# Automation for the Synthesis and Application of PET Radiopharmaceuticals

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## Introduction

The development of automated systems supporting the production and application of PET radiopharmaceuticals has been an important focus of researchers since the first successes of using carbon-11 (Comar *et al.,* 1979) and fluorine-18 (Reivich *et al.,* 1979) labeled compounds to visualize functional activity of the human brain. These initial successes of imaging the human brain soon led to applications in the human heart (Schelbert *et al.,* 1980), and quickly radiochemists began to see the importance of automation to support PET studies in humans (Lambrecht,1982; Langstrom *et al.,* 1983).

Driven by the necessity of controlling processes emanating high fluxes of 511KeV photons, and by the tedium of repetitive syntheses for carrying out these human PET investigations, academic and government scientists have designed, developed and tested many useful and novel automated systems in the past twenty years. These systems, originally designed primarily by radiochemists, not only carry out effectively the tasks they were designed for, but also demonstrate significant engineering innovation in the field of laboratory automation. These laboratory automation systems draw heavily on the chemical engineering concepts of unit operations and have evolved from isolated manually operated electro-mechanical devices to large-scale integrated systems utilizing

the latest in personal computer (PC) and laboratory robot technologies. The success of these initial engineering efforts carried out by radiochemists is an important reason for the recent growth of clinical PET procedures due to the increased availability of cost-effective PET radiochemicals that commercially available systems now provide.

This chapter will first briefly describe the evolution of these automated systems for PET, followed by a discussion of specific engineering design considerations. The scope of this chapter will focus on the design of automated systems for the rapid synthesis and application of PET radiotracers labeled with $^{15}O$, $^{13}N$, $^{11}C$, and $^{18}F$. Systems designed to use other important positron emitting nuclides will be described only in the context of the evolution of engineering design in PET radiopharmaceutical automation. Finally, this presentation will highlight current automated systems addressing automation of both the synthesis of radiotracers for PET, and the assay of radioactivity in plasma for input function determination needed for quantitative PET imaging. Methodological details of specific automated systems including schematic diagrams can be found in an excellent compilation of automated production methods by Crouzel *et al.*, (1993). Automated systems for accelerator, particle beam, and target control and will not be discussed. The reader is referred to a recent review article describing integrated, automated accelerator and target systems for clinical PET radiotracer production (Satyamurthy, 1999).

## Automation of Radiotracer Synthesis

Because of the unique operational and safety requirements of PET radiotracer synthesis, the motivation for the development of automated systems is clear and

compelling. These unique constraints include short synthesis (often limited to 2 or 3 half-lives) times and control from behind bulky shielding structures that make both access to, and visibility of, radiochemical processes and equipment difficult. Often Curie levels of positron emitting nuclides are required for synthesis of PET radiopharmaceuticals, making this potentially dangerous for a radiochemist or laboratory specialist. The use of short half-lived radionuclides also necessitates that many PET radiotracers (particularly those labeled with $^{11}C$, $^{13}N$, and $^{15}O$) be synthesized repetitively during the day, each dose being produced separately just before administration. Radiotracer synthesis must be reliable and efficient to keep the costs of PET procedures down. Furthermore, radiotracer synthesis procedures for human use must produce pharmaceutical quality products and be well documented and controlled to help satisfy requirements of federal and local regulations on human research.

Automation can help PET research institutions overcome all of these potential limitations. A look at the history of the development of successful automated PET radiotracer synthesis machines reveals a richness in engineering solutions to these problems that still exists today.

## A Historical Perspective: Chemistry First

Automated synthesis systems require no direct human participation to perform the various physical and chemical operations that comprise a synthesis. Scientists outside of PET radiopharmaceutical research were the pioneers of automated synthesis, with the most well known example being the work in solid phase peptide synthesis (SPPS) by Merrifield and co-workers (Merrifield *et al.*,1966). It was Merrifield's innovations in peptide chemistry that laid the foundation for the development of fully automated

commercially available (e.g Applied Biosystems, Foster City, CA) synthesizers of today. More recently, in 1981, Caruthers and others developed novel solid phase supported DNA chemistry (Beaucage & Caruthers 1981; Matteucci & Caruthers, 1981) that led to the development of modern DNA synthesizers that were used almost exclusively in mapping the human genome (Caruthers, 1985). Unfortunately, these highly successful automated benchtop synthesis systems were designed for a rather narrow range of chemistries and therefore did not lend themselves to adaptation by PET radiochemists for radiosynthesis automation. In general, PET radiosynthesis draws from a broader chemistry knowledgebase rooted in synthetic organic chemistry (Fowler & Wolf, 1982; Fowler & Wolf, 1997). However, these examples do serve to make an important point: that the success in synthesis automation requires first and foremost *innovative* chemistry.

Parallel to these important developments in the 70's and 80's in automated oligiopeptide and nucleic acid chemistry was the exploration in automation by traditional synthetic organic chemistry labs. Motivated by the desire to optimize organic synthesis yields efficiently, researchers outside of the field of PET developed the first automated systems for controlling more general-purpose laboratory-scale organic reactions. The control strategy employed by these systems progressed from hard-wired logic control (Legrand & Foucard, 1978), to microcomputer-based automation (Winicov *et al.*, 1978), to laboratory robot controlled organic synthesis (Frisbee *et al.*, 1984). Development of these systems was motivated primarily by the need to optimize synthetic yields in a synthesis containing several important controllable parameters. These automated organic synthesis systems derive optimum synthesis conditions automatically by applying an

optimization algorithm to results obtained from computer and robot controlled experiments (Winicov *et al.*,1978; Frisbee *et al.*, 1984).

Still, progress in automating optimization of organic synthesis reactions had minimal impact on the development of automated machines for PET radiotracer synthesis. The automated synthesis optimization systems often proceeded using standard laboratory equipment using reaction volumes of 50 mL – 5 L. Radiotracer synthesis, on the other hand, is most often carried out in volume range of 5 μL to 5 mL, and has special time constraints and shielding requirements defined by the short-lived isotopes that PET exploits. Hence, as the need to develop automated systems became urgent by the beginning of the 1980's, PET radiochemists were faced with limited or inappropriate functionality provided by automated peptide and DNA synthesis or robot controlled benchtop organic synthesis systems.

The design and development of automated radiotracer synthesis systems by PET radiochemists followed a similar evolution to the systems described above, starting as hard-wired, remotely controlled apparatus. Prompted by the success of using the radiopharmaceutical 2-deoxy-2-[$^{18}$F]fluoro-D-glucose (FDG) to measure localized cerebral (Reivich *et al.*, 1979) glucose metabolism in a living human subject, PET researchers quickly developed synthesis systems that could produce multidose batches of FDG safely, efficiently, and repeatedly (Barrio *et al.*, 1981; Fowler *et al.*, 1981). These systems were manually controlled by manipulator arms and electric switches connected to equipment such as solenoid valves, vacuum pumps, regulated pressure sources, motorized lab jacks, rotary evaporators, and temperature controllers. A skilled operator could manipulate glass vessels, switches, reagents, and solvents from behind the

protection of thick lead shielding. Remotely controlled synthesis systems for several carbon-11 compounds were also being developed around the same time (Berger *et al.*, 1979; Padgett *et al.*, 1982; Welch *et al.*, 1982; Welch *et al.*, 1983). Although sometimes referred to as "automated" systems (Berger *et al.*, 1979), these carbon-11 synthesis systems used devices controlled remotely by a human operator in a fashion similar to that described above for the synthesis of FDG (Fowler *et al.*, 1981).

The earliest fully automated systems appeared also by the early 1980's. A sampling of these pioneering systems includes hard-wired automatic syntheses of $^{11}$C-Glucose (Ishiwata *et al.*, 1982) and $^{13}NH_3$ (Ido & Iwata, 1981), a microprocessor based synthesis of $^{13}NH_3$ and L-[$^{13}$N]-glutamate (Suzuki *et al.*, 1982), an automatic production system for the synthesis of $^{75}$Br-labelled radiopharmaceuticals (Blessing *et al.*, 1982) based on the Kontron industrial microcomputer (Kontron Embedded Computers AG, Munchen, Germany), and a microcomputer controlled synthesis of the production of FDG (Iwata *et al.*, 1982; Iwata *et al.*, 1984). A closer look at these early systems reveals most of the important underlying characteristics of modern automated systems and how they are designed today. In fact, the complexity and sophistication of radiochemical hardware used in automated PET radiotracer synthesis has not changed significantly since these early designs. The greatest progress has come from defining the radiochemical processes themselves and creating the strategies for implementing automatic control.

In the automatic synthesis of 11C-glucose (Ishiwata *et al.*, 1982), the radiochemical hardware used for the synthesis of $^{11}$C-glucose (19 teflon solenoid valves, 2 reaction vessels, 2 heaters, 4 reagent resevoirs, a vacuum pump, a peristolic pump, 2

Sep-paks™, a purification column and 4 photo-level sensors) reflects accurately the complexity and functionality of hardware used in modern automatic synthesis machines. For example, the FDG machine sold today by Nuclear Interface (Muenster, Germany) has 23 solenoid valves, 1 reaction vessel, 1 heater, 2 Sep-Paks™, 7 reagent reservoirs, a vacuum pump, and two radioactivity sensors. In fact, most automatic radiotracer machines today are configured with two dozen or so valves, 1 or 2 reaction vessels, a heater, a half dozen or so reagent reservoirs, a vacuum pump, and several (or none) sensors for measuring localized radiation fields, vessel pressures, liquid levels, and temperatures.

Methods for automatic control of the physical parameters (pressure differences, temperature, and object displacement) needed to invoke a sequence of steps leading to the synthesis and purification of a particular radiotracer using this generic set of miniature chemistry hardware has evolved greatly in the past two decades. For example, at the same time that Ishiwata and colleagues (Ishiwata *et al.*, 1982) were using hardwired timers, limit switches, and photo sensors to control a 19 step production of 11C-glucose, other PET investigators were starting to take advantage of progress in semiconductor technologies leading to the creation of software programmable microprocessors. Suzuki and co-workers (Suzuki *et al.*, 1982) described the automation of the production of $^{13}NH_3$ and L-($^{13}N$)-glutamate using two general purpose microprocessors (6 kilobytes RAM, 32 kilobytes ROM, 7 digital outputs, 2 analog inputs each). The system described controlled the reaction of $^{13}NH_3$ with the immobilized enzyme glutamate dehydrogenase (Suzuki *et al.*, 1982) after the reduction of labeled nitrogen oxides with Davarda's alloy and sodium hydroxide. Both software timers and signals from radiation and conductivity sensors were

used to control the multistep synthesis. In addition, provisions were made for running the device automatically 4 times without replacing reagents or vessels.

Other early microprocessor based systems described how multiple processors could be connected in a distributed control fashion so that more flexible automated systems could be created (Alexoff *et al.*, 1986; Ferdeghini *et al.*, 1987; Russell *et al.*, 1987). These systems, built using 8-bit microprocessors (6511 Rockwell International, Z80 STD Bus Mostek), were designed with the intention of facilitating the automation of multiple radiotracers from a single system. Interfacing and data acquisition responsibilities were separated from broader context control problems like sequencing of steps and display of information. This modular design was intended to make automation of new radiotracer synthesis easier to implement.

Microcomputers offer simplified automatic synthesis programming compared to microprocessors (programmed in assembly language) by providing integrated disk, operating system and high-level software language capabilities. One of the earliest applications of the microcomputer to radiotracer synthesis automation was described by Blessing and co-workers (Blessing *et al.*, 1982). This system used the BASIC language to supplement assembly language routines (reserved for time-critical operations) to control the isolation of $^{75}$Br from a solid target, radiosynthesis, HPLC purification, and solvent evaporation using a rotary evaporator. A unique characteristic of this system was the design of a carrousel type design where the reaction vessel, reagent ports, and refluxing hardware where movable so that plumbing connections could be made in way that minimized dead volume, valves, and interconnected plumbing.

The description of the first automated synthesis of FDG by Iwata *et al.* represented the first milestone of automation efforts for PET radiopharmaceuticals (Iwata *et al.*, 1984). Iwata's FDG machine was similar in complexity to the automated devices described above and was built from 37 solenoid valves and 18 sensors. This radiochemical hardware was interfaced directly to a microcomputer that was programmed in HP-BASIC to execute sequentially 32 steps comprising the synthesis of FDG following the radiosynthesis described by Fowler *et al.* (Fowler *et al.*, 1981). This automated system controlled many organic synthesis operations ubiquitous to PET radiopharmaceutical production at the time. These operations included flash column chromatography, solvent evaporation, and radioactivity recovery from purification columns. Iwata's system was the culmination of early automation efforts. Using more than 18 sensors, it was highly instrumented and employed significant feedback control during operation. The system incorporated several types of transducers capable of detecting or measuring liquid levels, gas pressures and flow rates, vessel temperatures, and localized radiation fields.

The automated synthesis of $^{18}$F-fluoroestradiol by Brodack *et al* using a Zymate Laboratory Automation System (Zymark Inc., Hopkington, MA) represented a new approach to PET radiopharmaceutical automation (Brodack et al., 1986). Laboratory robots are interfaced with a microcomputer and can be programmed in high-level languages. Mimicking many human operations and using standard laboratory equipment, these robots could be quickly configured and programmed to carry out a radiopharmaceutical synthesis. In contrast to the highly instrumented "fixed plumbed" machines described previously, early robot-controlled radiosyntheses often lacked the use

of feedback sensing to control or monitor specific radiochemical operations like solvent evaporation. The focus of commercial laboratory robot manufactures at this time was in providing robust feedback control strategies for controlling gripping, interchanging hands, and other physical manipulations (Nelson & Lightbody, 1991).

By 1990 automated systems were common in many research PET facilities. This was in large part due to the success a novel synthesis of FDG reported by Hamacher *et al.* (Hamacher *et al.*, 1986). This stereospecific, high yield, one-pot synthesis based on the nucleophilic reagent $K^+[2.2.2]^{18}F^-$ lead to a proliferation of custom built automated FDG systems at research PET centers (Alexoff *et al.,* 1989; Padgett *et al.,* 1989; Hamacher *et al.,* 1990; Mader *et al.,* 1992) and became the synthetic pathway of most modern commercial FDG machines. The synthesis of FDG by nucleophilic substitution using Kryptofix 2.2.2 not only provided a simple, efficient, stereospecific route, but it also allowed the utilization of new high yield cyclotron targets for the production of $^{18}F^-$ from $H_2{}^{18}O$ (Kilbourn et al., 1984; Wieland *et al.* 1986).

During this time PET radiochemists were using a plethora of automatic control strategies. Goodman *et al.* report the automatic synthesis of $^{15}O$- butanol, $^{15}O$- water (Goodman *et al.*, 1991a), and 1-$^{11}C$-aminocyclobutane carboxylic acid (Goodman et al 1991b) using an 8085 microprocessor. Researchers at KFA Julich, Germany also describe an automated oxygen-15 labeled butanol system based on a Programmable Logic Controller (PLC) (described in Crouzel *et al.*,1993). These systems were designed for up to 8 repetitive syntheses using the reaction of n-butylborane with $^{15}O$-$O_2$ on an alumina Sep-Pak™ as first described by Kabalka *et al.* (Kabalka *et al.*, 1985). In both automated butanol systems, radiopharmaceutical quality formulations for clinical studies

were obtainable with simple in-line solid phase extraction (Berridge *et al.,* 1986), further simplifying automation of this efficient, fast reaction.

Still other PET radiochemists recognized that the functionality of PLCs matched well the sequential nature of even more complex syntheses of PET radiotracers. A Toshiba EX40 industrial PLC was used by Clark & Dowsett to control the synthesis of a variety of carbon-11 labeled compounds from $^{11}CH_3I$, including [O-methyl-$^{11}$C]raclopride, [N-methyl-$^{11}$C]SCH 23390, and S-[N-methyl-$^{11}$C]nomifensine (Clark & Dowsett, 1992).

At the same time the researchers were using microprocessors and industrial PLCs to automate their processes, Ruth and colleagues describe the use of a personal computer connected to an intelligent data acquisition system (Optomux™, Opto22, Temecula, CA) to synthesize L-6[$^{18}$F]Fluorodopa (Ruthe *et al.*, 1991a). A similar control stragegy was used by Hamacher *et al.* in the computer-controlled synthesis of FDG (Hamacher *et al.*, 1990). Both of these systems were programmed in high level languages common to personal computers. Personal computers provide more sophisticated user interfaces and programming capabilities than PLCs or microprocessors that can expedite software development. Finally, a variety of commercial laboratory robot systems were used to synthesize both $^{11}$C and $^{18}$F labeled compounds by several different groups (Brihaye *et al.,* 1994; Brodack *et al.,* 1988; Brodack *et al.*, 1991).

The latest milestone in the development of automated PET radiotracer synthesis machines was reached in the mid 1990's with the report of a high yield, high specific activity gas phase synthesis of $^{11}CH_3I$ (Link *et al.,* 1997; Larsen *et al.,* 1997). Gas phase synthesis of $^{11}CH_3I$ had several advantages over the popular wet chemistry method

(Langstrom & Lundqvist 1976) including rapid turnaround for multiple syntheses and simplified operation for automation. This method was quickly commercialized and evaluated for routine use in PET research environment (Fallis *et al.*, 1997). The commercial system (GE Medical Systems, Milwaukee, MN) was constructed using an industrial PLC with open loop timed control of synthesis steps.

Designs of modern automation systems for PET still reflect this richness in automatic control strategy. This diversity is no doubt in part a reflection of the breadth of chemical pathways the PET radiopharmaceutical production relies on, as well as an indication of the individual vitality of each group in the international PET radiochemistry community.

## Unit Operations Design

Although the evolution of automated chemistry systems for PET radiopharmaceutical has resulted in a proliferation of designs and control strategies, all of these systems were created using the modular design concept of laboratory unit operations (Padgett, 1982; Severns & Hawk, 1984). PET radiopharmaceuticals can not only be made using a set of generic hardware of solenoid valves and vessels as just described, but more important, each radiosynthesis can be broken down into a set of common laboratory operations. In radiotracer synthesis these general-purpose operations include manipulations common to the organic chemist like transferring reagents, evaporating solvents, regulating vessel temperature, and solid phase extraction (SPE). Unit operations design was first successfully applied to the design of remotely controlled syntheses of several $^{11}C$ (Berger 1979; Welch *et al.*, 1982; Padgett, *et al.*, 1982) and 18F labeled compounds including FDG (Barrio *et al.*, 1981; Fowler *et al.*, 1981). This

modular approach to remote apparatus construction has a parallel application to the design of automated equipment and computer software (Alexoff *et al.*, 1986; Felieu 1991; Russell *et al.*, 1987).

The utility of a unit operations approach is perhaps best demonstrated by laboratory robot systems (Severns & Hawk, 1984). In these robot systems, general purpose workstations like solid phase extraction or reaction vessel heating surround a central manipulator arm which can be programmed to execute unique sequences of steps to create a specific automated process like a radiotracer synthesis. In this instance, unit operations are distinct not only functionally, but also by physically separate, disconnected pieces of hardware. In non-robotic automated systems, unit operation boundaries are defined more algorithmically, although concomitant hardware exists and is plumbed together (Padgett *et al.*, 1982). In these fixed-plumbed automated systems, modular designs are used for intelligent interface hardware that connects laboratory and synthesis equipment to a controlling computer or microprocessor as well as software design of highly structured synthesis software. In fact, many modern laboratory automation machines use object oriented programming languages like Visual Basic (Cadavid *et al.*, 1997) that facilitate the creation of highly structured and modular automation tools (Echols and Russon, 1997; Feiglin & Russell, 1997).

In sum, although sometimes confused with the concept of fixed-plumbing automation, unit operations is a useful engineering concept that has been applied successfully to both robot systems (Brodack *et al.*, 1988; Brihaye *et al.*, 1994; Brihaye *et al.*, 1996; Krasikova 1998) and fixed plumbed "black-box" automation (Satymurthy *et al.*, 1999). Structured design techniques facilitate the development of automated systems

from manual methods by first providing plumbing building blocks for remotely-controlled systems (Clark & Dowsett, 1992; Crouzel *et al.*, 1993) and then providing a framework for both process control system and software design (Alexoff *et al.*, 1986; Russell *et al.*, 1987). The decision to use modular hardware and software design can mitigate the cost and time needed to develop new automated systems by providing generic solutions to focussed control problems (such as the evaporation of solvent from a reaction vessel or the isolation of a component by SPE) found radiotracer syntheses. Given a complete enough set of generic automation building blocks, the automation of any radiotracer sythesis could be carried out by radiochemists with a minimum of automation expertise (Alexoff, 1991; Felieu, 1991). This flexibility of both modern robot and fixed-plumbed automated systems in PET contrasts the one-of-a-kind nature of early hardwired automated systems built by PET radiochemists.

## Role of Feedback Control

While most PET radiochemists involved in automating their processes will agree on the virtues of the concept of unit operations and modular design, the extent of the use of feedback control in both custom and commercial automated systems has varied greatly. The utility of including sensors for feeding back information during synthesis has been debated (Link & Clark, 1994). Most of the arguments reflect concerns about reliability. For example, the well-established engineering design principle of "keep-it-simple" dictates that before the added complexity of incorporating sensors can be justified they must provide functionality and information that increases reliability and overall performance. In fact, one of the first successful commercial FDG machines was first designed with feedback control, only to be marketed without sensors required for

closed loop control of unit operations (Satyamurthy *et al.*, 1999). The following quote from a one of the developers of the prototype machine summarizes well the debate over incorporating sensors in automated PET radiopharmaceutical equipment:

> "The initial module incorporated self-diagnosis and feedback from sensors such as vapor pressure monitors, liquid level sensors, etc. However, the system worked quite well with a simple series of on/off commands and time waits. Thus, to maintain simplicity and reliability, the time of various tasks that took place during the synthesis was determined and a margin for variation incorporated in the final program."

Although many of the automated systems already described sequence the steps required to carry out radiotracer synthesis in the same open loop timed control strategy, it can be argued that appropriate feedback control strategies can increase reliability by automatically compensating for dynamic process variables. For example, variable volumes of solvent to be evaporated could change drying times considerably. Without any feedback, an evaporation step time would have to be set for the longest evaporation time (largest solvent volume). Alternatively, volume information could be input to the system and a previously calibrated lookup table mapping drying times to solvent volumes could be used to determine an appropriate evaporation time. This could be extended to account for changes in solvent composition. Even so, this strategy would require feedback from either an operator or an appropriate liquid sensing system. Fortunately, most processes automated for PET radiotracer production have well-defined parameters that lend themselves to open loop control strategies.

It is clear from this example that with the use of appropriate sensors and feedback control algorithms, more robust, general-purpose machines can be built. Furthermore, information gathered from sensors can be important for either pre-run diagnostics (Alexoff *et al.*, 1986; Iwata *et al.*, 1990) or computer assisted problem solving (Alexoff,

1991). Advanced features like these may be critical to future development of commercial $^{11}C$ labeled radiotracer machines, where it is even more important to minimize synthesis times, provide reliable control, and simplify operation and maintenance. In fact, the trend in modern automated systems is to include such feedback strategies (Jackson, 2000; Zigler, 2000).

**Real-time control of unit operations**

Feedback control strategies for PET radiotracer synthesis control can be classified as either continuous (regulatory) or discrete (step control). In continuous control, sampled data from process sensors is input to an algorithm that modulates an output device to achieve a desired value (setpoint) of the measured process variable. For example, reaction vessel temperature regulation is often achieved by using a Proportional Integral Derivative (PID) control algorithm that is either part of a separate commercial controller (Mader *et al.,* 1992) or synthesis control software (Alexoff *et al.,* 1989). Besides being used for feedback control of temperature regulation, regulatory type control in PET radiotracer manufacturing systems has been limited to a small number of applications, mostly gas flow systems. For example, Le Bars *et al.* described a PLC system that automatically adjusts a dilution gas flow rate to regulate a final radioactivity concentration flow to the patient. By including a feedback circuit that regulated gas radioactivity concentration, these designers minimized changes in radioactivity delivered to the patient due to disturbances in particle beam irradiation conditions, including momentary disruption of beam (Le Bars *et al.*, 1991).

Most unit operations for PET tracer synthesis do not require regulatory type feedback control action, but represent instead discrete or threshold type control problems. More recently, step control using a variable threshold or fuzzy logic approach has been proposed (Hichwa, 2000). Fuzzy logic strategies attempt to mimic human decisions by incorporating production trends or other information available to human operators (see also Alexoff, 1991). Most feedback control discussions in PET tracer synthesis have focussed on the utility of using sensors in this way to determine the status of discrete steps comprising a synthesis, whether it be a fixed threshold control or more sophisticated fuzzy logic approach. Solvent evaporations and liquid transfers are the two most common unit operations used in automated PET systems that have been subject to feedback control using sensors. These operations often represent more than 90% of control responsibilities comprising the execution of a typical radiotracer synthesis. At Brookhaven Lab, for example, an automated synthesis of FDG proceeds in 36 discrete steps, 5 of which are solvent evaporations, 22 of which are liquid transfers of some sort (extractions, vessel washes, transfers, etc.).

Solvent evaporations and liquid transfers can each be subdivided into two subtypes, each posing a slightly different control problem. Solvent evaporations, for example, may be used for drying or concentration. Liquid transfers, particularly for solvent delivery, are used either to move fixed volumes of liquid from one place to another or to dispense a programmable volume. While control of both drying and batch transfer require information about when a discrete volume of liquid is either evaporated or transferred respectively, concentration and dispensing control require feedback about the remaining volume in a reaction vessel of solvent reservoir. Most sensor applications

have been developed for drying or batch transfer control, although feedback control of dispensing solvent using a mass flow controller during synthesis has also been described. (Iwata *et al.*, 1990).

*Solvent evaporations*

Conductivity (Link *et al.*, 1994), temperature (Link *et al.*, 1994; Zeisler *et al.*, 1994) and solvent vapor pressure signals (Ducret *et al.*, 1994) have all been used as feedback signals to drying algorithms in automated synthesis equipment. Gas vapor pressure signals are obtained directly through pellistor type gas sensors (Ducret *et al.*, 1994) or indirectly using diaphragm type pressure transducers (Alexoff *et al.*, 1989). The most common feedback practice, however, is to use encapsulated thermistor or thermocouple inserted inside the reaction vessel. Robust signals for input to drying algorithms for both aqueous (Zeisler *et al.*, 1994) and organic (Link *et al.*, 1994) solvents are obtained by the effect of evaporative cooling on temperature sensors in liquids. The most common control algorithms include a simple comparison of current process variable values with empirically determined "dry" endpoint values (Link *et al.*, 1994). To help eliminate premature endpoint triggering due to electronic noise, signal averaging of sampled data in digital systems is often used before endpoint testing in software (Link *et al.*, 1994). In addition, simultaneous smoothing and differentiation using simple but robust digital filters (Savitsky & Golay, 1964) can provide drying control information for algorithms that are less dependent on empirical endpoints that could be susceptible to change. For example, thresholds based on rates of change are less dependent on absolute drying conditions and can be used in conjunction with signal magnitudes to create robust endpoint determination algorithms for solvent evaporation (Alexoff et al., 1986).

*Fluid transfers*

Since the earliest prototype automated systems, liquid sensing has been employed. Reservoir liquid levels can be monitored by optical detectors mounted exterior to the reservoir that give a digital signal indicating the presence of a liquid at some predefined level. Liquid presence sensors for tubes can be used to determine whether a tube is filled with a liquid or not. Most often these detectors rely on changes in reflected or transmitted light emanating from a solid state or incandescent energy source due to a change in refractive index inside the vessel filled with liquid compared to air or inert gas (Zeisler *et al.*, 1994; Alexoff *et al.*, 1994). A different type of liquid presence sensor has been designed to take advantage of changes in dielectric constant of fluids (e.g. water vs air in a tube (McKinney *et al.*, 1995). This design greatly improves the radiation hardness of the liquid detectors used in automated radiopharmaceutical production equipment (McKinney *et al.*, 1995). Note that these sensing strategies are limited to discrete type control problems associated with determining when the transfer of a fixed volume of liquid has been completed.

Other methods of determining the completion of a liquid transfer also have the potential of continuous control for dispensing applications. A method reported by Iwata *et al.* based on thermal mass flow controller can be used for both liquid transfer and liquid dispensing applications. In this feedback control strategy, measurements of instantaneous gas flow rate can be used to assess the completion of a liquid transfer while a real time integration of transfer gas flow rate can be used to dispense calibrated volumes of liquid (Iwata *et al.*, 1990). Another advantage of this system is that, depending on the plumbing of a specific automated system, a single sensor can be used as

feedback to control transfer and/or dispensing tasks from multiple reagent vessels or reservoirs. In a similar fashion, changes in pressure measured by a pressure sensor have also been shown to give robust signals indicating the completion of liquid transfers associated with solid phase extraction (Alexoff *et al.*, 1989). In this example, the changes in resistance due to the presence then absence of liquid between the vent and the vacuum source give rise to decreased pressure differentials across valves and tubing that signal clearly the completion of batch liquid transfers.

Radioactivity sensors can also be used to provide feedback for liquid transfer control (for detector examples, see Crouzel *et al.*, 1993). Radioactivity measurements for fluid transfer control can be especially useful when controlling the release and transfer of small volumes of gas, from, for example, irradiated cyclotron targets. These small volumes are often introduced with a carrier gas stream under a constant flow rate into a reaction vessel or trap. In this case, radioactivity may be the only measure, even in manual or remotely controlled syntheses, that allows an operator or algorithm to determine the end of the gas transfer. Ruth and co-workers have presented an example where $^{11}CO_2$ is released from a liquid nitrogen trap into a reaction vessel (Ruth *et al.*, 1989). In this example, transfer signal endpoints derived from integrated radioactivity signals can be used for transfer control (Ruth *et al.*, 1991b).

**Sensor data for diagnostics and documentation**

The trend in automated radiopharmaceutical synthesis is proceeding steadily to include more feedback control. For example, many second-generation commercial machines have some kind of feedback (e.g., Jackson 2000; Zigler 2000). One of the motivations for this is not only more robust and efficient real time control as just

discussed, but as importantly, information from process sensors aids in pre-run diagnostics, post-run troubleshooting, trend analysis, and process documentation.

Pre-run diagnostics built into automated PET radiotracer equipment include signal noise measurements, leak rates of vessels, heater performance, and automated PID tuning (Alexoff *et al.*, 1986). Iwata described the use of a mass flow controller to assess the presence of liquid in a vessel by measuring the head space in the vessel from gas flow rate measurements (Iwata *et al.*, 1990). Pre-run diagnostics still rely on careful operator inspection of liquid levels, tubing connections, and vessel connections. This visual inspection is often aided by automated or computer-assisted leak checking

Sensor information is also important to follow manufacturing trends in a radiopharmaceutical production line. It is often possible to for an experienced radiochemist to respond to subtle changes in precursor yield, age of reagents, or integrity of radiochemical hardware to maintain overall synthesis yields. Although the increased availability of PET synthesizers using totally disposable components (Mosdzianowski and Morelle, 2000) can help to minimize some of these problems, data from sensors can be used to schedule important maintenance of radiochemical equipment. For example, Ferrieri *et al.* have recently demonstrated that the strategic of placement of a single radiation detector external to the GEMS methyl iodide box can give useful information about the integrity of a major reagent supply ($I_2$ tube) used to make $^{11}$C-methyl iodide (Ferrieri *et al.*, 2000). Robust changes in radioactivity signal frequency, integrated activity, and overall curve shape (rates of changes and inflection points) are observed as the $I_2$ tube ages. These changes in radioactivity signal characteristics can be used to

schedule preventative maintenance tasks, thus avoiding unexpected radiolabeling failures due to low $^{11}$C-methyl iodide yields.

Other PET researchers have reported the use of trend data from an in-line conductivity sensor upstream of automated synthesis equipment to schedule target and delivery line maintenance (McKinney, 2000). This scheduled maintenance of important radiochemical systems feeding automated synthesizers avoids unexpected decreases in radiotracer yield due to changes in precursor purity, delivery time, and delivery line losses that are a function of cumulative target and delivery line use. (McKinney, 2000).

Incorporating automated or computer-assisted trouble shooting capabilities into PET radiotracer synthesis machines has been proposed as a solution to the problem of the disparity of knowledge and experience of OEM designers compared to end users (Alexoff 1991). Automated trouble-shooting may also may also be useful for institutions using in-house machine designs by empowering less experienced operators with the knowledge of equipment designers and veteran radiochemists. It is clear that incorporating more sophisticated software strategies such as artificial intelligence based troubling shooting or fuzzy logic unit operations control requires the increased use of sensors. Although advances in radiotracer chemistry will continue to provide simple and robust systems that minimize the need for increased intelligence of PET radiotracer synthesis machines, the development of sophisticated synthesizers with optimal control and autodiagnostic capabilities could facilitate the supply of cost-effective new radiotracers for clinical use.

## Modern Automatic Synthesizers

Design concepts for modern automatic synthesizers for PET have been discussed and various approaches to automation have been compared and contrasted (Crouzel *et al.*,

1993; Link *et al.*, 1992; Satyamurthy *et al.*, 1999). Some of the important design criteria to consider when building an automated synthesis system include multi-run capability, requirements for sterile disposable components, self-cleaning capability, auto-diagnostic functions, and process documentation. These design criteria effect both the choice of specific radiochemical process control hardware (e.g. valve type or tubing material) and overall control system design (e.g. robot, PC, PLC). Most modern machines share a highly structured, modular, unit operations based design of radiochemical processes, valve and tubing hardware, and intelligent (computer/microcomputer based) data acquisition and control hardware and software systems. Today's machines utilize highly modular, distributed intelligent industrial process control and data acquisition hardware such as OPTO-22™ (Opto22, Temecula, CA) and Fieldpoint™ (National Instruments Corporation, Austin, TX). These systems are modular and expandable, providing appropriate input/output (I/O) densities of common industrial I/O hardware (e.g. medium power DC output, PID control, analog to digital (A/D) conversion, analog filtering) for machine designers. These intelligent interface systems are often opto-isolated for high noise immunity, allowing user interfaces (PC) to be located large distances from the actual control area (e.g. shielded synthesis hood). Additionally, modern software engineers have available to them a rich palate of graphically based, object oriented user interfaces and software tools such as LabView™(National Instruments Corporation, Austin, TX), FactoryFloor™22™ (Opto22, Temecula, CA), and Visual Basic™(Microsoft, Seattle, WA).

A brief discussion of two modern machines serves to highlight these latest engineering design strategies as well as to illustrate the diversity of engineering solutions

to the problem of automated PET radiotracer synthesis that persists today. The reader is also directed to the web sites and product specifications of the major commercial suppliers of automated radiochemical production equipment (Sumitomo, CTI, Concurrent Microsystems, Nuclear Interface, GE, Ebco).

**Automated synthesis of 6-[$1^8$F]fluoro-L-DOPA**

Significant engineering innovation is demonstrated in the automated synthesis of 6-[$^{18}$F]fluoro-L-DOPA reported by de Vries *et al.* (de Vries *et al.*, 1999). Success of this machine depends first and foremost on the choice of synthetic route. As the authors discuss, the choice of electrophilic fluorodestannylation as a synthetic pathway gave high yields of labeled compound without the complication of labeled isomers that require separation. Furthermore, the route chosen did not require separation of a labeled intermediate and therefore allowed the synthesis to proceed in one pot. Finally, HPLC conditions were such that evaporation and reformulation of the purified product was not required. This work demonstrated that simplified chemical processes amendable to automation could be implemented without compromising radiopharmaceutical quality.

This successful system was constructed by modifying a commercially available PET radiotracer synthesizer (Nuclear Interface, Muenster, Germany) that was designed for the automated synthesis of FDG. Success of this system is a testimony to the flexibility of most modern radiotracer systems that use structured software designs, modular intelligent interfaces, and unit operations-based radiochemical processing. Utilizing pressure, radioactivity, and UV sensors incorporated in the commercial machine, the authors present robust process signals documenting most steps in the entire process (de Vries *et al.*, 1999). Although it is not clear that these signals are used directly

as feedback for step control, they do provide important process documentation and information for trouble shooting. As described in this work, the Nuclear Interface machine also includes automated cleaning and automated diagnostics for flow and leak checking.

Finally, a fluid sensor detecting the presence of liquid in tubing leading to the HPLC injector was incorporated so that HPLC injection could be automated. An interesting finding of the authors was the failure of the fluid sensor due to a sensitivity to metal ions used during neutralization of HBr used for hydrolysis. Proper function of the sensor was restored by changing the reagent used for neutralization from 10N NaOH to 25% ammonium hydroxide with phosphate buffer. In this instance, successful application of feedback control required a commitment of the radiochemists and modification of chemical processing to accommodate sensor characteristics.

**Robot synthesis of [$^{11}$C]flumazenil**

Krasikova *et al.* report the use of a commercially available Anatech RB-86 robot (Anatech, Husbyborg, Uppsala, Sweden; for detailed description see Krasikova, 1998) to prepare [$^{11}$C]flumazenil from [$^{11}$C]methyl iodide (Krasikova *et al.*, 2000). This laboratory robot system has also been used to automate other PET radiotracer syntheses including FDG and L-[C-11-methyl]methionine (Krasikova, 1998) and includes a personal computer (PC) and programmable logic controller (PLC). Robot workstations include hardware for solid phase extraction (SPE), solvent evaporation, and reaction vessel capping/dilution. Starting from trapped [$^{11}$C]$CH_3I$, the synthesis of labeled flumazenil proceeds in just 7 steps and is completed in 18 minutes. A novel feature of this system was the elimination of HPLC purification. Although HPLC injection and

purification can be automated reliably (see above), alternative purification strategies can simplify control and shorten overall synthesis times considerably. This is especially important for the synthesis of carbon-11 compounds. In this work, the authors demonstrated that through the careful determination of optimal conditions for both solid supported alkylation of the desmethyl compound Ro 15-5528 using [$^{11}$C]$CH_3I$, and the separation of [$^{11}$C]flumazenil from Ro 15-5528, HPLC purification could be eliminated altogether. Krasikova *et al* report a mass of Ro 15-5528 in the final product formulation of [$^{11}$C]flumazenil (7.5 mL) to be only 0.1 to 0.8 micrograms using this method .(Krasikova *et al.*, 2000).

It is clear from the success of these two different automatic control designs that consideration of a diversity of machine designs by radiochemists is appropriate when faced with the challenge of automating the synthesis of a PET radiopharmaceutical. This design diversity reflects the unique challenge facing PET radiochemists who draw upon the myriad of strategies and pathways inherent to organic chemistry. In fact, the power of the PET method in research is derived in part from this basis in organic chemistry and the concomitant plethora of biologically important molecules that can be labeled with the positron emitting nuclides $^{18}$F, $^{11}$C, $^{13}$N, and $^{15}$O. Automated synthesis designers must be prepared incorporate this flexibility when building machines in support of PET research.

## Automation for the application of PET radiopharmaceuticals

Development of automated systems for PET research has not been limited to the design of machines to carry out the syntheses of radiopharmaceuticals. Motivated by many of the same problems presented by routine, rapid syntheses of PET radiopharmaceuticals, PET researchers have also developed automated systems to

facilitate the *application* of PET radiotracers in basic and drug research and development. These systems include automated quality control of radiotracers (see Crouzel *et al.*, 1993), computer controlled infusion systems for automated injection of radiopharmaceuticals (Palmer *et al.*, 1995), automated dose dispensing systems (Jackson 2000; Plascjak *et al.*, 1997), and automated delivery of radiotracers using pneumatic transport systems (Dembowski and Gonzalez-Lepera, 1994).

In particular, significant progress has been made in automating plasma analyses required for quantitative PET studies (Alexoff *et al.*, 1995; Andersson & Schneider, 1998; Lindner *et al.*, 1995; Luthra *et al.*, 1992). Accurate assays of unchanged PET radiotracers in plasma (plasma input function) are important for the determination of model parameters that reflect specific biochemical properties of specific molecular targets (e.g. receptor availability or enzyme concentration). Determination of these input functions can be time consuming, labor intensive, as well as hazardous. In certain instances, input functions can be generated non-invasively using reference tissue regions (Logan et al., 1996). In general, however, new tracers are validated and new drug research is carried out with direct measurements of plasma radioactivity and its identity. These measurements are often carried out for multiple blood samples making up a discretely sampled function representing the time-course of radiotracer activity after bolus injection. Automated blood sampling devices (Grahm & Lewellen, 1993) may be used to obtain discrete blood samples for automated analysis. Flow counting systems generating continuous time-activity data have also been used to automate input function measurement, particularly in $^{15}O$ studies (Hutchins *et al.*, 1986).

Automated systems have been described for automating plasma assays for unchanged radiotracer in plasma using laboratory robots (Alexoff *et al.*, 1995; Andersson & Schneider, 1998), and programmable logic controllers (Luthra *et al.*, 1992). The latter system is based on HPLC and automates extraction of radioactivity from plasma followed by analysis by HPLC and therefore may be applied to any suitable HPLC method for any new radiotracer. The system requires only one person to operate (manual injections) and has been used successfully to determine the unchanged fraction of radiotracer in plasma for several compound including $^{11}$C-L-deprenyl, $^{11}$C-diprenorphine, $^{11}$C-flumazenil, $^{11}$C-raclopride, and $^{11}$C-SCH 23390. By contrast, the laboratory robot system described by BNL researchers to automate the same task requires no human participation. This system, however, is based on a validated solid phase extraction assay that eliminates HPLC and therefore may not be as universally applied to new radiotracer assays without revalidation. Selective assays for many radiotracers, however, have been developed and implemented using this robotic SPE-only strategy (Alexoff *et al.*, 1996).

Although performance of these automated systems is often reliable (a 6 year "uptime" in excess of 95% is reported by Andersson & Schneider, 1998), sample throughputs for the plasma assay robots can be 1/3 to1/2 throughputs achieved by an experienced human laboratory worker. At BNL, for example, robotic steady-state throughput of 14.3 samples/hour (2 minute counting interval) is 1/3 that of a human worker (1 minute counting interval). As first reported, this throughput rate is highly dependent on the range of whole blood volumes in a study because of an iterative gravimetric feedback algorithm used to obtain cell free plasma for counting. This algorithm uses a linearized model of a 1.5 mL tapered blood sampling tube (Eppendorf)

and an initial estimate of the subjects hematocrit and a maximum whole blood volume to calculate the cell/plasma interface in each tube. Using this technique, the robot's pipetting hand (1.0 mL syringe tip) was able to obtain sufficient cell-free plasma for good counting statistics using as little at 0.2 - 0.4 mL of whole blood (Alexoff *et al.*, 1997). Sample throughput of the system, however, depends strongly on sample volume uniformity. This is usually not a problem when using auto-sampled blood.

Using the Anatech RB-86 robot and a direct measure of the cell/plasma interface with an optical sensor, Andersson & Schneider report a throughput of 21 plasma samples/hour (30 second counting interval). This system also incorporates whole blood counting and bar coding of samples, but requires larger whole blood volumes (1-1.5 mL). Direct detection of the cell/plasma interface and the use of several cross-calibrated well counters allows for higher sample throughputs that are independent of sample volume (Andersson & Schneider, 1998).

## Future directions

As illustrated by past successful automated chemistry systems both within and beyond the field of PET radiochemistry, future advances in automated systems will once again reflect mostly the creativity of PET radiochemists and their ability to refine processes and characterize new radiolabeling pathways. Recently, PET radiochemists have continued this tradition by exploiting captive solvent techniques and solid phase reaction schemes to create very simple high yield radiochemical systems that are amendable to automation (Jewett & Kilbourn, 1999;Wilson *et al.*, 2000; Iwata *et al.*, 2000; Iwata et al 2001). In particular, captive solvent techniques have recently been used

by Wilson *et al* to make C-11 labeled raclopride, N-methylspiperone, Ro 15-1788, FLB 457, Rolipram, SCH 23390 and SKF 82957 from [$^{11}$C]-iodomethane (Wilson *et al.*, 2000). This method extends the pioneering work of Jewett and co-workers (Jewett *et al.*, 1991) by eliminating the need for solid supports and elevated temperatures. This streamlined "loop method" yields efficient trapping of $^{11}CH_3I$ and fast methylation reactions both at room temperature, greatly simplifying radiochemical processing. Recently, researchers in Japan have investigated the use of the "loop method" with [11C]methyl triflate in the radiosynthesis of [$^{11}$C]raclopride (Iwata *et al.*, 2001). Using this method, an automated synthesis system (starting from [$^{11}$C]methyl iodide) can be constructed with only 4 valves, 1 reservoir, a furnace, and an HPLC system (Iwata *et al.*, 2001).

In addition to impacting future advances in radiolabeling and purification, solid phase techniques can be expected to continue to simplify radiochemical processing that is needed for the formulation of radiopharmaceutical. Methods based on a C18 Sep-Pak™ are being developed to replace the need for rotary evaporators. Lemaire and colleagues report formulations of several C-11 and F-18 radiopharmaceuticals in 3-6 minutes with recoveries >97% using only solid phase extraction techniques (Lemaire *et al.*, 1999).

Taken together, these new strategies for radiolabeling, purification, and formulation of PET radiopharmaceuticals are likely to be utilized extensively in future automated systems, particularly for carbon-11 labeled compounds. PET radiochemists will continue to use the latest in personal computer, industrial control, and laboratory robot technologies to implement these radiochemical processes and others to create reliable, flexible, automated chemistry systems.

## Summary

It is clear that this current state of reliable, cost-effective commercially available PET radiochemicals is the result of the early engineering goundwork put down by a handful of pioneering radiochemists from around the world. These early pioneers had not only the prescience to see the benefits of automating their processes, but also had the vision to see the benefits of international collaboration. Those of us in the PET field today are greatly in debt to these early innovators whose world-view and breadth of knowledge has put the future of PET on firm ground for this the 21$^{st}$ century.

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