

Chapter 8

Synthesis of PET Radiopharmaceuticals

Introduction

PET radiopharmaceuticals are uniquely different from SPECT radiopharmaceuticals in that the former have radionuclides that are positron emitters and the majority of them have short physical half-lives. The most common PET radionuclides are ^{11}C , ^{15}O , ^{13}N , ^{18}F , and ^{82}Rb , which are short-lived (see Table 7.2) and put limitations on the synthesis time for PET radiopharmaceuticals and their clinical use. The attractive advantage of PET radiopharmaceuticals, however, is that the ligands used in radiopharmaceuticals are common analogs of biological molecules and, therefore, often depict a true representation of biological processes after in vivo administration. For example, ^{18}F -fluorodeoxyglucose (FDG) is an analog of glucose used for cellular metabolism and H_2^{15}O for cerebral perfusion.

Automated Synthesis Device

Conventional manual methods of synthesis of radiopharmaceuticals using a high level of radioactivity are likely to subject the personnel involved in the synthesis to high radiation exposure. This is particularly true with short-lived positron emitters such as ^{11}C , ^{13}N , ^{15}O , and ^{18}F , because the quantity of these radionuclides handled in the synthesis is very high. To minimize the level of exposure, automated modules have been devised for the synthesis of PET radiopharmaceuticals.

The automated synthesis device, often called the *black box*, is a unit controlled by microprocessors and software programs to carry out the sequential physical and chemical steps to accomplish the entire synthesis of a radiolabeled product. The unit consists of templates or vials prefilled with required chemicals attached to the apparatus via tubings that are connected to solenoid valves to switch on and off as needed. Most black boxes are small enough to be placed in a space of

20 × 20 × 20 in. and are capable of self-cleaning. In some units, disposable cassettes (cartridges) are employed so that new cassettes can be used for each new synthesis, thus minimizing contamination and radiation exposure. Various parameters for synthesis such as time, pressure, volume, and other requisites are all controlled by a remote computer using appropriate software. The unit has a graphic display showing the status of the ongoing process. After the synthesis, a report with the date and start and end time of the radiosynthesis and the calculated yield is printed out. Technologists can operate these units very easily. Automated synthesis modules for ^{18}F -FDG, ^{13}N - NH_3 , ^{11}C - CH_3I , ^{11}C -HCN, ^{11}C -acetate, and a few other PET tracers are commercially available. Versatile automated modules are commercially available to use for the synthesis of a variety of PET tracers in the single module. This is accomplished by simple exchange or modification of various segments inside the unit to suit the specific product synthesis. To minimize radiation exposure, often the synthesis box is placed inside a minicell (see Chap. 7). After each synthesis, the product is passed through a high-performance liquid chromatography (HPLC) described later to achieve a high-purity finished product. A schematic diagram of a black box for ^{18}F -FDG synthesis is shown in Fig. 8.1. Often the synthesis box is placed inside a minicell to minimize radiation exposure. An automated multi-synthesis module (FASTlab2) for the synthesis of different PET radiopharmaceuticals marketed by GE Healthcare is shown in Fig. 8.2. Other vendors include Siemens Medical Solutions, Inc. (Explora), IBA (Synthera), and Eckert & Ziegler (FDG-Plus).

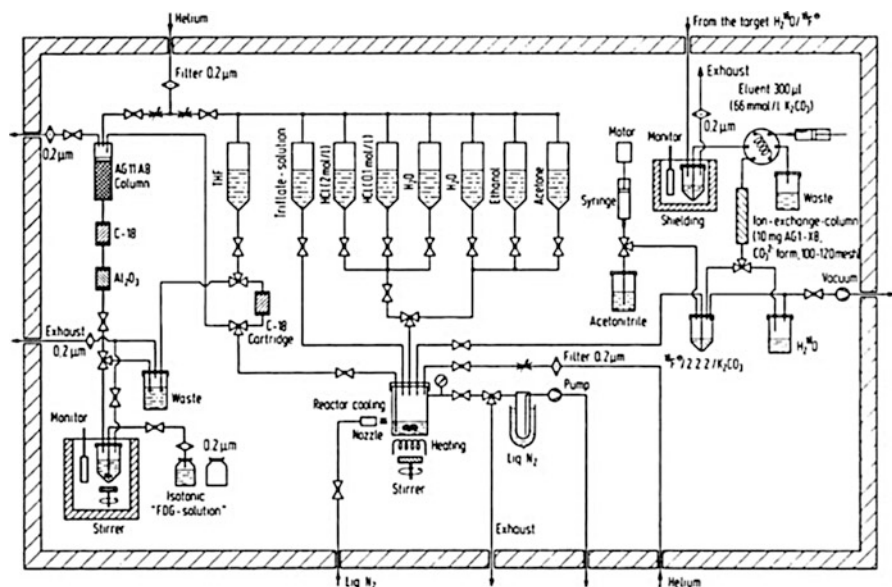


Fig. 8.1 A schematic block diagram showing different components in the ^{18}F -FDG synthesis box (Reproduced with kind permission of Kluwer Academic Publishers from Crouzel C et al. (1993) Radiochemistry automation PET. In: Stöcklin G, Pike VW (eds) Radiopharmaceuticals for positron emission tomography, Kluwer Academic, Dordrecht, the Netherlands, p 64. Fig. 9)



Fig. 8.2 Automated synthesis box, FASTlab2, from GE Healthcare (Courtesy of GE Healthcare)

PET Radiopharmaceuticals

Many radiopharmaceuticals have been used for PET imaging; however, only a few are routinely utilized for clinical purposes. Almost all of them are labeled with one of the four common positron emitters: ^{11}C , ^{13}N , ^{15}O , and ^{18}F . Of the four, ^{18}F is preferred most, since it has a relatively longer half-life ($t_{1/2} = 110$ min) that allows its supply to relatively remote places. In all cases, a suitable synthesis method is adopted to provide a stable product with good labeling yield, high specific activity, high purity, and, most importantly, high in vivo tissue selectivity. The following is a description of the syntheses of the common clinically used PET radiopharmaceuticals and a few with potential for future use.

^{18}F -Sodium Fluoride

Fluorine-18 ($t_{1/2} = 110$ min) is produced by irradiation of ^{18}O -water with 10–18 MeV protons in a cyclotron and recovered as ^{18}F -sodium fluoride by passing the irradiated water target mixture through a carbonate-type anion-exchange resin column. The water is forced out of the column with neon gas, whereas $^{18}\text{F}^-$ is retained on the column, which is recovered by elution with potassium carbonate solution. Its pH should be between 4.5 and 8.0. While ^{18}F -sodium fluoride is most commonly used for the synthesis of FDG, it is also used for other ^{18}F -labeled PET radiopharmaceuticals.

The US FDA has approved it for bone scintigraphy, since it localizes in bone by exchanging with PO_4^- ion in the hydroxyapatite crystal.

¹⁸F-Fluorodeoxyglucose

¹⁸F-2-fluoro-2-deoxyglucose (2-FDG) is normally produced in places where a cyclotron is locally available. Its molecular formula is C₈H₁₁¹⁸FO₅ with molecular weight of 181.3 Da. ¹⁸F-2-FDG can be produced by electrophilic substitution with ¹⁸F-fluorine gas or nucleophilic displacement with ¹⁸F-fluoride ions. The radiochemical yield is low with the electrophilic substitution, so the nucleophilic displacement reaction has become the method of choice for ¹⁸F-FDG synthesis. Deoxyglucose is labeled with ¹⁸F by nucleophilic displacement reaction of an acetylated sugar derivative followed by hydrolysis (Hamacher et al. 1986). In nucleophilic substitution, a fluoride ion reacts to fluorinate the sugar derivative. A solution of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethane-sulfonyl-β-D-mannopyranose in anhydrous acetonitrile is added to a dry residue of ¹⁸F-fluoride containing aminopolyether (Kryptofix 2.2.2) and potassium carbonate (Fig. 8.3). Kryptofix 2.2.2 is used as a catalyst to enhance the reactivity of the fluoride ions. The mixture is heated under reflux for about 5 min. The solution is then passed through a C-18 Sep-Pak column, and acetylated carbohydrates are eluted with tetrahydrofuran (THF), which are then hydrolyzed by refluxing in hydrochloric acid at 130 °C for 15 min. ¹⁸F-2-fluoro-2-deoxyglucose (2-FDG) is obtained by passing the hydrolysate through a C-18 Sep-Pak column. The yield can be as high as 60%, and the preparation time is approximately 50 min. The final solution is filtered through a 0.22-μm filter and diluted with saline, as needed. According to USP specifications, it should have pH of 4.5–7.5 and a specific activity of more than 1 Ci (37 GBq)/μmol. The chemical purity is limited to 50 μg/mL of Kryptofix 2.2.2 and 1 mg of 2-chloro-2-deoxy-D-glucose per total volume. Radiochemical purity should be >90%, as determined by the TLC method

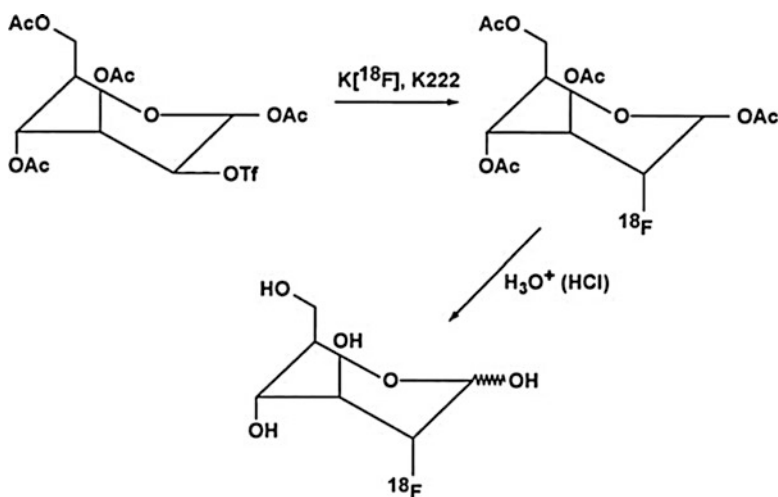


Fig. 8.3 Schematic synthesis of ¹⁸F-2-fluoro-2-deoxyglucose (FDG) (Reprinted with the permission of the Cleveland Clinic Center for Medical Art and Photography ©2009. All rights reserved)

using activated silica gel as the solid phase and a mixture of acetonitrile and water (95:5) as the liquid phase.

Since Kryptofix 2.2.2 is toxic causing apnea and convulsions, modifications have been made to substitute it with tetrabutylammonium hydroxide or bicarbonate, which have been adopted by many commercial vendors. Also, in some other methods, the C-18 Sep-Pak column separation has been eliminated so as to carry out the acidic hydrolysis in the same vessel. In methods where Kryptofix 2.2.2 is still used, several Sep-Pak columns are used to separate Kryptofix 2.2.2 and reduce it to practically a negligible quantity.

The FDA has approved ^{18}F -2-FDG for many clinical uses such as the metabolism in the brain and heart and the detection of epilepsy and various tumors. In metabolism, ^{18}F -2-FDG is phosphorylated by hexokinase to 2-FDG-6-phosphate which is not metabolized further. It should be noted that 3-fluorodeoxyglucose (3-FDG) is not phosphorylated and hence is not trapped and essentially eliminated rapidly from the cell. This is why 3-FDG is not used for metabolic studies. Detailed protocols of ^{18}F -FDG usage in humans are given in Chap. 13.

Because of the relatively longer half-life of ^{18}F among the PET radionuclides, commercial and institutional facilities having cyclotrons produce ^{18}F -FDG in bulk quantities and supply to nearby clinics and hospitals as needed. Supply can be made as far as 200 miles away with a loss of activity, which can be compensated by adding more activity. The details of ^{18}F -FDG distribution is given in Chap. 10.

6- ^{18}F -L-Fluorodopa

Like ^{18}F -2-FDG, 6- ^{18}F -L-fluorodopa is also produced in places where a cyclotron is available locally. There are several methods of synthesizing 6- ^{18}F -fluoro-3,4-dihydroxyphenylalanine (6- ^{18}F -L-fluorodopa), of which the method of fluorodemetalation using electrophilic fluorinating agents is most widely used. Electrophilic reactions involve the reaction of fluorine in the form of F^+ with other molecules. Only the L-isomer of dopa is important, because the enzymes that convert dopa to dopamine, which is targeted by the radiopharmaceutical, are selective for this isomer. Initially, a suitably protected organomercury precursor (*N*-[trifluoroacetyl]-3,4-dimethoxy-6-trifluoroacetoxymethylmercuriophenylalanine ethyl ester) of dopa is prepared. [^{18}F]-labeled acetylhypofluorite prepared in the gas phase is then allowed to react with the mercury precursor in chloroform or acetonitrile at room temperature. Other precursors using metals such as tin, silicon, selenium, and germanium have been reported. Acid hydrolysis with 47% HBr provides a relatively high yield (10–12%) of 6- ^{18}F -L-fluorodopa (Luxen et al. 1992) compared with other available methods. Substitution at position 6 is most desirable, because this does not alter the behavior of dopa, whereas substitutions at 2 and 5 do. It is sterilized by filtering through a 0.22- μm membrane filter and is supplied at pH between 6 and 7. Normally EDTA and ascorbic acid are added to the final preparation for stability. Its specific activity should be more than 100 mCi (3.7 GBq)/mmol and radiochemical purity >95% as

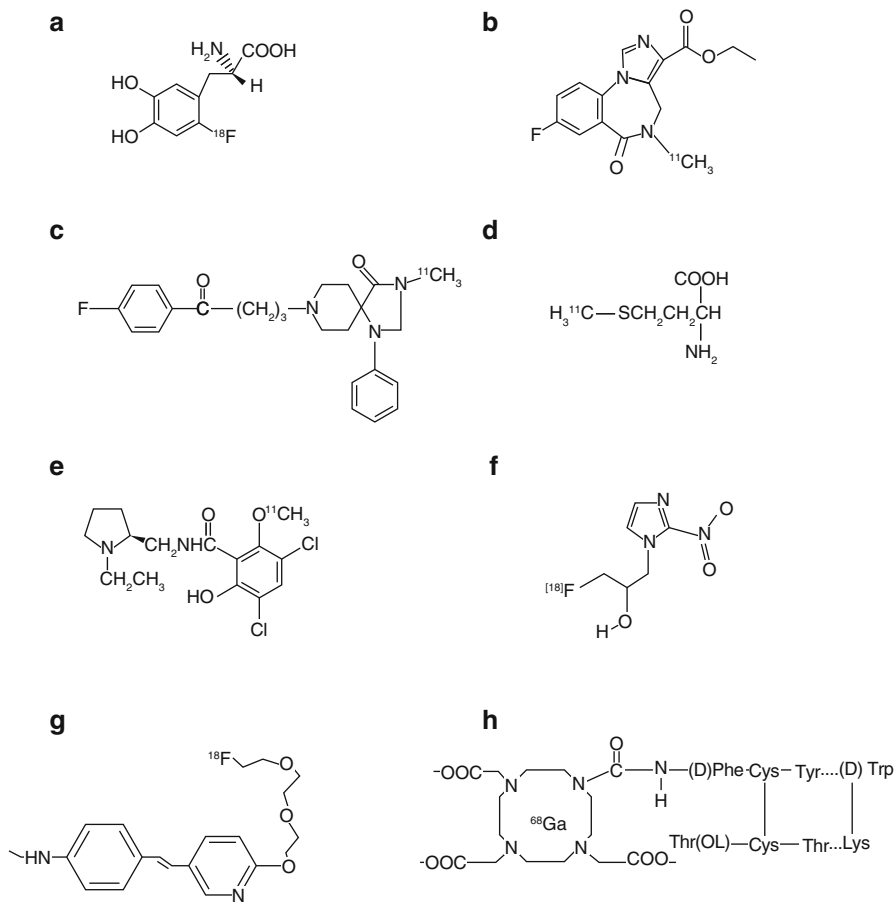


Fig. 8.4 Molecular structures of (a) 6- ^{18}F -L-fluorodopa, (b) ^{11}C -flumazenil, (c) ^{11}C -methylspiperone, (d) ^{11}C -L-methionine, (e) ^{11}C -raclopride, (f) ^{18}F -fluoromisonidazole, (g) ^{18}F -florbetapir, and (h) ^{68}Ga -DOTATOC (Reprinted with the permission of the Cleveland Clinic Center for Medical Art and Photography[©]2009. All rights reserved)

determined by HPLC. Mercury is the major chemical impurity (toxic) that originates from organomercuric precursor used in the synthesis, and its USP limit is 0.5 $\mu\text{g/mL}$ of L-dopa solution. The molecular structure of 6- ^{18}F -L-fluorodopa is shown in Fig. 8.4a.

It is particularly useful for the detection of Parkinson's disease.

¹⁸F-Fluorothymidine

¹⁸F-Fluorothymidine (FLT) is prepared by nucleophilic reaction between ¹⁸F-sodium fluoride and a precursor, 2, 3'-anhydro-5'-O-benzoyl-2'-deoxythymidine, which is prepared by standard organic synthesis (Machula et al. 2000). ¹⁸F-Sodium fluoride is added to a mixture of Kryptofix 2.2.2 and potassium carbonate in acetonitrile, and the mixture is dried to a residue by heating at 120 °C for 5 min. The precursor in dimethyl sulfoxide (DMSO) is added to the dried residue and heated at 160°C for 10 min. Hydrolysis of the 5'-O-protecting group is performed with sodium hydroxide. ¹⁸F-FLT is isolated by passing through alumina Sep-Pak and further purified by using high-performance liquid chromatography (HPLC). The overall yield is about 45% and the radiochemical purity is more than 95%. The synthesis time is about 60 min.

Since thymidine is incorporated into DNA and provides a measure of cell proliferation, ¹⁸F-FLT is commonly used for in vivo diagnosis and characterization of tumors in humans.

¹⁸F-O-(2-Fluoroethyl)-L-Tyrosine

The synthesis of ¹⁸F-O-(2-fluoroethyl)-L-tyrosine (FET) is carried out in two steps (Wester et al. 1999): First, ethylene glycol-1,2-ditosylate in acetonitrile is reacted with dry ¹⁸F-containing Kryptofix 2.2.2 and potassium bicarbonate at 90°C for 10 min. The product is purified by absorbing it on a polystyrene cartridge and then eluting with dimethyl sulfoxide. Second, the eluate is mixed with dipotassium sodium salt of L-tyrosine and heated at 90°C for 10 min. The mixture is purified by HPLC and cation exchange to obtain ¹⁸F-FET. The radiochemical yield is about 40–45% with purity between 97 and 99%.

¹⁸F-FET is used as a PET tracer for the detection of a variety of tumors.

¹⁸F-Fluoromisonidazole

¹⁸F-Fluoromisonidazole (FMISO) is synthesized in one-step reaction between the protected precursor, 1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonyl-propanediol (NITTP) and ¹⁸F-containing Kryptofix 2.2.2 in acetonitrile solution (Kamarainen et al. 2004). The labeled product is hydrolyzed with acid to give ¹⁸F-FMISO, which is further purified by column chromatography using a Sep-Pak cartridge. From automated synthesis, the radiochemical yield is 34% at EOS after a synthesis time of 50 min. HPLC shows a radiochemical purity of 97%. The molecular structure of ¹⁸F-FMISO is shown in Fig. 8.4f.

¹⁸F-FMISO is a specific tracer used for detection of hypoxic tissues by PET.

¹⁸F-1-(5-Fluoro-5-Deoxy- α -Arabinofuranosyl)-2-Nitroimidazole

¹⁸F-1-(5-Fluoro-5-deoxy- α -arabinofuranosyl)-2-nitroimidazole (FAZA) is synthesized by nucleophilic substitution reaction between the precursor 1-(2,3-di-*O*-acetyl-5-*O*-tosyl- α -D-arabinofuranosyl)-2-nitroimidazole in DMSO solution and ¹⁸F-fluoride containing mixture of Kryptofix 2.2.2 and potassium carbonate at 100°C for 5 min (Reischl et al. 2005). The product is hydrolyzed with NaOH solution at 20°C for 2 min, and the solution is neutralized with NaH₂PO₄. It is further purified by HPLC and sterilized by 0.22- μ m filtration. The radiochemical yield is about 61%.

¹⁸F-FAZA is used for the detection of tissue hypoxia by PET.

¹⁸F-Florbetapir

¹⁸F-Florbetapir (brand name ¹⁸F-AV-45 or Amyvid) has the molecular structure of (E)-4-(2-(6-(2-(2-(2-([¹⁸F]-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-*N*-methylbenzenamine. It is prepared by nucleophilic substitution reaction between ¹⁸F-containing mixture of Kryptofix 2.2.2 and potassium carbonate in acetonitrile solution and the precursor (E)-2-(2-(2-(2-tosyloxyethoxy)ethoxy)ethoxy)-5-(4-(tert-butoxycarbonyl (methyl)amino)styryl)pyridine (-OTs derivative) in DMSO solution at 115 °C for 10 min (Liu et al. 2010). The product is hydrolyzed by hydrochloric acid solution followed by neutralization with a solution of NaOH and ammonium acetate solution. The solution is loaded on a Sep-Pak C18 column, and unreacted ¹⁸F⁻ is removed by rinsing with deionized water, and ¹⁸F-florbetapir is eluted with acetonitrile. ¹⁸F-Florbetapir is purified by HPLC and finally taken up in ethyl alcohol for clinical use. The radiochemical yield is 34% and the radiochemical purity is nearly 95%.

¹⁸F-florbetapir binds to amyloid plaques in Alzheimer's disease (AD) and other dementia-related conditions and has been approved by the FDA for the detection of these diseases in patients. The molecular structure of ¹⁸F-florbetapir is shown in Fig. 8.4g.

¹⁵O-Water

¹⁵O-Oxygen ($t_{1/2}$ = 2 min) is produced in the cyclotron by the ¹⁵N(p, n)¹⁵O reaction, or the ¹⁴N(d, n)¹⁵O reaction, and the irradiated gas is transferred to a [¹⁵O] water generator in which ¹⁵O is mixed with hydrogen and passed over a palladium/charcoal catalyst at 170 °C (Meyer et al. 1986; Welch and Kilbourn 1985). The H₂¹⁵O vapor is trapped in saline, and the saline solution is filtered through a 0.22- μ m

membrane filter. The sample is then passed through a radiation detector for radioassay and ultimately injected online into a patient in a very short time.

H₂¹⁵O is commonly used for myocardial and cerebral perfusion studies.

***n*-¹⁵O-Butanol**

n-¹⁵O-Butanol is prepared by the reaction of ¹⁵O-oxygen, produced by the ¹⁵N(p, n)¹⁵O reaction, with tri-*n*-butyl borane loaded onto an alumina Sep-Pak cartridge (Kabalka et al. 1985). Carrier oxygen at a concentration of about 0.5% is added to the ¹⁵N target in order to recover ¹⁵O. After the reaction, *n*-¹⁵O-butanol is eluted from the cartridge with water. It is further purified by passing through C-18 Sep-Pak and eluting with ethanol–water.

n-¹⁵O-Butanol is used for blood flow measurement in the brain and other organs. It is a better perfusion agent than ¹⁵O-water, because its partition coefficient is nearly 1.0 compared to 0.9 for water.

¹³N-Ammonia

Nitrogen-13-labeled ammonia (*t*_{1/2} = 10 min) is produced by reduction of ¹³N-labeled nitrates and nitrites that are produced by proton irradiation of water in a cyclotron. The reduction is carried out with titanium chloride in alkaline medium. ¹³N-NH₃ is then distilled and finally trapped in acidic saline solution. Wieland et al. (1991) has used a pressurized target of aqueous solution of acetic acid and ethanol, in which ethanol acts as a hydroxyl free radical scavenger to improve the yield of ¹³N-NH₃. The mixture is passed through an anion-exchange resin to remove all anion impurities. It is filtered through a 0.22-μm membrane filter and its pH should be between 4.5 and 7.5. The radiochemical purity is greater than 95% as determined by HPLC.

The US FDA has approved it for measurement of myocardial and cerebral perfusion.

¹¹C-Sodium Acetate

¹¹C-Sodium acetate is produced by the reaction of the Grignard reagent, methylmagnesium bromide in diethyl ether, with cyclotron-produced ¹¹C-carbon dioxide at –15 °C (Oberdorfer et al. 1996). After reaction, the product is allowed to react with O-phthaloyl dichloride to produce ¹¹C-acetyl chloride, which is then hydrolyzed to ¹¹C-acetate with saline. The solution is filtered through a 0.22-μm

membrane filter. ^{11}C -Acetate has been found to be stable at pH between 4.5 and 8.5 for up to 2 h at room temperature. The overall yield is about 10–50%.

It is used for the measurement of oxygen consumption (oxidative metabolism) in the heart, since acetyl CoA synthetase converts ^{11}C -acetate to acetyl coenzyme A after myocardial uptake, which is metabolized to ^{11}C - CO_2 in the tricarboxylic acid cycle.

^{11}C -Flumazenil

^{11}C -Flumazenil is commonly labeled at the *N*-methyl position by *N*-methylation with ^{11}C -iodomethane, which is prepared from ^{11}C - CO_2 , and using the freshly prepared Grignard reagent, methylmagnesium bromide (Maziere et al. 1984). The specific activity is very important for this product and therefore is purified by HPLC to give an optimum value between 0.5 and 2 Ci/ μmol (18.5–74 GBq/ μmol). It remains stable for up to 3 h at room temperature at pH 7.0. The molecular structure of ^{11}C -flumazenil is shown in Fig. 8.4b.

Since it is a benzodiazepine receptor ligand, ^{11}C -flumazenil is primarily used for the neuroreceptor characterization in humans.

^{11}C -Methylspiperone

^{11}C -Methylspiperone (MSP) is prepared by *N*-methylation of commercially available spiperone with ^{11}C -methyl iodide in the presence of Grignard reagent, methylmagnesium bromide, using different solvents and bases (Mazière et al. 1992). Since spiperones are sensitive to bases and to radiolysis at high level of activity, the yield of ^{11}C -MSP has been variable for different investigators. Cold spiperone present in the preparation reduces its specific activity and should be controlled. Specific activity should be around 10–50 GBq/mol (270–1350 mCi/mol). High specific activity ^{11}C -MSP undergoes autodecomposition in saline due to radiation, and a hydroxyl radical scavenger (e.g., ethanol) is added to prevent it. The final preparation is filtered through a 0.22- μm membrane filter and its pH is adjusted to 7 ± 1 with a suitable buffer. The molecular structure of ^{11}C -methylspiperone is shown in Fig. 8.4c.

^{11}C -Methylspiperone is primarily used to determine the dopamine-2 receptor density in patients with neurological disorders, because of its high affinity for D-2 receptors in the brain.

¹¹C-L-Methionine

¹¹C-L-Methionine has ¹¹C at its methyl position and has two forms: L-[1-¹¹C] methionine and L-[S-methyl-¹¹C] methionine. The former is obtained by the reaction between ¹¹C-CO₂ precursor and carbanion produced by a strong base added to the respective isonitrile, followed by hydrolysis with an acid. The latter is the common method and obtained by alkylation of the sulfide anion of L-homocysteine with ¹¹C-iodomethane or ¹¹C-methyl triflate (Långström et al. 1987). The product is purified by HPLC yielding a purity of >98% and further filtered through a 0.22-μm membrane filter. The pH should be between 6.0 and 8.0 and it is stable for 2 h at room temperature. The molecular structure of ¹¹C-L-methionine is shown in Fig. 8.4d.

This compound is used for the detection of different types of malignancies, reflecting the amino acid utilization (transport, protein synthesis, transmethylation, etc.).

¹¹C-Raclopride

Raclopride is labeled with ¹¹C either by *N*-ethylation with [1-¹¹C] iodoethane or by *O*-methylation with [¹¹C] iodomethane, although the latter is more suitable for routine synthesis. Production of ¹¹C-labeled iodoethane and iodomethane are described in Chap. 7. A recent efficient method of synthesis of ¹¹C-raclopride utilizes a loop chemistry in which the precursor (raclopride) is dissolved in a small quantity of ethanol and loaded in an HPLC loop. ¹¹C-Methyl triflate is then passed through the HPLC loop for 3 min, whereby reaction occurs to produce ¹¹C-raclopride (Shao et al. 2013). The product is purified by HPLC giving a purity of greater than 98%. The specific activity should be in the range of 0.5–2 Ci/μmol (18.5–74 GBq/μmol). The product at pH between 4.5 and 8.5 remains stable for more than 1 h at room temperature. The molecular structure of ¹¹C-raclopride is shown in Fig. 8.4e.

¹¹C-Raclopride is primarily used to detect various neurological and psychiatric disorders, such as schizophrenia, Parkinson's disease, etc.

¹¹C-Choline

¹¹C-Choline is prepared by the reaction between the precursor ¹¹C-methyl iodide and dimethylaminoethanol at 130 °C for 5 min (Hara and Yuasa 1999). The reaction mixture is evaporated to remove the precursors leaving behind the residue of ¹¹C-choline, which is dissolved in water and further purified by the cation exchange

method. The final product is available as ^{11}C -choline chloride with a radiochemical yield of ~43% at the end of synthesis.

^{11}C -Choline is a highly specific PET tracer used for the detection of various tumors, specifically prostate cancer, and has been approved by the FDA for clinical use in some specific prostate cancer patients.

^{62}Cu -Pyrvaldehyde-Bis(N^4 -Methylthiosemicarbazonato) Copper(II)

^{62}Cu -Pyrvaldehyde-bis(N^4 -methylthiosemicarbazonato) copper(II) (PTSM) is prepared by mixing H_2 (PTSM) in ethanol and ^{62}Cu acetate solution at room temperature for 2–3 min (Green et al. 1990). The mixture is passed through C_{18} Sep-Pak column, and finally ^{62}Cu -PTSM is eluted from the column with ethanol followed by filtration through a 0.2- μm filter. The radiochemical yield is nearly 50% (without decay correction).

^{62}Cu -PTSM is used for measurement of myocardial perfusion by PET.

^{68}Ga -DOTA-Peptides

^{68}Ga -labeled peptides such as octreotide (DOTATOC) and octreotate (DOTATATE) are obtained by mixing the DOTA-peptides in 0.01 M acetic acid solution and $^{68}\text{GaCl}_3$ solution with 1.25 M Na-acetate in a small volume (Breeman et al. 2005). The reaction is allowed in a temperature-controlled heating block at a pH of 4. HPLC is employed to purify the labeled peptide, which is then filtered through a 0.22 μm Millipore filter for sterilization. The molecular structure is shown in Fig. 8.4g.

^{68}Ga -DOTATOC and ^{68}Ga -DOTATATE are used for PET imaging of neuroendocrine tumors and more advantageous than ^{111}In -octreotide because imaging time with the former is only a few hours compared to 2–3 days with the latter. The FDA has recently approved ^{68}Ga -DOTATOC as an orphan drug for specific use in neuroendocrine tumor imaging. An orphan drug is stipulated in relatively rare diseases that affect less than 200,000 people or for limited clinical use. This will ultimately lead to final approval for routine clinical use.

^{82}Rb -Rubidium Chloride

^{82}Rb -Rubidium chloride is available from the ^{82}Sr – ^{82}Rb generator, which is manufactured and supplied monthly by Bracco Diagnostics, Inc. The activity in

the column is typically 90–150 mCi (3.33–5.55 GBq) ^{82}Sr at calibration time. ^{82}Rb is eluted with saline and must be checked for ^{82}Sr and ^{85}Sr breakthrough daily before the start of its use for patient studies. The allowable NRC limit for ^{82}Sr is 0.02 $\mu\text{Ci}/\text{mCi}$ or 0.02 kBq/MBq of ^{82}Rb and the limit for ^{85}Sr is 0.2 $\mu\text{Ci}/\text{mCi}$ or 0.2 kBq/MBq of ^{82}Rb . Measurement of ^{82}S and ^{85}Sr breakthrough is described later in Chap. 14. Since ^{82}Rb has a short half-life of 75 s, it is administered to the patient by an infusion pump (see Chap. 14). The administered activity is the integrated activity infused at a certain flow rate over a set time, which is provided on the printout from a printer. The generator is supplied with calibrated activity enough for a month's or 6 weeks' study with ^{82}Rb . Because of the long half-life of ^{82}Sr , the generator can be shipped to remote places.

The FDA approved it in 1989 for clinical use. Now ^{82}Rb is routinely used for myocardial perfusion imaging to delineate ischemia from infarction. A detailed protocol for its clinical use in patients in the USA has been included in Chap. 13.

Quality Control of PET Radiopharmaceuticals

The FDA mandates that synthesis of all PET radiopharmaceuticals including those described above for human administration must be carried out in sterile and clean environment, following recommendations given in FDA 21CFR212, which is described in detail in Chap. 9. All PET radiopharmaceuticals must undergo a set of quality control tests prior to human administration, which are described below. [US Pharmacopeia \(USP\) 35](#) Chapter <823> provides the recommended methods for all these tests to ensure drug identity, strength, quality, purity, and patient safety. Because of the short half-lives of positron emitters used in PET imaging, some quality control tests have been grouped into two categories: those with nuclides with $t_{1/2} > 20$ min and those with nuclides with $t_{1/2} < 20$ min. In the case of the former, each production on a given day is considered a batch and all quality control tests must be performed for the batch; while in the latter group, a batch is defined as all related subbatches of PET radiopharmaceuticals compounded on a given day. The first subbatch (for $t_{1/2} < 20$ min) is designated as the *initial quality control subbatch* for the day, which is considered good for all quality control tests for all subsequent subbatches.

(a) The following quality control tests must be performed for all PET radiopharmaceuticals prior to release for human use:

- Visual inspection.
- pH.
- Radionuclidic purity.
- Radiochemical purity.
- Chemical purity.
- Specific activity.
- Isotonicity.

- Limits for residual solvents or toxic chemicals.
 - A bubble point test or filter integrity test to check the structural integrity of the membrane filter immediately after filtration but prior to release of the product. (This is done by applying air pressure through the filter until the validated bubble point is reached. Filter integrity is indicated by the absence of a steady stream of bubbles. Maximum pressure to be applied is specified on the filter.)
 - A 20-min endotoxin test on each batch ($t_{1/2} > 20$ min) or quality control subbatch ($t_{1/2} < 20$ min).
 - Sterility tests on each individual batch ($t_{1/2} > 20$ min) or quality control subbatch ($t_{1/2} < 20$ min) initiated within 24 h (USP 35 Chapter <823>) or 30 h (FDA 21CFR 212) of sterility filtration.
 - Toxicity.
- (b) Written procedures for each quality control test must be established.
- (c) Verification testing of all equipment (e.g., dose calibrator) and procedures must be carried out to comply with the acceptance limit. The results must be recorded, signed, and dated by the individual carrying out the test.

Methods of Quality Control

In this section, the methods of quality control tests for these products are briefly described below. Since PET radiopharmaceuticals are short-lived, some lengthy tests cannot be performed prior to release for human use and are performed within a short time after the release.

The quality control tests can be divided into two categories: physicochemical tests and biological tests. Refer to Saha (2010) for detailed description of these methods. These tests are briefly outlined below.

Physicochemical tests. Physicochemical tests include the tests for the physical and chemical parameters of a PET radiopharmaceutical, namely, physical appearance, isotonicity, pH, radionuclidic purity, chemical purity, and radiochemical purity.

Physical appearance. Physical appearance relates to the color, clarity, or turbidity of a PET radiopharmaceutical and should be checked by visual inspection of the sample.

pH. The pH of a PET radiopharmaceutical for human administration should be ideally 7.4, but both slightly acidic and basic pH values are tolerated due to the buffer capacity of the blood. The pH can be adjusted by adding appropriate buffer to the solution.

Isotonicity. Isotonicity is the ionic strength of a solution, which is mainly adjusted by adding appropriate electrolytes. Normally PET radiopharmaceuticals have appropriate isotonicity for human administration.

Radionuclidic purity. The radionuclidic purity of a radiopharmaceutical is the fraction of total activity in the form of the desired radionuclide in the sample. These impurities primarily arise from the radionuclides produced by various nuclear reactions in a target as well as the impurities in the target material. Using a multichannel spectrometer, one can determine the level of impurities in a sample of a positron-emitting radionuclide produced by a specific nuclear reaction in a cyclotron. Using highly pure target material and appropriate chemical separation techniques, the radionuclidic impurity can be minimized to an acceptable level. Short-lived radionuclides can be allowed to decay to have a pure long-lived radionuclide in question. Even though the impurities in the routine preparations of PET radionuclides do not vary significantly from batch to batch, periodic checkup is recommended to validate the integrity of the method of production. The radionuclidic impurities must be established in ^{11}C , ^{13}N , ^{15}O , and ^{18}F radionuclides, prior to their use in the synthesis of radiolabeled compounds.

Chemical purity. Chemical purity is the fraction of a radiopharmaceutical in the desired chemical form, whether all of it is radiolabeled or not. The presence of extraneous stable atoms may cause adverse reactions and is not desirable in a PET radiopharmaceutical. These impurities arise from the incomplete synthesis, addition of extraneous ingredients during the synthesis, and so on. Chemical methods such as the spectrophotometric method, ion exchange, solvent extractions, chromatography, etc., are applied to measure the level of these chemical impurities. Again, these tests can be performed a priori in many dry runs and thus the level of chemical impurities can be established, prior to human administration.

Radiochemical purity. The radiochemical purity of a radiopharmaceutical is defined as the fraction of the total activity in the desired chemical form in the sample. These impurities arise from incomplete labeling, breakdown of the labeled products over time due to instability, and introduction of extraneous labeled ingredients during synthesis. These impurities cause altered in vivo biodistribution after administration, resulting in an unnecessary radiation dose to the patient. For these reasons, the USP and the US Food and Drug Administration have set limits on the impurities in various radiopharmaceuticals, and these limits must not be exceeded in clinical operations.

Since most PET radiopharmaceuticals are produced on site daily, the radiochemical purity must be checked for each batch. For very short-lived radionuclides, however, the methodology must be validated beforehand by carrying out many dry runs so that the radiochemical purity of the product remains within the limit set for human administration.

Several analytical methods are employed to establish the radiochemical purity of PET radiopharmaceuticals. The most common method is HPLC, which gives separation of components with high resolution. The general principle of HPLC involves forcing a sample at high pressure (6000 psi) by a pump through a column (15–30 cm long and 2–5 mm inner diameter) of special packing material by an electric pump, whereby various components pass out of the column at different rates depending on their molecular weights. The eluate is passed through a sensitive ultraviolet

(UV) detector to identify different components, which are then quantitated by the UV absorbance peaks. Alternatively, fractions of the eluate are collected and the activity in each fraction is measured in a gamma counter.

Another common method of determining the radiochemical purity is the thin-layer chromatography (TLC). In this method, a drop of the radiopharmaceutical sample is spotted on a solid-phase paper strip (e.g., silica gel, Whatman), and then the paper is placed vertically in a small vial containing a small amount of an appropriate solvent, taking care that the spotted area remains above the solvent. The solvent flows along the paper strip, and different components of the sample will flow at different rates along the strip depending on their solubility in the solvent. The ratio of the distance traveled by a component to the distance traveled by the solvent front is called the R_f value. When the solvent front reaches the top of the strip, the strip is removed and scanned with radiochromatographic scanner for the distribution of components along the strip. Alternatively, the strip is cut into several segments (e.g., ten segments), and the activity in each segment is measured in a gamma counter. From the counts of the segments, the radiochemical purity can be calculated.

Biological tests. Biological tests include sterility testing, pyrogen testing, and toxicity testing.

Sterility. Sterility indicates the absence of any viable bacteria or microorganisms in a radiopharmaceutical preparation. All radiopharmaceuticals must be sterile prior to administration to humans, and it is normally accomplished by filtering the product through a 0.22- μm membrane filter or heating the sample to 120°C for 20 min at a pressure of 18 psi. PET radiopharmaceuticals are normally sterilized by filtration because of their short half-life.

Sterility tests are normally performed by incubating the sample with fluid thioglycollate medium at 30–35°C for 14 days or with soybean–casein digest medium at 20–25°C for 14 days. The sample volume should be as large as that for human dosage. If bacterial growth is observed in either test, the preparation is considered *asterile*. For PET radiopharmaceuticals, these tests must be started within 24 h (per USP 35 Chapter <823>) or 30 h (per FDA 21CFR212) after the release of the product.

Pyrogenicity. Pyrogens are bacterial endotoxins consisting of polysaccharides or proteins produced by the metabolism of microorganisms, and upon administration they cause undue symptoms such as fever, flushing, chill, sweating, malaise, etc. These symptoms typically set in 30 min to 2 h after administration and are rarely fatal. There are no specific methods of making a preparation pyrogen-free, and the only way to avoid pyrogens is to strictly follow the method of preparation employing meticulous aseptic technique so that microbes are not introduced into the sample.

Tests for pyrogens include a rabbit test, in which rabbits are administered with the radiopharmaceutical and their rectal temperatures are monitored. From the rise in temperature in the rabbits, pyrogenicity of a sample is determined. However, a

simpler and quicker method is the so-called limulus amebocyte lysate (LAL) test, also called the bacterial endotoxin test (BET). In this test, the lysate of amebocytes from the blood of the horseshoe crab (*Limulus polyphemus*) is mixed with the sample and incubated at 37°C. An opaque gel is formed within 15–60 min depending on the concentration of pyrogens.

Toxicity. The toxicity of a radiopharmaceutical causes alteration in the histology or physiologic functions of an organ or even death of a species after in vivo administration. It is commonly characterized by LD_{50/60}, which is defined as the quantity of a sample that kills 50% of the species within 60 days after administration. For a radiopharmaceutical, it must be established at least in two species (e.g., rats and rabbits) before human administration, and the dosage for the humans is decided by a large safety factor. Toxicity arises from the pharmaceutical part and most PET radiopharmaceuticals are not toxic for human administration.

Questions

1. Describe in detail the method of ¹⁸F-FDG synthesis.
2. Describe the method of synthesis of (a) 6-¹⁸F-L-fluorodopa, (b) ¹⁸F-fluorothymidine, and (c) ¹¹C-L-methionine.
3. What is the difference between the nucleophilic and electrophilic reaction?
4. What are the clinical uses of ¹⁸F-FDG, ¹⁸F-FLT, 6-¹⁸F-L-fluorodopa, ¹⁵O-water, ¹³N-ammonia, ¹¹C-sodium acetate, ¹⁸F-florbetapir, and ¹¹C-methylspiperone?
5. Describe the operational principles of an automated synthesis box.
6. Define (a) radionuclide purity, (b) radiochemical purity, and (c) chemical purity of a radiopharmaceutical.
7. Describe the method of determining the radiochemical purity of an ¹⁸F-FDG sample.
8. Describe the methods of sterilizing radioactive samples.
9. Describe the methods of sterility tests and pyrogen tests.
10. A sterile sample is always pyrogen-free. True ____; False ____.
11. A LAL test is required for every batch prior to release of PET radiopharmaceuticals ($t_{1/2} < 20$ min) for human use. True ____; False ____.
12. Sterility test of PET radiopharmaceuticals must begin (a) prior to release, (b) within 24 h of release, (c) anytime after release, or (d) is not required.

References and Suggested Reading

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