware. Next the instrument is set-up as described in the command file SETUPFDG. The operation SETUPFDG, which is simply a series of text statements, specifies all initial conditions such as oil bath temperatures and volume of each reagent solution or solvent to be placed in each reservoir. Also specified are the columns that need to be replaced before each run, and the reminder to replace the two used vessels with clean dry ones.

In practice, the CPCU can be set-up for a routine 2-[^{18}F]FDG synthesis in about 30–60 min. The tasks involved include filling replacing the reservoirs, cleaning and drying the two stopper tubing assemblies (rinse each tube with ethanol, blow dry with nitrogen), replacing the used vessels, and connecting the purification column and cartridges. If one wished to use the CPCU to prepare a different radiochemical than the one synthesized last, then the appropriate clean stopper tubing assemblies would be substituted for the dirty ones. Of course, to avoid unnecessary exposure, after a synthesis one would wait until the remaining activity (on the columns and the vessels) had decayed, usually overnight.

For all aqueous [^{18}F]fluoride ion-based syntheses, the CPCU is programmed to start with approximately 0.3 mL of water containing [^{18}F]fluoride ion. This is the working volume of the liquid water target holder which, when loaded with oxygen-18 enriched water and bombarded with 11 MeV protons, produces fluorine-18 according to the ^{18}O(p,n)^{18}F nuclear reaction with yields in the range of 90–100% based on the published excitation function (Ruth and Wolf, 1979). A similar target has been described recently (Luxen *et al.*, 1986a), and preliminary results from this target have been presented (Wieland *et al.*, 1986).

In addition to having all functions of the radiochemical synthesis process computer controlled, the [^{18}O]water target loading and unloading is also carried out using appropriate command files and an ^{18}O/^{18}F-target supply unit (TSU). The ^{18}O/^{18}F-TSU hardware consists primarily of a syringe pump,

four solenoid valves and associated small-bore tubing, which, in conjunction with the zero dead-volume pinch valve on the target delivery line, and a high (14 psi) and low (1.5 psi) source of nitrogen gas, prepares the target for loading, loads the target, provides target overpressure during irradiation, and then unloads the target at the beginning of the radiochemistry process. The programs for these operations are named F18LOAD and F18UNLOAD respectively, and consist of on/off commands for the valves controlling the two gas pressures, the pinch valve, and the power for the syringe pump. The load time for the syringe pump is precalibrated and aside from the target pressure feed-back sensor, all operations are carried out in sequence by simple timing. Using small-bore polyethylene tubing, the [^{16}O]water [^{18}F]fluoride ion may be delivered as far as 25 m with the loss of activity (and [^{18}O]water) at about 10%. The ^{16}O/^{18}F-TSU is shown schematically in Fig. 6. Early in the automated radiochemical synthesis development process, it was realized that the reagent stoichiometry reported for the aqueous [^{18}F]fluoride ion-based synthesis of 2-[^{18}F]FDG (Hamacher *et al.*, 1986) was not compatible with our synthesis unit. We found it necessary to use considerably less potassium carbonate (1.0 vs 4.6 mg), Kryptofix 222 (10.0 vs 26 mg), and mannose triflate (10 vs 20 mg) in our version of the synthesis.

The first step in the synthetic process, the azeotropic removal of the [^{16}O]/[^{18}O]water to leave a dry, reactive residue of [^{18}F]fluoride ion/K_2CO_3/K222, is achieved by the successive evaporation of three portions of acetonitrile. Our experiments have taught us that the single most important factor in the successful synthesis of 2-[^{18}F]FDG is this drying process. To assist in the total elimination of residual water in the fluorination vessel, we not only heat the vessel in a 110–115°C oil bath with a slow stream of nitrogen gas passing through the solution, but we have heated air flowing over and around the opening of the vessel. The fact that our fluorination yields (fluorination yield = total [^{18}F]fluoride ion activity delivered to the CPCU − (activity remaining on the fluorination vessel and tubings + activity trapped on the silica gel cartridge)) have averaged about 70% means that we are removing most of the water that can interfere with the nucleophilic [^{18}F]fluorination reaction. With this system and programming only one or two acetonitrile evaporations results in reduced [^{18}F]fluorination yields.

The next step, the [^{18}F]fluorination via a nucleophilic displacement reaction on the mannose triflate, is carried out as described by the literature procedure. To the dry [^{18}F]fluoride ion/K_2CO_3/K222 residue is first added a small portion of acetonitrile followed by the mannose triflate solution. The reaction mixture is heated at 80–85°C for 5 min. Again, the yields of [^{18}F]fluoride ion incorporation using our system have averaged about 70% with yields in the mid to high seventies being common.

The next step, the isolation and purification of the fluorinated intermediate, is achieved by the dilution of the [^{18}F]fluorination reaction mixture with ethyl ether and the passage of this solution through a silica gel cartridge. As verified by TLC (I_2 visualization), all Kryptofix 222 and unreacted [^{18}F]fluoride ions are retained on the silica gel cartridge, and the neutral nonpolar [^{18}F]fluorinated carbohydrate passes through this cartridge and is delivered to the hydrolysis vessel.

The next step in the synthesis, and acid-catalyzed hydrolysis of the hydroxyl-protecting acetyl groups, is carried out in the traditional manner. To the acetylated [^{18}F]fluorocarbohydrate residue, after evaporation of the acetonitrile/ether solvents, is added dilute aqueous hydrochloric acid and the resulting solution is heated to reflux for 15 min with occasional mixing by the passage of nitrogen gas through the solution. This mixing insures that unreacted intermediate that may be sticking to the walls of the vessel are washed down into the acid solution and allowed to react.

The final step, the purification and delivery of the 2-[^{18}F]FDG, is accomplished by passing the hydrolysis reaction mixture through successive columns of ion retardation resin (to neutralize the acid), neutral alumina (to adsorb any [^{18}F]fluoride ion present) and C-18 bonded silica gel (to retain relatively nonpolar species such as unhydrolyzed and partially unhydrolyzed [^{18}F]fluorinated intermediate). The product is delivered to a shielded vial located outside the synthesis unit.

An instrument designed for the routine, totally automated synthesis of radiochemicals is now a reality. For example, using the described chemistry process control unit, appropriately configured, and in conjunction with the necessary command file programming, 2-[^{18}F]FDG has been routinely prepared from aqueous [^{18}F]fluoride ion in 54.9% (±11.2%, corrected to EOB; $n = 125$) radiochemical yield after a

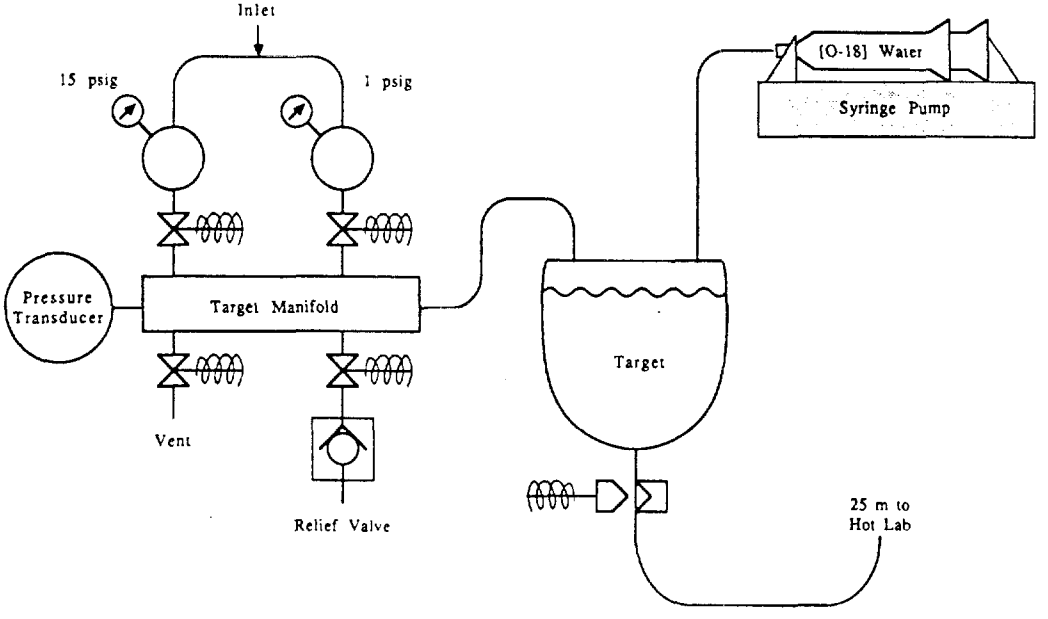

Fig. 6. Schematic of [^{18}O]water target supply unit (TSU).

Table 2. Radiochemicals produced using the CPCU

Product	Yield[a] (%)	Purity[b] (%)
FDM	48	>99[c]
FA	66[d]	>99[d]
FESP	29	[e]
FDG[f]	20	>99[c]

[a]FDM: 2-Deoxy-2-[^{18}F]fluoro-D-mannose; FA: [^{18}F]-Fluoroacetate; FESP: 3-(2′-[^{18}F]Fluoroethyl)-spiperone; FDG: 2-Deoxy-2-[^{18}F]fluoro-D-glucose.
[a]Based on total recovered activity, corrected to EOB.
[b]Radiochemical purity.
[c]As determined by radio-TLC.
[d]As determined by radio-HPLC.
[e]Final product not isolated.
[f]Using electrophilic [^{18}F] fluorination (Bida *et al.*, 1984) with gaseous AcO[^{18}F]F.

synthesis time of 50–55 min. A radiochemical purity of >99% was determined by radioTLC. The average total activity produced (for runs of 5–10 μA) is 28.1 mCi/μA ($\pm$5.03 mCi/μA). Thus, the amount of 2-[^{18}F]FDG produced from a 10 μA for 1 h bombardment was 154.3 mCi ($\pm$27.4 mCi). In addition, the CPCU has been used to prepare a variety of radiochemicals starting from either aqueous [^{18}F]fluoride ion or gaseous acetyl hypo[^{18}F]fluorite, and thus has been shown to be useful for the general production of labeled compounds.

Conclusion

The benefits that are derived from the application of this automated chemistry process control unit are numerous. The obvious ones, for the research center or commercial operation that depends on ready access to various radiochemicals, are reduced requirements for a large professional and technical staff, improved reliability of radiochemical production procedures, and lowered radiation exposures for all personnel.

Even though in this description of the CPCU emphasis has been given to the synthesis of 2-[^{18}F]FDG, with appropriate hardware and control program changes, such as described above, the unit is readily adaptable to employ a variety of procedures for the general synthesis of radiochemicals, for example, fluorine-18 labeled radiochemicals from aqueous [^{18}F]fluoride ion such as 2-[^{18}F]FDG by alternate synthetic routes (Levy *et al.*, 1982; Tewson, 1983a,b; Haradahira *et al.*, 1985a,b; Szarek, 1982, 1985; Olesker *et al.*, 1982; Dessinges *et al.*, 1984), 2-[^{18}F]FDM (Luxen *et al.*, 1986a), 3-(2′-[^{18}F]fluoroalkyl)spiperones (Satyamurthy *et al.*, 1986; Shiue *et al.*, 1987), and [^{18}F]fluoroacetate (Sykes *et al.*, 1986). It has also been demonstrated, by the synthesis of 2-[^{18}F]FDG via electrophilic [^{18}F]fluorination using gaseous acetyl hypo[^{18}F]fluorite, that it is possible to adapt this system for radiochemical syntheses using gaseous radiochemical precursors such as [^{18}F]F_2 (Barrio *et al.*, 1981; Fowler *et al.*, 1981), AcO[^{18}F]F (Luxen *et al.*, 1986b; Adam *et al.*, 1986), [^{11}C]CO_2 (Padgett *et al.*, 1982b; Pike *et al.*, 1981), H[^{11}C]CN (Padgett *et al.*, 1982a; MacGregor *et al.*, 1981; Barrio *et al.*, 1982a; Iwata *et al.*, 1987), and [^{11}H]CH_3I (Dannals *et al.*, 1985). In many cases, the final purification procedures for the products of these syntheses involve the application of preparative high pressure liquid chromatography. The CPCU, as currently configured, does not allow for these HPLC-based purification procedures to be automated; however, this should not prevent the chemical processes in these syntheses from being automated in the same fashion as those described here.

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APPENDIX 1

List of Purchased CPCU Hardware Components

Air flow heater:	Cat No. 125CH38A2X, 1–1/4 in. i.d., band heater. Watlow Electric Mfg Co., St Louis, MO 63146.
Oil bath heaters:	Cat No. B2C2AN1 band heater, type L, 2–1/8 in. i.d. × 2 in. wide. Watlow Electric Mfg Co., St Louis, MO 63146.
Oil bath temperature controllers:	
Air cylinders:	
16-Valve manifold:	
Teflon valves:	
Stepping motors:	
Air Flow Fan:	

[a]Assumes multi-user syntheses.
[b]IBM Corporation,
[c]AST Research, Inc
[d]ULTRALINK Int
[e]Kimtron Corporati
[f]Analog Devices, In
[g]Pro-Log Corporati
[h]Real-Time Comp California.
[i]Intel Corporation,

Oil bath temperature controllers:	No. CN9111 Microprocessor temperature controller. Omega Engineering, Stamford, CO 06907.
Air cylinders:	Model No. 3BDS-2 miniature cylinder. Clippard Instrument Laboratory, Cincinnati, OH 45239.
16-Valve manifold:	Interface system series 3, model BES-530-16-16-24-0-M-CTI, 3-way exhaust ported PC board. Bay Pneumatic, Inc., San Leandro, CA 94577.
Teflon valves:	No. 368-3-24-30 24 V 3-way. Asco/Angar Scientific Co., Inc., Cedar Knolls, NJ 07927.
Stepping motors:	Slo-Syn Model No. SS50 72 RPM. Superior Electric Co., Bristol, CT 06010.
Air Flow Fan:	Model No. 126 LH-0182 w/ball bearing. ETRI, Monroe, NC 28110.
Interruptor module (Optical limit switches):	Type No. H21B6. GE Semiconductor, Syracuse, NY 13221.

[a]Assumes multi-user system capable of two simultaneous syntheses.
[b]IBM Corporation, Boca Raton, Florida.
[c]AST Research, Inc., Irvine, California.
[d]ULTRALINK International, Gardnerville, Nevada.
[e]Kimtron Corporation, San Jose, California.
[f]Analog Devices, Inc., Norwood, Massachusetts.
[g]Pro-Log Corporation, Monterey, California.
[h]Real-Time Computer Science Corporation, Camarillo, California.
[i]Intel Corporation, Santa Clara, California.

APPENDIX 2

List of Control System Components[a]

Hardware

IBM[b] PC AT with 512K memory,
serial/parallel adapter,
20 Mb hard disk,
1.2 Mb floppy disk,
monochrome interface,
monochrome monitor,
80287 math coprocessor.

AST[c] Advantage! Multifunction board with 512K memory.
ULTRALINK[d] No. 102 PC link interface to STD bus.
ULTRALINK[d] No. 103 STD link interface card.
Kimtron[e] KT-7/PC data terminal.
Analog Devices[f] RTI-1260 A/D converter.
Analog Devices[f] expander No. 0A10 for RTI-1260 A/D converter.
Pro-Log[g] No. 701B-0L1 package including 22-slot STD but card rack, power supply, forced air cooling, and terminator card.
Pro-Log[g] No. 7508 TTL Input/Output card.

Software

IBM[b] DOS version 3.20 operating system.
RTCS[h] RTX286 real-time multi-tasking multi-user operating system.
Intel[i] AEDIT text editor for iRMX™ 286 systems.
Intel[i] Fortran-286 for iRMX™ 286 systems.

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6-[F-18]FLUORO-L-DOPA

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SYNTHESIS OF

6-[F-18]FLUORO-L-DOPA

6-[F-18]FLUORO-L-DOPA

The synthesis of 6-[F-18]fluoro-L-dopa follows the procedure reported in the literature [Luxen et al., Appl. Radiat. Isot. 41, 275-281 (1990). A copy of the paper is enclosed]. The procedure involves the production of [F-18]fluorine gas [Bishop et al., Nucl. Med. Biol. 23, 189-199 (1996). A copy of the paper is enclosed] and its subsequent utilization in the preparation of 6-[F-18]fluoro-L-dopa.

Description of the production of [F-18]fluorine gas

[F-18]fluorine is made by the $^{18}O(p,n)^{18}F$ nuclear reaction in an aluminum target body. The target is filled with [O-18]oxygen gas and irradiated with 11 MeV protons. The target gas is cryorecovered and then filled with 100 μmol of F_2 in argon. Subsequent proton irradiation releases [F-18]F_2.

Remote semiautomated production of 6-[F-18]fluoro-L-dopa

[F-18]F_2 produced as described above was passed through a cartridge (5 mm i.d. x 35 mm) containing powdered $NaOAc \bullet 3H_2O$ (340 mg). The effluent from the cartridge was bubbled into a reaction vessel containing 4,5-dimethoxy-2-trifluoroacetate-mercuri-N-trifluoroacetyl-L-phenylalanine, ethyl ester (101 mg, 153 μmol) in chloroform (10 mL); [F-18]AcOF escaping from the reaction mixture was contained within a soda lime trap. After the bubbling had ceased, the volume of the resultant organic solution was reduced under N_2 to 1 mL using the aluminum block heater (75°C). The aluminum block was rapidly cooled with water and diethyl ether (10 mL) was added in two portions to the solution. The ethereal solution was transferred through a column (7 mm i.d.) which has been packed with $Na_2S_2O_3$ (25 mm) and silica gel (75 mm) and had been previously equilibrated with diethyl ether. The fluoro compound was eluted from the

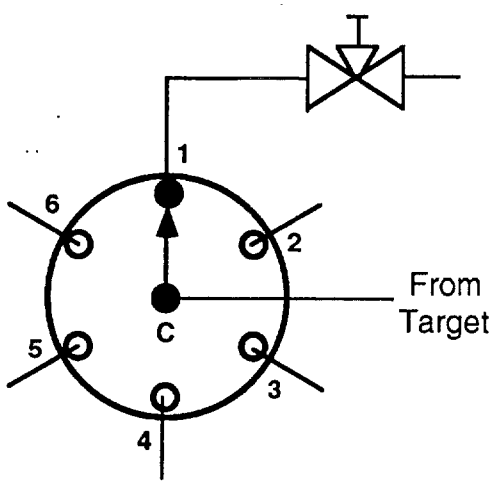
column and collected in test tube (16 x 125 mm). The solvent was then evaporated under N_2 with heat supplied from the thermostatically controlled oil bath (130°C). Hydrolysis of the intermediate began when HI (55%, 2.0 mL) was added in two portions and the solution was refluxed. Reflux was maintained by having a cool set of N_2 gas surrounding the upper portion of the test tube. After the solution was refluxed for 15 min, the oil bath was remotely lowered and the solution allowed to cool. Partial neutralization of HI was accomplished by using 0.75 equiv of 3N NaOH (~1.8 mL) followed by addition of the HPLC—mobile phase (see below) to a total volume of 4 mL. Prior to injection, the solution was transferred to a syringe (12 mL) and filtered [0.22 μm membrane filter (Millipore-Vented Millex-GS)]. The solution was then loaded into the 5.0 mL HPLC—loop by drawing the solution from its vessel to the Rheodyne 7124 injector by the use of negative pressure at the loop vent. The solution was loaded remotely onto a semi-preparative column (Adsorbosphere C-18, 7 μm, 10 x 300 mm, eluent 5 mM sodium acetate, 1 mM EDTA, 0.1% HOAc, 0.01% ascorbic acid; pH: 4; flow rate, 7 mL/min) and the fraction eluting from the HPLC column which corresponded to 6-[F-18]fluoro-L-dopa, as monitored by an in-line plastic scintillation detector, was collected by passing the eluent directly through a sterile 0.22 μm membrane filter (Millipore-Vented Millex-GS) in a sterile vial (20 mL) containing NaCl to insure the isotonicity of the final solution. The chemical (>99%) and radiochemical purity (>99%) of the final product was monitored by HPLC.

The step by step procedures for the operation of the fluorine gas target to produce [F-18]F_2 and the synthesis of 6-[F-18]fluoro-L-dopa are described in the following sections.

RDS 112 [F-18] FLUORINE GAS TARGET OPERATION

A. Target Evacuation

1. At the fluorine gas target control valve (Fig. 1) switch, move the valve to position **P4** (Vacuum).
2. On the computer terminal type **ON R84** (RDS #1) **R94** (RDS #2). Note the decrease in target pressure.
3. When target is < 8 psia, the target is evacuated.



1. To chemistry module
2. Not used
3. F_2/Ar supply
4. Vacuum
5. Argon supply
6. Not used

Figure 1 Fluorine gas target control valve

B. Pre-run Loading

CAUTION: Before changing position of the Target control Valve, turn off R84 (RDS #1) R94 (RDS #2).

1. At the computer terminal, type **OFF R84** (RDS #1) **R94** (RDS #2).
2. Open the 5% F_2/Ar tank.
3. At the 6-way valve, press controller to position **P3** (F2/Ar).
4. At the computer terminal, type **CYCLE R84,ON,0.1,5** (RDS #1) **CYCLE R94,ON,0.1,5** (RDS #2) and fill the target with 45-50 psia of F_2/Ar. (Net 45-50 psi F_2/Ar).
5. Type **CYCLE R84,OFF** (RDS #1) **CYCLE R94,OFF** (RDS #2) after the target has been filled.

6. At the fluorine gas target control valve switch, move the valve to position **P4** (Vacuum).
7. Allow approximately 1 min. to evacuate the Load/Delivery line.
8. At the fluorine gas target control valve switch, move the valve to position **P5** (Ar).
9. At the computer terminal, type **CYCLE R84,ON,0.1,5** (RDS #1) **CYCLE R94,ON,0.1,5** (RDS #2) and fill the target with 230 psia of Argon.
10. Type **CYCLE R84,OFF** (RDS #1) **CYCLE R94,OFF** (RDS #2) after the target has been filled.

    The target is now ready for bombardment. The pre-run bombardment is normally 10μA for 10 minutes. Repeat sections A and B if multiple pre-runs are requires, normally two pre-runs are sufficient.

C. Oxygen-18 Loading

1. At the end of the final pre-run, move the fluorine gas target control valve to position **P4** (Vacuum), at the computer terminal, type **ON R84** (RDS #1) **R94** (RDS #2), to evacuate the target.
2. Flush the target three times with Argon, move the Fluorine gas target control valve to position **P5** (Ar) for approx. 3 seconds and then back to position **P4** (Vacuum). Allow the flush gas to pump out, then repeat two more times.
3. Ensure the target is completely evacuated prior to [O-18]O_2 gas Loading.
4. On the computer terminal type **OFF R84** (RDS #1) **R94** (RDS #2).
5. Move the fluorine gas target control valve to the **No Flow** position.

WARNING: Make sure **R84** (RDS #1) **R94** (RDS #2) is **OFF**, and the fluorine gas target control valve is in the **No Flow** position prior to opening the [O-18]O_2 bottle valves.

3. Place the [O-18]O_2 bottle in liquid nitrogen until the pressure is <300 psig.
4. Open the [O-18]O_2 bottle valves and remove from the liquid nitrogen.

5. On the computer terminal, type **CYCLE R85 ON,0.2,10** (RDS #1) **CYCLE R95 ON,0.2,10** (RDS #1).
6. Fill the target with [O-18]O_2 gas until the pressure is between 210 to 220 psia.

WARNING: Do not fill the target to more than 230 psia because the beam on target pressure could exceed 650 psia and cause a rupture of the target foil.

NOTE: If the target is overfilled the pressure can be reduced by returning the [O-18]O_2 bottle to the liquid nitrogen to condense the oxygen. Then on the computer terminal typing **CYCLE R85 ON,0.2,10** (RDS #1) **CYCLE R95 ON,0.2,10** (RDS #1) until the pressure is reduced.

7. When the target pressure is between 210 to 220 psi type **CYCLE R85 OFF** (RDS #1), **CYCLE R95 OFF** (RDS #2).
8. Close [O-18]O_2 bottle valves.

The target is now ready for bombardment. The normal bombardment parameters are 40μA for 60 minutes.

D. O-18 Recovery

1. About 10 minutes prior to EOB place the [O-18]O_2 bottle in liquid nitrogen.
2. When the bottle has cooled, open the bottle valves.
3. At EOB, open the [O-18]O_2 bottle valves, and at the computer terminal type **ON R85** (RDS #1) **ON R95** (RDS #2). The target pressure should decrease at this time.
4. After 5 minutes type **OFF R85** (RDS #1) **OFF R95** (RDS #2).

CAUTION: The [O-18]O_2 bottle may be radioactive after O_2 recovery.

5. Close [O-18]O_2 bottle valves and remove from the liquid nitrogen.

E. F-18 Recovery

1. Move the fluorine gas target control valve to position **P4** (Vacuum), at the computer terminal, type **ON R84** (RDS #1) **R94** (RDS #2), to evacuate the target.
2. Flush the target three times with Argon, move the fluorine gas target control valve to position **P5** (Ar) for approx. 3 seconds and then back to position **P4** (Vacuum). Allow the flush gas to pump out, then repeat two more times.
3. Ensure the target is completely evacuated prior to F2/Ar gas Loading.
4. On the computer terminal type **OFF R84** (RDS #1) **R94** (RDS #2).
5. At the 6-way valve, press controller to position **P3** (F_2/Ar).
6. At the computer terminal, type **CYCLE R84,ON,0.1,5** (RDS #1) **CYCLE R94,ON,0.1,5** (RDS #2) and fill the target with 45-50 psia of F_2/Ar. (Net 45-50 psi F_2/Ar).
7. Type **CYCLE R84,OFF** (RDS #1) **CYCLE R94,OFF** (RDS #2) after the target has been filled.
8. At the fluorine gas target control valve switch, move the valve to position **P4** (Vacuum).
9. Allow approximately 1 min. to evacuate the Load/Delivery line.
10. At the fluorine gas target control valve switch, move the valve to position **P5** (Ar).
11. At the computer terminal, type **CYCLE R84,ON,0.1,5** (RDS #1) **CYCLE R94,ON,0.1,5** (RDS #2) and fill the target with 230 psia of Argon.
12. Type **CYCLE R84,OFF** (RDS #1) **CYCLE R94,OFF** (RDS #2) after the target has been filled.

Target is now ready for bombardment. The recovery bombardment parameters are 10μA for 10 minutes.

F. F-18 Delivery and reaction

1. At EOB, on the computer terminal type **CYCLE R84,ON,0.2,15** (RDS #1) **CYCLE R94,ON,0.21,15** (RDS #2)

2. At the fluorine gas target control valve switch, move the valve to position **P1** (To Chemistry), and adjust the metering valve for the desired reaction flow rate.
3. When the target pressure is approximately 100 psi, at the computer terminal type **CYCLE R84,OFF** (RDS #1) **CYCLE R94,OFF** (RDS #2). Then type **ON R84** (RDS #1) **R94** (RDS #2).
4. Adjust the metering valve to maintain the required flow rate.
5. When the target pressure has dropped to atmosphere the target can be flushed with Argon. Close the metering valve and move the fluorine gas target control valve to position **P5** (Ar) for approx. 3 seconds and then back to position **P1** (To Chemistry). Then readjust the metering valve for the required flow rate.
6. After all of the activity has been transferred to the chemistry module, close the metering valve and evacuate the remaining target gas and fill with F_2/Ar.

6 - [F-18]Fluoro-L-Dopa Synthesis Procedure

Introduction

The synthesis of 6 - [F-18]Fluoro-L-Dopa (FDopa) consists of five steps; fluorination of the precursor molecule using [F-18]acetlyhypofluorite, silica column purification of the fluorinated intermediate, hydrolysis of the intermediate, filtration, and HPLC purification of the final product.

Materials

1. 18M ohm water
2. Methanol (CH_3OH)
3. Glacial Acetic Acid (CH_3COOH)
4. Ethyl Ether ($C_2H_5OC_2H_5$)
5. Ascorbic Acid
6. EDTA
7. Sodium Acetate ($CH_3COONa\ 3H_2O$)
8. Chloroform ($CHCl_3$)
9. 4,5-Dimethoxy-2-trifluoroacetate-mecuri-N-trifluoroacetyl-L-phenylalanine, ethyl ester (BOZ Chemical)
10. Two 0.22μm vented sterilizing filters
11. 30ml sterile vial
12. Chromatography grade silica gel
13. Sodium Thiosulfate
14. Sodium Acetate Cartridge

Sodium Acetate Cartridge Preperation

1. Take a 1.0ml syringe barrel and cut at the 0.6ml position, keeping the luer end of the barrel.
2. Insert the barrel into a 0.25in stainless steel Swagelok nut.
3. Place a small glass wool plug into the barrel and clamp on the Swagelok nut.

4. Fill the barell to the bottom of the Swagelok nut with dried sodium acetate trihydrate powder. Tap the barrel to pack the powder.
5. Insert a second glass wool plug on top of the powder to prevent it from spilling out. Do not pack the contents tightly.
6. Screw into the Swagelok nut a 1/4in to 1/8in reducing union without the front and back ferrules. Tighten the union until just barely snug.

NOTE: Sodium Acetate Cartridges can be prepared ahead of time and stored in a canister over Drierite. If this is done, be sure to cover the ends of the cartridge with Parafilm.

HPLC System Preparation

The HPLC is normally stored in 100% methanol. System preparation requires equilibration with 18M ohm water, followed by equilibration with the Dopa Buffer Solution (see below) prior to injection of the sample.

Caution: Mixing methanol and the Dopa Buffer Solution in the HPLC system can cause salts to precipitate and clog the column.

A. Dopa Buffer Preparation
   The Dopa Buffer is 5mM Sodium Acetate, 1mM EDTA, 0.1% Acetic Acid, and 0.01% Ascorbic Acid in water. The pH is 4.0-4.4.
   1. In 2 liters of 18M ohm water dissolve:
      a. 1.361gm of Sodium Acetate
      b. 0.744gm of EDTA
      c. 2.0ml of Acetic Acid
      d. 0.20gm Ascorbic Acid
   2. Thoroughly mix all ingredients
   3. Filter the solution
   4. Degas the solution
B. Pass 300ml of 18M ohm water through the HPLC system to flush out the methanol.
C. Pass 300ml of the Dopa Buffer Solution through the HPLC system the flush out the water. The system is now ready for use. Always maintain a flow through the system before use.

Synthesis Module Set-Up

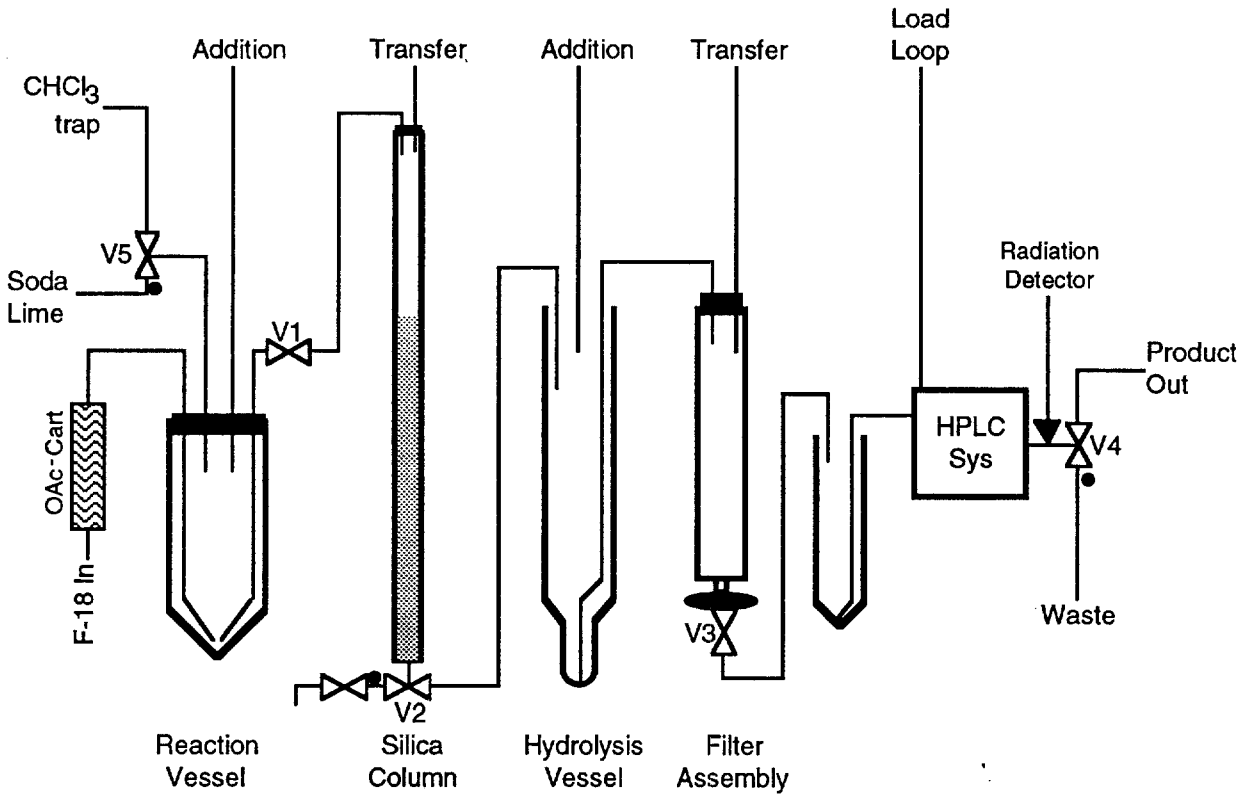


Figure 1. Fluorodopa system diagram

A. Install clean glassware.
   1. A large conical glass tube in the reaction vessel holder.
   2. A 20ml concentrator tube (Ace Glass Inc.) in the hydrolysis heating block and insert the half stopper into the top of the tube.
   3. A 10ml conical tube under the filter assembly, and place the outlet tube from **Filter Assembly Valve (V3)** and the HPLC load line into the tube.

B. Install the filter assembly
   1. Attach a 0.22µm vented sterilizing filter to the barrel of a 10ml syringe.
   2. Insert the male luer fitting of the filter into the female fitting on **Filter Assembly valve (V3)**.
   3. Insert the stopper into the top of the syringe barrel.

C. Silica column preparation
   1. Take a 1 x 30cm liquid chromatography column and place approx. 1.5cm of sodium thiosulfate in the bottom.

2. Fill the column about 2/3 full with chromatography grade silica gel, and top with a wad of glass wool.
3. Insert the column luer fitting into the female luer fitting on **Silica Column Out Valve (V3)**.
4. Insert the stopper in the top of the column.
5. Wash the column with approx. 30ml of ethyl ether. Open the stop cock on the flush line and inject the ether through the Silica Column Transfer Line (Fig. 2).

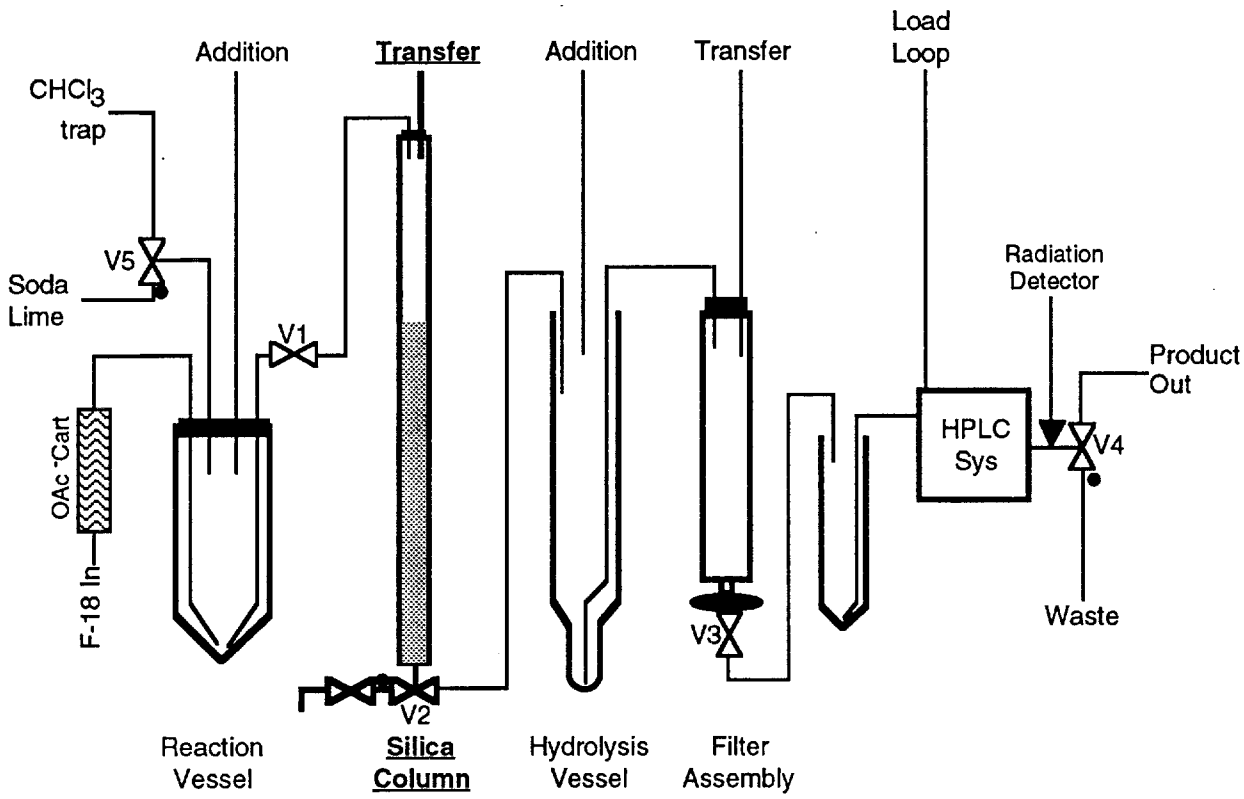


Figure 2. Silica column ether wash

6. Leave 2cm of ether above the silica, and close the stop cock.

D. Reaction vessel preparation

1. About 10 minutes prior to End Of Bombardment (EOB) dissolve 105 mg of the precursor (4,5-Dimethoxy-2-trifluoroacetate-mecuri-N-trifluoroacetyl-L-phenylalanine, ethyl ester) in 10 ml of chloroform.
2. Place in the Reaction Vessel Heater block and insert the stopper assembly into the Reaction Vessel.

E. About 10 minutes prior to EOB install the Sodium Acetate Cartridge on the gas delivery line. Connect the target delivery line to the Swagelok fitting, and the reaction vessel line to the male luer fitting.

F. Prepare a sterile vial with a 0.22μm vented filter, place in a lead pig, and attach the product out line to the filter.

G. Radiation detector and strip chart recorder
   1. Set the radiation meter scale to 1 x 10^5 cts/min.
   2. Set the strip chart recorder pen #1 for:
      a. 1.0 cm/min
      b. 100 mVolts

Fluorination of Precursor

NOTE: Please refer to the F-18 Target Operating Procedure for the details of the target operation.

A. Target gas delivery
   1. Cycle the $^{18}F_2$ Target Bolus Valve on for 0.1 seconds and off for 10 seconds.
   2. Adjust the target gas needle valve to maintain the flow rate through the Reaction Vessel.

Caution: Radioactivity is now present in the synthesis module.

   3. Observe the target pressure, when the pressure decreases to about 100psia, stop cycling the Bolus Valve, then turn it on.
   4. When the target pressure decreases to approx. 40psia stop R72 cycling, and turn it on.
   5. When the target pressure reaches 35psia begin the argon flush of the target. Cycle R74 on for 0.1 seconds and off for 45 seconds. After two cycles of R74 stop the cycling and turn the valve off.
   6. When the target pressure decreases to about 40psia the fluorination step is finished.

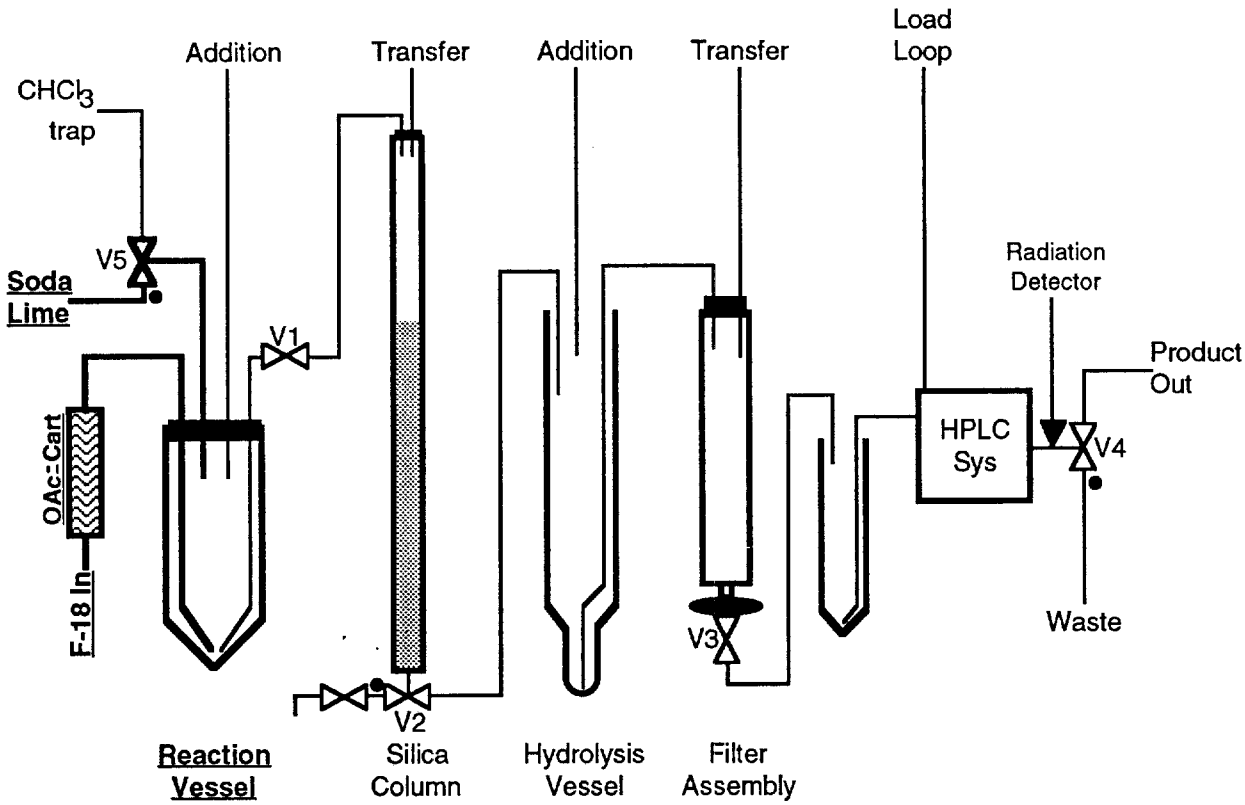


Figure 3. Precursor fluorination

Chloroform Evaporation

A. At the completion of the fluorination step turn on the Reaction Vessel Heater, check the temperature setting at 100°C.

B. Turn ON the **Chloroform Trap Valve (V5).**

C. Turn ON the **To Silica Valve (V1).**

D. Attach a dry nitrogen gas line to the Silica Column Transfer Line and adjust the flow rate to keep the chloroform from refluxing in the Reaction Vessel.

E. Observe the Reaction Vessel, when the chloroform solution is reduced by about 2/3 - 3/4.

   1. Turn OFF the dry nitrogen flow.
   2. Turn OFF To **Silica Valve (V1).**
   3. Turn ON the **Cooling Water Valve** and cool the reaction vessel to less than 40°C. Then turn OFF the **Cooling Water Valve,** and blow out the cooling water lines by turning ON the **Air Purge Valve** for several seconds.

NOTE: If all of the chloroform evaporates, add 3 - 4 ml of chloroform to the Reaction Vessel and bubble dry nitrogen through the solution to redissolve the fluorinated precursor. Do not use diethyl ether as the fluorinated precursor is not very soluable in diethyl ether.

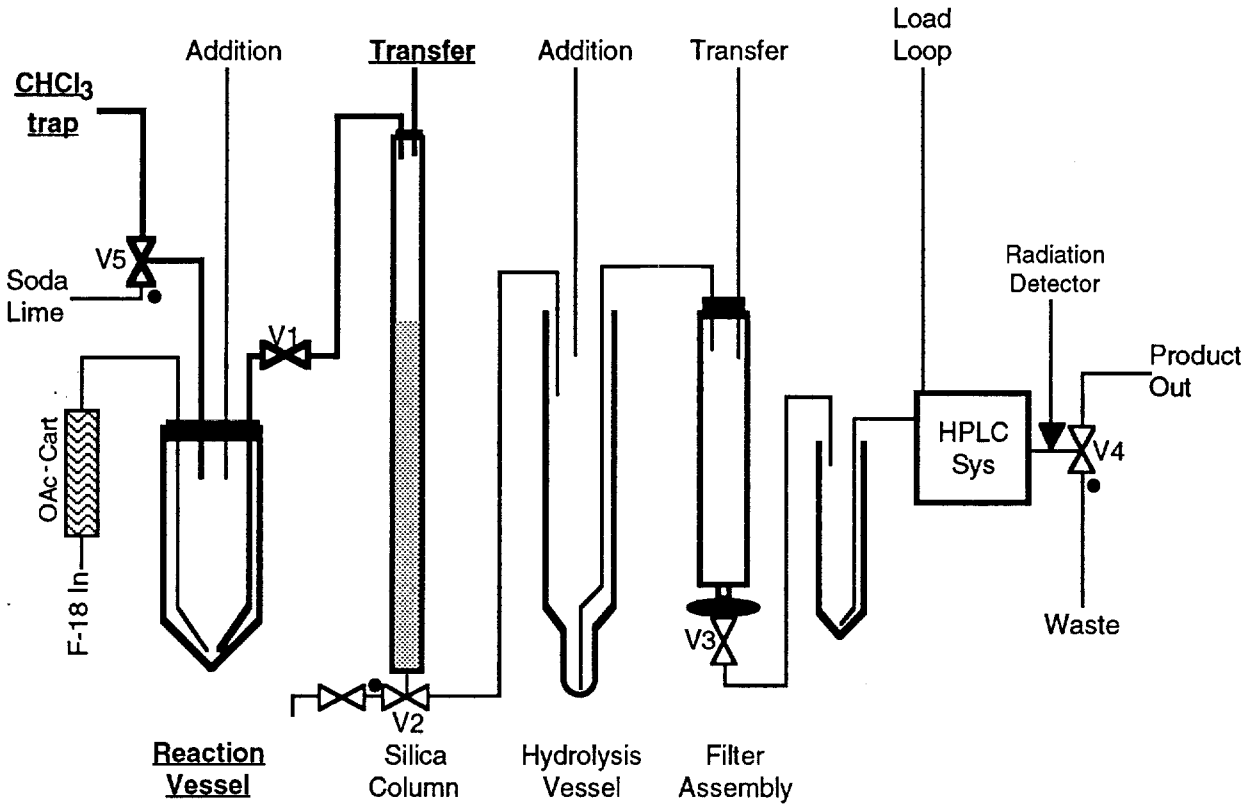


Figure 4: Chloroform Evaporation

Silica Column Purification

A. At the completion of fluorination turn on the Hydrolysis Vessel Heater and set the temperature to 135°C.

B. Attach a nitrogen line to the Filter Assembly Transfer Line and establish a low flow of nitrogen through the Filter Assembly into the bottom of the Hydrolysis Vessel.

C. Turn ON the **To Silica Column Valve (V1)** and using a syringe gently pull a vacuum in the Silica Column Transfer Line (Fig. 4) causing the remaining chloroform solution to move to the top of the Silica Column.

NOTE: When pulling the vacuum the silica may move up in the column due to the evaporation of the ether caused by the partial vacuum.

D. When the liquid level reaches the plastic top of the column break the vacuum and Turn OFF the **To Silica Column Valve (V1)**.

Caution: Overfilling of the Silica Column can cause radioactive material to be pulled into the transfer syringe.

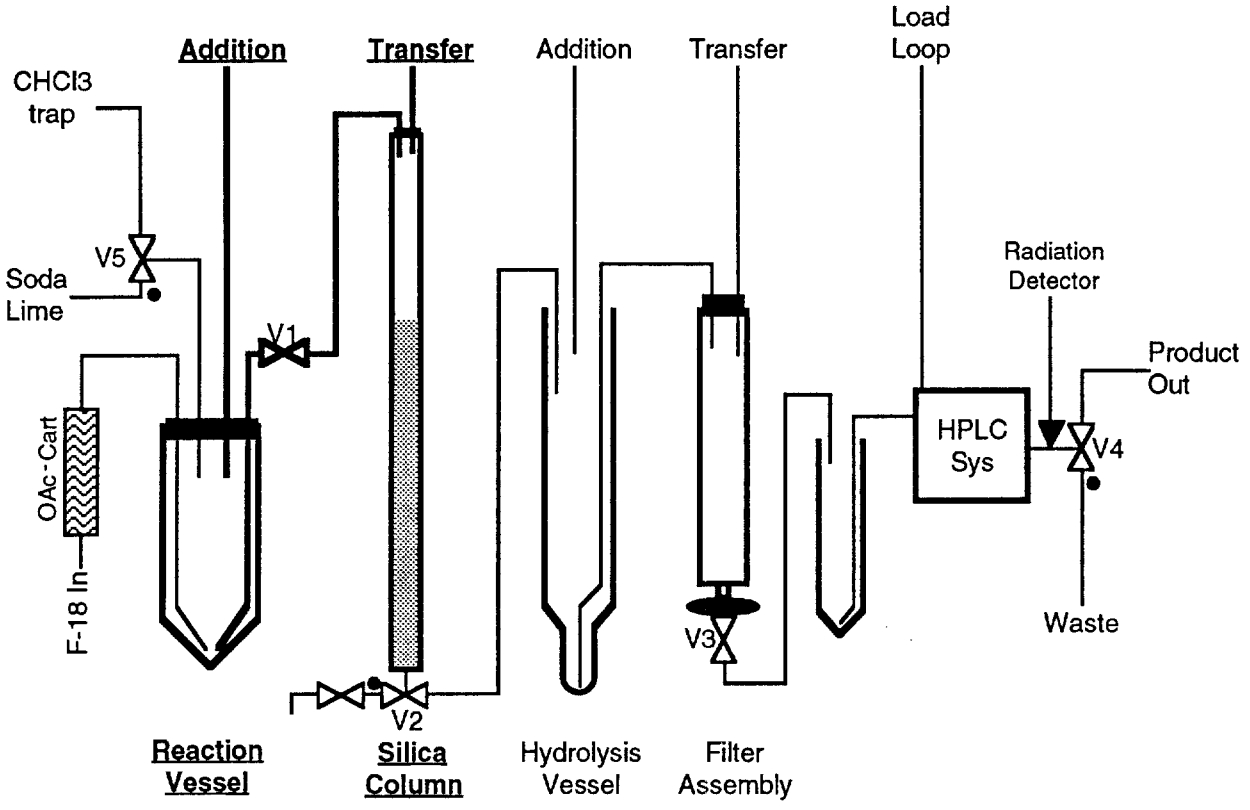


Figure 4. Liquid transfer from the reaction vessel to the silica column

E. Turn ON **To Hydrolysis Vessel Valve (V2)** and apply light pressure, using the syringe, to the Silica Column Transfer Line washing the liquid into the Hydrolysis Vessel (Fig. 5).

F. When the liquid level is just above the top of silica release the pressure and Turn OFF the **To Hydrolysis Vessel Valve (V2)**

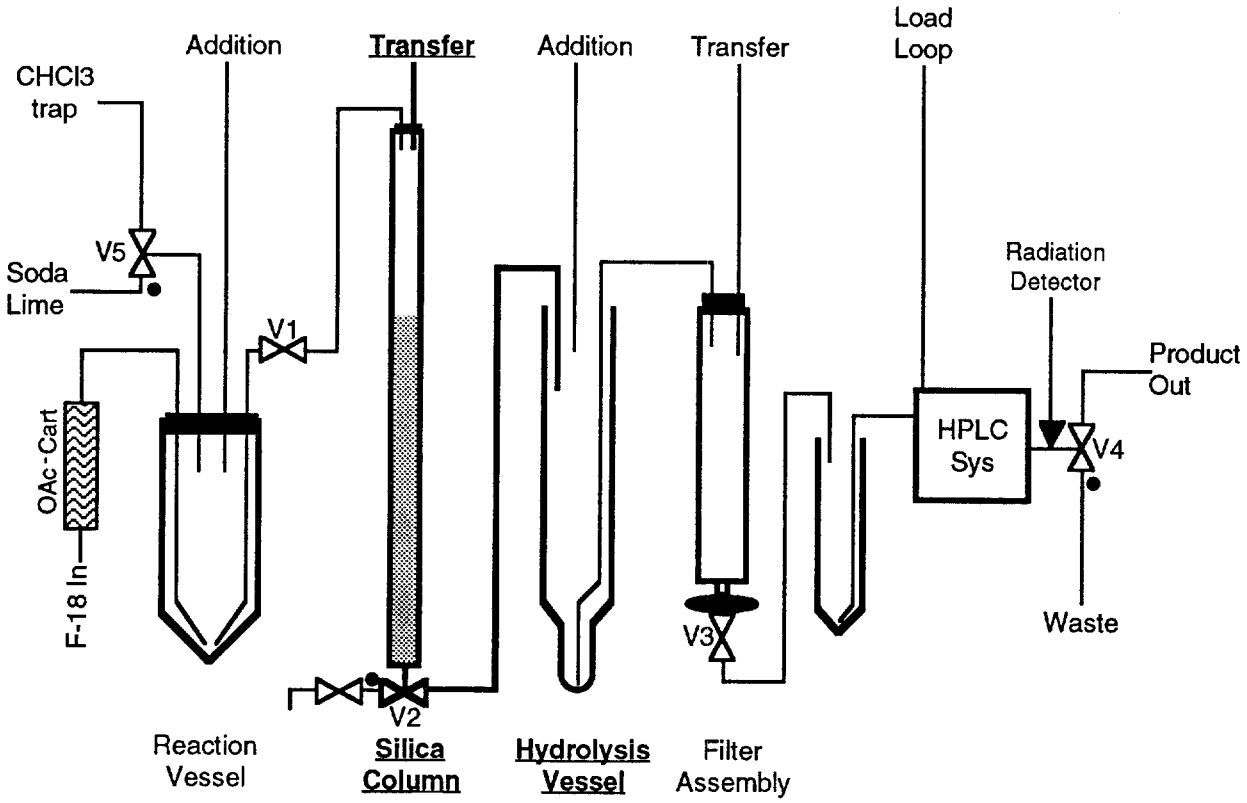


Figure 5. Transfer of liquid from the silica column to the hydrolysis vessel

G. Repeat steps C - F until all of the chloroform solution has been transferred through the Silica Column into the Hydrolysis Vessel.

H. Add 10ml of ethyl ether to the Reaction Vessel and again repeat C - G until all of the ether has been transferred through the Silica Column to the Hydrolysis Vessel.

Caution: Watch the Hydrolysis Vessel liquid level do not allow liquid to splash out of the vessel. Adjust the nitrogen bubble rate or wait until liquid has evaporated before adding more solution.

1. Add another 10ml aliquot of ethyl ether to the Reaction Vessel and repeat the transfer steps C- G.
2. Repeat Step 1 above until the Silica Column has been washed with a total of 30ml of diethyl ether.
3. After the last aliquot of ether is on the Silica Column, the column can be pushed dry.

J. Allow all of the liquid to evaporate from the Hydrolysis Vessel.

Hydrolysis

A. Remove a vial of redistilled 46% HI solution from the refrigerator just prior to use.
B. Using a syringe and hypodermic needle remove 2.0ml from the vial.

Caution: The volumes of HI, NaOH solution and buffer solution should be measured accurately to insure the final volume of the solution after hydrolysis is 5.0ml, as this is the volume of the HPLC loop.

C. Turn off the nitrogen bubbling flow.
D. Inject the HI into the Reaction Vessel through the Hydrolysis Vessel Addition Line.
E. Hydrolyze for 15 minutes.
F. Turn off the Hydrolysis Vessel Heater.
H. Establish a low flow of dry nitrogen flow through the solution from the Filter Assembly Transfer Line.
G. Turn ON the **Cooling Water Supply**, cool the Hydrolysis Vessel to less than 40°C.
I. In a 3ml syringe draw up 1.5ml of the Dopa Buffer Solution, and inject into the Hydrolysis Vessel.
J. In a 3ml syringe draw up 1.5ml of 3N NaOH Solution and inject slowly into the Hydrolysis vessel.

Caution: The hydrolyzed Dopa is very pH sensetive, the solution must be kept acidic or the compound will decompose. Be sure there is a flow of nitrogen through the solution to keep it mixed, and add the NaOH sloution very slowly in order to prevent localized areas of high pH.

K. Allow the solution to mix for several seconds.
    1. Turn off the nitrogen flow.

Filtration

A. Using a syringe pull a suction on the Filter Assembly Transfer Line, pulling the contents of the Hydrolysis Vessel into the Filter Assembly (Fig. 6)

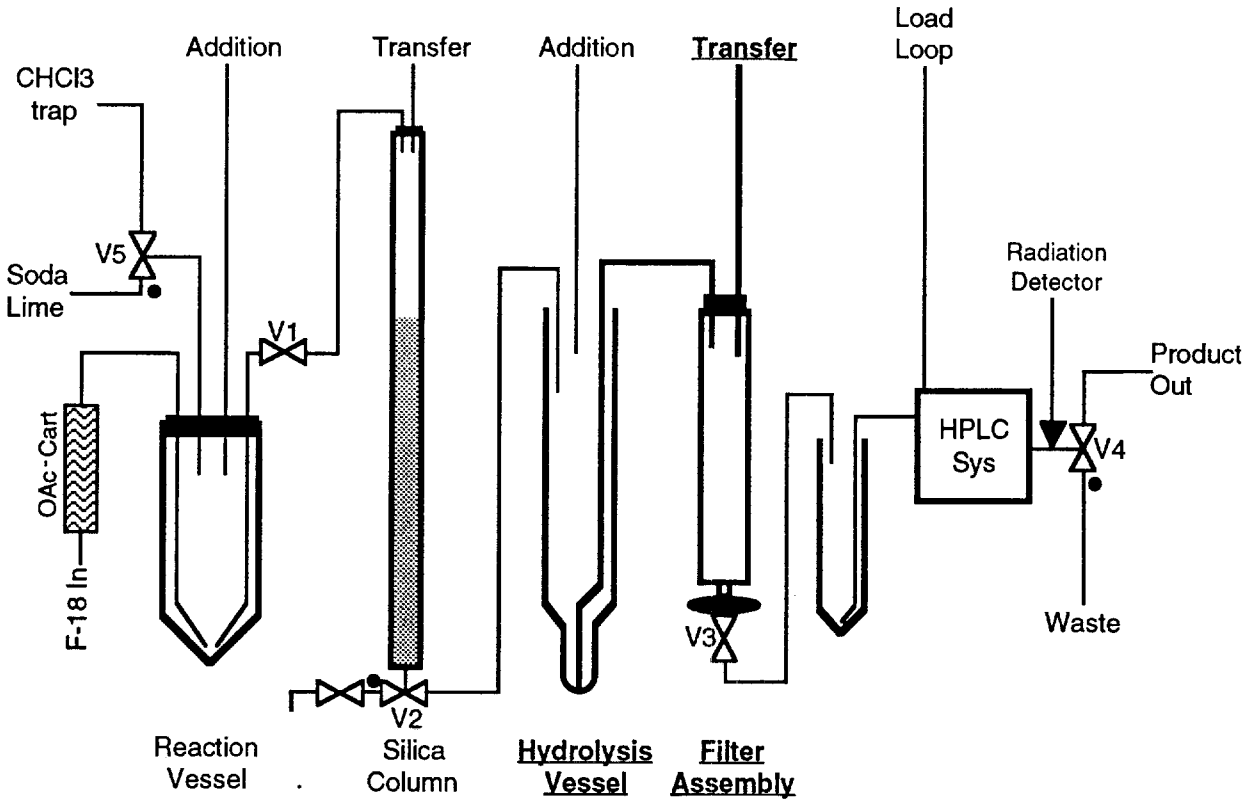


Figure 6. Liquid transfer from the hydrolysis vessel to the filter assembly

B. Open **Filter Assembly Valve (V3)** and using the syringe apply pressure to the Filter Assembly Transfer Line and push the solution through the filter into the HPLC CollectionTube (Fig. 7).

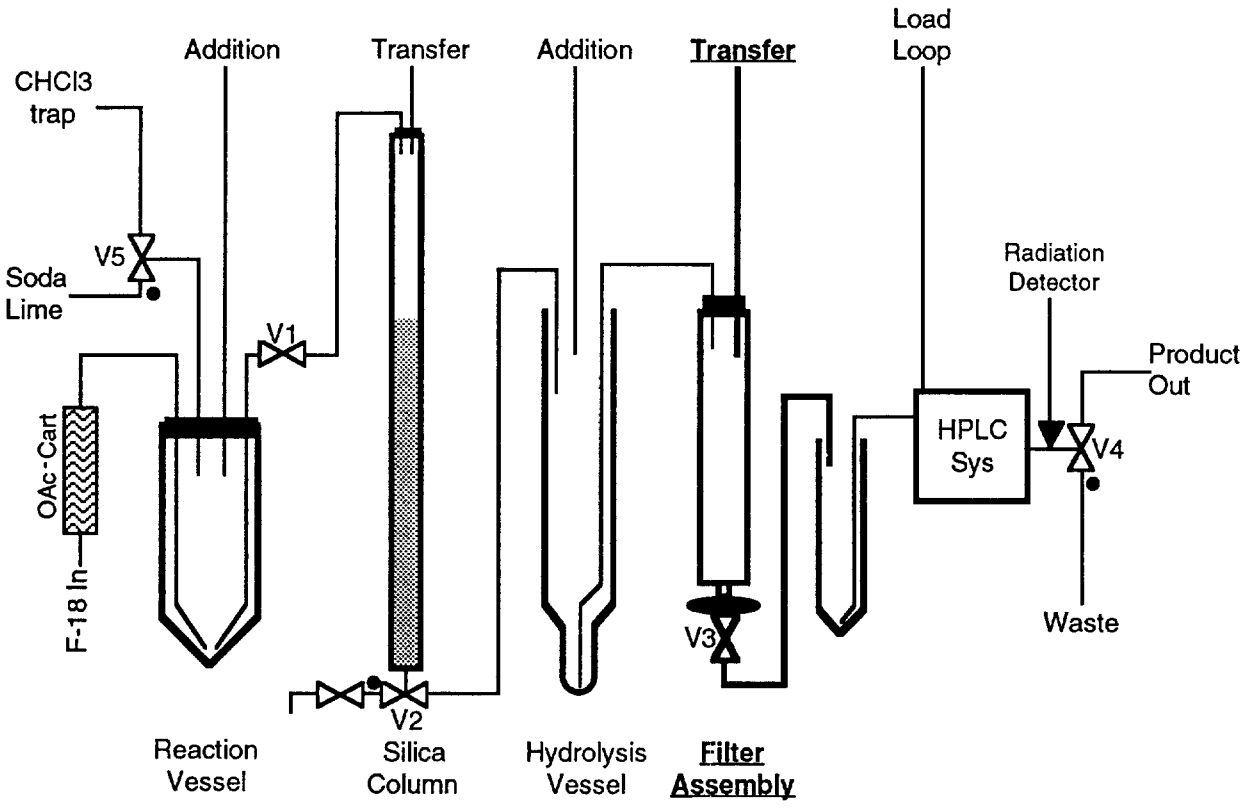


Figure 7. Transfer of liquid from the filter assembly to the HPLC collection tube

HPLC Purification

A. Set the HPLC Flow Rate to 7.0ml/min of the Dopa Buffer Solution.
B. Check the **HPLC Injector Valve** is in the LOAD position
C. Using the HPLC Load Syringe pull a suction on the HPLC Load Line, pulling the contents of the collection tube into the 5.0ml injection loop (2.0ml HI, 1.5ml dopa buffer, 1.5ml 3N NaOH) (Fig. 8).

Caution: Carefully observe the HPLC loading syringe, do not pull more then 5.1ml of liquid into the syringe. Doing so could pull air into the HPLC loop and pull radioactive material into the syringe.

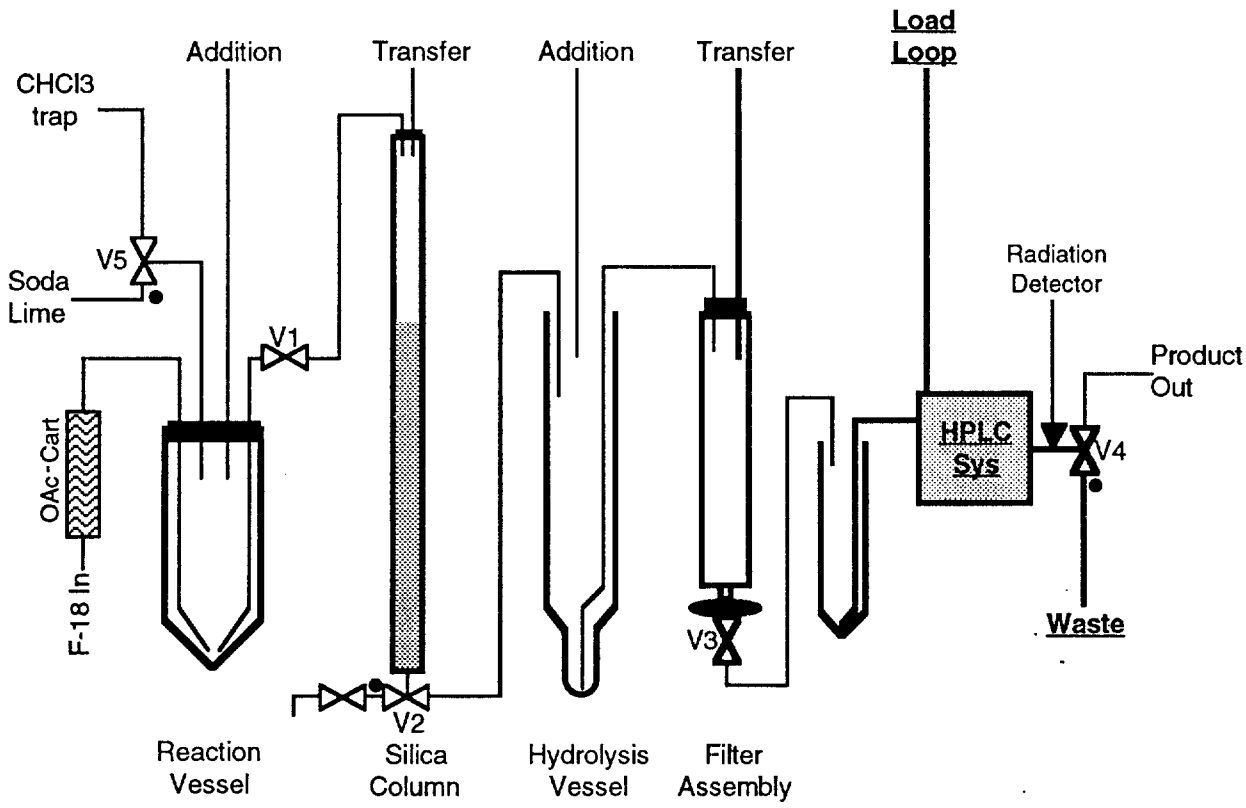


Figure 8. Loading the HPLC injection loop.

D. When the HPLC Loop is loaded:
   1. Switch the **HPLC Injection Valve** to the INJECT position
   2. Start the strip chart recorder
   3. Start a stopwatch

Collection of the Final Product

A. Observe the strip chart recorder, the Fluorodopa peak usually appears between 4 and 6 minutes.

B. When the peak appears turn ON the **Waste/Collect Valve (V4)** to send the HPLC flow to the sterile vial (Fig 9).

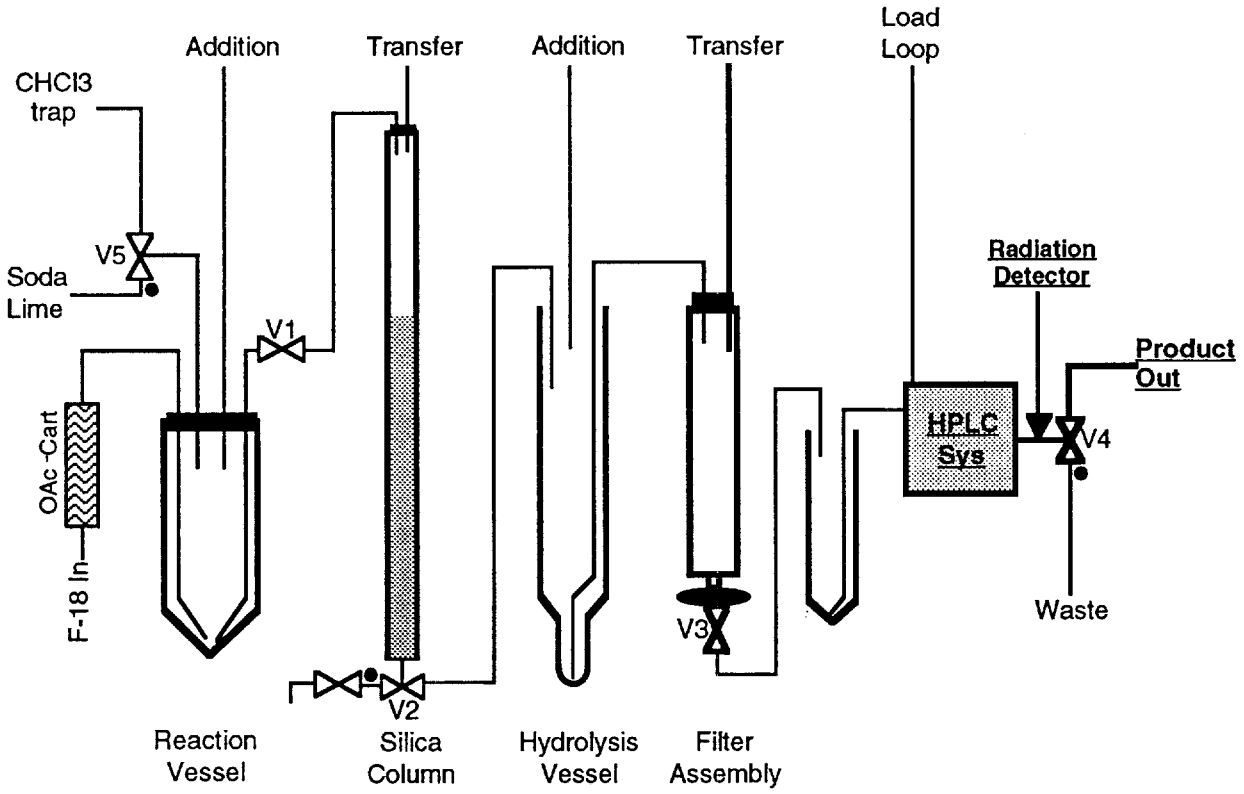


Figure 9. Final product collection

C. Collect the peak until the strip chart trace returns to the base line voltage, approx. 2 minutes, 2cm on the strip chart.
   1. Turn OFF the **Waste/Collect Valve (V4).**
D. Remove the needles from the sterile vial, and read the activity in the dose calibrator.

HPLC Shutdown

A. Change the HPLC input from the Dopa Buffer to 18M ohm water, allow at least 300ml to flow through the system.
B. Change the HPLC input from 18M ohm water to methanol, allow at least 300ml to flow through the system. Then shut off the pump.

System Shutdown

Make sure, in the end, the following are shutdown

A. The heaters
B. Water and compressed air
C. Nitrogen

D. HPLC pump
E. Strip chart recorder

QUALITY CONTROL PROCEDURES

FOR

6-[F-18]FLUORO-L-DOPA

RADIOCHEMICAL QUALITY CONTROL FOR 6-[F-18]FLUORO-L-DOPA

Introduction

The following protocol is used to check the purity of a sample of 6-[F-18]fluoro-L-dopa, utilizing a radioactivity detector.

Materials

1) Freshly made vial of 6-[F-18]fluoro-L-dopa
2) μ Bondapak C-18 HPLC column (30 x 0.46 cm)
3) CH_3OH; 18M ohm water; HOAc
4) HPLC injector with 20 μl load loop
5) Rainin Rabbit-HP Pump
6) Beckman Model 170 Radioisotope Detector
7) Macintosh Computer running Rainin Dynamax HPLC controller and data collection system
8) 1 ml syringe and an injection needle

6-[F-18]Fluoro-L-dopa

For [F-18]fluorodopa synthesis procedure refer to the Production Protocol described earlier. With a 1 ml syringe withdraw ~0.2 ml of the [F-18]fluorodopa sample.

Preparation of HPLC Mobile Phase

Mix 1000 mL of 18M ohm water with 1 mL of glacial HOAc. To 970 mL of this acetic acid solution add 30 mL of CH_3OH and mix well. Filter the solution

through a 0.45 micron nylon filter to remove any particulate matter and then vacuum degas it.

Note: give the HPLC system sufficient time to equilibrate with the solvent system (usually 30 min at a flow rte of 1 ml/mm).

HPLC System Preparation and Shutdown

After use, the HPLC system is flushed with 18M ohm water for 30 min at a flow rate of 1.0 mL/min followed by 100% CH_3OH for 30 min at a flow rate of 1.0 mL/min.

Computer Program Settings

1) Click twice on the hard drive symbol on the Mac screen. At this point a menu will appear.
2) Double click on the "Dynamax" folder
3) Choose "DA" folder and double click
4) Go to "Control" on top of the screen and pull it down. Select "Manual Control" and set the pump flow rate to 0.8 mL/min. Drag the box to the lower left-hand corner using a mouse.
5) Go to "Data" menu and select "Show Report". Drag the box to the lower right-hand corner. This window provides quick information on the retention time and the area under the peaks.
6) From the same "Date" menu select "Data Parameters"
    a) Under "Aquire Data" choose "A" (crosshairs signify confirmation of selection)
    b) Under "Process Data" choose "A" again. Leave the rest of the parameters unchanged and click "OK".

7) From the "Data" menu once again select "Report Format". Click off the "Auto Range Data" function. Leave the rest of the parameters unchanged. Shut down the window by clicking the mouse arrow on a small square in the upper left-hand corner of that window.

8) Go to "Data" menu and select "Show Data". This will bring up an empty window on which the chromatogram will be displayed.

9) As a last task, go to the lower left-hand side of the screen and click once on the "Manual Control" window. This will bring it up to the forefront of the page. Leave the screen in this mode until the moment you are ready to inject.

Sample Injection

With a 1 ml syringe draw about 0.1 ml of sample. Place the injection needle on the syringe. Make sure to evacuate all of the air bubbles from the syringe by tapping it gently on the sides.

Turn the injector to the 'LOAD" position and insert the needle. Gently inject the contents of the syringe.

At this point the sample is in the injector ready to be processed.

Position the mouse pointer on "Aquire Data" and press the mouse button at the same time as you turn the injector lever from "Load" to "Inject" position. Everything from now on is done automatically.

Data Analysis and Discussion

6-[F-18]fluoro-L-dopa elutes in the analytical HPLC as a single peak in the radioactivity detection with ~6.0 min retention time.

RADIOCHEMICAL QUALITY CONTROL
RADIOANALYTICAL HPLC OF
6-[F-18]FLUORO-L-DOPA

Column: μ Bondapak C-18 HPLC column 5 μm, 30 x 0.46 cm.

Eluent: 3% CH_3OH + 97% (0.1% HOAc in water).

Flow rate: 0.8 mL/min.

Chart Speed: 1 cm/min.

Radioactivity detection

CHEMICAL QUALITY CONTROL FOR 6-[F-18]FLUORO-L-DOPA

Introduction

The following protocol is used to check the purity of a sample of 6-[F-18]fluoro-L-dopa.

Materials

1) Freshly made vial of 6-[F-18]fluoro-L-dopa
2) μ Bondapak C-18 HPLC column (30 x 0.46 cm)
3) CH_3OH; 18M ohm water; HOAc
4) HPLC injector with 20 μl load loop
5) Rainin Rabbit-HP Pump
6) Kratos Spectroflow 773 UV detector
7) Macintosh Computer running Rainin Dynamax HPLC controller and data collection system
8) 1 ml syringe and an injection needle

6-[F-18]Fluoro-L-dopa

For [F-18]fluorodopa synthesis procedure refer to the Production Protocol described earlier. With a 1 ml syringe withdraw ~0.2 ml of the [F-18]fluorodopa sample.

Preparation of HPLC Mobile Phase

Mix 1000 mL of 18M ohm water with 1 mL of glacial HOAc. To 970 mL of this acetic acid solution add 30 mL of CH_3OH and mix well. Filter the solution

through a 0.45 micron nylon filter to remove any particulate matter and then vacuum degas it.

Note: give the HPLC system sufficient time to equilibrate with the solvent system (usually 30 min at a flow rte of 1 ml/mm).

HPLC System Preparation and Shutdown

After use, the HPLC system is flushed with 18M ohm water for 30 min at a flow rate of 1.0 mL/min followed by 100% CH_3OH for 30 min at a flow rate of 1.0 mL/min.

Computer Program Settings

1) Click twice on the hard drive symbol on the Mac screen. At this point a menu will appear.
2) Double click on the "Dynamax" folder
3) Choose "DA" folder and double click
4) Go to "Control" on top of the screen and pull it down. Select "Manual Control" and set the pump flow rate to 0.8 mL/min. Drag the box to the lower left-hand corner using a mouse.
5) Go to "Data" menu and select "Show Report". Drag the box to the lower right-hand corner. This window provides quick information on the retention time and the area under the peaks.
6) From the same "Date" menu select "Data Parameters"
    a) Under "Aquire Data" choose "A" (crosshairs signify confirmation of selection)
    b) Under "Process Data" choose "A" again. Leave the rest of the parameters unchanged and click "OK".

7) From the "Data" menu once again select "Report Format". Click off the "Auto Range Data" function. Leave the rest of the parameters unchanged. Shut down the window by clicking the mouse arrow on a small square in the upper left-hand corner of that window.

8) Go to "Data" menu and select "Show Data". This will bring up an empty window on which the chromatogram will be displayed.

9) As a last task, go to the lower left-hand side of the screen and click once on the "Manual Control" window. This will bring it up to the forefront of the page. Leave the screen in this mode until the moment you are ready to inject.

Sample Injection

With a 1 ml syringe draw about 0.1 ml of sample. Place the injection needle on the syringe. Make sure to evacuate all of the air bubbles from the syringe by tapping it gently on the sides.

Turn the injector to the 'LOAD" position and insert the needle. Gently inject the contents of the syringe.

At this point the sample is in the injector ready to be processed.

Position the mouse pointer on "Aquire Data" and press the mouse button at the same time as you turn the injector lever from "Load" to "Inject" position. Everything from now on is done automatically.

Data Analysis and Discussion

6-[F-18]fluoro-L-dopa elutes in the analytical HPLC as a single peak in the UV detection with ~6.0 min retention time. The salts in the preparative HPLC solvent system produced a small peak with a retention time of ~4 min in the analytical HPLC.

CHEMICAL QUALITY CONTROL
ANALYTICAL HPLC (UV TRACE) OF
6-[F-18]FLUORO-L-DOPA

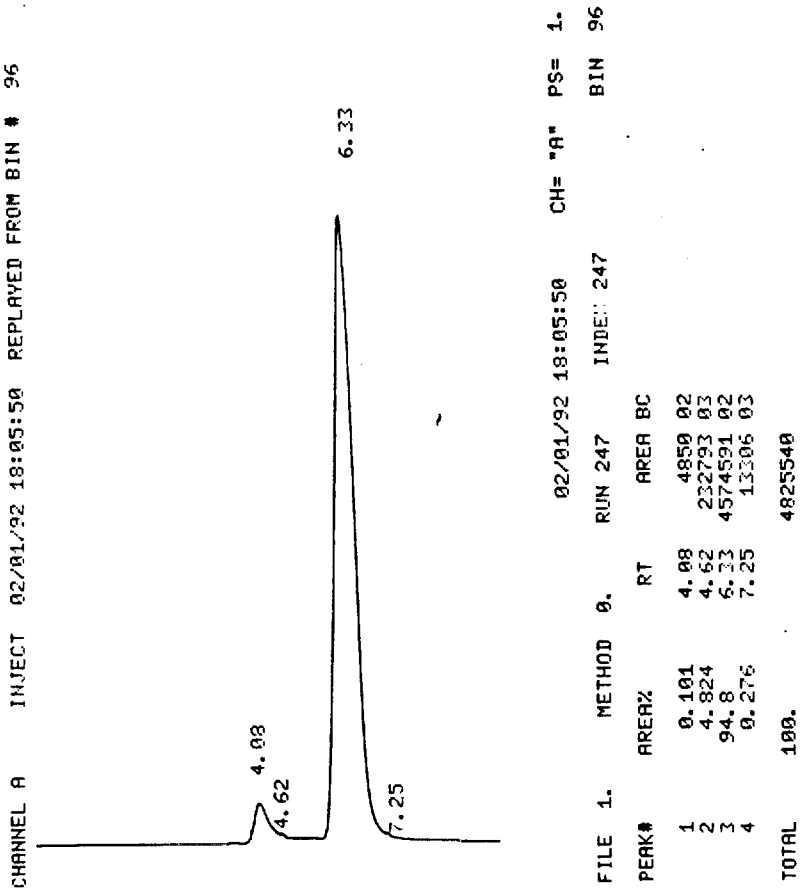
Column: μ Bondapak C-18 HPLC column 5 μm, 30 x 0.46 cm.

Eluent: 3% CH_3OH + 97% (0.1% HOAc in water).

Flow rate: 0.8 mL/min.

Chart Speed: 1 cm/min.

UV wave length: 282 nm.



RADIONUCLIDIC QUALITY CONTROL OF 6-[F-18]FLUORO-L-DOPA

Introduction

The following protocol is used to determine the radionuclidic purity of a sample of 6-[F-18]fluoro-L-dopa.

Materials

1) ORTEC Gamma-Ray Spectrometer
2) Gamma Vision Software
3) Capintec Radioisotope Calibrator Model CRC-15R

6-[F-18]Fluoro-L-dopa Sample Preparation

For [F-18]fluorodopa synthesis procedure refer to the Production Protocol described earlier.

Analysis and Discussion

The γ-ray spectrometer was first calibrated with a NBS standard radioactive source for energy and efficiency. A 1 μL capillary tube was dipped into a sample of 6-[F-18]fluoro-L-dopa and the capillary tube was placed in a 5 mL glass beaker and kept in the counting chamber of the γ-ray spectrometer. The γ-ray spectrum showed only 511 keV peak attesting to the F-18 radionuclidic purity in the sample as 100%.

A 0.1 mL sample of 6-[F-18]fluoro-L-dopa taken in a glass test tube was placed in the radioisotope calibrator chamber. The decrease of radioactivity with time was

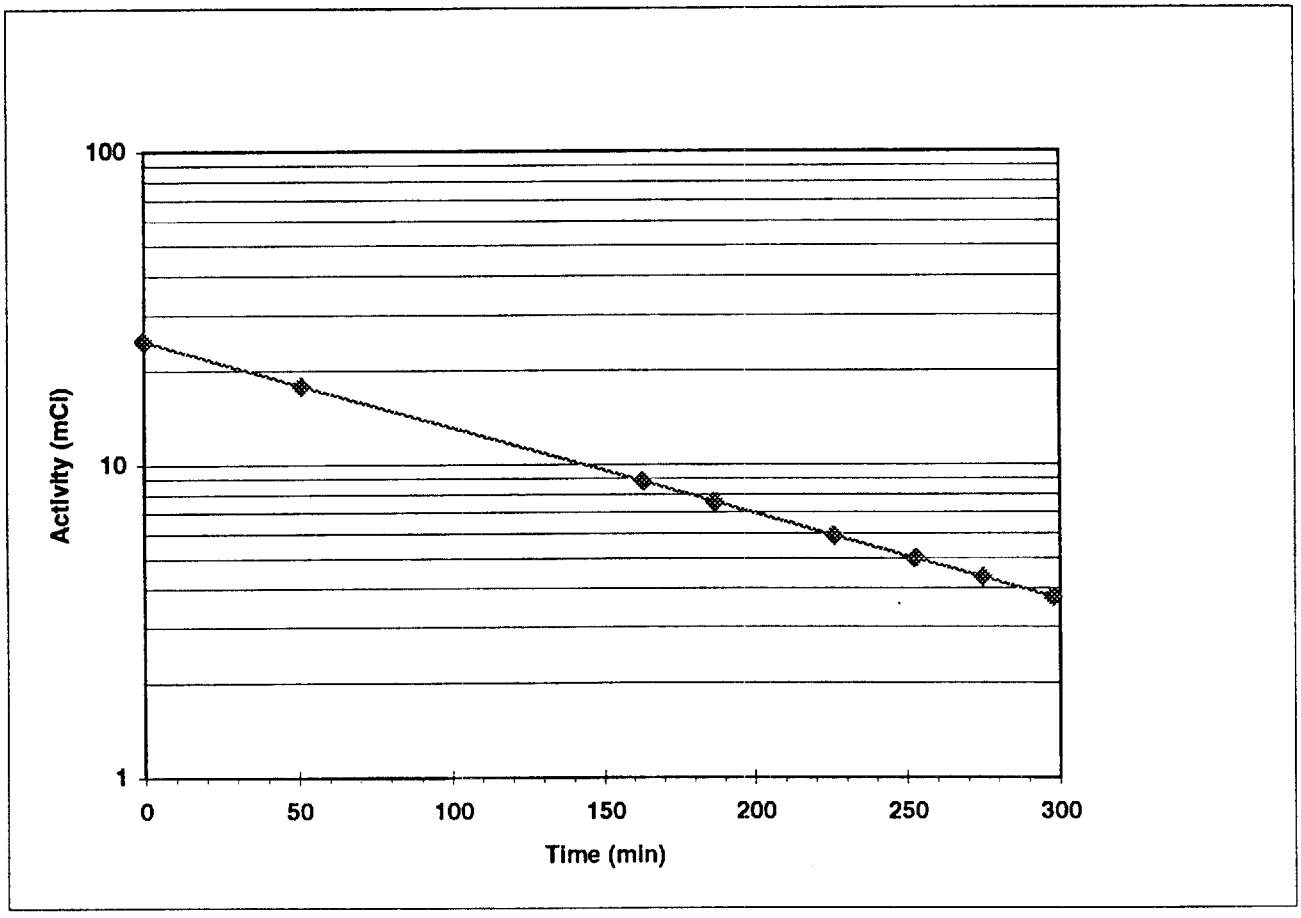
measured and from which the half-life of the isotope was determined to be 109.8 min consistent with the half-life of F-18 isotope.

DECAY CURVE ANALYSIS FOR

[F-18] HALF LIFE

Time (min)	Observed Activity (mCi)	Calculated Half-Life (min)	Corrected Activity (mCi)
0	24.7		
51	17.8	107.9	24.56
163	8.80	109.5	24.62
187	7.55	109.3	24.58
226	5.90	109.4	24.57
253	4.97	109.3	24.54
275	4.33	109.4	24.56
298	3.74	109.4	24.53

Average Half-life **109.2** min
% Error From 109.8 min **-0.57%**



PROCEDURE FOR TESTING STERILITY OF 6-[F-18]FLUORO-L-DOPA

Materials

1. Incubator set at 34°C
2. Thioglycollate medium, enriched with vitamin K1 and Hemin, 8 ml (Baxter Scientific)
3. 0.5 ml of sterile water (as negative standard), bacteria contaminated solution (as saliva for positive standard) and test specimens

Procedure

1. To use optimally: before use, boil thioglycollate tubes for 10 min to rid media of oxygen (have tubes slightly open to prevent any cracking of tubes)
2. Allow tubes to cool to room temperature
3. Add approximately 0.5 ml of specimen to thioglycollate tubes
4. Close tube leaving them slightly loose and incubate
5. Examine cultures for 7 days for evidence of growth
6. Report results as "No growth" or negative or positive if there is any growth

Reference

1. Saule, B.M. (ed.), 1983. The APIC Curriculum for Infection Control Practice Association for Practitioners in Infection Control.

2. Widmann, F.K. (ed.), 1985 AABB Technical Manual, Ninth edition, Arlington, VA, American Association of Blood Banks; pp 325-343.

PROCEDURE FOR TESTING PYROGENICITY OF 6-[F-18]FLUORO-L-DOPA

Materials

1. Limulus amebocyte lysate (LAL) lymphylized
2. E-coli endotoxin 0.55: B5 10 mg lymphylized Kit Cat # N 284 from Whittaker Bioproducts, Inc. 8830 Biggs Ford Road, Walkersville, MD 21793-0127
3. Pyrogen free water-commercially available sterile water for injection (USP) is usually satisfactory
4. Pipettes: 1, 5, 10 ml and 100 µl - pyrogen free
5. 10 x 75 ml glass reaction tubes, pyrogen free - available from Whittaker Bioproducts, Inc. or may be rendered pyrogen free by heating at 180°C for 4 hr

Procedure

1. Set up pyrogen free reaction tubes (10 x 75 m) accordingly for standards and unknown samples in triplicate
2. Reconstitute control standard endotoxin (CSE) with required amount of pyrogen free water as stated on vial. Reconstituted CSE maybe stored at 2-8°C for up to 4 weeks
   a. Vortex well and dilute to concentrations of 1 EU/ml
   b. Make series of dilution of 0.5, 0.25, 0.12, 0.06 and 0.03 EU/ml for standards
   c. Use pyrogen free water as negative control
3. Reconstitute the lysate with pyrogen free water according to vial instructions. Swirl gently but thoroughly for at least 30 sec
4. Carefully transfer 100 µl of water, standards and test samples into appropriate reaction tubes
   (NOTE: Test specimens should be in the pH range of 6.0-7.5. If necessary pH adjust with pyrogen free acid or base. Do not adjust unbuffered solutions)
5. Add 100 µl of reconstituted LAL to each reaction tube, immediately mix and place in a 37°C water bath. After exactly 1 hr of incubation, remove tube from both and examine for gelation
6. Record results as either positive (gelation) or negative (no gelation)

Initial Quality Control - for lysate sensitivity

Endpoint dilution is determined as the last dilution of endotoxin which still yields a positive result

Assay Results
CSE DILUTIONS (EU/ml)

Replicates	0.5	0.25	0.12	0.6	0.3	H_2O	Endpt.
1	+	+	+	-	-	-	0.12
2	+	+	+	-	-	-	0.12
3	+	+	+	+	-	-	0.06
4	+	+	+	-	-	-	0.12

Lysate sensitivity is calculated by determining geometric mean of the end point. Each end point is converted to log 10. Individual log 10 values are averaged and the lysate sensitivity is taken as the antilog 10 of this average log value.

Calculation of Geometric Mean Endpoint

Endpoint EU/ml	Log 10 Endpoint
0.12	-0.921
0.12	-0.921
0.06	-1.222
0.12	-0.921

Mean = -0.996
Antilog 10 mean = 0.10 EU/ml
Data of endotoxin is unknown
Lysate sensitivity = 0.10 EU/ml

Determination of Endotoxin in Unknown Sample
Lysate Sensitivity = 0.10 EU/ml

Sample Dilution

Replicate	1/2	1/4	1/8	1/16	1/32	1/64
1	+	+	+	-	-	-
2	+	+	+	+	-	-

Endpoint dilution	Log 10 endpoint
1/8 (0.125)	-0.903
1/16 (0.0625)	-1.204
	Mean -1.054

Antilog 10 mean = 0.088 = 1/11.3

Endotoxin Conc. = lysate sensitivity x endpoint dilution
= 0.10 x 11.3 = 1.13 EU/ml

Reference

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UCLA RADIOPHARMACY DELIVERY SERVICE

PRODUCT: 6-[F-18]Fluoro-L-Dopa DATE: 4-3-96

LOT NO.: 4-3-96

FORMULATION: Fluorine-18 6-Fluoro-L-Dopa

PURPOSE: PET Research/Clinic

CONTAINER: 30 ml. sterile glass vial

CLOSURES: Rubber stopper
Aluminum seal

Test	Sampling Requirements (mL)	Limits	Results
pH	0.1	3.5-8.5	3.6
Appearance	stock	clear, colorless	√
Bacterial Endotoxin	0.5	175 EU/v	negative
Sterility	1.0	sterile	no growth

LITERATURE REFERENCES

FOR

6-[F-18]FLUORO-L-DOPA

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Remote, Semiautomated Production of 6-[^{18}F]Fluoro-L-dopa for Human Studies with PET

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Regioselective radiofluorodemercuration of the 6-mercurio derivative **5** with [^{18}F]acetylhypofluorite afforded, after acidic hydrolysis, 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine (6-FD, **1**) with a radiochemical yield of 11% (decay corrected and based on the total amount of [^{18}F]F_2 recovered from the target). 6-FD was obtained with a chemical and radiochemical purity of >99% and with a level of mercury in the final preparation of less than 20 ppb. Utilization of a remote, semiautomated production system, resulted in the preparation of a sterile, pyrogen-free product suitable for human injection after a synthesis time of 50 min.

Introduction

In the last decade, enormous efforts have been devoted to the synthesis of 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine (6-[^{18}F]fluoro-L-dopa, 6-FD, **1**) primarily due to its use as a probe of central dopamine metabolism *in vivo* in humans with positron emission tomography (PET) (Garnett *et al.*, 1983). Several methods for the synthesis of **1** have been described (Adam *et al.*, 1986a,b; Chirakal *et al.*, 1984, 1986; Chaly and Diksic, 1986). However, these methods, owing to the lack of regioselectivity, afforded 6-FD in low yield frequently after an extensive purification procedure by high pressure liquid chromatography (HPLC).

The need for dependable and reliable production of **1** necessitated the development of new synthetic pathways which could be adapted to radiolabeling with ^{18}F. We have recently investigated the fluorodemercuration reaction and applied successfully this methodology for the preparation of fluorosubstituted veratroles (Luxen *et al.*, 1987; Luxen and Barrio, 1988). In the present paper, we describe a high yield synthesis of **1** based on a radiofluorodemercuration procedure followed by an acidic hydrolysis (Luxen *et al.*, 1986; Adam and Jivan, 1988) (Fig. 1). We also describe a remote, semi-automated system used to perform this synthesis, which includes the following processing steps, (a) fluorination of the organomercury derivative **5**; (b) purification of **7** by column chromatography; (c) acid hydrolysis of **7**; and (d) HPLC purification and sterilization of **1**.

Material and Methods

Proton nuclear magnetic resonance spectra (^{1}H NMR) and ^{19}F NMR were recorded on a Bruker AM-360 spectrometer. Proton chemical shifts are reported in parts per million (ppm) relative to the internal standards tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). ^{19}F chemical shifts were referenced to an external standard ($CFCl_3$). Infrared spectra (i.r.) were recorded on a Perkin–Elmer 710 B spectrophotometer. Mass spectral data were collected on a AEI MS-902 mass spectrometer. Melting points (m.p.) were determined on an Electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith, Microanalytical Laboratories, Knoxville, Tenn.

TLC analysis was performed on Merck silica gel 60F-254 plates and visualized by u.v. illumination or

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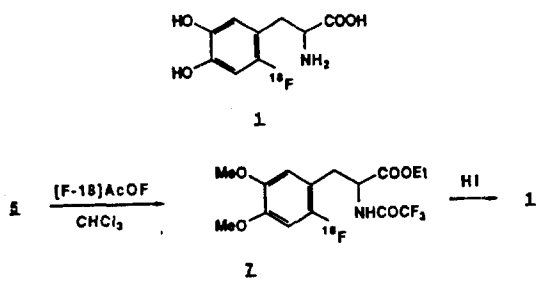

Fig. 1. Synthesis of 6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine (1).

analyzed for radioactivity (Radio TLC Berthold Model LB 2832). TLC solvent systems employed were: (A) ethyl acetate; (B) petroleum ether–ethyl ether (1:1). Silica gel column chromatography was performed on Merck Kieselgel 60 (70–230 mesh); L-dopa, D-dopa, formic acid, dimethylsulfate, acetic anhydride, mercuric trifluoroacetate, 1-octanesulfonic acid sodium salt, trifluoroacetic anhydride (Aldrich), and gaseous hydrogen chloride (Matheson) were used as received. Hydriodic acid (55%) (Aldrich) was distilled under hydrogen and sealed in ampules with 0.04% hypophosphorus acid as stabilizer.

3,4-Dimethoxy-L-phenylalanine ethyl ester-hydrochloride (3)

A solution of *N*-formyl-3,4-dimethoxy-L-phenylalanine (Schrecker and Hartwell, 1957) (8.0 g, 31.6 mmol) in absolute ethanol (200 mL) was saturated with dry hydrogen chloride, refluxed for 3 h and the solvent evaporated *in vacuo*. The residual solid material was recrystallized from ethanol–ethyl ether (1:6) to afford the hydrochloride (3) as a white solid (8.34 g, 91%). R_f: 0.16 (Solvent A), m.p. 174–175°C; ^{1}H NMR (D_2O/DSS): δ 1.25 (t, 3H, CH_3), 3.15–3.30 (m, 2H, CH_2), 3.85 (s, 6H, OCH_3), 4.28 (q, 2H, OCH_2), 4.38 (t, 1H, CH), 6.87 (dd, J = 7.7 Hz, J < 1 Hz, 1H, H-6), 6.90 (d, J < 1Hz, 1H, H-2), 7.02 (d, J = 7.7 Hz, 1H, H-5). The above product (100 mg) was dissolved in water (10 mL) and sodium bicarbonate (200 mg) was added. After extraction with ethyl acetate (10 mL) the organic layer was dried ($MgSO_4$) and concentrated to afford the free base, 3,4-dimethyloxy-L-phenylalanine ethyl ester as an oil: ^{1}H NMR (CD_3OD/TMS): δ 1.21 (t, 3H, CH_3), 2.84–2.97 (m, 2H, CH_2), 3.67 (t, 1H, CH), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.13 (q, 2H, OCH_2), 6.75 (dd, J = 8.2 Hz, J = 1.5 Hz, 1H, H-6), 6.79 (d, J = 1.5 Hz, 1H, H-2), 6.87 (d, J = 8.2 Hz, 1H, H-5). The above NMR data were not in agreement with those reported by Diksic and Farrokhzad (1985).

N-(Trifluoroacetyl)-3,4-dimethoxy-L-phenylalanine ethyl ester (4)

To trifluoroacetic anhydride (50 mL) cooled in an ice bath was added compound 3 (8.0 g, 27.6 mmol), and the reaction mixture was stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure and the residue was co-evaporated with ethanol (3 × 20 mL). Recrystallization of the residual solid from diethyl ether–petroleum ether afforded 4 as a white solid (9.05 g, 94%). R_f: 0.23 (Solvent B); m.p. 74–75°C; i.r. ϑ_{max} ($CHCl_3$) 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS): δ 1.30 (t, 3H, CH_3), 3.11–3.22 (m, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.25 (q, 2H, OCH_2), 4.80–4.85 (m, 1H, CH), 6.58–6.80 (m, 4H, aromatic H's, NH). MS (m/e, relative intensity): 349 (M^+, 14), 304 (1), 236 (9), 164 (1), 152 (14), 151 (100), 107 (3), 106 (2), 105 (2), 91 (2), 90 (1), 78 (2), 77 (2), 69 (1). Anal. calcd for $C_{15}H_{18}F_3NO_5$: C, 51.57; H, 5.19; N, 4.01. Found: C, 51.55; H, 5.19; N, 3.98.

N-(Trifluoroacetyl)-3,4-dimethoxy-6-trifluoroacetoxymercurio-L-phenylalanine ethyl ester (5)

To a solution of 4 (8.0 g, 22.9 mmol) in absolute ethanol (250 mL) was added mercuric trifluoroacetate (10.48 g, 24.6 mmol), and the reaction mixture was stirred at room temperature for 5 days. The solvent was concentrated under reduced pressure and the residue was recrystallized twice from diethyl ether–petroleum ether to afford compound 5 as a pale yellow solid (10.0 g, 66%); m.p. 123–125°C; ^{1}H NMR ($CDCl_3$/TMS): δ 1.26 (t, 3H, CH_3), 3.1–3.3 (m, 2H, CH_2), 3.88 (s, 6H, OCH_3), 4.22 (dq, 2H, OCH_2), 4.82 (q, 1H, CH), 6.75 (s, $J_{^1H-^{199}Hg}$ = 87 Hz, 1H, H-2), 6.79 (s, $J_{^1H-^{199}Hg}$ = 232 Hz, 1H, H-5), 7.2 (m, 1H, NH). Anal. calcd for $C_{17}H_{17}F_6HgNO_7$: C, 30.85; H, 2.59; N, 2.12. Found: C, 29.97; H, 2.44; N, 1.99.

N-(Trifluoroacetyl)-3,4-dimethoxy-6-fluoro-L-phenylalanine ethyl ester (7)

Acetyl hypofluorite (AcOF) (prepared from 500 μmol of fluorine, 0.5% in neon) generated in the gas phase (Bida *et al.*, 1984) was bubbled over a period of 40 min into a solution of 5 (0.569 g, 860 μmol) in chloroform (40 mL) at room temperature. The organic phase was successively washed with 1M $Na_2S_2O_5$ (20 mL), water (2 × 20 mL), dried over anhydrous $MgSO_4$, concentrated and chromatographed (Silica gel, CH_2Cl_2/MeOAc, 90:10) to afford the fluorinated derivative 7 as a white solid (73 mg, 40%); m.p. 90–91°C, ^{1}H NMR ($CDCl_3$/HMDS): δ 1.31 (t, 3H, CH_3), 3.15–3.35 (m, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.24, 4.25 (dq, 2H, OCH_2), 4.75–4.85 (m, 1H, CH), 6.53 (d, J = 7.0 Hz, 1H, H-2), 6.61 (d, J = 11.3 Hz, 1H, H-5), 6.8–7 (m, 1H, NH); ^{19}F NMR ($CDCl_3$/$CFCl_3$): δ-124.9 (q, J = 7.0 Hz, J = 11.0 Hz), −76.3 (s). MS, m/e (relative intensity) 367 (M^+, 4), 294 (1), 254 (6), 209 (1), 182 (2), 170 (11), 169 (100), 166 (2), 155 (1), 153 (2), 126 (4), 125 (8), 124 (3), 123 (3), 109 (3), 108 (3), 107 (2), 97 (3), 96 (5), 95 (2), 83 (4), 75 (1), 69 (6), 57 (1).

N-(Trifluoroacetyl)-3,4-dimethoxy-6-iodo-L-phenylalanine ethyl ester (6)

Method (a). Silver trifluoroacetate (0.64 g, 2.9 mmol) and iodine (0.56 g, 2.2 mmol) were added to a solution of compound **4** (0.75 g, 2.2 mmol) in carbon tetrachloride (50 mL) and the reaction mixture was stirred at room temperature for 48 h. The precipitate was removed by filtration, washed with dichloromethane, and the combined filtrate was washed with 1M $Na_2S_2O_5$ (2 × 50 mL) and then with water (2 × 50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to a solid residue. Recrystallization of this residue from diethyl ether–petroleum ether (95:5) afforded **6** as a white solid (0.71 g, 68%). R_f: 0.25 (Solvent B); m.p. 160–161°C; ^{1}H NMR ($CDCl_3$/TMS): δ 1.27 (t, 3H, CH_3), 3.14–3.36 (m, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.25 (dq, 2H, OCH_2), 4.84–4.90 (m, 1H, CH), 6.68 (s, 1H, H-2), 6.89 (d, 1H, NH), 7.21 (s, 1H, H-5). MS m/e, (relative intensity): 475 (M^+, 19), 278 (8), 277 (100), 276 (1), 275 (2), 236 (2), 235 (2), 207 (3), 151 (7), 150 (2), 107 (1), 91 (1), 77 (2). Anal. calcd for $C_{15}H_{17}F_3I\,NO_5$: C, 37.91; H, 3.61; N, 2.95. Found: C, 38.01; H, 3.56; N. 2.93.

Method (b). A solution of iodine (0.05 g, 200 μmol) in $CHCl_3$ (5 mL) was added at room temperature to a solution of the mercurio derivative **5** (0.145 g, 220 μmol) in $CHCl_3$ (10 mL) and the solution was stirred for 10 min. The organic layer was washed with 1M $Na_2S_2O_5$ (20 mL), water (2 × 20 mL), dried over $MgSO_4$ and concentrated to yield pure **6** (85 mg, 89%). The product had the same spectral characteristics as those described above for **6** [Method (a)].

6-Fluoro-3,4-dihydroxy-L-phenylalanine (6-fluoro-L-dopa) (1)

L-*N*-(Trifluoroacetyl)-3,4-dimethoxy-6-fluoro-L-phenylalanine ethyl ester (**7**) (10 mg) was dissolved in 55% HI (2 mL) and the solution was refluxed for 30 min. After evaporation of HI under vacuum, the residue was dissolved in D_2O (2 mL) and evaporated: Yield 95%; ^{1}H NMR (DCl/DSS): δ 3.1–3.4 (m, 2H, CH_2) 4.2–4.4 (m, 1H, CH) 6.65 (d, J = 10.0 Hz, H-5), 6.75 (d, J = 7 Hz, H-2); ^{19}F NMR (DCl/$CFCl_3$) δ: −125.6 (decoupled). Analysis of the product by HPLC (Ultrasphere ODS, 5 μm, 4.6 × 250 mm; eluent: 83.5% 0.1 M NaH_2PO_4, 2.6 mM octanesulfonic acid sodium salt, 0.1 mM EDTA; pH: 3.35; 16.5% methanol; flow rate 1 mL/min; u.v. detection: 282 nm) revealed a single peak having a retention time at about 6 min (Fig. 2).

Remote semiautomated production of 6-[^{18}F]fluoro-L-dopa (Fig. 3)

[^{18}F]Fluorine was produced from the deuteron bombardment of 0.1% F_2–Neon mixture by the ^{20}Ne (d, α) ^{18}F nuclear reaction (9.4 MeV on target at 25 μA for 30–60 min). At the end of bombardment,

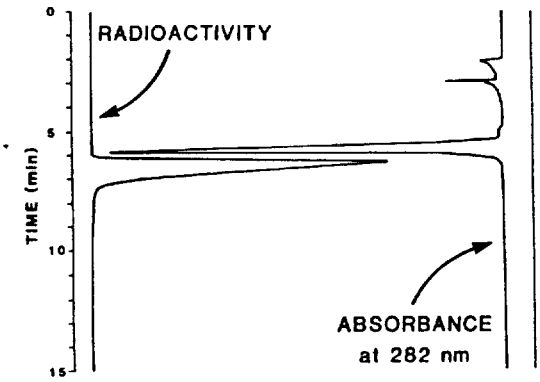

Fig. 2. Chemical and radiochemical purities of the 6-[^{18}F]fluoro-L-dopa preparations are routinely monitored by HPLC analysis using a reverse phase column coupled with u.v. and radioactivity detection of the column effluent (see Material and Methods).

[^{18}F]F_2 (100 μmol) contained in the target chamber was passed through a cartridge (5 mm i.d. × 35 mm) containing powdered NaOAc·$3H_2O$ (340 mg) (Bida *et al.*, 1984). The effluent from the cartridge was bubbled into a reaction vessel (A) containing the mercurio derivative **5** (101 mg, 153 μmol) in chloroform (10 mL); [^{18}F]AcOF escaping from the reaction mixture was contained within the soda lime trap. After the bubbling had ceased, the volume of the resultant organic solution was reduced under N_2 to 1 mL using the aluminum block heater (75°C). The aluminum block was rapidly cooled with water and diethyl ether (10 mL) was added in two portions to the solution. The ethereal solution was transferred through a column (B) (7 mm i.d.) which has been packed with $Na_2S_2O_3$ (25 mm) and silica gel (75 mm) and had been previously equilibrated with diethyl ether. The fluoro compound ([^{18}F]labeled **7**) was eluted from the column and collected in test tube (16 × 125 mm) (C). The solvent was then evaporated under N_2 with heat supplied from the thermostatically controlled oil bath (130°C). Hydrolysis of **7** began when HI (55%, stabilized with 0.04% hypophosphorus acid, 1.6 mL) was added in two portions and the solution was refluxed. Reflux was maintained by having a cool set of N_2 gas surrounding the upper portion of the test tube. After the solution was refluxed for 10 min, the oil bath was remotely lowered and the solution allowed to cool. Partial neutralization of HI was accomplished by using 0.75 equiv of 3N NaOH (~1.8 mL) followed by addition of the HPLC—mobile phase (see below) to a total volume of 4 mL. Prior to injection, the solution was transferred to a syringe (D) (12 mL) and filtered (0.22 μm membrane filter (Millipore-Vented Millex-GS)). The solution was then loaded into the 5.0 mL HPLC—loop (F) by drawing the solution from its vessel (E) to the Rheodyne 7124 injector by the use of negative pressure at the loop vent. The solution was loaded remotely onto a semi-preparative column (Adsorbosphere C-18, 7 μm, 10 × 300 mm, eluent 5 mM

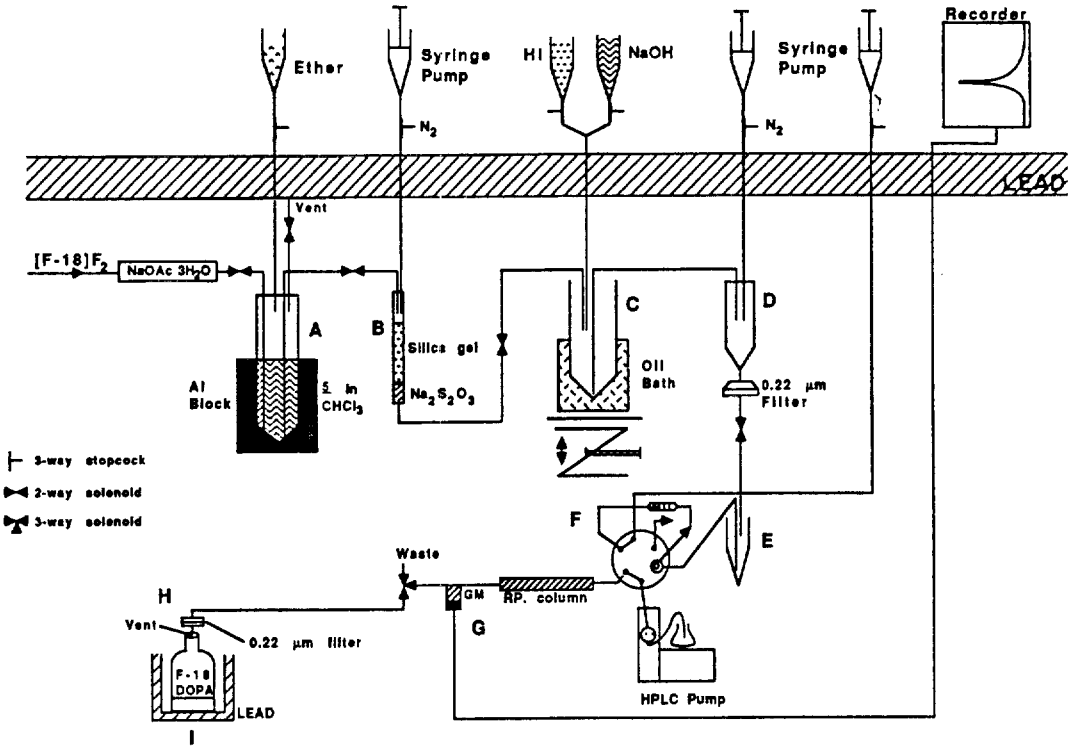

Fig. 3. Remote, semiautomated system for producton of 6-[^{18}F]fluoro-L-dopa. It is conceptually divided into 5 operational units: (a) fluorination; (b) purification by column chromatography on silica gel; (c) acidic hydrolysis; (d) purification by HPLC; and (e) sterilization. Specific components are; A, reaction vessel; B, silica gel column; C, hydrolysis vessel; D, syringe (12 mL); E, test tube; F, HPLC loop (5.0 mL); G, in-line plastic scintillation detector; H, 0.22 μm-pore membrane filter; and I, injection vial.

sodium acetate, 1 mM EDTA, 0.1% HOAc, 0.01% ascorbic acid; pH: 4; flow rate, 7 mL/min) and the fraction eluting from the HPLC column which corresponded to 6-[^{18}F]fluoro-L-dopa, as monitored by an in-line plastic scintillation detector (G), was collected by passing the eluent directly through a sterile 0.22 μm membrane filter (Millipore-Vented Millex-GS) (H) in a sterile vial (20 mL) (I) containing NaCl to insure the isotonicity of the final solution. The chemical (>99%) and radiochemical purity (>99%) of the final product was monitored by HPLC using the procedure described above for the unlabeled compound (Fig. 2). The enantiomeric purity of the final preparation was determined by ligand exchange chromatography (Chiral Pro = Si 100 Polyol (Serva) 4.6 × 250 mm; eluant: 50 mM KH_2PO_4, 1 mM Cu SO_4; pH: 4; flow rate, 1 mL/min) and found to be greater than 99%, which indicated that no racemization occurs during the preparative process (Fig. 6). 6-[^{18}F]Fluoro-D-dopa was obtained from D-dopa using the same reaction sequence than that described in this work for its L-enantiomer (Luxen *et al.*, 1987).

Discussion

Electrophilic substitution of activated aromatic rings with elemental fluorine, or other fluorinating agents, is frequently difficult to control (Purrington *et al.*, 1986). Therefore, it is not surprising that direct fluorination of dopa or dopa derivatives with F_2 (Chirakal *et al.*, 1986; Firnau *et al.*, 1984) or with AcOF (Adam *et al.*, 1986a,b) has been described to afford a mixture of isomers (2-, 5- and 6-fluoro-L-dopa) in low yields. Reactions of aryltrimethylsilanes with [^{18}F]F_2 or with [^{18}F]AcOF have also been reported to afford low yields of monofluorinated materials that were frequently present with other polyfluorinated products (Purrington *et al.*, 1986). Nevertheless, a synthesis based on radiofluorodesilylation has been proposed (Diksic and Farrokhzad, 1985), but the difficulty in the preparation of the starting material, based on a Grignard reaction, makes this method unattractive for the routine preparation of 1.

Recently, Visser *et al.* (1984, 1986) reported that selective aromatic radiofluorination can be performed in high yields using a fluorodemercuration reaction. Electrophilic mercuration allows for the presence of many sensitive organic functionalities (Larock, 1985) whereas classical metallation with elements of Group 14 (Sn, Ge, Si) requires the use of lithium and Grignard intermediates. The mercurio derivative 5 was prepared from commercially available L-dopa by a 4 step sequence in an overall yield of 36%. The synthetic sequence for the preparation of 5, depicted in Fig. 4, was utilized to prevent

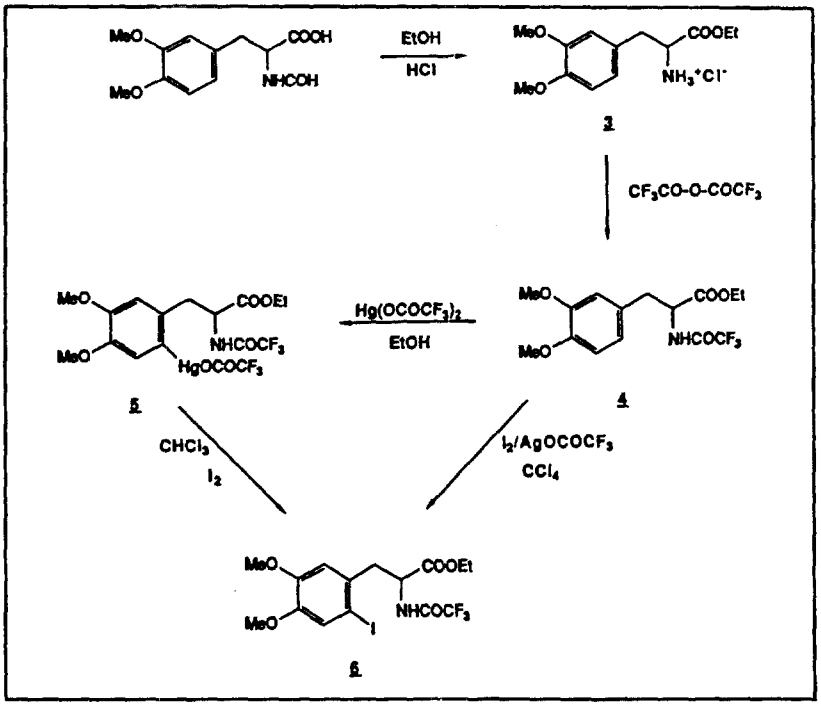

Fig. 4. Reaction sequence for the preparation of *N*-(trifluoroacetyl)-3,4-dimethoxy-6-trifluoroacetoxymercurio-L-phenylalanine ethyl ester (**5**).

racemization (i.e. retention of the L-configuration). A key step is the preparation of **2** by methylation of the formylaminocatechol precursor, that under the conditions described (Schrecker and Hartwell, 1957), produced enantiomerically pure **2**. In addition, the trifluoroacetamido protecting group was selected for its relative ease of deprotection (Bodanszky, 1984).

The mercuration reaction is highly solvent dependent. For example, mercuration of **4** with mercuric trifluoroacetate in ethanol afforded exclusively the 6-regioisomer whereas reaction of **4** with mercuric trifluoroacetate in acidic solvents such as acetic acid or trifluoroacetic acid produced a mixture of aromatic mercurio regioisomers. In addition, the transesterification product can also be obtained when the mercuration reaction is performed in methanol.

The purity of **5** and the regioselectivity of the mercuration reaction were assessed by spectroscopic and chemical means. The two singlets at 6.75 and 6.79 ppm in the ^{1}H NMR spectrum of **5** were attributed to the aromatic protons H-2 and H-5 with their respective satellites ($J_{^1H-^{199}Hg} = 87$ Hz and $J_{^1H-^{199}Hg} = 232$ Hz). Furthermore, the product of the iodination of **5** with iodine [Method (a)] was identical in all respects with an authentic standard **6** prepared by an alternative route [**4** and iodine/silver trifluoroacetate, Method (b)].

Electrophilic radiofluorination of **5** in chloroform with [^{18}F]AcOF (Fig. 1), generated in the gas phase, (Bida *et al.*, 1984) at room temperature afforded the 6-[^{18}F]fluorodopa derivative **7** in good yields. After solvent evaporation and purification of the crude reaction mixture by column chromatography on silica gel, the radiofluorinated derivative **7** was identical in all respects with a standard example prepared using unlabeled fluorine, as determined by ^{1}H NMR, ^{19}F NMR, and mass spectroscopy. The pair of doublets ($J = 11.3$ Hz, $J = 7.0$ Hz) observed for the aromatic protons in the ^{1}H NMR spectrum of **7** as well as the quadruplet observed in the ^{19}F NMR spectrum provided unequivocal evidence for the regioselective aromatic fluorination at position 6. Furthermore, the mass spectrum of **7** (70 eV ionization) revealed the presence of the [M$^+$], and [M$^+$-ethyl] peaks; the peak of greatest intensity (base peak) was at m/e = 169 which corresponded to the benzylic moiety **8b**. This was also observed in the mass spectra

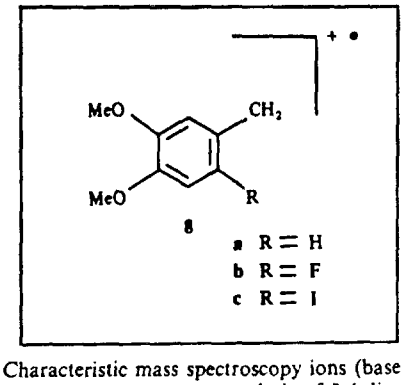

Fig. 5. Characteristic mass spectroscopy ions (base peaks) observed by mass spectroscopy analysis of 3,4-dimethoxyphenylalanine derivatives (i.e. base peaks for **4**, **6** and **7** are **8a**, **8c**, and **8b**, respectively.

Table 1. Effect of solvent on the radiochemical yield of the electrophilic radiofluorination of organomercury derivative 5 with [^{18}F]AcOF generated in the gas phase

Solvent	Radiochemical yield (%) (RTLC)
$CHCl_3$/Freon 9:1	53
$CHCl_3$	55
$CHCl_3$/10% HOAc	62
HOAc	65

of 4 and 6 which showed m/e = 151 (8a) and 277 (8c), repectively (Fig. 5).

The radiofluorination reaction was conducted in various solvents and the results, after analysis by radio-TLC, are summarized in Table 1. Similar radiochemical yields were obtained in acidic and in non polar solvents, for which, as a matter of convenience, routine radiofluorination reactions are better carried out in chloroform or in a mixture of chloroform/freon (1:9). Moreover, the radiofluorination reaction was very sensitive to the stoichiometric ratio of the substrate/fluorinating agent and to the nature of the fluorinating agent. A ratio AcOF/mercury derivative (5) of 1:1 afforded a complex mixture, which was very difficult to purify, whereas a ratio of 1:1.5 afforded a clean reaction. In addition, when [^{18}F]F_2 was employed as the fluorinating agent in place of [^{18}F]AcOF, complex reaction mixtures are obtained and radiolabeled 7 was produced in low yields.

Acid deprotection of 7 with 55% HI at 130°C for 10 min afforded, after purification by HPLC on a semi-preparative reverse phase C-18 column and sterilization (see Materials and Methods) 6-FD (1) with a radiochemical yield of >95%. The ^{19}F- and ^{1}H NMR chemical shifts of the 6-FD sample recorded after radioactive decay were in good agreement with these reported for 6-fluoro-D,L-dopa (Grierson and Adam, 1986; Creveling and Kirk, 1985). Prior to injection in humans, the chemical and radiochemical purity of the 6-FD preparation was routinely monitored by HPLC analysis and was typically found to be >99% (Fig. 2). The pH of the final preparation (approx. 4.0) assures the stability of 6-FD. Oxidative decomposition of 6-FD takes place at pH values at or above neutrality. For ionic mercury levels in the final preparations, an aliquot of the solution was analyzed by HPLC (Luxen et al., 1988). Levels less than 20 ppb (detection limit = 10 ppb) were obtained in all 6-[^{18}F]-fluoro-L-dopa samples, and these results were comparable to those obtained by atomic absorption.

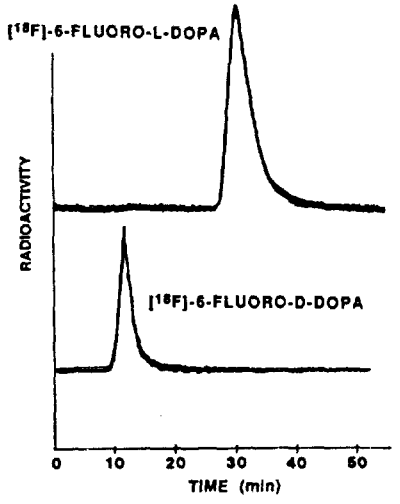

Fig. 6. Radiochromatographic profile of the final preparations of 6-[^{18}F]fluoro-D-dopa and 6-[^{18}F]fluoro-L-dopa as analyzed by HPLC using a chiral proline derivatized column (see Material and Methods). The k' values determined are 2.1 and 7.3 for the D and L isomers, respectively.

Using the experimental procedure and the remote, semiautomated system (Fig. 3) described in this work, 10.7 ± 3.6 mCi (N = 22) were routinely obtained with 1 h deuteron bombardment of the ^{20}Ne target. Similarly, 30 min target bombardment produced 7.5 ± 2.6 (N = 13) mCi of pure 6-[^{18}F]fluoro-L-dopa ready for human injection which corresponds to a radiochemical yield of 11% (decay corrected and based on the total amount of [^{18}F]F_2 recovered from the target). Moreover, all reactions and procedures described in this work for production of 6-[^{18}F]fluoro-L-dopa can be easily automated. A first automated microprocessor controlled synthesis prototype has been built as an extension of the remote, semiautomated principles originated in our Laboratory and described in this work.

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Proton Irradiation of $[^{18}O]O_2$: Production of $[^{18}F]F_2$ and $[^{18}F]F_2$ + $[^{18}F]OF_2$

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ABSTRACT. The production of ^{18}F electrophilic reagents via the $^{18}O(p,n)^{18}F$ reaction has been investigated in small-volume target bodies made of aluminum, copper, gold-plated copper and nickel, having straight or conical bore shapes. Three irradiation protocols—single-step, two-step and modified two-step—were used for the recovery of the ^{18}F activity. The single-step irradiation protocol was tested in all the target bodies. Based on the single-step performance, aluminum targets were utilized extensively in the investigation of the two-step and modified two-step irradiation protocols. With an 11-MeV cyclotron and using the two-step irradiation protocol, >1Ci $[^{18}F]F_2$ was recovered reproducibly from an aluminum target body. Probable radical mechanisms for the formation of OF_2 and $FONO_2$ (fluorine nitrate) in the single-step and modified two-step targets are proposed based on the amount of ozone generated and the nitrogen impurity present in the target gases, respectively. NUCL MED BIOL 23;3:189–199, 1996.

KEY WORDS. $[^{18}F]F_2$, $[^{18}F]OF_2$, Single-step irradiation, Two-step irradiation, Aluminum target, Mechanism of OF_2 formation

INTRODUCTION

Fluorine-18 labeled F_2 has maintained a high profile in the continually expanding development of substrates and analogs for positron emission tomography (PET) (32). Previously, the major emphasis for the production of $[^{18}F]F_2$ has been the $^{20}Ne(d,\alpha)^{18}F$ reaction in nickel target bodies (22). The higher cross sections for the $^{18}O(p,n)^{18}F$ reaction (50) have spurred the development of targetry designed for use with this relatively expensive enriched isotope (4, 6, 12, 41, 62, 66). This interest has been further enhanced by the advent of low-energy, proton-only, hospital-based cyclotrons and, thus, targetry to produce electrophilic ^{18}F fluorinating agents via these means has been developed (66). As in the case of the neon reaction, these investigations also utilized a nickel target body (4, 12, 41, 62). There has also been experimentation with a gold-plated target body for the production of ^{18}F electrophilic fluorinating agents using the $^{18}O(p,n)^{18}F$ nuclear reaction (66).

Among the positron emitting reagents for PET, production of ^{18}F electrophilic fluorinating agents is known to be the most intractable. Drawbacks include the high maintenance and sensitive targetry that has generally yielded inconsistent quantities of ^{18}F isotope (22). Yet there has been no comprehensive investigation of target body materials and their roles, if any, in the formation of the fluorinated species. Thus, we have recently evaluated the suitability of a variety of metals for the production of ^{18}F electrophilic fluorinating agents (7). Among them we observed, for the first time, aluminum as a suitable metal for fabrication of fluorine gas targets (6). Aluminum target bodies have been in use for the routine production of $[^{18}F]F_2$ using $^{20}Ne(d,\alpha)^{18}F$ as well as $^{18}O(p,n)^{18}F$ reactions in our laboratories since 1990 (39, 40). In this article we describe the results of the first comprehensive investigation of the $^{18}O(p,n)^{18}F$ nuclear reaction in the gas phase in target bodies made of aluminum, silver, copper, nickel and gold-plated copper. Three different irradiation protocols—namely, single-step, two-step and modified two-step—were used with on-target proton beam energies of 13.5 and 10.3 MeV. A mechanistic rationalization is also provided for postirradiated fluorinated species recovered from various target bodies.

EXPERIMENTAL

Irradiations were performed on CS-22 (The Cyclotron Corporation) and RDS 112 (Siemens/CTI) cyclotrons. The emergent 22-MeV proton beam of the CS-22 cyclotron was degraded with an appropriate carbon degrader, and the experimentally observed on-target beam energy (65), after passing through aluminum (0.025 mm) vacuum and Havar (0.025 mm) target windows, was 13.5 MeV. The 11-MeV proton beam from the RDS 112 cyclotron was degraded to 10.3 MeV on gas by aluminum (0.025 mm) vacuum isolation and Havar (0.025 mm) target body foils.

Target Material

Isotopically enriched $[^{18}O]O_2$ (≥97 atom %; Isotec and EG & G Mound Applied Technologies), 5% fluorine in helium or argon (Matheson and Spectra Gases), 99.998% $[^{16}O]O_2$ (Matheson and Liquid Carbonic), oxygen difluoride (Allied Signal), neon (99.999%), helium (99.995%) and argon (UHP) were obtained from commercial sources. Hydrogen fluoride contamination was removed from the carrier fluorine gas mixtures by passing through an activated NaF trap

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Oxygen difluoride was purified by passing through a NaF trap followed by a cryotrap held at −130°C.

Target Bodies

Parameters critical to the design of targets for PET isotope production include the beam strike volume and geometry. Specifically, a low or zero dead volume beam strike that accommodates small-angle, multiple scattering has made possible the economic use of isotopically enriched materials. Multiple scattering results from long-range Coulomb forces between charged particles and target atoms. The theoretical treatment of multiple scattering and the calculation of approximate scattering angles have been discussed amply (1, 16, 29, 55). Further, an intercomparison of some of these methods has been reported (52). The dimensions of the conical beam strike of the targets used in this investigation derived from a calculation of the scattering angle coupled with empirical refinements based on $^{11}CO_2$ recovery. The scattering angle was calculated using the computer code TRIM, a Monte Carlo program that calculates the slowing and scattering of energetic ions in materials (71). The results obtained using TRIM compared favorably with other methods (52).

All target bodies used in this investigation followed, in general, the gas target design used for the production of O-15 and C-11 isotopes (66). Other important characteristics of the targets are provided in Table 1. During irradiation, the target bodies were cooled by water circulation (temperature 15–20°C). The target foils were cooled by recirculating helium (temperature 15–20°C). Before the first use, target bodies were cleaned by sonication (1 h) at room temperature in four successive baths: (a) chloroform, (b) methanol, (c) de-ionized water and (d) methanol. The targets were then dried in an oven overnight. An identical treatment was followed if the target was exposed to air after use (e.g., after target foil failure). Fluorine (1% in argon, 100 psi) was left in the target when not in use.

Irradiation Protocols

PASSIVATION BOMBARDMENT. The target bodies were all passivated at least once before use by the beam-induced plasma process (22). The passivation process constituted irradiation of 100 μmol of fluorine and [^{16}O] oxygen (~250 psi) with protons (10 μA × 10 min). None of the targets were subjected to a harsh thermal passivation (41).

SINGLE-STEP IRRADIATION. The single-step method (4) involved the irradiation of a mixture of carrier fluorine (normal concentration range 70–250 μmol) in helium or argon and [^{18}O]O_2 (200–250 psi). The irradiated gases were released from the target by an on–off cycle of the target containment valve regulated by a computer. The residual gas phase activity in the target was recovered with an inert gas fill (100–150 psi) and release cycle.

TWO-STEP IRRADIATION. The previously described two-step method (41) was modified for convenience. The schematic of the simplified set-up used for the two-step approach for the 13.5-MeV proton irradiations is shown in Fig. 1. All solenoid valves in the system were remotely controlled by an IBM PC. The [^{18}O]O_2 (~1000 psia) in a stainless steel cylinder (50 cc) containing 4 Å molecular sieves (8/12 beads, 11 gm, Alltech) was cooled to −20°C (to condense trace amounts of moisture in the gas). The target was evacuated and then isolated from the vacuum system. The [^{18}O]oxygen was bled into the target to the desired pressure, and the solenoid valve 1 was closed and the first step of the irradiation conducted. The target material (>98%) was then cryopumped back into the oxygen reservoir by cooling it first to −196°C (liquid nitrogen bath) and opening valve 1. The residual oxygen gas in the target was evacuated and the target was refilled with appropriate amounts of carrier fluorine (30–120 μmol) and argon or neon (total pressure ~300 psi), and the second step of the irradiation was performed. The ^{18}F-labeled products were then released from the target through solenoid valves 2 and 6. The two-step irradiation protocol with a 10.3-MeV proton beam utilized the commercially available three-port target (66).

MODIFIED TWO-STEP PROCESS. For this method, $^{18}O_2$ (200–250 psi) was irradiated with 10.3-MeV protons in a conical aluminum target body at the desired conditions of beam current (25–35 μA) and time (15–45 min). At the end of the irradiation, rather than recovering the charge of $^{18}O_2$, an appropriate amount of fluorine (100–250 μmol) was added to the oxygen and a second irradiation was conducted under milder irradiation conditions (typically 5–15 μA × 5–10 min). The ^{18}F activity was then recovered as described for the single-step method.

TABLE 1. Target Bodies for ^{18}O(p,n)^{18}F Reaction

Target body	Bore shape[a]	Diameter (mm) Beam entrance	Diameter (mm) Beam exit	Target volume (cc)
Aluminum (6061T6)	conical	10	14	11.4
Aluminum (6061T6)	straight	10	10	7.9
Silver	conical	10	17	14.6
Silver	straight	10	10	7.9
Copper	straight	10	10	7.9
Nickel (electroformed)[b]	conical	10	17	14.6
Nickel (200)	straight	10	10	7.9
Gold plated copper	conical	10	17	14.6

[a] The lengths of the target bore were all 10 cm.
[b] Wall thickness: 0.025 mm (58). Obtained commercially from Optical Radiation Corporation, Azusa, California.

^{18}F Yields

After irradiation, the target gases were collected for radioassay by passage through soda lime or 1.0 M potassium iodide solution (to trap the reactive gas fraction: 80–100% [^{18}F]F_2 + 0 – 20% [^{18}F]OF_2 + 0 – 3% [^{18}F]$FONO_2$) followed by a gas sampling bag or activated charcoal trap at −78°C (to collect the inert gas fraction: [^{18}F]CF_4) (5, 6). The use of 1.0 M KI solution (35 mL) also allowed for the determination of the amount of the reactive fluorinated fraction of the irradiated gases after decay via iodometric titration against a standard solution of $Na_2S_2O_3$ (10). Knowing the volume of sodium thiosulfate solution and the ratio of F_2/OF_2 from the ^{19}F nuclear magnetic resonance (NMR) analyses (6, 7), the quantity of each reactive fluorine specie was determined. An identical volume of KI was used as a blank, and the corresponding titer value was applied as a correction factor to the KI trap titer volume. A complementary way of determining the fluorine content of the target load was accomplished by establishing pressure (5% F_2 in helium or argon) versus quantity of fluorine (μmol) calibration curves for each of the target bodies. The curves were generated by filling the target bodies with known pressures of dilute F_2 and delivering the gas into KI solution for iodometric titration analysis. This provided an accurate measurement of the amount of fluorine added. Thus, the amount of fluorine

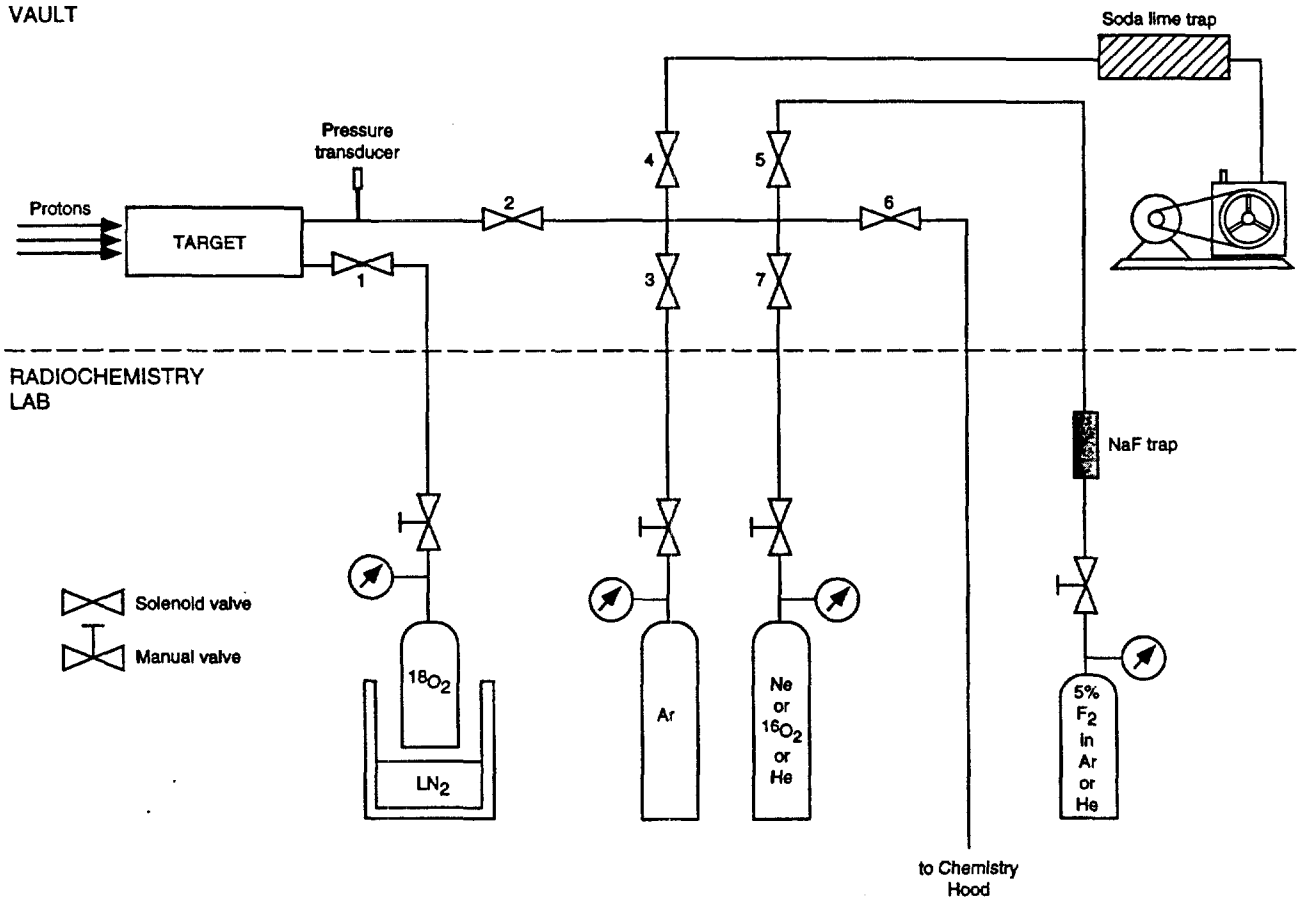


FIG. 1. Gas manifold for the two-step $[^{18}O]O_2/F_2$ target system.

in a target load could be reasonably estimated when direct titration of the reactive fraction was not possible.

Determination of 13.5-MeV Proton Beam Penetration in $[^{18}O]O_2$

A test target system modeled after a literature report (67) (Fig. 2) consisting of a straight-bore aluminum body (1) 10 cm long with 1-cı beam entrance diameter was used for this purpose. The back end of th target was a 0.0025-cm-thick aluminum foil (3) with two holes, on above and one below the aluminum flange (2), such that both hol were away from the beam path but allowed an equilibration of g pressures between the target chamber and the aluminum beam stop (5

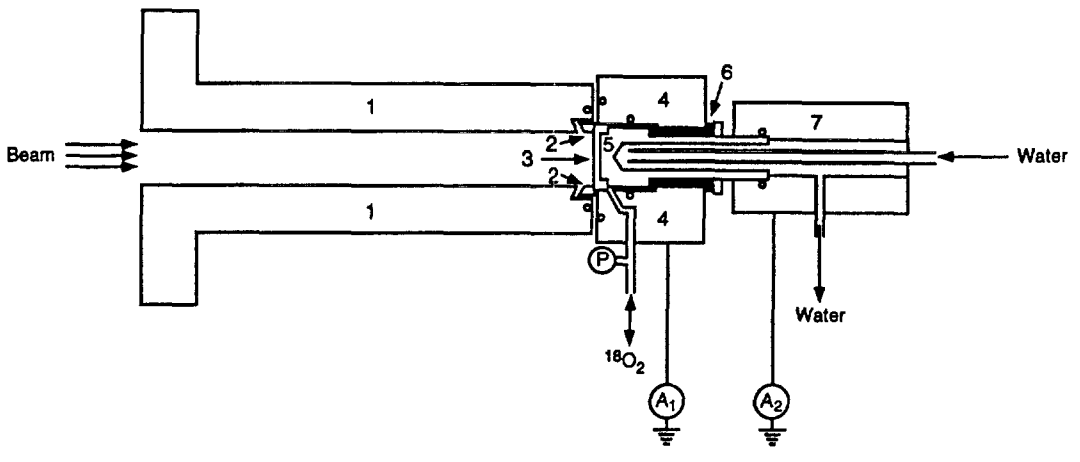


FIG. 2. Schematic of the test target system for measuring particle beam penetration. (1) Target body, (2) Retainer flange, (3) Aluminum exit foil, (4) Rear flange, (5) Beam stop, (6) Vespel insulator, (7) Beam stop retainer with water cooling finger for the beam stop. P—Pressure transducer, A_1 and A_2—Microammeters, ○—Buna-N O-rings. The front end assembly and the target cooling water jacket are not shown in this figure.

The beam stop was held in place by the aluminum rear flange (4) but electrically isolated from it as well as the target body by the vespel insulator (6). The beam stop was cooled by a cold-water finger, which also formed part of the beam stop retainer (7) made of aluminum. Gas-tight seals between interfaces were achieved with O-rings (Buna-N). The gas pressure in the target system was measured with a pressure transducer (Kulite XTM-190). The microammeter A_1 measured the target beam current, while A_2 recorded the current induced in the beam stop (5) when ions were generated in the 1.3-mm gas gap between the aluminum foil (3) and the beam stop.

The target was first evacuated and filled with $[^{18}O]O_2$ (~50 psia; beam-off pressure) and irradiated with the proton beam (13.5 MeV) at a constant beam current. At this low pressure, the beam penetrated the 10 cm of $[^{18}O]O_2$, aluminum foil (3) and ionized the $[^{18}O]O_2$ in the space between the foil and the beam stop (5). The current (usually called gap current) thus generated in the gas gap was measured with the ammeter A_2, and simultaneously the pressure (beam-on) was recorded.

The pressure in the target was then gradually increased and the corresponding gap current was recorded until it reached a plateau, signifying that the gas pressure in the target was high enough to stop the beam from penetrating the aluminum foil. A plot of the gap current versus beam-off pressure showed a gradual drop in the gap current with an increase of pressure and reached a sudden inflection and stayed relatively constant thereafter. The pressure corresponding to this point of inflection represents the pressure just sufficient to stop the beam penetrating the aluminum foil and is the minimum pressure that would be required to make the target range thick at that beam current (67).

Ozone

IDENTIFICATION. The presence of ozone in the irradiated target gas effluent was determined by the formation of biperoxide 2 from the reaction of ozone with tetramethylethylene (TME; 1), as shown in Scheme 1. For these reactions, the target body was filled with either oxygen plus the typical concentration of carrier fluorine (100 μmol) or neat oxygen. For the experiments without carrier fluorine, the target body used had either no prior fluorine exposure or had been thoroughly cleaned. Further, ozone generated using a high-frequency generator (Tesla coil) and research-purity oxygen was used as a standard.

Post proton irradiation, the target gas or ozone standard was gently bubbled into TME (150 mg, 1.79 mmol) in freon (5 mL) at room temperature. After decay of the radioactivity, the samples were analyzed by fast atom bombardment mass spectroscopy. Attempts were also made to identify ozone in the postirradiation effluent and the standard prepared with the Tesla coil using a residual gas analyzer (RGA).

QUANTITATION. The concentration of ozone present in the proton-irradiated target effluent was quantitated using iodometric titration (14). In these experiments, target bodies completely free from fluorine were filled with oxygen and irradiated with protons. The postirradiation effluent was bubbled into buffered KI solution (1 M, pH = 7.0; phosphate buffer) and was immediately titrated against a dilute (0.0098 N) sodium thiosulfate solution.

RESULTS AND DISCUSSION

Proton Beam Penetration and Density Reduction in $[^{18}O]$Oxygen Gas

The optimum performance of a gas target depends on the density reduction phenomenon caused by particle beam penetration (24, 25, 67). The density reduction, in turn, is related to the beam energy, beam current, nature of the target gas and the volume of the target. A thorough examination of these factors on the deuteron beam penetration in neon gas targets for the production of $[^{18}F]F_2$ has been reported (67). No such information is yet available for the $^{18}O(p,n)^{18}F$ reaction at 13.5 MeV proton beam energy. Thus, a characterization of 13.5 MeV proton beam penetration in $[^{18}O]$oxygen gas under one of the irradiation conditions utilized was conducted. The gap current method developed for the $^{20}Ne(d,\alpha)^{18}F$ reaction (67) was chosen for its ease, accuracy and convenience.

The beam-off and beam-on pressures versus gap current data at various beam currents (see "Experimental") are provided in graphical form in Fig. 3. It is evident from Fig. 3 that beam-on pressures well in excess of 700 psia would be developed for beam currents ≥20 μA, which is the normal range of beam currents used for the production of ^{18}F activity using this nuclear reaction. Pressures of this magnitude would cause severe strain on the target foils and could lead to frequent foil failures. Increasing the volume of the target as a means of accommodating the pressure increase with beam current would, in fact, lead to more beam penetration and hence more density reduction (25). Larger volume targets are undesirable from the standpoint of the expense and scarcity of $[^{18}O]O_2$. Moreover, an increase in volume would concomitantly increase the surface area for adsorption of ^{18}F, leading to less efficient recovery of radioactivity. Since production of ^{18}F isotope was the main goal, a compromise between the 13.5-MeV proton beam energy for higher ^{18}F yields and density-reduction-related problems was reached. The targets for the 13.5-MeV experiments were thus consciously filled with only ~250 psi of $[^{18}O]$oxygen based on stopping power calculations (68) for a thick target. On the other hand, it has been reported that density reduction was not a problem with 10.3-MeV proton beam penetration even at 40-μA beam current in gas target bodies of design identical to those utilized in this study (66). Hence, it is presumable that the targets were thick for the 10.3-MeV experiments conducted herein (*vide infra*).

Single-Step Irradiation Protocol

The results from the single-step irradiation protocol using the $^{18}O(p,n)^{18}F$ reaction are listed in Table 2. A judicious selection of single-

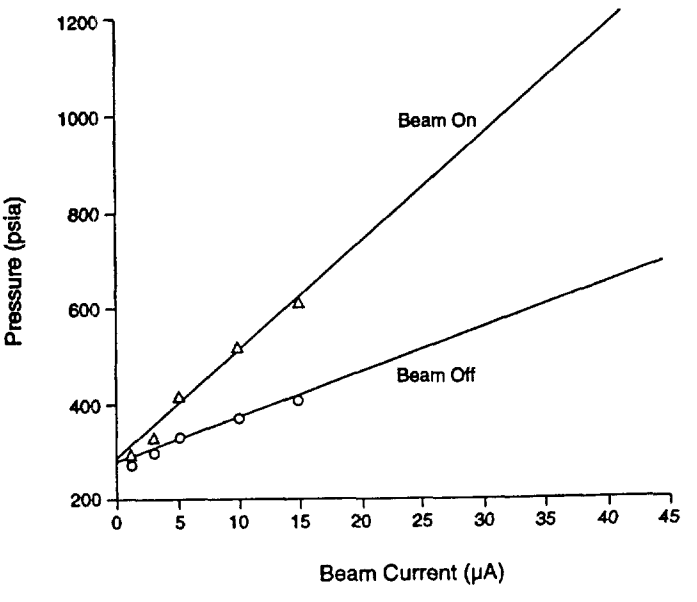


FIG. 3. Beam current versus pressure for $[^{18}O]$oxygen gas target. The principle behind this technique has been reported (67).

TABLE 2. Single-Step Irradiation of $[^{18}O]O_2/F_2$

Target body[a]	Beam[b] Current (μA)	Beam[b] Time (min)	^{18}F Activity recovered[c,d,e] (mCi)	Saturation yield (mCi/μA)
El Ni c	15	60	629	133
Ni s	15	60	317	67
	25	60	593	75
Cu s	15	60	267	56
	25	60	697	88
Ag c	15	60	350	74
	25	60	772	98
	30	120	907	57
Al s	15	30	176	68
	15	60	419	89
	20	60	397	63
	25	60	533	68
	30	60	615	65
	30	120	961	60
Al c	25	60	513	65
	30	60	582	61
	30	120	930	58
Au c	10	10	78	128
	15	60	367	78
	25	60	638	81
	30	120	1171	73
Al c[f]	10	10	59	97
	30	60	741	79
	35	45	639	74
Au c[f]	20	10	204[g]	167
	30	10	269[g]	147
	40	10	397[g]	163
	30	30	511[g]	99
	30	45	759[g]	102
	30	60	1006[g]	107

[a] El: Electroformed. Target bore shape c: conical; s: straight.
[b] Experiments conducted with 13.5-MeV proton beam energy, unless noted otherwise.
[c] Average of up to 6 experiments.
[d] Amount of carrier fluorine used: 75–200 μmol, unless noted otherwise.
[e] Of the activity recovered, 2–6% constituted inert fluorinated products (5). The Ni s target yielded a slightly higher (7–12%) proportion of inerts.
[f] 10.3-MeV proton beam energy.
[g] Amount of carrier fluorine used: 20 μmol.

step experiments was required due to the expense of $[^{18}O]$oxygen gas. Nonetheless, enough experiments were carried out to establish a target body performance based hierarchy for the $^{18}O(p,n)^{18}F$ reaction. In general, longer irradiation periods (≥30 min) were selected, mimicking those commonly employed for ^{18}F radiopharmaceutical productions. The reactive fraction from these irradiations was generally trapped in KI solution for iodometric titrations (10). On average, the constitution of this fraction, as determined by ^{19}F NMR and RGA mass spectroscopy, was F_2 (85%), OF_2 (15%) and $FONO_2$ (0–3%) (6, 7). Thus, determination of the total concentration of the reactive fraction by iodometric titration depends on the proportion of each component in the mixture. Knowing the percentages of each of these components and the stoichiometry of their reactions with KI (26, 46), the amount of the reactive fluorine fraction was determined. The small quantities of $FONO_2$ (0–3%) found in these single-step irradiations were generally ignored in the reactive fluorine determinations. Occasionally, the concentration of the reactive fluorine fraction was estimated based on the amount of carrier F_2 used. The recovery of reactive fluorinated fraction ranged between 30 and 90% based on the amount of carrier F_2 added in the target.

Based on a limited number of experiments conducted with 13.5-MeV proton beam energy, the highest saturation yield (133 mCi/μA) was obtained with the electroformed nickel target. The calculated thick target yield for this beam energy is 236 mCi/μA (50). The relatively low saturation yields (Table 2) observed could probably be due to the density reduction of the target material during irradiation. On the other hand, at 10.3 MeV proton beam energy, even with a beam current of 40 μA, a saturation yield of 163 mCi/μA was obtained in the case of the gold-plated target body. These data agree favorably with the calculated thick target yield value of 172 mCi/μA (50), suggesting strongly that density reduction was not a problem for the condition of this irradiation. Nevertheless, for a given target system, as observed previously (10, 49), the higher fluence irradiations generally resulted in lower saturation yields (Table 2).

The ^{18}F inert fractions observed in the single-step $^{18}O(p,n)^{18}F$ reactions on average ranged from 5 to 10% and essentially constituted CF_4, as judged by ^{19}F NMR (7). It is interesting to compare the inert fractions obtained in this investigation with those of the $^{20}Ne(d,\alpha)^{18}F$ reaction (5). For example, even though the enriched $[^{18}O]O_2$ contained 50–350 ppm of nitrogen and 50–3700 ppm of CO and CO_2, which are larger than the amounts present in ^{20}Ne, a greater fraction of ^{18}F inerts was not recovered. Further, the nitrogen impurity in enriched $[^{18}O]O_2$ resulted in reactive $[^{18}F]FONO_2$ rather than in $[^{18}F]NF_3$, as in the case of the neon gas target system (5). A rationale for this observation is given later in this discussion.

A systematic investigation of the effect of carrier fluorine concentration on the ^{18}F activity recovery or the specific activity was not performed. However, the computed range of specific activities based on the data given in Table 2 is 2–9 mCi/μmol, the same range as obtained with the neon gas target system (10). The highest specific activity (~45 mCi/μmol) was obtained with the gold-plated target at a beam energy of 10.3 MeV (Table 2).

The good performance characteristics of aluminum target bodies with the single-step protocol (Table 2) are noteworthy. Further, aluminum has excellent thermal conductivity, exhibits high resistance toward fluorine, displays low nuclear activation and is easy to machine, properties that are ideal for ^{18}F gas targets. Thus, a greater emphasis was placed on aluminum target bodies for the investigation of other irradiation protocols.

Two-Step Irradiation Protocol

The $[^{18}O]O_2$ cryorecovery-based two-step method (41) was conducted in a conical bore aluminum target body with 10.3-MeV protons (see "Experimental"). We have previously demonstrated the suitability of aluminum for the construction of fluorine gas target bodies (6). The two-step method can equally be conducted in an aluminum target (47), as evidenced by the data given in Table 3. The efficiency of the cryorecovery of $[^{18}O]O_2$ was excellent (>98%) with the modified setup described in the "Experimental" section. A small amount of ^{18}F activity (<5%) was invariably recovered with the oxygen. The bulk of the ^{18}F activity subsequently recovered in the second step was identified as >99% F_2 by ^{19}F NMR and RGA mass spectroscopy (6, 7). Further, this ^{18}F activity has been used successfully in the synthesis of 6-$[^{18}F]$fluoro-L-dopa (39) and $[^{18}F]$fluoro-m-tyrosines (40).

Recently, it has been shown that a repeated recovery of $[^{18}F]F_2$ is possible from a nickel target body (62). This interesting potential was tested in aluminum as well as in gold-plated target bodies. In step one of this process, the targets were filled with $[^{18}O]O_2$ (~250 psi) and

TABLE 3. $[^{18}F]F_2$ Production with 10.3-MeV Protons in a Conical Aluminum Target Body by Two-Step Irradiation Method

Expt.	Beam[a] Current (μA)	Beam[a] Time (min)	^{18}F Activity recovered[b] (mCi)	Saturation yield (mCi/μA)
1	40/20	60/10	1451[c]	112
2	35/20	45/10	1065[d]	123

[a] Beam conditions for step 1/step 2.
[b] Total activity; the inert gas fraction constituted <10%.
[c] Average of six experiments with a range of 1060–1614 mCi. Carrier fluorine concentration: 67–83 μmol.
[d] Average of eight experiments with a range of 916–1155 mCi. Carrier fluorine concentration: 33–113 μmol.

irradiated with protons (13.5 MeV) for 45 min. The target gas was then bubbled into a KI solution followed by a charcoal trap for ^{18}F activity balance. Both reactive as well as inert ^{18}F species were always present in this irradiated target gas, even though carrier fluorine was not added in the first step. Subsequent to the removal of [^{18}O]oxygen, the target was flushed with neon and filled with the same along with carrier fluorine. The target was re-irradiated for 15 min (13.5-MeV protons) and the ^{18}F activity was trapped in the KI/charcoal system. The recovery step was repeated three times to examine the potential of total ^{18}F recovery. For each experiment, all four steps of the irradiations were conducted at the same beam current:

The ^{18}F activity (decay corrected to EOB of step 1) recovered from each step is conveniently evaluated in graphical form (Fig. 4). Approximately 10% of the total ^{18}F activity recovered was released along with [^{18}O]oxygen in the first step. The second step of the recovery process always provided the highest amount of ^{18}F activity (average ~55%). Succeeding steps yielded roughly half of that of the immediately preceding step. Overall, a relatively higher percentage of ^{18}F inert species was observed in the target gases from step 1 (11–16% versus 1–3% for steps 2–4). This is not surprising considering the levels of impurities present in the [^{18}O]oxygen (as stated previously) combined with the absence of carrier fluorine competing for ^{18}F. Interestingly, these results also indicate that the multiple recovery process can be used advantageously with aluminum and gold-plated targets.

Modified Two-Step Irradiation Protocol

The intent of the modified two-step procedure was to determine the effect, if any, of adding carrier fluorine after significant ^{18}F production on the observed product spectrum and recovered radioactivity. The ^{18}F observed yields are summarized in Table 4. The composition of the reactive as well as the inert products was identical to that of the single-step method described earlier (6). Due to the expense of $[^{18}O]O_2$, modified two-step experiments were conducted without regard to locating optimum extraction conditions. However, the ^{18}F activities recovered (85% $[^{18}F]F_2$ + 15% $[^{18}F]OF_2$) using the single-step and modified two-step methods were utilized successfully in [^{18}F]fluorodestannylation reactions (39, 40).

Fluorinated Products Generated in the Targets

The reactive fraction of the single-step and modified two-step irradiated target gases constituted, on average, 85% F_2, 15% OF_2 and 0–3% $FONO_2$ (fluorine nitrate), while the two-step method (in an aluminum

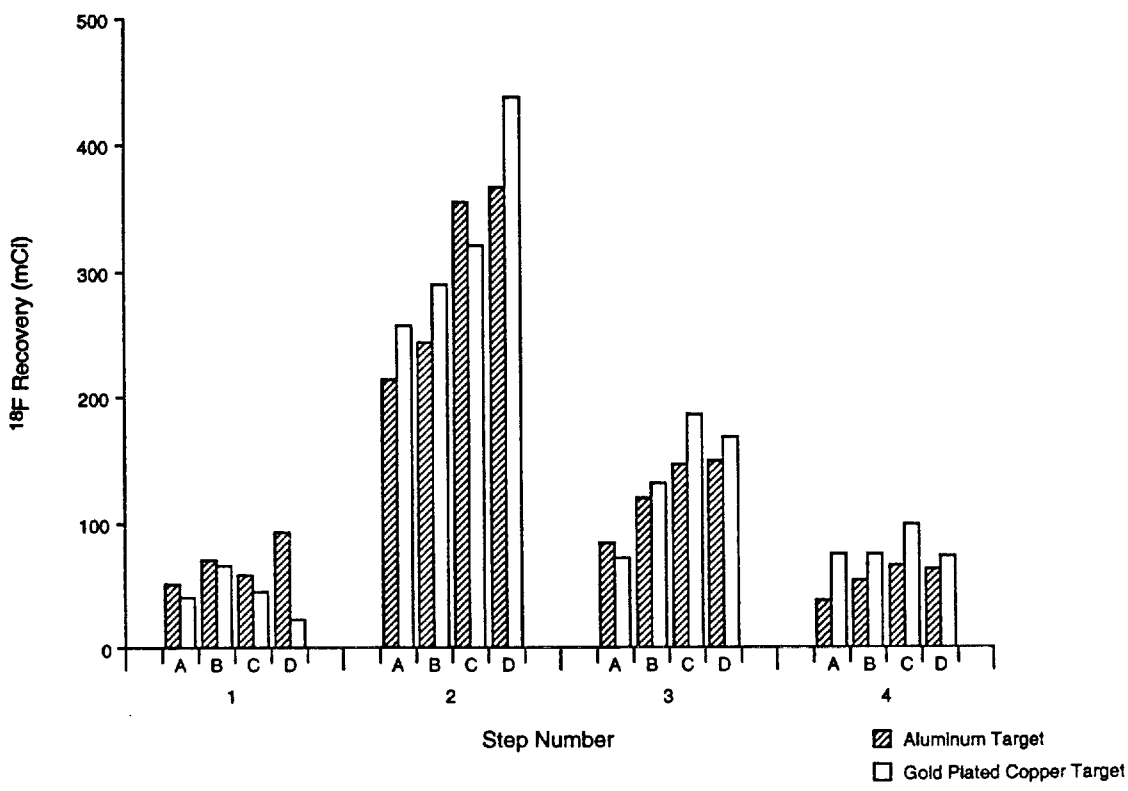


FIG. 4. Two-step $^{18}O(p,n)^{18}F$ reaction yields for 13.5-MeV proton irradiation experiments. Data shown in order of increasing beam current for each target body A: 15, B: 20, C: 25 and D: 30 μA.

TABLE 4. F-18 Production with 10.3-MeV Protons in a Conical Aluminum Target by Modified Two-Step Method

Expt.	Beam[a] Current (μA)	Beam[a] Time (min)	^{18}F Activity recovered[b,c,d,e] (mCi)	Saturation yield[f] (mCi/μA)
1	25/5	15/5	228	101
2	25/10	15/10	293	112
3	25/10	30/10	397	92
4	35/10	30/10	511	85
5	35/15	45/10	865	98

[a] Beam conditions for step 1/step 2.
[b] Total activity; the inert gas fraction constituted <10%.
[c] Carrier fluorine concentration 100–250 μmol.
[d] Corrected to EOB of second irradiation.
[e] Average of up to four experiments.
[f] Accounts for production and decay of ^{18}F activity from time of recovered ^{18}F measurement to each EOB.

target) provided >99% F_2, as determined by ^{19}F NMF (6, 7). The inert gas fraction from the oxygen gas targets under all conditions of irradiations contained essentially CF_4 (7). In this part of the discussion, the likely pathways for the formation of oxygen difluoride, fluorine nitrate and carbon tetrafluoride are suggested based on the amount of ozone generated in the targets and the nitrogen and carbon dioxide impurities present in the target gases. The mechanistic processes invoked (17, 30) are in terms of probable beam-induced chemical reactions involving distinct intermediates generated in oxygen/fluorine gas target systems.

Ozone

The presence of ozone in irradiated oxygen, in general, has been well documented (33, 43). The existence of ozone in the oxygen target systems described herein was demonstrated by its reaction with tetramethylethylene (TME) (1). The reaction of O_3 with TME yields the biperoxide **2** (27) as shown in Scheme 1. The aluminum, silver, gold-plated copper and nickel target bodies were filled with oxygen, with and without carrier F_2, and irradiated between 1.7 and 7.5 μAhr with protons, and the target gases were then bubbled into TME in freon. A large excess of TME was generally used to trap all the possible products generated during the irradiation process. The products were conveniently analyzed by fast atom bombardment mass spectroscopy (FAB MS). As control experiments, O_3 was generated with a Tesla coil and reacted with TME, and the products were analyzed as discussed earlier. In all cases, the expected (M^+ + 1) signal for the biperoxide **2** at 149 was observed.

All attempts to identify ozone in the irradiated target gases directly using RGA met with limited success. Even O_3 generated with a Tesla coil did not show the presence of the molecular ion, and the fragments from ozone were indistinguishable from that of the oxygen background. The relative abundance of M^+ observed for even neat O_3 is only 20% (23). Thus, the absence of the molecular ion peak for the relatively low concentration of ozone present in the irradiated oxygen was not surprising.

The quantitation of ozone in the postirradiated oxygen gas was accomplished by iodometric titration. The measurement of ozone concentration with this technique was applied only to target systems that were completely free of fluorine. The concentration of ozone was calculated based on the following stoichiometry (35):

$$O_3 + 2I^- + 2H^+ \rightarrow O_2 + H_2O + I_2$$

In each of 11 experiments ranging in fluence from 0.08 to 20 μAhr and using three different target bodies (aluminum, silver and copper), the resulting ozone content was between 5 and 10 μmol.

Oxygen Difluoride

Fluorine, the most reactive element known, and oxygen, a highly reactive gas, would be expected to form binary oxygen fluorides under irradiation conditions. A variety of oxygen fluorides OnF_2, $n = 1$ to 6, and oxygen fluoride radicals OnF, $n = 1$ to 4, are known (19). Among these oxygen fluorides, OF_2 is perhaps the only compound likely to survive under the irradiation conditions (61). The rest of the oxygen fluorides are extremely temperature-sensitive products, and even if they are formed in the target, they will decompose immediately into their elements (19). Oxygen difluoride is thermally stable up to temperatures of 200–250°C and less reactive than fluorine; hence, it is not surprising that the single-step oxygen gas target products constituted OF_2.

The formation of OF_2 is known under photolytic conditions (57), ultraviolet light irradiations (2, 34) and 3-MeV bremmstrahlung (21). However, the formation of OF_2 under high-pressure and high-energy irradiation conditions has not been reported. Nevertheless, a probable scheme for the formation of OF_2 can be formulated based on the known radiolysis of oxygen (42, 56) and the dissociation of molecular fluorine under electron impact (11, 20, 69). Of the number of reactions observed in these processes, the following steps are important, and most of them could also take place efficiently in the single-step oxygen target system. Thus, the interaction of the proton beam with oxygen could cause the following principal reactions:

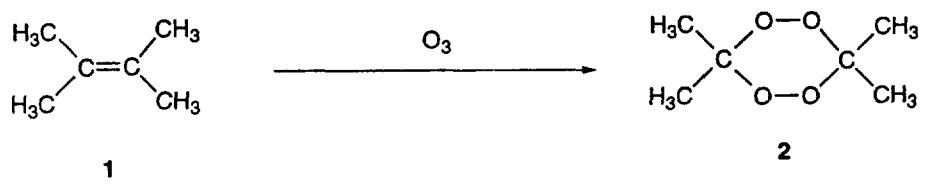
$$O_2 \rightarrow O + O \quad (1)$$

$$\rightarrow O_2^+ + e \quad (2)$$

$$\rightarrow O_2^* \quad (3)$$

$$\rightarrow O^+ + O + e \quad (4)$$

$$O + O_2 \rightarrow O_3 \quad (5)$$



SCHEME 1. Reaction of tetramethylethylene with ozone.

$$O_2 + e \rightarrow O_2^- \quad (6)$$

$$O_2^+ + e \rightarrow 2O \quad (7)$$

The predominant reactions of F_2 under irradiation conditions could be as follows:

$$F_2 \rightarrow F + F \quad (8)$$

$$F_2 + e \rightarrow F + F^- \quad (9)$$

$$F_2 + e \rightarrow F_2^* + e \rightarrow F + F + e \quad (10)$$

$$F_2 + e \rightarrow F^+ + F^- + e \quad (11)$$

$$F_2 + e \rightarrow F^+ + F + 2e \quad (12)$$

The various energetically possible interactions between these radicals (atomic fluorine and oxygen) and ions and other atoms and molecules should be responsible for oxygen difluoride formation.

The key intermediate initially invoked in the formation of OF_2 under ultraviolet light conditions has been the OF radical (34, 60).

$$O_3 + F \rightarrow OF + O_2 \quad (13)$$

$$F + OF \rightarrow OF_2 \quad (14)$$

$$F_2 + OF \rightarrow OF_2 + F \quad (15)$$

Since then, the OF radical has been matrix isolated (3) and also examined extensively over the years (63, 64). Reactions (13)–(15) are equally possible in the single-step oxygen gas target systems. For example, the presence of ozone in the irradiated target gases has been confirmed. The other reactant that is necessary for the formation of OF_2 is atomic fluorine, which is likely to be present in the target gas (reactions 8, 9 and 10).

Other possible pathways for the formation of OF radicals involving atomic fluorine or oxygen can also be envisioned. For example, the reaction of molecular fluorine with atomic oxygen ($F_2 + O \rightarrow OF + F$) can yield OF radicals. However, this reaction has a large energy of activation (10.4 kcal/mol, about 3.5 times higher than that for reaction 13) (19, 36). Further, the rate of reaction of atomic oxygen with ozone ($O + O_3 \rightarrow 2O_2$) is four orders of magnitude higher than that with F_2 (59). Hence, this process may not be a facile channel for the formation of OF radicals. The reaction of atomic fluorine with O_2 ($F + O_2 \rightarrow OF + O$) can be contemplated for an alternate source of OF. In this case, the rate constant for the reverse reaction (i.e., $FO + O \rightarrow F + O_2$) is much larger than that for the forward reaction (36). Thus, it is likely that reaction 13 represents the most prolific pathway for the production of OF radicals (19).

It was rather surprising that F_2 constituted >80% of the total fluorinated products recovered from the single-step oxygen gas target systems (6, 7). Since the bulk of the target gas was oxygen, a higher conversion of carrier F_2 into OF_2 under irradiation conditions was anticipated. One of the main reasons for the limited formation of OF_2 is the low amount (5–10 μmol) of ozone formed in these targets, as described earlier. The decomposition of ozone in the pressurized targets is directly responsible for its low concentrations (56).

$$O_3 + O \rightarrow 2O_2 \quad (16)$$

$$O_3 + O \rightarrow 2O + O_2 \quad (17)$$

In the oxygen gas targets, the ozone that is formed undergoes decomposition readily by reacting with the more abundant oxygen atoms. The competition of reaction 17 with 16 can lead to a chain reaction enhancing the decomposition of ozone in the target. Consequently, the formation of OF radical via reaction 13 is restricted, ultimately causing a low output of OF_2.

The beam-induced decomposition of OF_2 can also play a major role in this regard, as experimentally determined in this work (*vide infra*). The kinetics and mechanism of the thermal and electron impact decomposition of OF_2 have been investigated thoroughly (3, 15, 18, 28, 37). Based on these studies, the beam-induced decomposition of OF_2 can be represented by reactions 18–20. In any event, the near constant proportion of OF_2 (15 ± 4%), as observed (6, 7) under the single-step as well as the modified two-step irradiation protocols,

$$OF_2 \rightarrow F + OF \quad (18)$$

$$2OF \rightarrow 2F + O_2 \quad (19)$$

$$2F \rightarrow F_2 \quad (20)$$

irrespective of fluence (Tables 2 and 4), strongly suggests the existence of a dynamic equilibrium between oxygen difluoride and its constituents in the targets.

Fluorine Nitrate

Fluorine nitrate was only a minor component among the reactive fluorinated products observed. Nitrogen is present as an impurity in the target gases (5) and is responsible for the formation of $FONO_2$. The amount of $FONO_2$ observed was seldom more than 3% of the total reactive fluorinated species recovered from any of the oxygen target systems. Based on the radiolysis of oxygen-nitrogen systems (43, 56) and the reaction of nitrogen atoms (9), a probable reaction sequence for the interaction of nitrogen with oxygen under a charged particle environment can be formulated:

$$N_2 \rightarrow N + N \quad (21)$$

$$O_2 \rightarrow O + O \quad (1)$$

$$N + O_2 \rightarrow NO + O \quad (22)$$

$$2NO + O_2 \rightarrow 2NO_2 \quad (23)$$

The formation NO_2 through reactions involving ozone has been reported (43). However, those reactions require a large excess of ozone compared with the concentration of nitrogen. The ozone concentrations observed in this investigation (5–10 μmol) were in the same range as that of nitrogen and, hence, the role of ozone in the formation of NO_2 was not invoked. The formation of $FONO_2$ from NO_2 can then be represented by reaction 24 (60):

$$NO_2 + OF \rightarrow FONO_2 \quad (24)$$

It is interesting to note that while stable ternary compounds of nitrogen, oxygen and fluorine, such as nitrosyl fluoride (FNO) and nitryl fluoride (FNO_2), are known (70), only fluorine nitrate was observed in the irradiated oxygen targets. The following known reactions for the formation of FNO (45) and FNO_2 (60) are also theoretically possible in the oxygen gas target systems:

$$NO + F_2 \rightarrow FNO + F \quad (25)$$

$$NO_2 + OF_2 \rightarrow FNO_2 + OF \quad (26)$$

However, nitric oxide formed via reaction **22** will immediately be consumed by the large amount of oxygen present in the target (reaction **23**) (14). This essentially curtails any probability of reaction **25** and, thus, the presence of nitrosyl fluoride was never observed in any of the irradiated oxygen target gas samples. The lack of formation of FNO_2 may be indicative of the presence of a higher concentration of OF than OF_2 during irradiation. Thus, reaction **24** would dominate over reactions **25** and **26** in yielding $FONO_2$.

Carbon Tetrafluoride

Carbon tetrafluoride is a normal contaminant in all the fluorine gas mixtures. In addition, fluorine combines with CO_2 (an impurity present in $[^{18}O]O_2$) under irradiation conditions to provide CF_4. The role of CO_2 in the formation of CF_4 has been addressed (5). However, a possible mechanism of formation of CF_4 under charged particle irradiation conditions has not been reported. On the other hand, an in-depth kinetic and mechanistic analysis of the photochemical reaction between fluorine and carbon dioxide has been undertaken (31). Thus, a likely pathway for the generation of CF_4 could be deduced knowing the resistivity of gaseous CO_2 to radiolysis (56).

$$F_2 \rightarrow F + F \quad (8)$$

$$F + CO_2 \rightarrow FCO_2 \quad (27)$$

$$FCO_2 + 3F \rightarrow CF_3OF + O \quad (28)$$

$$CF_3OF + F \rightarrow CF_4 + OF \quad (29)$$

Interestingly, the formation of $[^{18}F]CF_4$ can proceed through two distinct pathways. A thermal ^{18}F atom can partake in any of the reactions **27–29** to form $[^{18}F]CF_4$. In addition, a hot ^{18}F atom can undergo a substitution reaction with CF_4 (13, 48). The vibrationally excited $CF_3{}^{18}F^*$ molecule can either fragment due to the internal energy or deactivate by collision with the surrounding molecules (reaction **31**).

$$^{18}F^* + CF_4 \rightarrow CF_3{}^{18}F^* + F \quad (30)$$

$$CF_3{}^{18}F^* + M \rightarrow CF_3{}^{18}F \quad (31)$$

M = a third body

However, the thermal ^{18}F atom induced reactions **27–29** would dominate the hot atom reaction sequence (38).

In general, radical mechanisms constitute a major part of the explanations offered for radiation-induced chemical reactions (56) (see also 17, 30). However, under high dose rates, ions can also play a role in the generation of products. The magnitude of the individual contribution of each radical, ion or excited specie to the product distribution is difficult to assess. Meanwhile, a clear and dominant role of atoms and radicals in the generation and decomposition of fluorinated products has been emphasized (17, 30). For example, the radiation-induced formation of OF_2 has been previously rationalized entirely on radical interactions (2, 21, 34, 59). Nevertheless, a few exploratory experiments of beam-induced decomposition of OF_2 (Table 5) were conducted to test the hypothesis of the involvement of radicals. For instance, when a mixture of 1% OF_2 in argon was irradiated (Table 5; experiment 1), there was a near complete decomposition of OF_2 into fluorine. A dominant radical involvement in this reaction is certain due to the nonpolar nature of the medium (8, 18, 28, 37, 54). On the other hand, in an atmosphere of oxygen instead of argon (experiment 2), under identical conditions of irradiation, there was an apparent decrease in the decomposition of OF_2. Whereas this result could be at least partially attributed to the suppression of radical reactions involved in the decomposition of OF_2 in an oxygen-rich environment, the simultaneous transformation of F_2 into OF_2 under these conditions (*vide supra*) would also reflect the radical scavenging effects of O_2. These results thus strongly suggest the existence of a dynamic equilibrium conducive to a balance of products (e.g., F_2 and OF_2) under the irradiation conditions.

TABLE 5. Proton (13.5-MeV) Irradiation of OF_2

Expt.	Target body[a]	Proton beam Current (μA)	Proton beam Time (min)	^{19}F NMR F_2 (%)	^{19}F NMR OF_2 (%)	Gas mix[b]
1	Au c	1	5	95	5	Ar
2	Au c	1	5	69	31	O_2
3	Au c	10	10	58	42	O_2
4	Ag s	10	10	73	27	O_2

[a] Target bore shape c: conical; s: straight.
[b] Preirradiation gas mixture contained the gas listed plus 1% OF_2 (180 μmol) in helium; experiment 4: 1.4% OF_2 (155 μmol) in helium. Total pressure: 210–230 psi.

CONCLUSION

The production of ^{18}F electrophilic agents via $^{18}O(p,n)^{18}F$ reaction is gaining popularity, especially due to the development of low-energy, proton-only cyclotrons for the preparation of positron emitting radionuclides. This investigation, a comprehensive evaluation of $^{18}O(p,n)^{18}F$ reaction in target bodies made of a variety of metals has, for the first time, resulted in aluminum for the production of ^{18}F electrophilic reagents (6). For nearly 5 years, aluminum target bodies have remained as the mainstay in our laboratories for the production of $[^{18}F]F_2$ and related products to synthesize a number of ^{18}F-labeled dopamine presynaptic probes (51). The relatively large cross section of the $^{18}O(p,n)^{18}F$ reaction (50), the ability to recycle the $[^{18}O]O_2$ target material (41,44) and the good recovery of ^{18}F activities from aluminum target bodies make an ideal combination for an efficient production of $[^{18}F]F_2$.

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[N-13]AMMONIA

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PREPARATION OF

[N-13]AMMONIA

[N-13]AMMONIA

The production of [N-13]ammonia follows the procedure reported in the literature [Wieland et al., App. Radiat. Isot. 42, 1095-1098 (1991). A copy of the paper is enclosed].

Description of the production of [N-13]ammonia

The proton irradiation of pressurized dilute ethanol (USP) solution (1 mM) in sterile water provides [N-13] labeled ammonia via $^{16}O(p,\alpha)^{13}N$ nuclear reaction. The cyclotron target consists of a water cooled aluminum chamber of 3.0 ml volume. The target is loaded with the above mentioned 1 mM ethanol solution using a HPLC pump and maintained at 150 psig beam-on pressure. At the end of beam the target contents are flushed out with 9 mL of 1 mM EtOH. The solution is passed through an anion exchange resin cartridge (Alltech) followed by a 0.22 μm sterilizing filter (Millipore) into a sterile vial (10 ml) containing a sterile solution of sodium chloride (90 mg in 1.0 ml of sterile water).

The step by step procedure for the production of [N-13]ammonia is described in the following section.

Preperation of [N-13]Ammonia

Introduction

The target system consists of an HPLC type pump, water-ethanol solution reservoir, target, flow control valve, and teflon tubing. The HPLC pump fills the target, maintains the target pressure during irradiation, and flushes the target at the end of bombardment. The flow rate is 1.5 ml/min. The target pressure is 150 ±10 psig during bombardment; the pressure is maintained by pumping against the closed flow control valve and by using a 150 psig relief valve to prevent overpressurization of the target or damage to the pump. The target consists of a water cooled aluminum chamber with a volume of approximately 3.0 ml and a target window of 0.001 in titanium. The aqueous solution pumped through the target is prepared by adding 0.06 ml anhydrous USP ethanol to a 1 liter IV bag of sterile water for injection, then connected to the inlet of the pump.

The target system is operated automatically by the RDS-112 radioisotope delivery system. The target is loaded and pressurized to150 psig and bombardment started. The irradiation parameters are normally 40μA for 8 mins. At the end of bombardment the flow control valve is opened and the target contents are flushed for 6 min, through an ion retardation column and a 0.22μm sterilizing filter into a sterile 10 ml vial. The final volume of the sample is approximately 9.0 ml.

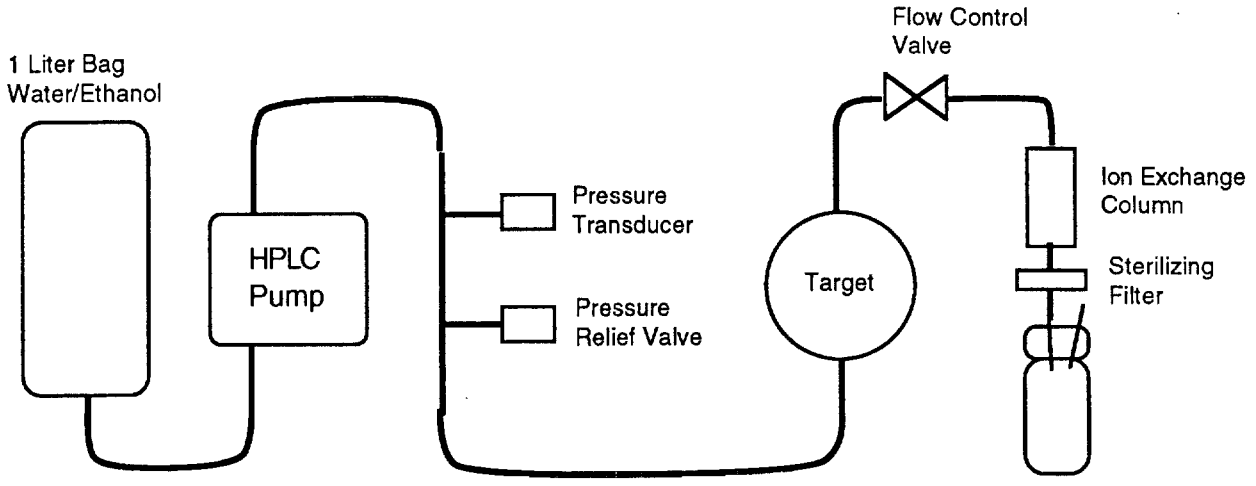


Figure 1. [N-13]Ammonia system

Materials

1. 10 ml sterile empty vial
2. Three 1" yellow needles
3. One 0.22 μm vented sterilizing filter
4. Waxed string
5. Alcohol swab
6. Ion retardation column, Alltech Maxi-Clean IC-OH, Stock #30262
7. 3 ml plastic syringe

Chemicals

1. 90 mg/ml NaCl solution
2. 1 liter sterile water bag
3. USP Ethanol

RDS Cyclotron Automatic Operation

1. The cyclotron system is assumed to be in the standby condition, ready to generate beam. If it is not ready refer to the cyclotron operation documents for further information.

Automatic [N-13]Ammonia Production

1. Select **Produce Labeled Product** from the **RDS Function Selection Menu**. Press **ENTER** to continue.
2. From the **Produce Labeled Product Menu**, for **Target Zone 1** select **Ammonia**. Press **ENTER** to continue.
3. On the **Target Zone 1 Setup Page**; enter the beam current, normally 40μA, and the bombardment time, normally 8 min.
4. Press **ENTER** to begin the automated production sequence; target loading, bombardment and chemistry.

Chemistry Process

1. The computer will turn on the target pump and pressurize the target. The pump will then be turned off and the target pressure monitored for 15 seconds to check for leaks. The target pressure should not drop more than 30 paig with the pump off. The pump is turned back on and the bombardment proceedes.
2. At EOB the target flow control valve is opened. The target pressure will drop to less than 40psig, and the target will be flushed into the collection vial for 5 minutes.
3. At the end of the unloading step, remove the needles, assay for activity and ship to the clinic.

Sterile Vial Preperation

1. Tie string to the top of the vial, knot a loop at the end, fill out and affix label.
2. Swab the top of the vial septum with an alcohol wipe and insert two 1" 20 guage needles into the vial, attach a 0.22μm sterilizing filter to one of the needles.
3. Pass 1.0 ml of 90 mg/ml NaCl solution through the filter into the sterile vial.
4. Attach an OH^- ion retardation column to the sterilizing filter.

5. Place vial in grey lead pig, position in holder located in the GPU and attach delivery line to ion column. Check that the water overflow cup next to the HPLC pump is not overflowing. Also check that sufficient water/ethanol remains in the bag located on the side of the chemistry enclosure.
6. Bombard target at 40μA for 8 minutes, 12 minutes for a double run. For a single run the total production time will be 15 minutes.
7 After delivery, use tongs to remove needles and filter assembly, assay and ship to clinic.

Target Material Preparation

Inject 0.06 ml of USP ethanol into a one liter sterile water bag using a one milliliter syringe. Mix the bag well and attach to HPLC pump inlet.

QUALITY CONTROL PROCEDURES

FOR

[N-13]AMMONIA*

*Due to the short half-life of [N-13], the chemical, radiochemical and radionuclidic quality control procedure were not conducted on every preparation. Instead, batches of [N-13]ammonia identical in all respects to those of the clinical doses were prepared separately, with which the chemical, radiochemical and radionuclidic quality control tests were performed.

Radiochemical Quality Control for [N-13] Ammonia

Introduction

The following protocol is used to check the purity of a sample of [N-13] ammonia, utilizing a radioactivity detector.

Necessary Materials

1) [N-13] Ammonia sample

2Acetic Acid ; Ammonium Acetate

4) Adsorbosphere SCX 5U HPLC column (Alltech)

5) 18 M ohm H_2O

6) HPLC injector with 20ul load loop

7) SSI 222D HPLC Pump

8) Beckman Model 170 Radioisotope Detector

9) Macintosh Computer running Rainin Dynamax HPLC controller and data collection system

10) 1ml syringe and an injection needle.

[N-13]Ammonia sample preparation

For ammonia production procedure refer to the High Pressure Ammonia Target

Preparation of Acetic Acid - Ammonium Acetate Mobile Phase

Weigh out 0.1541 g of CH_3COONH_4 and 0.0600 g (56.3ul) of CH_3COOH for every 1L of 18M ohm water, for a resultant concentrations of 2mM and 1mM , respectively. Balance the pH to 5.0 by adding base or acid accordingly, and filter the buffer through a 0.45 micron nylon filter to remove any particulate matter. Degas the solvent with vacuum. Note: give the HPLC system time to equilibrate with every new batch of buffer. It is advisable to make a larger quantity of buffer to start with and avoid the unnecessary equilibration periods. It saves time and produces more consistent results.

HPLC System Preparation and Shutdown

Set the flow rate on the SSI HPLC Pump to 1.0ml per min. Always flush the HPLC system with 18 M ohm water before swithing between different solvent systems. Use at least 10 column volumes for each flush. Allow the system to equilibrate with the buffer for at least 30 min before analyzing the [N-13] ammonia sample.

Computer Program Settings

1)Click twice on the hard drive symbol on the Mac screen. At this point a menu will appear.

2)Double click on the "Dynamax" folder

3)Choose "DA" folder and double click

4)Go to "Control" on top of the screen and pull it down. Select "Manual Control"

Drag the box to the lower left-hand corner using a mouse.

5) Go to "Data" menu and select "Show Report." Drag the box to the lower right-hand corner.

This window provides quick information on the retention time and the area under the peaks.

6)From the same "Data" menu select "Data Parameters"

a) Under "Aquire Data" choose "A" (crosshairs signify confirmation of selection)

b)Under "Process Data " choose "A" again

Leave the rest of the parameters unchanged and click "OK"

7) From the "Data" menu once again select "Report Format". Click off the "Auto Range Data" function.

Leave the rest of the parameters unchanged. Shut down the window by clicking the mouse arrow on a small square in the upper left-hand corner of that window.

8) Go to "Data" menu and select "Show Data". This will bring up an empty window on which the chromatogram will be displayed.

9) As a last task, go to the lower left-hand side of the screen and click once on the "Manual Control" window. This will bring it up to the forefront of the page. Leave the screen in this mode until the moment you are ready to inject.

Sample Injection

With a 1ml syringe draw about 0.6ml of sample. Place the injection needle on the syringe. Make sure to evacuate all of the air bubbles from the syringe by tapping it gently on the sides.

Turn the injector to the "LOAD" position and insert the needle. Gently inject the contents of the syringe. At this point the sample is in the injector ready to be processed.

Position the mouse pointer on "Aquire Data" and press the mouse button at the same time as you turn the injector lever from " Load" to "Inject" position. Everything from now on is done automatically.

Data analysis and discussion

The [N-13] ammonia should elute as a single peak at roughly the 6.3 minute mark. Impurities such as [N-13] nitrate and [N-13] nitrite will elute at 2-3 min., if they are present in the sample. No impurities have ever been found in any of the [N-13] ammonia samples. In all the analyses, the chromatogram always contains a single peak at 4.5 minutes.

RADIOCHEMICAL QUALITY CONTROL

RADIOANALYTICAL HPLC OF [N-13]AMMONIA

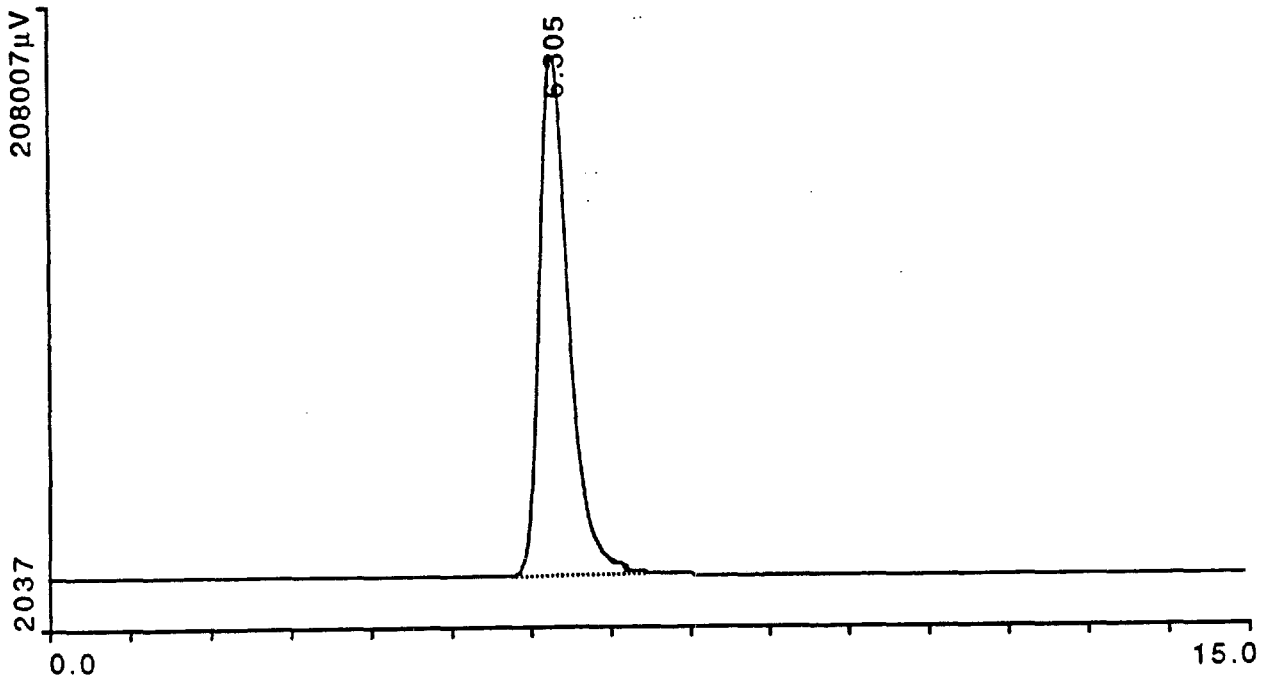
Column: Adsorbosphere SCX 5 μ column (25 x 0.4.6 cm)

Eluent: 4 mM Ammonium Acetate + 2 mM acetic Acid (1:1)

Flow rate: 1.0 mL/min

Chart speed: 1 cm/min

Detector: Beckman Model 170 Radioisotope detector



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	6.305	N	172209	4126820	100.000
Total Area				4126820	100.000

Chemical Quality Control for [N-13] Ammonia

Introduction

The following protocol is used to determine the chemical purity of a sample of [N-13] ammonia.

Materials

1) Ammonia Vial:

   a) 10ml empty sterile vial

   b) two 20G yellow needles

2) 18M ohm water (for column and detector flush)

3) Mobile phase:

   a) Citric Acid

   b) 18M ohm water

4) Alltech Universal Cation 7U HPLC column and pre-column

5) Waters 432 Conductivity Detector

6) SSI 222D HPLC Pump

7) Macintosh Computer running Rainin Dynamax HPLC controller and data collection system

8) 1ml syringe and an injection needle.

[N-13]Ammonia sample preparation

For ammonia production procedure refer to the High Pressure Ammonia Target Protocol . The addition of saline to the final product should be omitted for this analysis (the large concentration of NaCl would otherwise mask the minute signal of ammonia in the conductivity detection).

Preparation of Citric Acid Mobile Phase

Weigh out 1.3448 g of citric acid for every 1L of 18M ohm water for a resultant concentration of 7mM. Stir the contents well and filter through a 0.45 micron nylon filter to remove any particulate matter and then vacuum degass the solvent.

HPLC System Preparation and Shutdown

Set the Waters 432 Detector to the following settings:

a)Swith off **Remote** (light off)

b)Depress the **Auto Base** button to set the appropriate baseline level

c)Set **Integrator Sensitivity** to 50uS/

d)Set **Recorder Sensitivity** to 0.01

e)Set **Response** to STD (standard)

f)Set **Temperature** to 40C

g)Set **Polarity** to negative (light off)

h)Set **Balance** to 0%

If in doubt as to any of the settings, consult the Waters 432 Detector Manual.

The day before analysis, perform the following tasks (refer to the appropriate manuals if in doubt):

1) Connect the Universal Cation Column to the system between the injector and the conductivity detector (make sure that the pre-column is installed)

2) In case it is not already in the system, install the Pulse Dampener between the HPLC pump and the injector (this is essential for proper functioning of the conductivity detector)

3) Make sure that a 100ul injection loop is being used

4) Flush the system for 2-3 hours with 18M ohm water at a pump setting of 0.8ml/min. At this point the conductivity on the readout of the Waters 432 Detector should read "0"

5) Switch to 7mM citric acid mobile phase at 0.05 ml/min and leave over night to equilibrate. Refer to "Preparation of Citric Acid Mobile Phase" paragraph on how to prepare the citric acid solution.

6) An hour to two hours before sample injection, increase the flow rate to 0.8ml/min and maintain it throughout the procedure.

7) Upon the completion of the procedure disconnect the column and cap it. Do not leave the column in the system. Important: Store the column in 7mM citric acid

8) Flush the detector with 18M ohm water (at 0.8ml/min) until conductivity reads "0" (about 1-2 hours) then flush for another hour with 100% Methanol. At this point the conductivity detector can be switched off.

Desired Computer Program Settings

1)Click twice on the hard drive symbol on the Mac screen. At this point a menu will appear.

2)Double click on the "Dynamax" folder

3)Choose "DA" folder and double click

4)Go to "Control" on top of the screen and pull it down. Select "Manual Control"

Drag the box to the lower left-hand corner using a mouse.

5) Go to "Data" menu and select "Show Report." Drag the box to the lower right-hand corner.

This window provides quick information on the retention time and the area under the peaks.

6)From the same "Data" menu select "Data Parameters"

a) Under "Aquire Data" choose "A" (crosshairs signify confirmation of selection)

b)Under "Process Data " choose "A" again

c) Change "Peak Width" from 30 to 50

d) Change "Min Peak Area" from 10,000 to 100

Leave the rest of the parameters unchanged and click "OK"

7) From the "Data" menu once again select "Report Format". Click off the "Auto Range Data" Function

Leave the rest of the parameters unchanged. Shut down the window by clicking the mouse arrow on a small square in the upper left-hand corner of that window.

8) Go to "Data" menu and select "Show Data". This will bring up an empty window on which the chromatogram will be displayed.

9) As a last task go to the lower left-hand side of the screen and click once on the "Manual Control" window. This will bring it up to the forefront of the page. Leave the screen in this mode until the moment you are ready to inject.

Sample Injection

With a 1ml syringe draw about 0.6ml of sample. Place the injection needle on the syringe. Make sure to evacuate all of the air bubbles from the syringe by tapping it gently on the sides.

Turn the injector to the "LOAD" position and insert the needle. Gently inject the contents of the syringe. At this point the sample is in the injector ready to be processed.

Position the mouse pointer on "Aquire Data" and press the mouse button at the same time as you turn the injector lever from " Load" to "Inject" position. Everything from now on is done automatically.

Data Analysis and Discussion

The chromatogram showed a peak for ammonia around 5.9 min. Two other peaks due to potassium ($R_T \sim$ 7.5 min) and sodium ($R_T \sim 5.1$ min) were also observed. Both sodium and potassium were derived from the sterile water used for the production of [N-13] ammonia. This was verified by analyzing the unirradiated sterile water under identical HPLC conditions.

CHEMICAL QUALITY CONTROL

ANALYTICAL HPLC (CONDUCTIVITY DETECTION) OF [N-13]AMMONIA

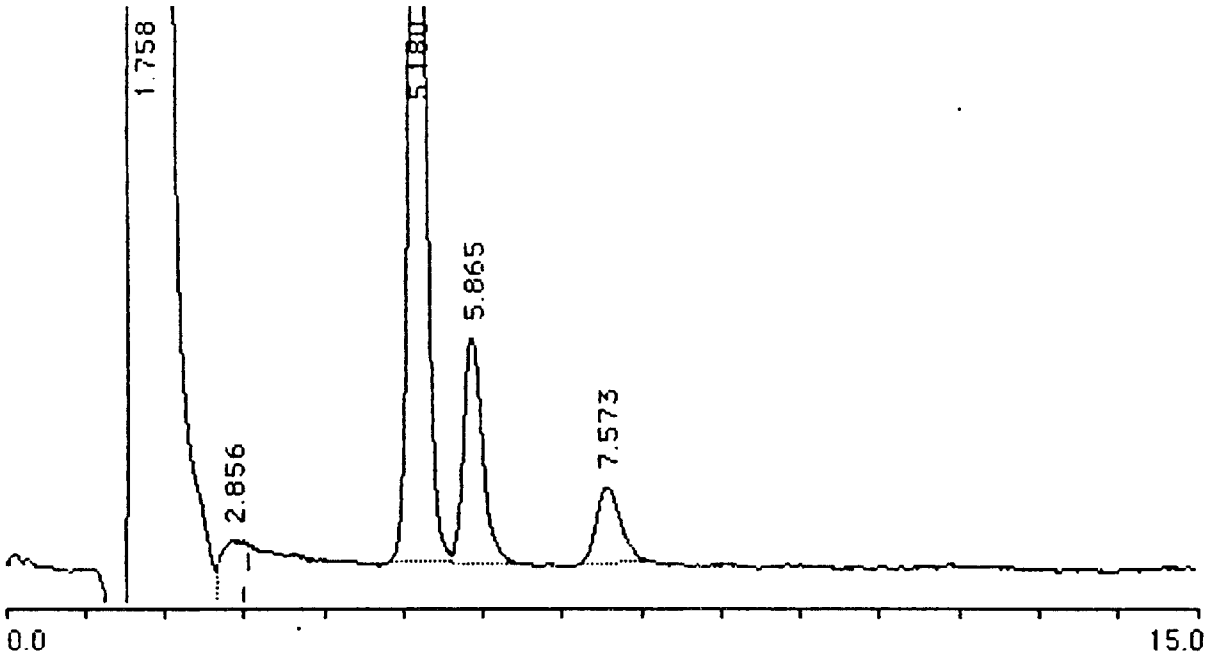
Column: Alltech Universal Cation 7 U column

Eluent: 7 mM citric acid in water

Flow rate: 0.8 mL/min

Chart speed: 1 cm/min

Detector: Waters Model 432 Conductivity detector



Analysis: Channel A

Peak No.	Time	Type	Height(µY)	Area(µY-sec)	Area%
1	1.758	Err!	1091459	24823430	96.644
2	2.856	N2	5619	135307	0.526
3	5.180	N1	41884	519780	2.023
4	5.865	N2	9615	143583	0.559
5	7.573	N	3223	63222	0.246
Total Area				25685322	99.998

RADIONUCLIDIC QUALITY CONTROL OF [N-13]AMMONIA

Introduction

The following protocol is used to determine the radionuclidic purity of a sample of [N-13]ammonia.

Materials

1) ORTEC Gamma-Ray Spectrometer
2) Gamma Vision Software
3) Capintec Radioisotope Calibrator Model CRC-15R

[N-13]Ammonia Sample Preparation

For [N-13]ammonia synthesis procedure refer to the Production Protocol described earlier.

Analysis and Discussion

The γ-ray spectrometer was first calibrated with a NBS standard radioactive source for energy and efficiency. A 1 μL capillary tube was dipped into a sample of [F-18]fluoride ion and the capillary tube was placed in a 5 mL glass beaker and kept in the counting chamber of the γ-ray spectrometer. The γ-ray spectrum showed only 511 keV peak attesting to the N-13 radionuclidic purity in the sample as 100%.

A 0.1 mL sample of [N-13]ammonia taken in a glass test tube was placed in the radioisotope calibrator chamber. The decrease of radioactivity with time was

measured and from which the half-life of the isotope was determined to be 10.0 min consistent with the half-life of [N-13] isotope.

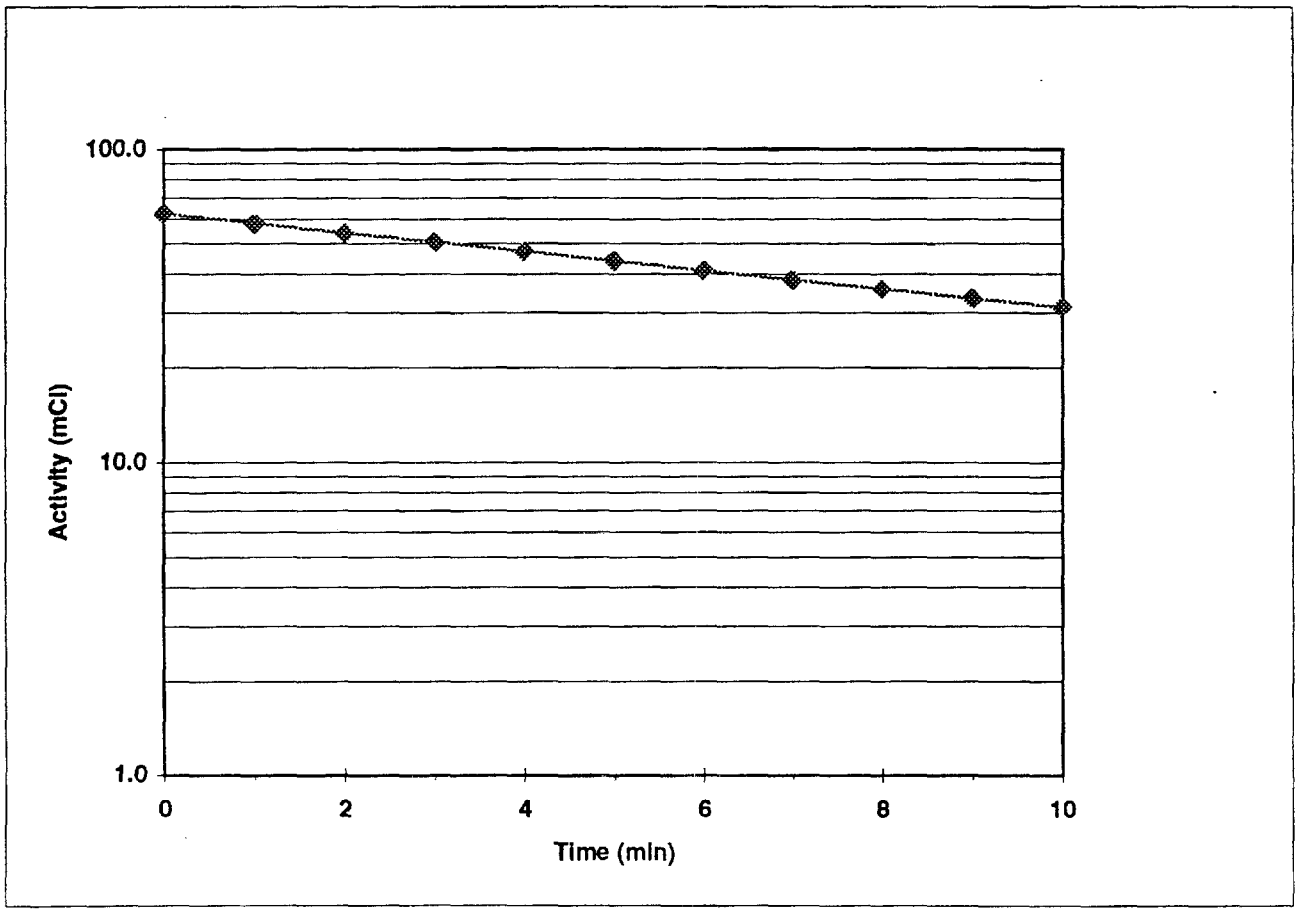
DECAY CURVE ANALYSIS FOR

[N-13] HALF LIFE

Time (min)	Observed Activity (mCi)	Calculated Half-Life (min)	Corrected Activity (mCi)
0	62.2		
1	58.0	9.9	62.2
2	54.1	9.9	62.2
3	50.5	10.0	62.2
4	47.1	10.0	62.2
5	44.0	10.0	62.3
6	41.0	10.0	62.2
7	38.2	10.0	62.1
8	35.7	10.0	62.2
9	33.3	10.0	62.2
10	31.1	10.0	62.3

Average Half-life **9.97** min

% Error From 9.98 min **-0.14%**



PROCEDURE FOR TESTING STERILITY OF [N-13]AMMONIA

Materials

1. Incubator set at 34°C
2. Thioglycollate medium, enriched with vitamin K1 and Hemin, 8 ml (Baxter Scientific)
3. 0.5 ml of sterile water (as negative standard) bacteria contaminated solution (as saliva for positive standard) and test specimens

Procedure

1. To use optimally: before use, boil thioglycollate tubes for 10 min to rid media of oxygen (have tubes slightly open to prevent any cracking of tubes)
2. Allow tubes to cool to room temperature
3. Add approximately 0.5 ml of specimen to thioglycollate tubes
4. Close tube leaving them slightly loose and incubate
5. Examine cultures for 7 days for evidence of growth
6. Report results as "No growth" or negative or positive if there is any growth

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PROCEDURE FOR TESTING PYROGENICITY OF [N-13]AMMONIA

Materials

1. Limulus amebocyte lysate (LAL) lymphylized
2. E-coli endotoxin 0.55: B5 10 mg lymphylized Kit Cat # N 284 from Whittaker Bioproducts, Inc. 8830 Biggs Ford Road, Walkersville, MD 21793-0127
3. Pyrogen free water-commercially available sterile water for injection (USP) is usually satisfactory
4. Pipettes: 1, 5, 10 ml and 100 µl - pyrogen free
5. 10 x 75 ml glass reaction tubes, pyrogen free - available from Whittaker Bioproducts, Inc. or may be rendered pyrogen free by heating at 180°C for 4 hr

Procedure

1. Set up pyrogen free reaction tubes (10 x 75 m) accordingly for standards and unknown samples in triplicate
2. Reconstitute control standard endotoxin (CSE) with required amount of pyrogen free water as stated on vial. Reconstituted CSE maybe stored at 2-8°C for up to 4 weeks
   a. Vortex well and dilute to concentrations of 1 EU/ml
   b. Make series of dilution of 0.5, 0.25, 0.12, 0.06 and 0.03 EU/ml for standards
   c. Use pyrogen free water as negative control
3. Reconstitute the lysate with pyrogen free water according to vial instructions. Swirl gently but thoroughly for at least 30 sec
4. Carefully transfer 100 µl of water, standards and test samples into appropriate reaction tubes
   (NOTE: Test specimens should be in the pH range of 6.0-7.5. If necessary pH adjust with pyrogen free acid or base. Do not adjust unbuffered solutions)
5. Add 100 µl of reconstituted LAL to each reaction tube, immediately mix and place in a 37°C water bath. After exactly 1 hr of incubation, remove tube from both and examine for gelation
6. Record results as either positive (gelation) or negative (no gelation)

Initial Quality Control - for lysate sensitivity

Endpoint dilution is determined as the last dilution of endotoxin which still yields a positive result

Assay Results
CSE DILUTIONS (EU/ml)

Replicates	0.5	0.25	0.12	0.6	0.3	H_2O	Endpt.
1	+	+	+	-	-	-	0.12
2	+	+	+	-	-	-	0.12
3	+	+	+	+	-	-	0.06
4	+	+	+	-	-	-	0.12

Lysate sensitivity is calculated by determining geometric mean of the end point. Each end point is converted to log 10. Individual log 10 values are averaged and the lysate sensitivity is taken as the antilog 10 of this average log value.

Calculation of Geometric Mean Endpoint

Endpoint EU/ml	Log 10 Endpoint
0.12	-0.921
0.12	-0.921
0.06	-1.222
0.12	-0.921

Mean = -0.996
Antilog 10 mean = 0.10 EU/ml
Data of endotoxin is unknown
Lysate sensitivity = 0.10 EU/ml

Determination of Endotoxin in Unknown Sample
Lysate Sensitivity = 0.10 EU/ml

Sample Dilution

Replicate	1/2	1/4	1/8	1/16	1/32	1/64
1	+	+	+	-	-	-
2	+	+	+	+	-	-

Endpoint dilution	Log 10 endpoint
1/8 (0.125)	-0.903
1/16 (0.0625)	-1.204
	Mean -1.054

Antilog 10 mean = 0.088 = 1/11.3

Endotoxin Conc. = lysate sensitivity x endpoint dilution
= 0.10 x 11.3 = 1.13 EU/ml

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UCLA RADIOPHARMACY DELIVERY SERVICE

PRODUCT: [N-13]Ammonia DATE: 7-1-96

LOT NO.: 7-1-96 (11:00)

FORMULATION: Nitrogen-13 Ammonia injection

PURPOSE: PET Research/Clinic

CONTAINER: 10 ml. sterile glass vial

CLOSURES: Rubber stopper
Aluminum seal

Test	Sampling Requirements (mL)	Limits	Results
pH	0.1	3.5-8.5	6.9
Appearance	stock	clear, colorless	√
Bacterial Endotoxin	0.5	175 EU/v	negative
Sterility	1.0	sterile	no growth

LITERATURE REFERENCE

FOR

[N-13]AMMONIA

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In-target Production of [^{13}N]Ammonia via Proton Irradiation of Dilute Aqueous Ethanol and Acetic Acid Mixtures

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(Received 15 January 1991)

A reliable, reproducible method has been developed for the *in situ* production of decicurie quantities of [^{13}N]ammonia by proton irradiation of pressurized, dilute aqueous solutions of acetic acid and ethanol. Some of the parameters investigated for their effect on the ^{13}N product distribution included dose, dose rate, solute concentration, target body material, beam strike volume and proton energy. For the conditions investigated, [^{13}N]NH_3 was produced directly in the target in yields >75%.

Introduction

Compounds labeled with the short-lived positron emitter ^{13}N($t_{1/2}$ = 9.97 min) have found widespread utility in a number of disciplines (Root and Krohn, 1981). In the field of positron emission tomography, ^{13}N-labeled-ammonia is routinely used as a tracer of myocardial blood flow (Schelbert and Schwaiger, 1986) and as a precursor for synthesis of various L-[^{13}N]amino acids (Barrio *et al.*, 1983). Until recently, the most common method used for preparation of [^{13}N]NH_3 has been by chemical reduction of the species resulting from proton irradiation of water (Parks and Krohn, 1978). However, the emergence of low energy, proton only accelerators coupled with the low saturation yields below 10 MeV for the ^{16}O(p,α)^{13}N reaction (Sajjad *et al.*, 1986) dictated that alternative target strategies be considered for production of [^{13}N]NH_3. To this end, we have reported (Bida *et al.*, 1986) the in-target production of clinically useful quantities of [^{13}N]NH_3 from the proton irradiation of a slurry of ^{13}C amorphous carbon powder in natural water. Others (Mulholland *et al.*, 1990) have reported high yields of [^{13}N]ammonia using a hydrogen-pressurized degassed water target. Previous reports (Welch and Straatmann, 1973; Tilbury and Dahl, 1979; Heselius *et al.*, 1989) of *in situ* chemical species produced by proton and deuteron irradiation of aqueous solutions containing additives (e.g. diamond dust, acetic acid, ethanol) indicated [^{13}N]ammonia as a product, but did not result in a method for direct in-target production of [^{13}N]ammonia for medical imaging applications.

Although the ^{13}C amorphous carbon slurry target has demonstrated reliable performance at low beam currents ($\leqslant 10\,\mu$A), we felt it desirable to produce larger quantities of ^{13}N while retaining the attractive feature of *in situ* ammonia production. Variations of the original slurry target approach proved successful in this regard, but again, the method was plagued with some unattractive mechanical difficulties. The most notable problem with all of the slurry target variations was difficulty in post-irradiation water transfer due to blockage of the transfer lines and/or the stainless steel frits used to isolate the carbon powder in the target beam strike. In investigating several variations of the hydrogen overpressure target (Mulholland *et al.*, 1990), we felt that solution pH may be an important parameter affecting [^{13}N]NH_3 yields. We have observed that pressurized, non-degassed dilute, aqueous acetic acid solutions produced favorable and reproducible product distributions at high proton currents. However, irradiation of a sodium hydroxide neutralized acetic acid solution produced a similar fraction of activity as ammonia, thus indicating that ammonia yields are insensitive to pH over the narrow range investigated. Guided by previous reports (Tilbury and Dahl, 1979), we observed that irradiation of dilute aqueous

*Author for correspondence.

Table 1. ^{13}N yields and product distributions for proton irradiation of dilute aqueous acetic acid and ethanol solutions

Run No.	Ep(MeV)	Target body	Pressure (MPa)	Solute (mM)	Beam (μA)	Time (min)	Total ^{13}N (mCi)	Sat. yield (mCi/μA)‡	[^{13}N]NH_3 (%)	[^{13}N]NO_x^- (%)	[^{13}N]N_2 (%)
1	11	Al	1.31	1	40	10	95	7.0	76	6	18
2	11	Al	1.31	5	40	10	129	6.8	96	4	0
3	11	Al	1.31	10	40	10	132	6.9	95	5	0
4	11	Al	1.31	100	40	10	136	7.1	96	4	0
5	11	Au	1.31	10	10	10	32	5.5	97	3	0
6	11	Au	2.40	10	20	10	61	6.0	92	4	4
7	11	Ag	1.31	10	20	10	72	7.1	93	5	2
8	11	Ag	2.06	10	20	10	76	7.4	88	7	5
9	11	Ag	2.06	10	20	10	73	7.2	94	5	1
10	11	Ag	2.06	10	30	10	108	7.3	78	5	17
11	11	Ag	2.06	0	40	10	140	7.1	0.3	99.4	0.3
12	11	Al	2.75	1	40	20	197	6.8	92	5	3
13	11	Al	2.75	5	40	10	138	6.9	95	5	0.04
14	11	Al	2.75	10	40	8	130	7.6	96	4	0
15	11	Al	2.75	5	40	20	198	6.9	94	6	0.3
16	11	Al	2.75	10	40	20	182	7.3	94	6	0.4
17†	10	Al	2.40	5	30	20	142	6.3	>99	<0.5	<0.1
18	18	Al	§	5	15	20	374	33.2	>99	<1	0
19	18	Al	§	5	25	20	651	34.7	>97	<3	<0.2
20	18	Al	§	5	25	20	695	37	98	2	0
21	18	Al	§	5	30	20	730	32.4	96	3	<0.5

*For experiments 1–4, the solute was acetic acid. All others used ethanol.
†Average of 13 experiments.
‡For experiments 1–16, yields were calculated from the accumulated charge and irradiation time.
§Target pressure in the range of 1.31–2.75 MPa was used.

ethanol solutions proved equally successful. Thus, we report herein the results of our preliminary investigations into high yield [^{13}N]ammonia production via proton irradiation of pressurized aqueous mixtures of ethanol and acetic acid. Some of the parameters investigated for their effect on the ^{13}N product distribution included dose, dose rate, solute concentration, target body material and beam strike volume.

Materials and Methods

Irradiations were performed on either the Siemens RDS 112 proton cyclotron with a fixed energy 11 MeV beam or the IBA Cyclone 30 proton accelerator with variable energy from 15 to 30 MeV. The variable energy of the latter cyclotron allowed for irradiations to be performed at 18 MeV with subsequent degradation from 15 MeV through 0.65 mm of aluminum for irradiations at 10 MeV. In this regard, the energies listed in Table 1 above are the accelerator energies impingent upon the target body vacuum isolation foils. For both accelerators, the proton beams were collimated to 10 mm dia.

Target chambers

Four different target body and foil combinations were used for these experiments. These combinations included (1) a 10 mm dia by 2 mm deep gold-plated copper body fitted with a 0.025 mm titanium foil; (2) a 10 mm dia by 5 mm deep silver body fitted with a 0.0125 mm Havar foil; (3) a 10 mm dia by 50 mm deep aluminum body fitted with 0.0125 mm Havar foil; and (4) a 13 mm dia by 12 mm deep aluminum body fitted with a 0.025 mm Havar foil. Each of the four target bodies was fitted with a 0.025 mm aluminum vacuum isolation foil upstream of the target body foil. All target designs provided both water cooling of the target body and helium cooling of the target foils.

Experimental procedure

For the experiments performed on the RDS 112, the target system used was a modification of the ^{13}N target system described elsewhere (Wieland *et al.*, 1990). Aqueous solutions (prepared using reagent grade acetic acid and ethanol and deionized or HPLC grade water) of the desired molarity of either acetic acid or ethanol were introduced through a low dead volume valve located at the bottom of the target. Loading was continued through the target beam strike volume until excess solution was discharged from a vent located at the top of the target. After target solution loading was completed, the target was pressurized with helium (99.995%) and the vent valve closed. Target pressure was monitored with a piezoelectric pressure transducer (Kulite Model XTM-190). After irradiation, the solutions were collected for radioassay by passage first through a strong anion exchange resin (Alltech Maxi-Clean IC-OH cartridge), followed by a sampling bottle for collection of the aqueous solution and then a gas collection bag. This approach allowed for rapid assessment of how the radioactivity was partitioned among the forms [^{13}N]NH_3, [^{13}N]anions (e.g. nitrite and nitrate) and [^{13}N]N_2 or other gases (*vide infra*).

For the experiments performed on the Cyclone 30 accelerator, target (4) was used. With the vent valve located at the top of the target open to atmosphere, the target was filled with 1.9 mL of solution through a valve located at the bottom of the target, leaving about 0.35 mL dead volume. This dead volume was filled with helium to atmospheric pressure and the valves were then closed prior to irradiation. During irradiation, the system pressure was not monitored,

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[^{13}N]NO_x^- (%)	[^{13}N]N_2 (%)
6	18
4	0
5	0
4	0
3	0
4	4
5	2
7	5
5	1
5	17
99.4	0.3
5	3
5	0.04
4	0
6	0.3
6	0.4
<0.5	<0.1
<1	0
<3	<0.2
2	0
3	<0.5

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Product identification

The ^{13}N-labeled products from these experiments were analyzed by radio–HPLC and radio-gas chromatography. Conditions for HPLC anion analyses were: Wescan Anion/R column, 4 × 250 mm, 4 mM *p*-hydroxybenzoic acid, pH = 8.4, 1.5 mL/min, Wescan Model 215100 conductivity detector coupled to a NaI radiation detector. Conditions for HPLC cation analyses were: Vydac 400IC column, 4.6 × 50 mm, 2 mM HNO_3, 4 mL/min, detectors as above. Conditions for the gas chromatographic analysis were: Alltech CTR I column, 1.83 m × 6.4 mm, ambient, 50 mL/min helium, thermal conductivity detector coupled to a NaI radiation detector. Retention times of the radiolabeled products were compared with the calibrated retention times of known standards. In addition, for the identification of [^{13}N]NH_3, an irradiated dilute ethanol solution was analyzed by radio–cation chromatography and the product retention time was compared with that of a sample, similarly analyzed, that had been prepared via Devarda's reduction of an irradiated water sample (Parks and Krohn, 1978). Further, no peak broadening was observed when the two irradiated samples were mixed together and subsequently analyzed. For these analytical conditions, the only major products observed were [^{13}N]NH_3, [^{13}N]NO_2^- and [^{13}N]NO_3^- (hereafter [^{13}N]NO_x^-) and [^{13}N]N_2. A small amount of [^{18}F]F^- is always observed in the radio–anion chromatograms due to proton irradiation of the natural abundance [^{18}O]H_2O in the target water.

Results and Discussion

Results for the proton irradiation of dilute aqueous acetic acid and ethanol solutions are summarized in Table 1. From the data, the following limited conclusions are drawn:

(1) Successful irradiations at currents $\geqslant 20\ \mu$A required operation at elevated pressure. This condition mitigates vapor voiding and boiling that occurs at these power levels (Heselius *et al.*, 1989).

(2) For either solute, [^{13}N]NH_3 was produced in greater than 75% yields for concentrations as low as 1 mM.

(3) It was observed that lowered [^{13}N]NH_3 yields occurred at the expense of a higher nitrogen gas fraction, but the saturation yield remained relatively unaffected. While the dependence of the change in product distribution on the parameters of the experiment is not clear from the data in Table 1, in general we have found that (a) little or no [^{13}N]N_2 is generated from the irradiation of pure water, the majority of the activity being [^{13}N]NO_x^-; and (b) the ammonia fraction decreases with increasing beam current and lower beam strike volume.

(4) The product distribution was unaffected by the target body and foil materials used.

(5) For the three different proton energies used with ethanol as solute, the ammonia yield was found to be invariant with dose over the calculated range of 7.5–125 eV/molecule.

(6) Finally, with the exception of experiments 5, 6 and 17, the saturation yields are on the order of 65–75% of the reported thick target yields for the ^{16}O(p,α)^{13}N reaction at the degraded on-target energy (Sajjad *et al.*, 1986). This fraction is consistent with previously reported saturation yields for the proton irradiation of water (Parks and Krohn, 1978; Tilbury and Dahl, 1979). Part of the discrepancy in yields, discussed to a certain extent elsewhere (Kilbourn *et al.*, 1984), is due to the difference in target material, i.e. water versus oxygen gas. For experiments 5 and 6, the lower observed saturation yields are probably due to a thin target as the depth of the beam strike was only 2 mm. The average saturation yield for experiment 17, within experimental error, is in agreement with the published value for oxygen gas at the degraded proton energy.

The proton and deuteron irradiation of dilute aqueous ethanol solutions has been previously reported (Welch and Straatmann, 1973; Tilbury and Dahl, 1979). However, some important differences in experimental conditions, and hence results, exist between the previous work and the results reported herein. The mechanism proposed for ammonia formation via proton irradiation of water involves formation of hydroxyl radicals generated when the nascent ^{13}N hot atom reaches chemical bond energies (Tilbury and Dahl, 1979; Patt *et al.*, 1991). As such, addition of an hydroxyl radical scavenger such as ethanol was postulated to result in an increase in the observed [^{13}N]NH_3 fraction. For 1 mM ethanol, [^{13}N]NH_3 was found to be the major product up to a system dose of 2.5 eV/molecule (Tilbury and Dahl, 1979). Beyond this point, the ammonia fraction was observed to decrease, reportedly due to consumption of the added radical scavenger. In the present work, for the experimental conditions summarized in Table 1, high ammonia fractions were observed for system doses in excess of 100 eV/molecule and for absolute amounts of ethanol on the order of 30 times less than the previous work (Tilbury and Dahl, 1979). One important difference between these two experimental conditions is that we operated a closed target system at elevated helium pressures whereas Tilbury

and Dahl (1979) operated their system at atmospheric pressure for the ethanol experiments. The possibility of radical scavenger consumption is possible under the conditions reported by Tilbury and Dahl (1979). In fact, our data for acetic acid as additive (see experiment 1) are suggestive of this occurrence. However, given the results observed involving such large differences in dose and ethanol quantities, it is also likely that evaporative loss of solute due to boiling may account for part of the reason for the premature decrease in ammonia yield in the open-target system (Tilbury and Dahl, 1979). Another important difference observed for these results concerns the ^{13}N-labeled product spectrum. Our preliminary search has identified four labeled products. Tilbury and Dahl have identified only labeled ammonia, nitrite and nitrate anions. Others (Welch and Straatmann, 1973) have observed the presence of ^{13}N-labeled acetamide and cyanide in the deuteron irradiation of dilute ethanol water solutions. In yet another report involving the effect of added hydroxyl radical scavenger (Patt *et al.*, 1991), ^{13}N-labeled hydroxylamine, nitric oxide and nitrogen dioxide have been detected. Further, as discussed above, our results showed that a decrease in the ammonia fraction was always at the expense of an increase in the nitrogen gas fraction when either an acetic acid or ethanol solution was used as target. This occurred even for target chambers where vapor voiding was not an issue. These results are in contrast to previously reported results wherein no gas fraction was observed (Tilbury and Dahl, 1979). It is also noted that in the absence of added scavenger, negligible gas was produced in a target geometry that most likely suffered poor heat transfer for the beam conditions used (see experiment 11). Efforts are currently underway to reconcile some of these discrepancies. In addition, we are investigating the fate of added ethanol after irradiation in order to elucidate the mechanism of ammonia formation in these systems. We are also exploring the ^{13}N product distribution in the concentration range of 0–1 mM.

Conclusion

The proton irradiation of pressurized, dilute aqueous ethanol solutions provides a new method for *in situ* production of large quantities of ^{13}N-labeled ammonia for medical imaging applications. The preliminary information presented here has prompted the development of a new production level target system for radiolabeled ammonia that can accommodate a reasonably wide range of proton energies and irradiation conditions. The results are consistent with the behavior expected in an environment involving scavenging of hydroxyl radicals that would otherwise limit the production of ammonia.

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Int. J. Radiat. Appl. Instrum.
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[F-18]FLUORIDE ION

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PREPARATION OF

[F-18]FLUORIDE ION

[F-18]FLUORIDE ION

The production of [F-18]fluoride ion follows the method reported in the literature [Wieland et al., J. Labelled Comp. Radiopharm. 23, 1205-1207 (1986). A copy of the paper is enclosed].

Description of the production of [F-18]fluoride ion

Eleven MeV proton irradiation of [O-18]H_2O in a silver target body produces [F-18]fluoride ion via $^{18}O(p,n)^{18}F$ nuclear reaction. The irradiated solution is passed through a cation exchange resin column. The resin column is eluted with sterile water. The eluent from the column containing [F-18]fluoride ion was passed through a MIllipore filter (0.22 μm) into a sterile vial contain sterile NaCl solution.

The step by step procedure for the production of [F-18]fluoride ion is described in the following section.

[F-18] Fluoride Ion Preparation

Introduction

The following is the procedure for the production of [F-18] Fluoride ion from [O-18] water by the RDS112 Cyclotron.

Materials

1) 10ml empty sterile vial (Hollister - Stier, Cat#7515ZA)
2) Vented 0.22 micron filter (Millipore, CAT# SLGSV255F)
3) 2 yellow 20 gauge needles (Owens & Minor, CAT#305175)
4)8 inch / 15ml Biorad chromatography column
5) Biorad Cation Exchange Resin (AG 50W-X12)
6) Two port stopper assembly (constructed in house)
7) 12 cc sterile Syringe (UCLA Storehouse #550607-302)
8) Vial of 10ml sterile water for injection
9) 0.5 ml of 180mg/ml saline solution
10) One luer tipped stopcock

Procedures

Before bombardment

1)Thoroughly clean the Biorad column and stopper assembly with pure water and rinse with methanol. The two items should then be oven dried and cooled just prior to use.

2) Swab a sterile empty vial with an alcohol prep pad and insert two twenty gauge yellow needles (gloves are to be worn at this time). To one of the needles, attach a 0.22 micron filter; the other needle will serve as a vent. Pass 0.5 ml of 180 mg/ml saline solution through the sterile filter, leaving some in the filter. Affix a label with date and the details of the isotope on the vial.

2) Fill the 15ml Biorad column 1/3 full with the cation exchange resin, flush the resin with 20ml of sterile water, and connect the two port stopper assembly.

The water level within the column should be at the level of the resin. The bottom of the resin column is then to be attached to the sterile filter.

3)This set up is to be secured to the appropriate CPCU mounting brackets found on the front exterior of the unit. Attach the tube designated "Delivery" to the delivery line from the target. The 1/8" tubing with the male luer fitting serves both as a vent during delivery and the water addition line subsequent to delivery of the [F-18] fluoride ion. A female end of the stopcock is to be attached to the male fitting of the vent/water addition line. To the other female end, attach a 12ml sterile syringe filled with 8 ml of sterile water. Prior to and during delivery, the stopcock is to be closed to the syringe and open to the column allowing flow of the helium sweep gas. The sterile vial should be placed in a lead pig provided in the CPCU.

Automated Production of [F-18] Fluoride Ion

1)The cyclotron is assumed to be in the standby mode, ready to generate beam.If this is not the case, refer to the cyclotron operation protocol for advisement. Make sure the [O-18] load syringe in the top drawer of the USS contains over 1ml of [O-18] water. If this is not the case, refer to [O-18] syringe refill protocol.

2) Select **Produce Labeled Product** from the RDS Function Selection Menu, using the arrow keys. Press Enter on the key board to continue.

3)From the **Produce Labeled Product Menu,** for Target Zone 4, select either **[F-18] FDG CPCU 1 or [F-18] FDG CPCU 2.** Press "Enter" to continue.

4) On the **Target Zone 4 Set Up Page,** enter the beam current which is typically 20uA, and the bombardment time, usually 10 minutes.

5) Press "Enter" to begin the automated production sequence: target loading, bombardment and delivery.

At End of Bombardment

1) At EOB the program will start unloading the activity directly into the chromatography column in the selected CPCU. This process is approximately 4 minutes in duration.

2) When the computer indicates a complete delivery process, go from the **Maintenance Main Page** to the **Target 2 Page** by typing **P Targ2.** Type "off r70" and note the target pressure bleed down to less than 1 psia, indicating the absence of a bolus in the delivery lines. Type "on r71". This high pressure valve (20 psia) will clear any residual product from the lines. Type "off r71", again noting the drop in pressure and with an appropriate meter check for the presence of radioactivity in the column. If confirmed, simultaneously press on the "control" and "C" keys. In so doing, the automated process is brought to closure.

3) Open the stopcock to the syringe and column and gently pass the 8ml of sterile water completely through the column and into the vial resting in a lead pig. With tongs, remove both yellow needles and transfer to another lead pig with a top. Bring this over to the capintec, assay, and ship the product to the clinic in the usual manner.

QUALITY CONTROL PROCEDURES

FOR

[F-18]FLUORIDE ION

Radiochemical Quality Control For [F-18] Fluoride Ion

Introduction

The following protocol is used to determine radiochemical purity of the [F-18] fluoride ion produced at the cyclotron.

Materials

1) 1 yellow 20 gauge needle (Owens & Minor, CAT# 305175)

2) 0.003N H_2SO_4 (made from Fisher 36N H_2SO_4, Cat #A300-500)

3) Macintosh computer running Dynamax Version 1.3.1 HPLC Method Manager

4) Fisher Model 222D HPLC Pump

5) Beckman Model 170 Radioisotope Detector

6) Alltech MF-Plus Anion Exclusion Column (Stock# 269062)

7) Rainin Microman Pipette

[F-18] Fluoride Ion Preparation

For [F-18] fluoride ion production procedure, refer to the [F-18] fluoride ion Synthesis Protocol

HPLC Solvent Preparation

The HPLC solvent, 0.003N H_2SO_4, is prepared by adding 0.167 ml of the concentrated H_2SO_4 to 2 Liters of 18 M ohm H_2O in a volumetric flask. The Rainin Microman Pipette is used to accurately measure the acid. Note that this solvent is also the column's storage solution. The solvent is filtered through a 0.45 micron nylon filter to remove any particulate matter and degassed. Prior any analysis, give the system ample time to equilibrate as indicated by a stable baseline on the conductivity detector.

HPLC System Preparation and Shutdown

Set the flow rate on the SSI HPLC Pump to 0.8 ml per min. Always flush the HPLC system with 18 M ohm water before switching between different solvent systems. Use at least 10 column volumes for

each flush. Allow the system to equilibrate with the eluent for at least 60 min. before analyzing the [F-18] fluoride ion sample.

Dynamax Program Settings

Control Menu:

Control: Manual

Acquisition Time: 15 min.

Data Menu:

Show Report (marks peak retention time and area)

Show Data (Actual Chromatograph)

Data Parameters

Acquire Data: Channels A
Process Data: Channel A
Peak Width: 30
Minimum Peak Area: 1000 (Leave rest unchanged)

Report Format

Auto range Data: off
Channels: A
Other: Composition
Show Scale: Data + Composition (Leave rest unchanged)

Note:

Be sure to save each chromatograph by double clicking on "Save Data As". Also, confirm that the integrator is plugged into the appropriate and labeled input source

System Parameters

1) The radioisotope detector output is read as a background adjusted kcpm*N. Ensure the folowing settings on the Beckman Radioisotope Detector: Norm Factor- $9X10^5$, Flow- 0.8ml/min, Scale-9999, Background- Variable, Cell length- 12.7, Exit length-0,Fraction Time- 0, Tube ID- 0.8. Consult manual for programming if power is lost to the instrument.

2) A 20μL load loop is to be used on the injector. Downstream from the column, volume is minimized (and therefore mixing is minimized) by using the shortest length of tubing and smallest inner diameter (10mil) possible.

3) Flow is set to 0.8 ml/min., during analysis. Back pressure will be approximately 2300psi. If it runs significantly higher, consult the instructions provided with the column for regeneration procedures

and test chromatograms. To protect the column, set the high pressure limit to 3500 on the 222D SSI HPLC Pump. The lower limit may be set at 0.

Sample Injection

With a 1ml syringe draw about 0.4 ml of sample. Make sure to remove all of the air bubbles from the syringe by tapping it gently on the sides. Turn the injector to the "LOAD" position and insert the needle. Gently inject the contents of the syringe. At this point the sample is in the injector ready to be processed. Position the mouse pointer on "Acquire Data" and press the mouse button at the same time as you turn the injector lever from " Load" to "Inject" position. Under the Control Menu of the previously opened "DA" file, click on "Start Acquisition". Fill out the "Sample Notes" accordingly.Use the "Print Report" command from the "File" Menu to make a hard copy of the chromatograph and use the "Save Data" file to save the chromatograph to the hard drive. Remove column and flush system with 300ml of 18 M ohm H_2O and 200ml of Methanol at a flow rate of 1.0 ml/min.

Data Analysis and Discussion

The [F-18] fluoride ion should elute as a single peak at roughly the 8.2 minute mark. No other radiochemical traces have ever been found in any of the [F-18] fluoride ion samples. In all the analyses, the chromatogram always contains a single peak at 8.2 minutes.

RADIOCHEMICAL QUALITY CONTROL

RADIOANALYTICAL HPLC OF [F-18]FLUORIDE ION

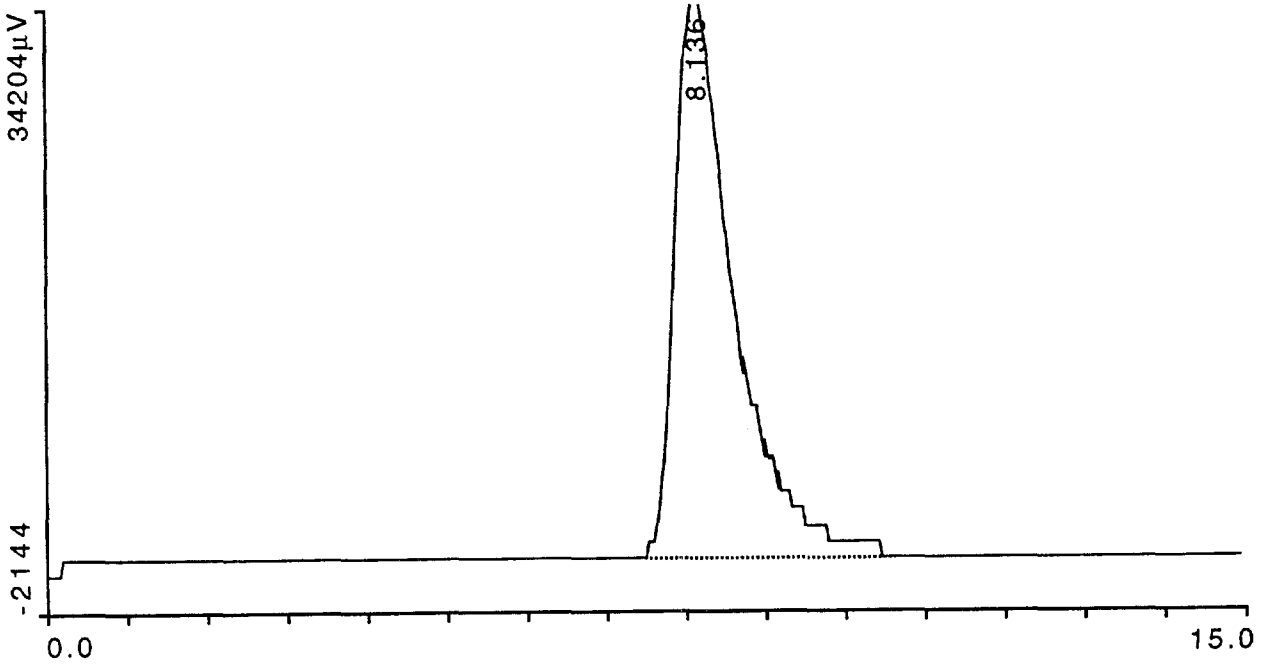
Column: Alltech MF-Plus Anion Exclusion column

Eluent: 0.003N Sulfuric acid in water

Flow rate: 0.8 mL/min

Chart speed: 1 cm/min

Detector: Beckman Model 170 Radioisotope detector



Analysis: Channel A

Peak No.	Time	Type	Height(µV)	Area(µV-sec)	Area%
1	8.136	*N	33681	1732878	100.000
Total Area				1732878	100.000

Chemical Quality Control For [F-18] Fluoride Ion

Introduction

The following protocol is used to determine the chemical purity of the [F-18] fluoride ion produced at the cyclotron.

Materials

1) 1 yellow 20 gauge needle (Owens & Minor, CAT# 305175)

2) 0.003N H_2SO_4 (made from Fisher 36N H_2SO_4, Cat #A300-500)

3) Macintosh computer running Dynamax Version 1.3.1 HPLC Method Manager

4) Fisher Model 222D HPLC Pump

5) Waters 432 Conductivity Detector

6) Alltech MF-Plus Anion Exclusion Column (Stock# 269062)

7) Potassium fluoride dihydrate (Aldrich 22,187-2)

8) Rainin Microman Pipette

[F-18] Fluoride Ion Preparation

For [F-18] fluoride ion production procedure, refer to the [F-18] fluoride ion Synthesis Protocol The addition of saline to the final product should be omitted for this analysis (the large concentration of NaCl would otherwise mask the minute signal of fluoride ion in the conductivity detection).

HPLC Solvent Preparation

The HPLC solvent, 0.003N H_2SO_4, is prepared by adding 0.167 ml of the concentrated H_2SO_4 to 2 Liters of 18 M ohm H_2O in a volumetric flask. The Rainin Microman Pipette is used to accurately measure the acid. Note that this solvent is also the column's storage solution. The solvent is filtered through a 0.45 micron nylon filter to remove any particulate matter and degassed. Prior any analysis, give the system ample time to equilibrate as indicated by a stable baseline on the conductivity detector.

HPLC System Preparation and Shutdown

Set the flow rate on the SSI HPLC Pump to 0.8 ml per min. Always flush the HPLC system with 18 M ohm water before switching between different solvent systems. Use at least 10 column volumes for each flush. Allow the system to equilibrate with the eluent for at least 60 min. before analyzing the [F-18] fluoride ion sample.

Dynamax Program Settings

Control Menu:

Control: Manual

Acquisition Time: 15 min.

Data Menu:

Show Report (marks peak retention time and area)

Show Data (Actual Chromatograph)

Data Parameters

Acquire Data: Channel A
Process Data: Channel A
Peak Width: 30
Minimum Peak Area: 1000 (Leave rest unchanged)

Report Format

Auto range Data: off
Channels: A+B
Other: Composition
Show Scale: Data + Composition (Leave rest unchanged)

Note:

Be sure to save each chromatograph by double clicking on "Save Data As". Also, confirm that the integrator is plugged into the appropriate and labeled input source

System Parameters

1)The HPLC system is to be equilibrated with 0.003N H_2SO_4 until a steady baseline is reached on the conductivity detector. Preferably, the system should equilibrate overnight at a flow rate of 0.05 ml/min. The detector should have the following settings: Temp- 35 degrees C, Base- 2000 µS/cm/FS, Response Time- 2 sec (STD), and the Conductance will be read in µS/cm. When the polarity indicator light is activated, positive ions will be read as negative peaks and negative ions will be read as positive peaks. The Balance is to be set at 0%. A properly inserted pulsed dampener is required for accurate conductivity readings. After use, make sure the detector is flushed with 300ml of H_2O, then 200ml of Methanol. This is to be performed with the column off line.

2) A 20μL load loop is to be used on the injector. Downstream from the column, volume is minimized (and therefore mixing is minimized) by using the shortest length of tubing and smallest inner diameter (10mil) possible.

3) Flow is set to 0.8 ml/min., during analysis. Back pressure will be approximately 2300psi. If it runs significantly higher, consult the instructions provided with the column for regeneration procedures and test chromatograms. To protect the column, set the high pressure limit to 3500 on the 222D SSI HPLC Pump. The lower limit may be set at 0.

5) Prior to use, the system is tested with a sample of KF in water. The retention time for fluoride ion is ~ 8.2 minutes.

Sample Injection

With a 1ml syringe draw about 0.4 ml of sample. Make sure to remove all of the air bubbles from the syringe by tapping it gently on the sides. Turn the injector to the "LOAD" position and insert the needle. Gently inject the contents of the syringe. At this point the sample is in the injector ready to be processed. Position the mouse pointer on "Acquire Data" and press the mouse button at the same time as you turn the injector lever from " Load" to "Inject" position. Under the Control Menu of the previously opened "DA" file, click on "Start Acquisition". Fill out the "Sample Notes" accordingly.Use the "Print Report" command from the "File" Menu to make a hard copy of the chromatograph and use the "Save Data" file to save the chromatograph to the hard drive. Remove column and flush system with 300ml of 18 M ohm H_2O and 200ml of Methanol at a flow rate of 1.0 ml/min.

Data Analysis and Discussion

In test chromatograms, fluoride ion standard elutes as a single peak at roughly the 8.2 minute mark. However, in all the analyses, no trace of cold fluoride ion was ever observed. This is quite consistent with the high specific activity nature of the [F-18] fluoride. The chromatogram showed only a large negative peak (R_T ~ 4.5 min) due to cations present in the sterile water.

CHEMICAL QUALITY CONTROL

ANALYTICAL HPLC (CONDUCTIVITY DETECTION) OF

[F-18]FLUORIDE ION

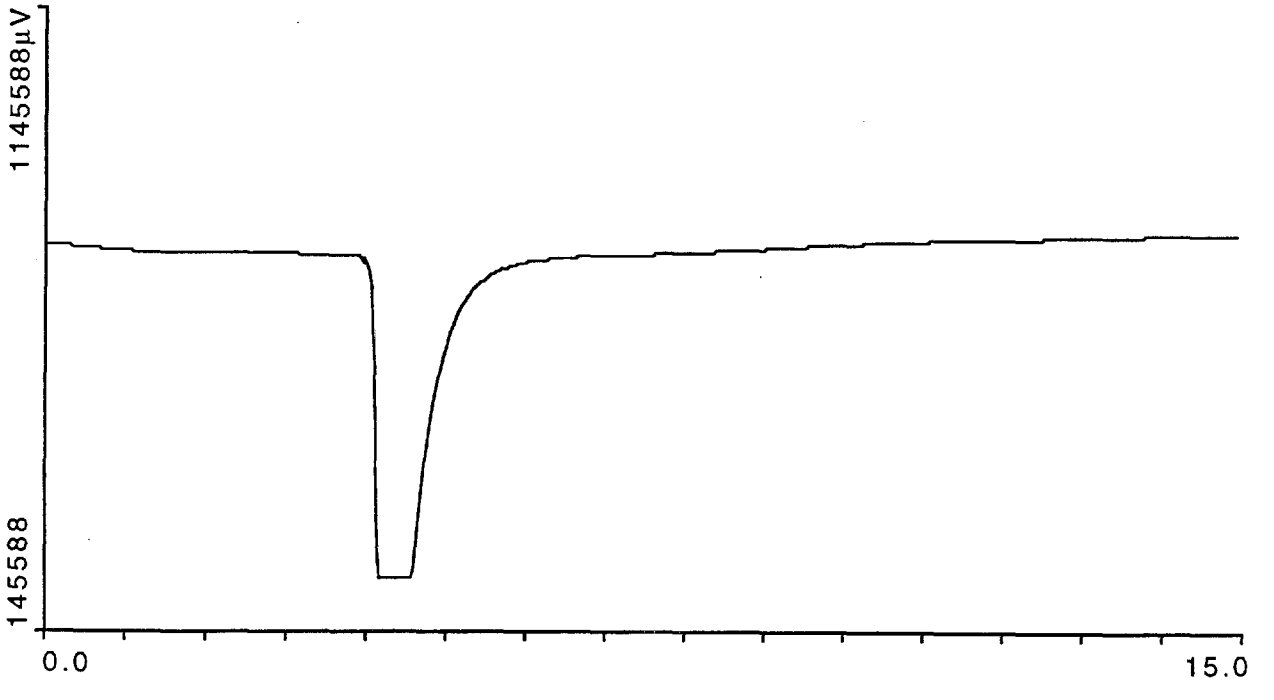
Column: Alltech MF-Plus Anion Exclusion column

Eluent: 0.003N Sulfuric acid in water

Flow rate: 0.8 mL/min

Chart speed: 1 cm/min

Detector: Waters Model 432 Conductivity detector



RADIONUCLIDIC QUALITY CONTROL OF [F-18]FLUORIDE ION

Introduction

The following protocol is used to determine the radionuclidic purity of a sample of [F-18]fluoride ion.

Materials

1) ORTEC Gamma-Ray Spectrometer
2) Gamma Vision Software
3) Capintec Radioisotope Calibrator Model CRC-15R

[F-18]Fluoride Ion Sample Preparation

For [F-18]fluoride ion preparation refer to the Production Protocol described earlier.

Analysis and Discussion

The γ-ray spectrometer was first calibrated with a NBS standard radioactive source for energy and efficiency. A 1 μL capillary tube was dipped into a sample of [F-18]fluoride ion and the capillary tube was placed in a 5 mL glass beaker and kept in the counting chamber of the γ-ray spectrometer. The γ-ray spectrum showed only 511 keV peak attesting to the F-18 radionuclidic purity in the sample as 100%.

A 0.1 mL sample of [F-18]fluoride ion taken in a glass test tube was placed in the radioisotope calibrator chamber. The decrease of radioactivity with time was

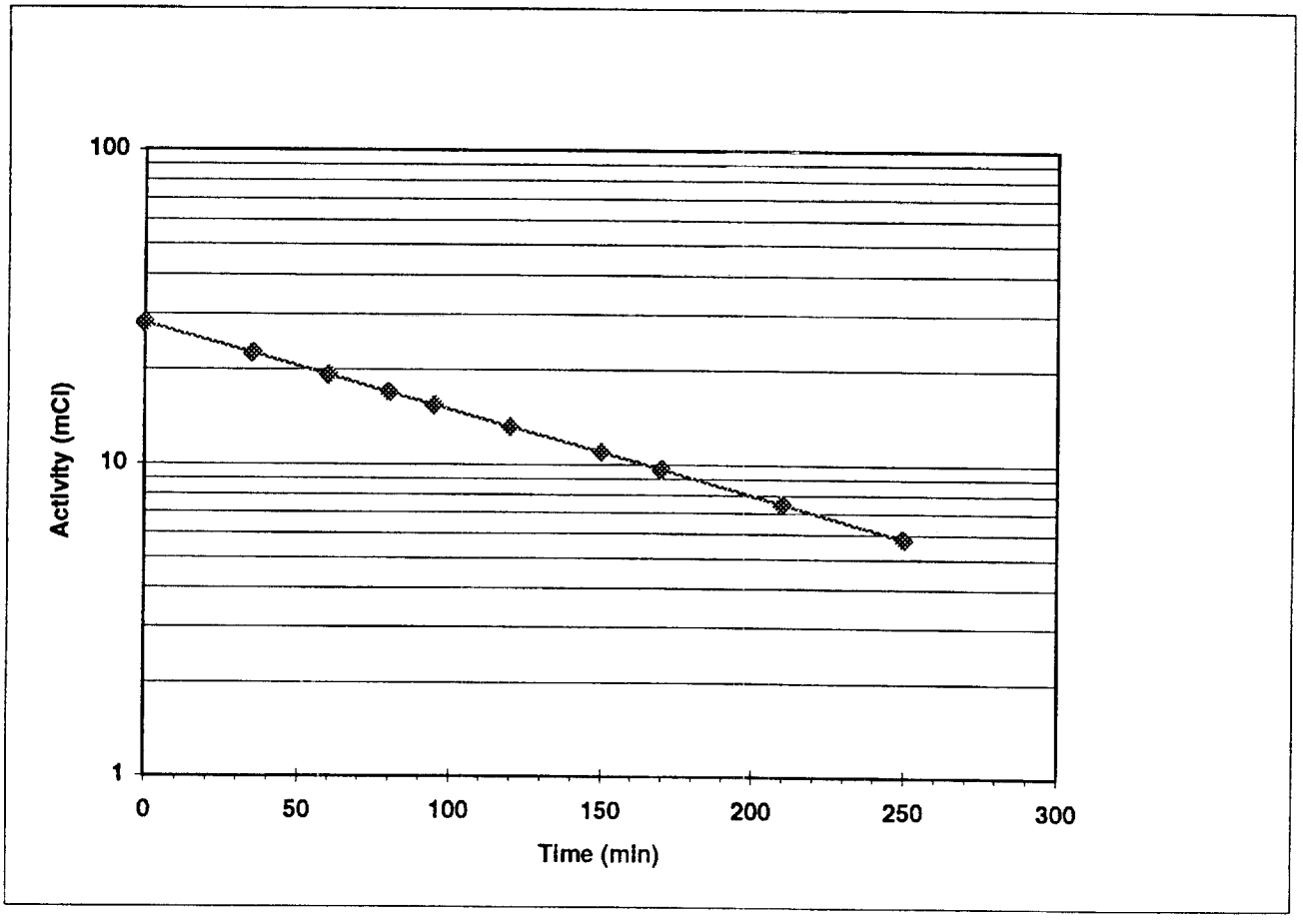
measured and from which the half-life of the isotope was determined to be 109.8 min consistent with the half-life of F-18 isotope.

DECAY CURVE ANALYSIS FOR

[F-18] HALF LIFE

Time (min)	Observed Activity (mCi)	Calculated Half-Life (min)	Corrected Activity (mCi)
0	27.9		
35	22.5	112.8	28.06
60	19.20	111.3	28.04
80	16.90	110.6	28.00
95	15.40	110.8	28.05
120	13.10	110.0	27.94
150	10.90	110.6	28.09
170	9.60	110.4	28.07
210	7.45	110.2	28.04
250	5.79	110.2	28.05

Average Half-life **110.8** min
% Error From 109.8 min **0.87%**



PROCEDURE FOR TESTING STERILITY OF [F-18]FLUORIDE ION

Materials

1. Incubator set at 34°C
2. Thioglycollate medium, enriched with vitamin K1 and Hemin, 8 ml (Baxter Scientific)
3. 0.5 ml of sterile water (as negative standard), bacteria contaminated solution (as saliva for positive standard) and test specimens

Procedure

1. To use optimally: before use, boil thioglycollate tubes for 10 min to rid media of oxygen (have tubes slightly open to prevent any cracking of tubes)
2. Allow tubes to cool to room temperature
3. Add approximately 0.5 ml of specimen to thioglycollate tubes
4. Close tube leaving them slightly loose and incubate
5. Examine cultures for 7 days for evidence of growth
6. Report results as "No growth" or negative or positive if there is any growth

Reference

1. Saule, B.M. (ed.), 1983. The APIC Curriculum for Infection Control Practice Association for Practitioners in Infection Control.

2. Widmann, F.K. (ed.), 1985 AABB Technical Manual, Ninth edition, Arlington, VA, American Association of Blood Banks; pp 325-343.

PROCEDURE FOR TESTING PYROGENICITY
[F-18]FLUORIDE ION

Materials

1. Limulus amebocyte lysate (LAL) lymphylized
2. E-coli endotoxin 0.55: B5 10 mg lymphylized Kit Cat # N 284 from Whittaker Bioproducts, Inc. 8830 Biggs Ford Road, Walkersville, MD 21793-0127
3. Pyrogen free water-commercially available sterile water for injection (USP) is usually satisfactory
4. Pipettes: 1, 5, 10 ml and 100 µl - pyrogen free
5. 10 x 75 ml glass reaction tubes, pyrogen free - available from Whittaker Bioproducts, Inc. or may be rendered pyrogen free by heating at 180°C for 4 hr

Procedure

1. Set up pyrogen free reaction tubes (10 x 75 m) accordingly for standards and unknown samples in triplicate
2. Reconstitute control standard endotoxin (CSE) with required amount of pyrogen free water as stated on vial. Reconstituted CSE maybe stored at 2-8°C for up to 4 weeks
   a. Vortex well and dilute to concentrations of 1 EU/ml
   b. Make series of dilution of 0.5, 0.25, 0.12, 0.06 and 0.03 EU/ml for standards
   c. Use pyrogen free water as negative control
3. Reconstitute the lysate with pyrogen free water according to vial instructions. Swirl gently but thoroughly for at least 30 sec
4. Carefully transfer 100 µl of water, standards and test samples into appropriate reaction tubes
   (NOTE: Test specimens should be in the pH range of 6.0-7.5. If necessary pH adjust with pyrogen free acid or base. Do not adjust unbuffered solutions)
5. Add 100 µl of reconstituted LAL to each reaction tube, immediately mix and place in a 37°C water bath. After exactly 1 hr of incubation, remove tube from both and examine for gelation
6. Record results as either positive (gelation) or negative (no gelation)

Initial Quality Control - for lysate sensitivity

Endpoint dilution is determined as the last dilution of endotoxin which still yields a positive result

Assay Results
CSE DILUTIONS (EU/ml)

Replicates	0.5	0.25	0.12	0.6	0.3	H_2O	Endpt.
1	+	+	+	-	-	-	0.12
2	+	+	+	-	-	-	0.12
3	+	+	+	+	-	-	0.06
4	+	+	+	-	-	-	0.12

Lysate sensitivity is calculated by determining geometric mean of the end point. Each end point is converted to log 10. Individual log 10 values are averaged and the lysate sensitivity is taken as the antilog 10 of this average log value.

Calculation of Geometric Mean Endpoint

Endpoint EU/ml	Log 10 Endpoint
0.12	-0.921
0.12	-0.921
0.06	-1.222
0.12	-0.921

Mean = -0.996
Antilog 10 mean = 0.10 EU/ml
Data of endotoxin is unknown
Lysate sensitivity = 0.10 EU/ml

Determination of Endotoxin in Unknown Sample
Lysate Sensitivity = 0.10 EU/ml

Sample Dilution

Replicate	1/2	1/4	1/8	1/16	1/32	1/64
1	+	+	+	-	-	-
2	+	+	+	+	-	-

Endpoint dilution	Log 10 endpoint
1/8 (0.125)	-0.903
1/16 (0.0625)	-1.204
	Mean -1.054

Antilog 10 mean = 0.088 = 1/11.3

Endotoxin Conc. = lysate sensitivity x endpoint dilution
= 0.10 x 11.3 = 1.13 EU/ml

Reference

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12. Cooperstock, M.S. (1975) The Lancet, June 1, 1975, 1272.

UCLA RADIOPHARMACY DELIVERY SERVICE

PRODUCT: [F-18]Fluoride ion DATE: 4-3-96

LOT NO.: 4-3-96

FORMULATION: F-18 Fluoride injection

PURPOSE: PET Research/Clinic

CONTAINER: 10 ml. sterile glass vial

CLOSURES: Rubber stopper
Aluminum seal

Test	Sampling Requirements (mL)	Limits	Results
pH	0.1	3.5-8.5	6.9
Appearance	stock	clear, colorless	√
Bacterial Endotoxin	0.5	175 EU/v	negative
Sterility	1.0	sterile	no growth

LITERATURE REFERENCE

FOR

[F-18]FLUORIDE

EFFICIENT SMALL-VOLUME O-18 WATER TARGETS FOR PRODUCING F-18 FLUORIDE WITH LOW ENERGY PROTONS

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Small, high-performance, static water targets for the production of aqueous F-18 fluoride from enriched O-18 water have been developed for use with a collimated 11 MeV proton beam extracted from a negative ion cyclotron. The beam current density distribution at the target entrance has been measured by activating 1 mm copper pins at the centerline and on 4 mm and 8 mm diameter circles. Counting the 38.1 min Zn-63 from the p,n reaction on Cu-63 indicates a uniform circular beam distribution with intensity of 84% of maximum at the 4 mm diameter and 49% at the 8 mm diameter. The 10 mm collimator is split assuring a centered beam when the collimator currents are balanced.

Targets were constructed of copper and plated with electroless nickel. A vacuum window of 25.4 micrometer aluminum and a target window of 12.7 micrometer Havar reduce the energy entering the target water to 10.5 MeV. 10 mm diameter cavity depths of 1.0 and 2.0 mm were used in the experiments, resulting in centerline water thicknesses of 1.15 and 2.15 mm due to outward deflection of the window with the target pressurized. The range of 10.5 MeV protons in water is 1.32 mm. This results in one target with predicted exit energies of 3.0 MeV centerline and 4.7 MeV at the perimeter, and one target which is 163% range thick at the centerline and 151% range thick at the perimeter. Both targets have a 0.300 mL reflux chamber above minimum loaded water level. Extra target water was loaded to insure make-up for radiolysis and reflux redistribution.

The targets have been remotely loaded through 15 meters of polyethylene tubing (0.86 mm I.D.). For these experiments the target was loaded through the bottom port via a zero dead volume Teflon slider-valve (necessary for pressurization). The radioactive water was recovered remotely in all runs. Transit time was approximately two minutes and the activity was recovered in a vessel located in a dose calibrator.

The "thick" target of Table 1 shows a relatively linear decrease in yield of about 1.6 mCi/uA per μA of current increase (29% yield decrease at 25 μA). The "thin" target of Table 2 shows 2.4 mCi/μA per μA (43% decrease at 25 μA). There is no significant difference between short runs (5 - 12 min) and long runs (26-113 min).

Table 3 indicates there was no significant increase in yield for the "thick" target run at 10 atmosphere pressure versus the 2 atmosphere condition of Table 1.

The target water loading was accurately weighed and compared to the recovered weight with and without bombardment. No decrease was observed without bombardment and an average decrease of 5% was observed with bombardment. The 10 mL volume of the pressurization tubing was valved off during each run and a pressure transducer was used to record the pressure increase. The increases in pressure correlated well with a calculation of hydrogen and oxygen resulting from the radiolysis of 5% of the target water.

Separate rinses were made of the "thin" target and of the recovery line after a 10 min run at 20 μA (0.195 mL initial loading). 91% of the activity was recovered before the rinses, 7% from the target rinse, and 2% from the line rinse.

The target loadings used in these initial experiments were considerably above the amount needed to bring the water level to the top of the beam strike (185% for the "thick" target and 253% or the "thin" target). The cost of the 0.330 mL loading of Table 1 is $24.

Table 1. Experiments with 2 mm Thick Water Target using 2 atm Helium Overpressure (average O-18 water loading of 0.330 mL with 0.157 mL beam strike and 0.157 mL refluxing space above liquid level).

Beam Current (microamps)	Bombardment Duration (minutes)	Activity at EOB (millicuries)	Saturation Yield at EOB (millicuries/microamp)
1.0	10	7.9	129.4
5.2	10	45.3	142.2
10.0	10	76.6	125.2
10.0	60	408.3	129.4
12.5	10	91.4	119.5
12.4	33	273.2	117.1
15.0	10	101.2	110.2
15.2	30	290.7	110.8
20.0	10	131.8	107.7
19.7	30	409.4	120.4
19.6	113	1170.2	117.0
25.0	10	150.3	98.2

Table 2. Experiments with 1 mm Thick Water Target using 2 atm Helium Overpressure (average O-18 water loading of 0.225 mL with 0.079 mL beam strike and 0.164 mL refluxing space above liquid level).

Beam Current (microamps)	Bombardment Duration (minutes)	Activity at EOB (millicuries)	Saturation Yield at EOB (millicuries/microamp)
1.0	10	8.9	144.8
5.0	5	20.8	132.7
5.2	10	43.0	134.9
8.7	12	70.9	111.7
15.0	10	89.8	97.8
15.2	26	264.0	114.6
20.0	10	124.2	101.4
20.0	60	546.5	86.6
23.5	10	129.7	90.2

Table 3. Experiments with 2 mm Thick Water Target using 10 atm Helium Overpressure (average O-18 water loading of 0.340 mL with 0.157 mL beam strike and 0.147 mL refluxing space above liquid level).

BEAM Current (microamps)	Bombardment Duration (minutes)	Activity at EOB (millicuries)	Saturation Yield at EOB (millicuries/micoramp)
25.0	10	171.1	111.8
25.0	29	371.7	88.8

Tables 1 and 2 give results for the "thick" (2 mm) target and the "thin" (1 mm) target as a function of beam current. These experiments were done with a 2 atmosphere initial pressure on the target chamber to keep the target window deflected outward against the helium window cooling pressure. Table 3 gives results for a 10 atmosphere pressure on the "thick" target.

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The F-18 fluoride produced has been used to prepare 2-(F-18)-fluoro-2-deoxy-D-glucose (FDG) in approximately 50% radiochemical yield (EOB) using the synthesis described by Hamacher et al[1]. The final product, analyzed by TLC, gave appropriate R_f values [2].

The results of these initial experiments indicate that an 11 MeV proton accelerator can economically produce sufficient quantities of F-18 fluoride to support a clinical PET program.

1) Hamacher, K., Coenen, H.H., Stocklin, G., J Nucl Med 27, 235, 1986.

2) Bida, G.T. Satyamurthy, N., Barrio, J.R., J Nucl Med 25, 1327, 1984