Application of Robotics to Radiopharmaceutical Preparation: Controlled Synthesis of Fluorine-18 16α-Fluoroestradiol-17β

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A commercially available robot system, the Zymark Zymate Laboratory Automation System, has been utilized for the preparation of a positron-emitting radiopharmaceutical, 16α-[^{18}F] fluoroestradiol-17β. This radiopharmaceutical is prepared in a three-step synthesis (preparation of [^{18}F]-tetrabutylammonium fluoride, S_N2 displacement of a triflate, and ketone reduction) and is purified by high performance liquid chromatography (HPLC). All steps in the synthesis and HPLC purification are controlled by the robot system with no manual intervention. This represents a new approach to the complete automation of radiopharmaceutical production.

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In the synthesis of radiopharmaceuticals labeled with short-lived positron-emitting radioisotopes (oxygen-15, nitrogen-13, carbon-11 (^{11}C), fluorine-18 (^{18}F), gallium-68), large initial amounts of radioactivity are often required to produce sufficient amounts of high specific activity products for clinical applications. There are three basic approaches to the preparation of such radiopharmaceuticals: (a) manual syntheses, (b) manual operation of a remote, well-shielded apparatus, and (c) using an automated radiopharmaceutical apparatus. Manual syntheses are usually the simplest and are inherently as versatile as the radiochemist, but they are labor intensive and inevitably lead to excessive cumulated radiation doses, especially for routine preparations. A shielded remote apparatus that is operated manually (using switches and/or manipulator arms) is more desirable, and can significantly reduce radiation doses. Such an approach has been taken in the preparation of 2-[^{18}F]fluoro-2-deoxyglucose (*1*), and we have reported remote apparatus for the preparation of [1-^{11}C]palmitic acid (*2*) and [^{11}C]glucose (*3*). These apparatus are still labor intensive and are usually restricted to the preparation of a single, or a few very closely related, radiopharmaceuticals. For truly routine radiopharmaceuticals, a totally automated apparatus, where all manipulations are controlled by a microprocessor, may be warranted. For example, automated methods for the preparation of 2-[^{18}F]fluoro-2-deoxyglucose (*4*) and [^{11}C]methionine (*5*) have been reported. Such automated apparatus have several drawbacks, however. They are often very complicated, and entail considerable time and labor in construction of the hardware and development of the software for the microprocessor. Again, they are restricted to one or at best a few related radiopharmaceuticals. In a program using many radiopharmaceuticals, separate apparatus have to be constructed. Finally, changes in the synthetic procedure (perhaps due to improvements, or new ideas, for the chemistry) are difficult to implement without considerable work.

With the recent advent of laboratory robots, numerous applications have been reported for these devices that can perform many sample preparation techniques with little or no operator intervention (*6*). For example, a recently described (*7*) robot system has been used for the optimization of organic reactions through the systematic manipulation of the reactants and the chromatographic analysis of the products. Superbly suited for repetitive tasks, such as multiple sample prepara-

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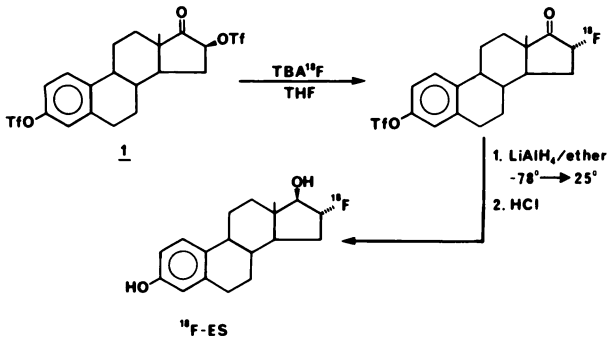
tions for chemical analyses, robots can also be employed to perform tasks in a hostile environment, such as under high radiation exposure. We have investigated the potential of a laboratory robot to synthesize complex organic positron-emitting radiopharmaceuticals, where the combination of half-life (<2 hr), low radiochemical yields and long synthesis times (at least one half-life) require the use of several hundred millicuries of activity at the start of synthesis. As a model synthesis, we have chosen the preparation of 16α-[^{18}F]-fluoro-estradiol-17β, (*8*) [^{18}F-ES], to be performed by the robot since the preparation requires many of the chemical procedures needed to produce most ^{11}C and ^{18}F radiopharmaceuticals. In this synthesis, several complex manipulations are required to label the precursor, 3,16β-bis(tri-fluoromethanesulfonyloxy) - estra-1,3,5 (10)-trien-17-one *1*, with ^{18}F fluoride ion produced from the proton bombardment of oxygen-18-enriched water (*9*). These manipulations consist of converting the radionuclide to its tetrabutylammonium salt in an anhydrous organic solvent, evaporating solvents to dryness, handling air-sensitive reagents, heating and cooling, liquid-liquid extractions, and injecting onto a high performance liquid chromatography (HPLC) column for final purification. This paper will discuss the application of the Zymate Laboratory Automation System to the synthesis of ^{18}F-ES.

MATERIALS AND METHODS

Synthesis of ^{18}F-ES

Before describing the robot system used for the preparation of ^{18}F-ES, a review of the synthesis is needed in order to discuss requirements and constraints to be imposed on the robot. The preparation of ^{18}F-ES (*8*), shown in Scheme 1, begins with the resolubilization of ^{18}F into THF for the labeling step. Here, the target water is evaporated to dryness under nitrogen in the presence of 2 μmol tetrabutylammonium hydroxide. The residue is then further dried azeotropically by the addition of two 300 μl portions of acetonitrile.



SCHEME 1
Reaction scheme of ^{18}F-ES starting from bistriflate precursor 3,16β-bis(trifluoromethanesulfonyloxy)-estra-1,3,5-(10)-trien-17-one

Upon final evaporation of the solution, the residue is dissolved in 1 ml dry THF.

A THF solution of the bistriflate precursor 1 is then added to the resolubilized ^{18}F, where after 5 min reaction time, the solution is evaporated to dryness under nitrogen and the residue is dissolved in 1 ml dry diethyl ether. The ethereal solution is cooled to −78°C in a dry ice/acetone bath, and 100 μmol $LiAlH_4$ in ether is added. After 3 min, the cold reaction solution is allowed to warm to room temperature for 5 min, followed by quenching with 300 μl 6 N HCl. The mixture is extracted with ether and the extract is dried through a Na_2SO_4 column. After evaporation of the extract and resolubilization of the residue into 0.5 ml methylene chloride, the mixture is purified on a preparative silica HPLC column (0.94 × 50 cm, 70/28/2 hexane/methylene chloride/isopropanol, 5 ml/min) and fractions are collected to isolate ^{18}F-ES.

Robot Hardware

The Zymate Laboratory Automation System* used in this application consists of a Z100E Corrosion Resistant Zymate System, Z150 Bench Top, Z900E Corrosion Resistant General Purpose Hand, Z960 Pipet Kit (1 ml), Z530 Remote Dispenser, Z510 Master Laboratory Station, Z520 Fraction Collector Station, Z830 Power and Event Control Station, Z620 Vortex Station, Z820 Printer, and a Z310 HPLC Injector Station. A custom turntable rack (42-13 × 100 mm tubes) and a Zymate Teaching Module were also supplied.* Additional equipment was added to the above hardware or constructed to fit the needs of the robot for this application. A plastic test tube rack (14-mm diameter holes) served both as a workstation for the synthesis and as a holder for the solvents and reagents. Vacutainers† (#6434, 5 ml) were used for the reaction vessels and solvent reservoirs. A 1 ml Reacti-Vial‡ with a teflon septum was used to contain the reducing agent (1*M* $LiAlH_4$ in diethyl ether)§. The drying column was made from the bottom of a 3-ml disposable syringe barrel and was packed with glass wool and Na_2SO_4. The column was manually placed on top of another empty Vacutainer for the extraction step and was removed by the robot during the synthesis.

Two oil baths (100°C and 50°C) were made from laboratory hot plates and 50-ml beakers that contained enough oil to cover the bottom 6 mm of the reaction vessel when it was placed in the bath. The vessels were held upright in the oil baths by glass sleeves cut from 16 × 100 mm culture tubes and secured with clamps. For the nitrogen purge line, a length of stainless steel tubing (1/16 in. o.d.) was welded to the bottom of a small lead pig (1 in. × 2.5 in.) and bent so as to direct the flow of gas into the desired vessel when the lead pig was placed alongside by the robot (Fig. 1). The purge line was attached to the robot-controlled gas valve on the Master Laboratory Station through a flexible piece of rubber tubing. A V-notch cut into the opposite end of the lead pig allowed the device to be positioned reproducibly in the robot hand by aligning the pig correctly between the robot's fingers when the device was dropped onto a horizontal metal bar. With the use of this device, the robot hand was free to perform other tasks during solvent evaporation procedures.

The General Purpose Hand was fitted with the Remote Dispenser in a position 180° to the fingers. The hand was also fitted with a Teflon HPLC Luer adapter to accommodate

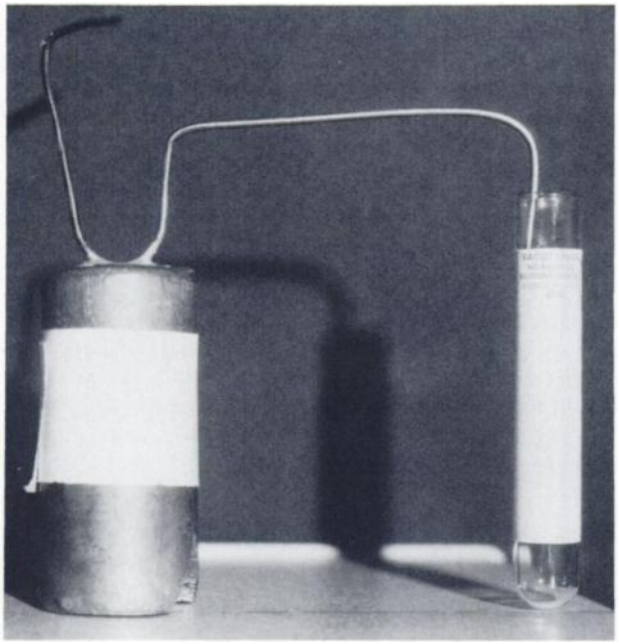


FIGURE 1
Device used by robot to direct flow of nitrogen into reaction vessel during solvent evaporations

disposable syringe needles that were used for handling the reducing agent (Fig. 2). The dispenser nozzle and the Luer adapter were both attached to syringes on the Master Laboratory Station through coiled teflon tubing.

The pipette tips used in this application were constructed from shortened lengths of disposable glass Pasteur pipettes (3.5 in.) and the tops of polypropylene pipette tips (9/16 in.) (Fig. 3). During preliminary experiments, it was discovered that the use of commercial polypropylene pipette tips rendered the [^{18}F]fluoride ion inactive before the labeling step, possibly due to an organic-extractable material that sequestered the radionuclide. Use of the above glass pipette tips circumvented this problem. The collars cut from the polypropylene pipette tips were fitted onto the glass pipettes as shown in Fig. 3, allowing the glass tips to be handled in the same manner as the plastic ones.

To verify the presence of a glass pipette tip on the robot hand, a small contact switch was mounted near the pipette tip rack. This switch, when depressed by a glass pipette tip attached to the remote dispenser on the hand, closed a circuit that was sensed by the robot electronics. A software program accepted or rejected the pipette tip attached to the hand depending on the status of the contact switch.

For the reduction step, a dry ice/acetone bath was constructed from two crystallizing dishes (2 in. × 3 in. and 2.25 in. × 2.5 in.) with insulating fiberglass sandwiched between them. The reaction vessel was held upright in this bath by a lead pig fitted with a 15 mm i.d. plastic sleeve to prevent the glass vessel from freezing to the lead pig. The lead pig was held secure by copper wire bent over the sides of the cold bath so as to immobilize the vessel holder.

An SP8700 HPLC System[1] was used for the chromatographic separations. By connecting the "run gradient" and "pump stop" terminals on the back of the pump controller to the robot-controlled closure switches on the HPLC Injector

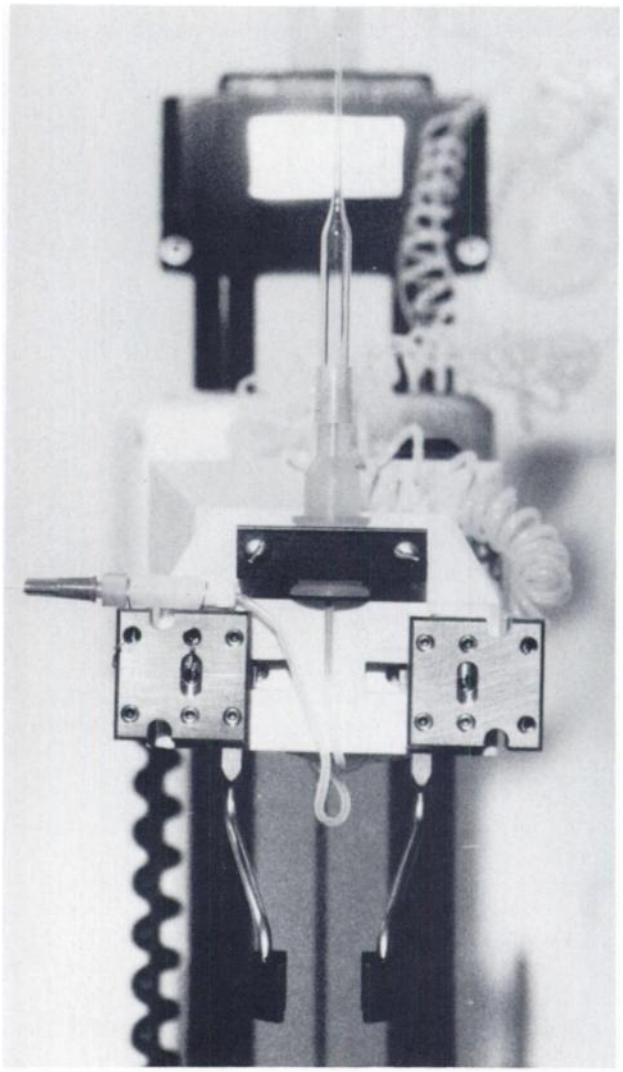


FIGURE 2
Modified General Purpose Hand used in robot synthesis of ^{18}F-ES with mounted HPLC luer adapter for attaching disposable syringe needles and remote dispenser nozzle for attaching disposable glass pipette tips

Station, the HPLC pump was able to be turned on and off through software commands programmed into the Zymate Controller. Similarly, the chart recorder was also integrated with the robot allowing software control over the chart recorder motor and the injection mark on the chromatogram. Injections were made by the robot as follows. After moving the injection valve to the load position by software-controlled solenoid, the robot loaded the sample with a pipette tip into a stainless steel conical well on top of the Injector Station. The sample was drawn into the injector loop by means of a Master Laboratory Station syringe attached to the vent port on the injector. After the sample was drawn into the injection loop, the robot injected the sample onto the HPLC column by means of another software command written into the synthesis program.

A radiometric detector consisting of a NaI(Tl) scintillation crystal, high voltage power supply, amplifier, single channel analyzer, and ratemeter was used to monitor the HPLC ef-

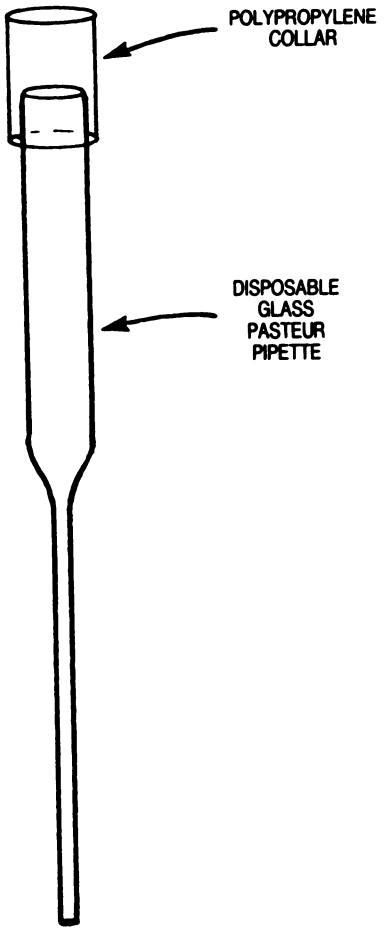


FIGURE 3
Disposable glass pipette tip used in the robot synthesis of ^{18}F-ES

fluent. The 10 mV analog output from the ratemeter was used to provide a signal for both the chart recorder and the Injector Station. To control the number of fractions collected by the robot, a 115 VAC 3-way valve was installed after the HPLC detectors and was controlled through the Power and Event Controller by software commands.

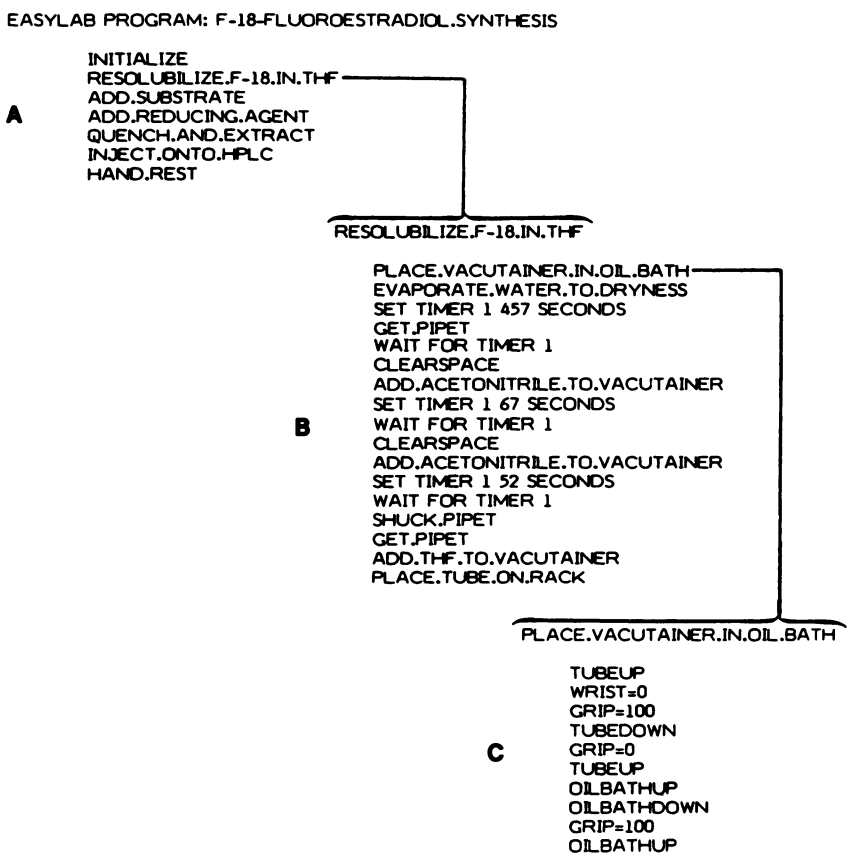
A software program was then written to allow the robot to monitor the radioactive effluent from the column and then select the fraction with the highest amount of activity and place it aside in a shielded vessel. This was achieved by setting up an array in the software to keep track of individual fractions and their relative amounts of radioactivity. By using integration parameters already incorporated into the Injector Station software, a program was written to monitor the analog signal from the ratemeter during the time that a fraction was being collected by the robot. At the end of each collection time, the Injector Station sent the data to the robot controller, where the result was then stored in an array. This process was continued for each fraction collected by the robot. At the end of the fraction collection, another program was used that compared each fraction's relative amount of radioactivity and then selected that tube which contained the highest result. The robot hand removed this tube from the turntable rack and then placed it aside in a shielded vessel. This selected fraction was then able to be removed by the operator and be converted into an injectable form (evaporation to dryness under N_2, followed by dissolution into 90/10 saline/ethanol and filtration (0.22 μm)). Although not presently programmed into the robot, this final workup of the radiopharmaceutical can readily be automated.

Robot Software

Programming the robot to perform the ^{18}F-ES synthesis was achieved by using the Easylab Software inherent in the Zymate Controller (*10*). This software permits the user to write programs in any descriptive language he chooses, simplifying the task of debugging his software at a later date. Programs are written using a "top-down" approach, i.e., by breaking down an overall reaction scheme into smaller segments, or subroutines, then breaking these subroutines down further into smaller ones, etc., until up to seven levels of subroutines have been written for a particular application. In our application, the ^{18}F-ES program uses five levels of subroutines. Each subroutine consists of program text, module commands and variables, and real data variables. Program text refers to lines of programming that describe a certain action. Module commands and variables are direct links to robotic actions and refer to any movement made by the robot. Real data variables are used in programs that require mathematic manipulations of data received by the robot. Programs are written for the robot by using various combinations of the above commands, variables, and text.

Scheme 2 shows a partial breakdown of the ^{18}F-ES program used in our application. In *A* we have a series of program lines that make up the zero level subroutine. These represent the major steps in the chemical synthesis: preparing anhydrous ^{18}F-tetrabutylammonium fluoride in THF, adding a solution of triflate 1, adding reducing agent, quenching and liquid-liquid extraction, and injecting onto the HPLC. If we expand the program line RESOLUBILIZE.F-18.IN.THF, we come to the first level subroutine in *B*, which consists of more program text that describes the robots actions to resolubilize the radionuclide. Here, the water is evaporated and the residue is azeotroped dry using acetonitrile, followed by dissolution of the ^{18}F-TBAF into THF.

By listing the program text of PLACE.VACUTAINER. IN.OIL.BATH. as in *C*, we come to the second level subroutine, which consists of specific module commands and variables used by the robot hand to place the reaction vessel in the 100°C oil bath. In this subroutine, TUBEUP refers to a defined position that the robot hand is at above the reaction vessel in the test tube rack. The command OILBATHDOWN defines the position of the robot hand after it has placed the reaction vessel in the oil bath. Positions such as TUBEUP and OILBATHDOWN are defined during robot programming as follows. Using keyboard control, the robot arm is moved to the desired location; when in place, that particular position is then given a specific name (e.g., TUBEUP). The variables of that position (arm height, extension, angle of rotation, wrist angle and grip opening) are then stored in memory. The variables WRIST and GRIP refer to the orientation of the robot hand (0–360°) and to the size of the opening between the robot's fingers (0–200 units), respectively. By writing a program using these types of module commands and variables, the robot is able to perform various tasks using the sequence of defined positions in the program, moving to each defined position as it is called up in the program.



SCHEME 2
Partial breakdown of ^{18}F-ES program used by robot. A: Zero level subroutines. B: First level subroutines. C: Module commands and variables used by robot in second level subroutine

For the robot to be able to perform certain steps of the synthesis, several software timers were incorporated into the program. For example, in the resolubilization step of the synthesis, the robot must wait until the target water has evaporated before proceeding with the next step. Since the robot cannot "see" when the water has evaporated to dryness, it must be programmed to wait a given amount of time, which is dependent on a specific set of parameters, such as temperature, volume, and nitrogen flow. These parameters must be kept constant to ensure proper evaporation by the robot. Changes in any of these conditions without an adjustment in the software timers would result in an improper or inefficient resolubilization of the radionuclide. Other timed pauses that are required in the synthesis are similarly incorporated into the ^{18}F-ES program.

Hardware Layout

Figure 4 shows a block diagram of the Zymate System and additional hardware needed to perform the ^{18}F-ES synthesis. Not shown are the Zymate Controller, HPLC pump controller and the uv and radiometric detectors, which are situated either off or under the table. Due to constraints imposed by the shielded hood in which the robot is situated (28 in. × 58 in.), the hardware had to be arranged in a 180° arc around the robot, or half of its available working radius. Although the robot has a maximum reach of 32 cm and a maximum working height of 34 cm, the total available working area of the robot is not used in this application. The close proximity of the hardware minimizes travel movements by the robot and helps increase overall efficiency. Figure 5 shows an overall view of the robot system used in this application.

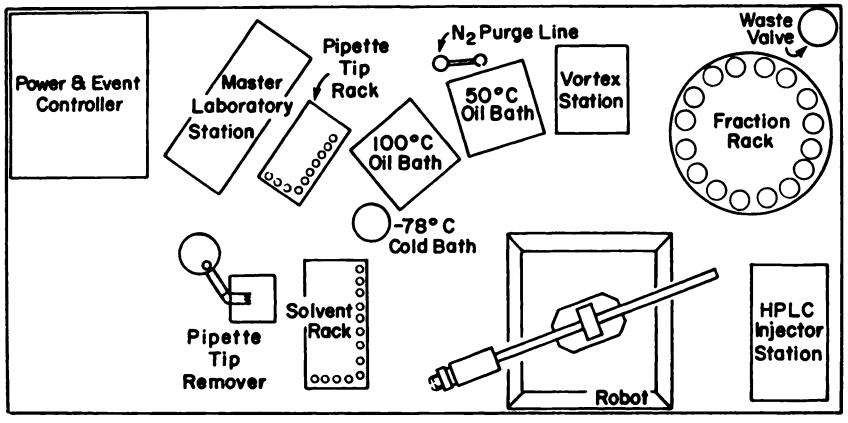


FIGURE 4
Block diagram of robot hardware layout used in synthesis of ^{18}F-ES. Zymate Controller is situated on another table, while HPLC pump and detectors are placed underneath robot bench

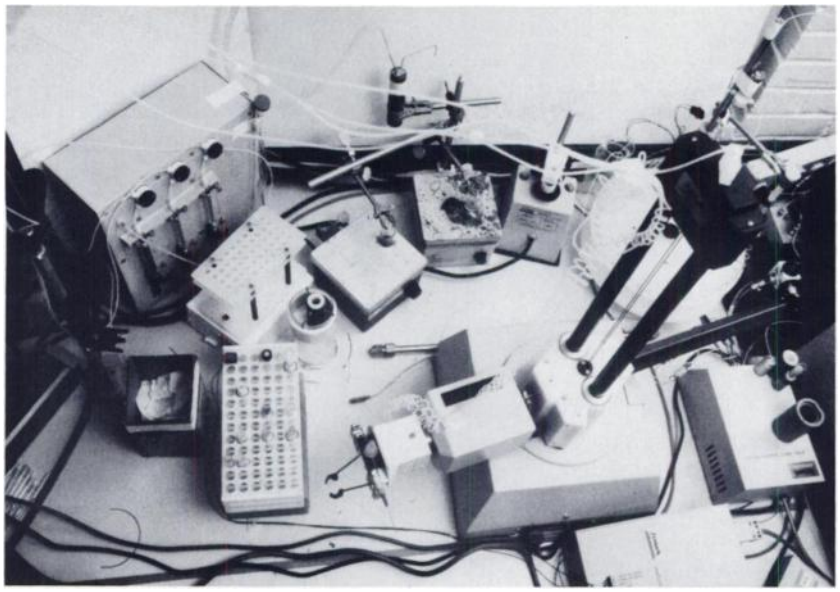


FIGURE 5
Overall view of robot and associated hardware used in ^{18}F-ES synthesis. HPLC pump, Zymate Controller, and uv and radiometric detectors are not shown

Before each synthesis, the robot system was replenished with the following items: fresh solutions, reagents, and solvents; dry ice and acetone for the cold bath; a new syringe needle on the robot hand; glass pipette tips; and a new rack of tubes for the fraction collector. After the robot was set up, the fluorine-18 was transferred remotely to the reaction vessel containing 2 μmol base and the synthesis program was initiated through the Zymate Controller. This replenishment procedure takes ~15 min to perform.

RESULTS AND DISCUSSION

In adapting the robot to perform the ^{18}F-ES synthesis using various complex manipulations, several problems needed to be overcome in order to automate the reaction. The first of these was in programming the robot to perform the resolubilization step in a Vacutainer to yield ^{18}F-TBAF in THF. The yield obtained when this step is performed manually is 90% (*11*). During the manual resolubilization of ^{18}F, the chemist can monitor the rate of evaporation by sight and add solvents at the appropriate times without concern for exact temperature or nitrogen flow conditions. The robot does not have the capability for sight in the usual sense, however, and therefore must rely on programming to proceed with the necessary steps. In this application, it was determined manually how long it would take for a given amount of target water to evaporate to dryness under apparently fixed conditions of temperature and gas flow. This evaporation time was then incorporated into the robot software in the resolubilization subroutine. During several automated runs, however, the evaporation rate was found to vary and resulted in the solvents (acetonitrile or THF) being added too early or too late. This is probably due to a variable temperature range on the hot plate at a given setting and/or a change in the air flow around the hot plate (changing the rate of cooling of the plate surface). These external factors would cause difficulty in maintaining a constant temperature for the resolubilization oil bath unless they could be controlled to a higher degree. In any event, the robot was able to resolubilize the radionuclide in at least a 50% yield.

The next difficulty was in finding a way for the robot to handle the moisture-sensitive reducing agent, $LiAlH_4$ in ether. At first, the robot was programmed to puncture a septum-sealed bottle of the reagent using a sharpened cannula on its hand. Over time, however, the reagent slowly decomposed as moisture gradually entered the reagent bottle through the punctured septum. A solution to this problem was to use smaller vials to contain the required amount of the reagent, eliminating the need for a larger stock solution. A number of small (1 ml) Reacti-vials could be charged with the reducing agent in a glove bag, providing the robot with a fresh aliquot of the reagent for each synthesis. Another advantage in using the Reacti-vials was the conical well at the bottom, allowing all of the reagent to be drawn up into the syringe needle during the withdrawing step.

During solvent handling procedures, it was noted that organic solvents tended to drip from the glass pipette tips on the remote dispenser nozzle. This was remedied in part by programming the Master Laboratory Station to draw back on the pipette syringe during transfer of the solvent from the reservoir to the vessel. Throughout the ^{18}F-ES synthesis, however, all but one liquid transfer was with a nonradioactive solution, and therefore any loss of solvent or solution through dripping was not critical. During the loading of the HPLC injector loop with the radioactive sample, though, the vessel containing the sample to be transferred was placed right next to the injector port well. This arrange-

ment allowed any radioactive solution to drip from the pipette tip into either the HPLC injector port or back into the sample vessel.

In spite of these minor problems, the Zymate robot was able to perform the synthesis of ^{18}F-ES. The total line for the robotic synthesis was 80 min, which is slightly less than that for the manual synthesis (~90 min) (*8*). The overall yield that we are obtaining is between 5 and 6% (decay corrected), that is less than that obtained manually using the same reaction vessel (22%) (*11*). The lowering of the yield occurs in three areas. As discussed previously, the resolubilization yield is ~50% rather than 90% because exact control of evaporation time is only possible under manual control. Lowering of the yield also occurs due to a less than efficient sample extraction of the organic phase under robotic control than when manually performed. The robotic controlled injection onto the HPLC is also less efficient than that carried out manually. We are currently studying the robotic performance during each of the above steps in order to improve the yield of ^{18}F-ES. It should be noted that with robot control and the ^{18}F target currently in use (*9*), up to 1 Ci of ^{18}F activity can be used at the start of the synthesis, allowing the production of >30 mCi of ^{18}F-ES by the synthesis described.

The flexibility of the Easylab programming means that any changes needed in the synthesis are readily incorporated into the software. This is also important if the robot is needed to carry out a completely different synthesis, as hardware in the form of various work stations can readily be added to or deleted from the robot layout to improve particular applications. This is in contrast to most automated systems that have a fixed layout of valves and pumps where modifications are difficult to make without disrupting all or part of the system involved (*4,5*).

We are currently planning a second worktable to be placed inside the hot cell with the robot raised to the second level using a programmed Lab-Jack. In this way, the robot and controller can be used to control another set of equipment arranged to carry out a completely different synthesis. The robot has particular advantages for this since the software written for the ^{18}F-ES application could be used for another synthesis. For example, if the other synthesis were the production of a ^{18}F radiopharmaceutical using $^{18}F^-$ as a precursor, the resolubilization step developed for the ^{18}F-ES synthesis could be readily used. This would be useful in the preparation of ^{18}F-labeled fluorodeoxyglucose using the cyclic sulphate as a precursor (*12*), or in the production of ^{18}F-labeled spiroperidol (*13*) or ^{18}F-labeled *N*-methylspiroperidol (*14*), where the initial synthetic step involves a nucleophilic substitution reaction. In the above two examples, nearly all of the chemical procedures required could be carried out by utilizing software already written. It should be noted that the ^{18}F-ES program utilizes ~40% of the computer memory, so that another program could either be stored directly in memory or on a separate disk.

Another advantage of the robotic control system is that a second run of the same synthesis could be performed immediately. By arranging a second set of vessels and reagents in another position on the board and having the Master Laboratory Station remotely clean valves and parts of the apparatus, a second synthesis could be carried out without the need for the chemist to clean out the radioactive hot cell. Although this is not important for the ^{18}F-ES synthesis we have developed, it may be for the synthesis of compounds such as ^{11}C-labeled 2-deoxyglucose (*15*) or ^{11}C-labeled palmitic acid (*16*), where repeat studies at relatively short time intervals are required.

In conclusion, we believe that the application of a laboratory robot costing ~$35,000 has major advantages over apparatus currently in use for the remote and automatic synthesis of short-lived radiopharmaceuticals. The robot takes less time to carry out the synthesis than manual procedures, and the robot can be adapted to the synthesis of many positron emitting radiopharmaceuticals, making it a versatile and useful instrument in the radiopharmaceutical laboratory. Using one robot, several syntheses could be carried out in one hot cell. We believe that this instrument will widen the application of positron emission tomography because of its ability to carry out syntheses of ^{11}C or ^{18}F-labeled radiopharmaceuticals in a simpler, safer, and less expensive manner.

FOOTNOTES

* Zymark Corp., Inc., Hopkinton, MA.
† Becton, Dickinson & Co., Rutherford, NJ.
‡ Pierce Chemical, Rockford, IL.
§ Aldrich Chemical Co., Milwaukee, WI.
¶ Spectra Physics, San Jose, CA.

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