Synthesis of PET Radiopharmaceuticals

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 6.2) 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.

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 \( (t_{1/2} = 110\text{min}) \) that allows its supply to 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. For example, \(^{82}Rb\) is used as a PET radiotracer in the form of \(^{82}Rb\)-RbCl that is available from the \(^{82}Sr\)-\(^{82}Rb\) generator. The following is a description of the syntheses of the common clinically used PET radiopharmaceuticals and a few with potential for future use.

PET Radiopharmaceuticals

\textbf{\(^{18}F\)-Sodium Fluoride}

Fluorine-18 \( (t_{1/2} = 110 \text{ minutes}) \) is produced by irradiation of \(^{18}O\)-water with 10 to 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 passes through, whereas \(^{18}F^-\) is...
retained on the column, which is recovered by elution with potassium carbonate solution. Its pH should be between 4.5 to 8.0. While $^{18}$F-sodium fluoride is most commonly used for the synthesis of $^{18}$F-fluorodeoxyglucose, it is also used for other $^{18}$F-labeled PET radiopharmaceuticals. The U.S. FDA has approved it for bone scintigraphy, since it localizes in bone by exchanging with $\text{PO}_4^-$ ion in the hydroxyapatite crystal.

$^{18}$F-Fluorodeoxyglucose (FDG)

$^{18}$F-2-fluoro-2-deoxyglucose (2-FDG) is normally produced in places where a cyclotron is locally available. Its molecular formula is $\text{C}_8\text{H}_{11}\text{FO}_5$ with molecular weight of 181.3 daltons. $^{18}$F-2-FDG can be produced by electrophilic substitution with $^{18}$F-fluorine gas or nucleophilic displacement with $^{18}$F-fluoride ions. The radiochemical yield is low with the electrophilic substitution, so the nucleophilic displacement reaction has become the method of choice for $^{18}$F-FDG synthesis. Deoxyglucose is labeled with $^{18}$F 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-$\text{O}$-acetyl-2-$\text{O}$-trifluoromethane-sulfonyl-$\beta$-D-mannopyranose in anhydrous acetonitrile is added to a dry residue of $^{18}$F-fluoride containing aminopolyether (Kryptofix 2.2.2) and potassium carbonate (Figure 7-1). 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 minutes. The solution is then passed through a C-18 Sep-Pak column, and acetylated carbohydrates are eluted with tetrahydrofuran (THF), which are then

![Schematic synthesis of $^{18}$F-2-Fluoro-2-deoxyglucose (FDG). (Reprinted with the permission of the Cleveland Clinic Foundation.)](image-url)
hydrolyzed by refluxing in hydrochloric acid at 130°C for 15 minutes. \(^{18}\text{F}\)-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 minutes. The final solution is filtered through a 0.22 \(\mu\)m filter and diluted with saline, as needed. It should have a pH of 7.0.

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.

\(^{18}\text{F}\)-2-FDG is used primarily for the study of metabolism in the brain and heart, and for the detection of epilepsy and various tumors. In metabolism, \(^{18}\text{F}\)-2-FDG is phosphorylated by hexokinase to 2-FDG-6-phosphate which is not metabolized further. It should be noted that 3-fluoro-deoxyglucose (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.

### 6-\(^{18}\text{F}\)-L-Fluorodopa

Like \(^{18}\text{F}\)-2-FDG, 6-\(^{18}\text{F}\)-L-fluorodopa is also produced in places where a cyclotron is available locally. There are several methods of synthesizing 6-\(^{18}\text{F}\)-fluoro-3,4-dihydroxyphenylalanine (6-\(^{18}\text{F}\)-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-trifluoroacetoxymercuriophenylalanine ethyl ester) of dopa is prepared. \([^{18}\text{F}]\)-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-\(^{18}\text{F}\)-L-fluorodopa (Luxen et al., 1987), compared to 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 \(\mu\)m membrane filter, and is supplied at pH between 4.0 and 5.0. Normally EDTA and ascorbic acid are added to the final preparation for stability. The molecular structure of 6-\(^{18}\text{F}\)-L-fluorodopa is shown in
Figure 7-2A. 6-18F-L-fluorodopa is used for the assessment of the presynaptic dopaminergic function in the brain.

18F-Fluorothymidine (FLT)

18F-fluorothymidine (FLT) is prepared by nucleophilic reaction between 18F-sodium fluoride and a precursor, 2,3¢-anhydro-5¢-0-benzoyl-2¢-deoxythymidine, which is prepared by standard organic synthesis (Machulla 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 minutes. The precursor in dimethyl sulfoxide (DMSO) is added to the dried residue and heated at 160°C for 10 minutes. 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 HPLC. The overall yield is about 45% and the radiochemical purity is more than 95%. The synthesis time is about 60 minutes.

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.

15O-Water

15O-oxygen ($t_{1/2} = 2$ minutes) is produced in the cyclotron by the $^{15}$N(p, n)$^{15}$O reaction, or the $^{14}$N(d, n)$^{15}$O reaction, and the irradiated gas is transferred over a palladium/charcoal catalyst at 170°C (Meyer et al., 1986; Welch and Kilbourn, 1985). The H$_2^{15}$O vapor is trapped in saline, and the saline solution is filtered through a 0.22 μm membrane filter. The sample is then passed through a radiation detector for radioassay and ultimately injected on-line into a patient in a very short time.

H$_2^{15}$O 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 $^{15}$N(p, n)$^{15}$O 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 $^{15}$N target in order to recover $^{15}$O. 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 ($t_{1/2} = 10$ minutes) 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-NH$_3$ is then distilled and finally trapped in acidic saline solution. Wieland et al. (1991) have used a pressurized target of aqueous ethanol, in which ethanol acts as a hydroxyl free radical scavenger
to improve the yield of $^{13}$N-NH$_3$. The mixture is passed through an anion-exchange resin to remove all anion impurities. It is filtered through a 0.22 $\mu$m membrane filter and its pH should be between 4.5 and 7.5. The US FDA has approved it for measurement of myocardial and cerebral perfusion.

$^{11}$C-Sodium Acetate

$^{11}$C-sodium acetate is produced by the reaction of the Grignard reagent, methylmagnesium bromide in diethyl ether, with cyclotron-produced $^{11}$C-carbon dioxide at ambient temperature. After reaction, the product is hydrolyzed with water or aqueous acid, followed by further purification using the solvent extraction or ion exchange method. The solution is filtered through a 0.22 $\mu$m membrane filter. $^{11}$C-acetate has been found to be stable at pH between 4.5 and 8.5 for up to 2 hours at room temperature. The overall yield is about 75%. 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. The specific activity is very important for this product and therefore is analyzed by HPLC to give an optimum value between 0.5 and 2 Ci/µmol (18.5 to 74 GBq/µmol). It remains stable for up to 3 hours at room temperature at pH 7.0. The molecular structure of $^{11}$C-flumazenil is shown in Figure 7-2B.

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

$^{11}$C-Methylspiperone (MSP)

$^{11}$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 to 50 GBq/µmol (270 to 1350 mCi/µmol). 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 $\mu$m membrane filter and its pH
is adjusted to $7 \pm 1$ with a suitable buffer. The molecular structure of $^{11}$C-methylspiperone is shown in Figure 7-2C.

$^{11}$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.

$^{11}$C-L-Methionine

$^{11}$C-L-methionine has $^{11}$C at its methyl position and has 2 forms: L-[1-$^{11}$C] methionine and L-[S-methyl-$^{11}$C] methionine. The former is obtained by the reaction between $^{11}$C-CO$_2$ precursor and carbanion produced by a strong base added to the respective isonitrile, followed by hydrolysis with an acid. The latter is obtained by alkylation of the sulfide anion of L-homocysteine with $^{11}$C-iodomethane. The product is purified by HPLC yielding a purity of $>98\%$ and further filtered through a 0.22 $\mu$m membrane filter. The pH should be between 6.0 and 8.0 and it is stable for 2 hours at room temperature. The molecular structure of $^{11}$C-L-methionine is shown in Figure 7-2D.

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

$^{11}$C-Raclopride

Raclopride is labeled with $^{11}$C either by N-ethylation with [1-$^{11}$C] iodoethane or by O-methylation with [$^{11}$C] iodomethane, although the latter is more suitable for routine synthesis. Both $^{11}$C-labeled iodoethane and iodomethane are prepared from $^{11}$C-CO$_2$. The product is purified by HPLC giving a purity of greater than 98%. The specific activity should be in the range of 0.5 to 2 Ci/µmol (18.5 to 74 GBq/µmol). The product at pH between 4.5 and 8.5 remains stable for more than 1 hr at room temperature. The molecular structure of $^{11}$C-raclopride is shown in Figure 7-2E.

$^{11}$C-raclopride is primarily used to detect various neurological and psychiatric disorders, such as Parkinson’s disease, schizophrenia, etc.

$^{82}$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. The activity in the column is typically 90 to 150 mCi (3.33 to 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 limit for $^{82}$Sr is 0.02 μCi/mCi or 0.02 kBq/MBq of $^{82}$Rb and the limit for $^{85}$Sr is 0.2 μCi/mCi or 0.2 kBq/MBq of $^{82}$Rb. Since $^{82}$Rb has a short half-life of 75 seconds, it is administered to the patient by an infusion pump (see Chapter 11). The administered activity is the integrated activity infused at
a certain flow rate for a period of time set by the operator, which is pro-
vided on the printout by a printer.

$^{82}$Rb is approved by the FDA for myocardial perfusion imaging to delin-
eate ischemia from infarction.

Automated Synthesis Devices

Conventional manual methods of synthesis of radiopharmaceuticals using
a high level of radioactivity are likely to subject the persons 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 con-
trolled by microprocessors and software programs to carry out the sequen-
tial physical and chemical steps to accomplish the entire synthesis of a
radiolabeled product. The unit consists of templates or vials pre-filled with
required chemicals attached to the apparatus via tubings that are connected
to solenoid values to switch on and off as needed. Most black boxes are
small enough to be placed in a space of 20 × 20 × 20 inches, and are capable
of self-cleaning. In some units, disposable cassettes are employed so that
new cassettes can be used for each new synthesis. Various parameters for
synthesis such as time, pressure, volume and other requisites are all con-
trolled by a remote computer. The unit has a graphic display showing the
status of the on-going process. After the synthesis, a report with the date,
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$_3$I, $^{11}$C-HCN, $^{11}$C-acetate, and a few
other PET tracers are commercially available. A schematic diagram of a
black box for $^{18}$F-FDG synthesis is shown in Figure 7-3. An automated FDG
synthesis module marketed by CTI Molecular Imaging, PET NET is shown
in Figure 7-4. Other vendors include GE Medical Systems (Tracerlab),
Siemens, Bioscan, EBCO and Sumitomo.

Quality Control of PET Radiopharmaceuticals

As with conventional drugs, PET radiopharmaceuticals must be tested for
chemical purity, radionuclidic purity, radiochemical purity, pH, isotonicity,
sterility, apyrogenicity, and toxicity prior to administration to humans.
Because PET radiopharmaceuticals are short-lived, many tests cannot be
performed in a short time just before administration. In these cases, quality
control tests are performed on the products using “dry runs” without
administration to patients and the method of production is validated. At
Figure 7-3. 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. Radiochemistry automation PET. In: Stöcklin G, Pike VW, eds. Radiopharmaceuticals for Positron Emission Tomography. Dordrecht, The Netherlands, Kluwer Academic; 1993; p. 64. Fig. 9.)

Figure 7-4. An automated $^{18}$F-FDG synthesis box, Explora-FDG4, manufactured by CTI Molecular Imaging, PET NET. (Courtesy of CPS Innovations, Knoxville, TN, USA.)
times, tests for very short-lived PET tracers may need to be performed on an "after-the-fact" basis.

The quality control tests can be divided into two categories: physicochemical tests and biological tests. Refer to Saha (2004) 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 purity can be minimized to an acceptable level. Short-lived radionuclides can be allowed to decay to have a pure relatively 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 form of the desired chemical molecule 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 United States Pharmacopeia (USP) and the United States 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.

Various analytical methods are employed to establish the radiochemical purity of PET radiopharmaceuticals. The most common method is high-performance liquid chromatography (HPLC), which gives separation of components with high resolution. The general principle of HPLC involves forcing a sample at high pressure through a column 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 fractions are collected at different times. The different components can then be identified and quantitated either by counting the radioactivity in each fraction with a counter, or by measuring their absorbance with an ultraviolet (UV) monitor.

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 jar 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 for the distribution of components along the strip. Alternatively, the strip is cut into several segments (e.g., 10 segments), and the activity in each segment is measured by a 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 minutes at a pressure of 18 pounds per square inch. 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° to 35°C for 14 days or with soybean-casein digest medium at 20° to 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 are performed “after the fact” because of their short half-life.

**Pyrogenicity:** Pyrogens are polysaccharides or proteins produced by the metabolism of microorganisms, and upon administration, cause undue symptoms such as fever, flushing, chill, sweating, malaise, etc. These symptoms typically set in 30 minutes to 2 hours 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. 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 to 60 minutes depending on the concentration of pyrogens. For PET radiopharmaceuticals, these tests are performed “after the fact”.

**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*<sub>50/60</sub>, which is defined as the quantity of a sample that kills 50% of the species within 60 days after administration. It must be established at least in two species before human administration, and the dosage to 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.

**USP Specifications for Routine PET Radiopharmaceuticals**

*<sup>18</sup>F-fluorodeoxyglucose*

**Appearance:** Clear

**pH:** 4.5 to 7.5
Specific Activity: Not less than 1Ci (37GBq)/μmol
Radionuclidic Purity: Not less than 99.5% should correspond to 511keV, 1.022MeV or Compton scatter peaks of $^{18}$F.
Chemical Purity: Major impurities are Kryptofix 2.2.2 and 2-chloro-2-deoxy-D-glucose, which are determined by TLC. Kryptofix 2.2.2 should not exceed 50μg/ml of the sample volume, and 2-chloro-2-deoxy-D-glucose should not exceed 1mg per total volume of the batch produced.
Radiochemical Purity: It is determined by TLC using activated silica gel as the solid phase and a mixture of acetonitrile and water (95:5) as the solvent. The $R_f$ value of $^{18}$F-FDG is 0.4. The radiochemical purity should be more than 90%.

$^{18}$F-L-Fluorodopa
Appearance: Clear
pH: 6 to 7
Specific Activity: Not less than 100mCi (3.7GBq)/mmol
Radionuclidic Purity: Not less than 99.5% correspond to 511 keV, 1.022 MeV or Compton scatter peaks of $^{18}$F, with no individual impurity present more than 0.1%.
Chemical Purity: Since the most common method of production utilizes organo mercury precursor, mercury is the major toxic impurity. It is determined by atomic absorption spectrometry and its USP limit is 0.5μg/ml of L-dopa solution.
Radiochemical Purity: It is determined by the HPLC method or ion pair chromatography. The USP limit is 95% of the total radioactivity in the form of $^{18}$F-L-fluorodopa.

$^{13}$N-Ammonia
Appearance: Clear
pH: 4.5 to 7.5
Specific Activity: no carrier added
Radionuclidic Purity: Not less than 99.5% should correspond to 511 keV, 1.022 MeV and Compton scatter of $^{13}$N.
Chemical Purity: Aluminum and titanium are the common impurities, determined by colorimetric methods. The USP limit of Al$^{3+}$ is 10μg/ml of the solution.
Radiochemical Purity: It is determined by the HPLC method. The radiochemical yield should be greater than 95%.
Questions

1. Describe in detail the method of $^{18}$F-FDG synthesis.
2. Describe the method of synthesis of: (a) $^{6-18}$F-L-Fluorodopa; (b) $^{18}$F-fluorothymidine; (c) $^{11}$C-L-methionine.
3. What is the difference between the nucleophilic and electrophilic reaction?
4. What are the clinical uses of $^{18}$F-FDG, $^{18}$F-FLT, $^{6}$-18F-L-Fluorodopa, $^{15}$O-Water, $^{13}$N-Ammonia, $^{11}$C-Sodium acetate, and $^{11}$C-Methylspiperone.
5. Describe the operational principles of an automated synthesis box.
6. Define (a) radionuclide purity, (b) radiochemical purity, (c) chemical purity of a radiopharmaceutical.
7. Describe the method of determining the radiochemical purity of a $^{18}$F-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 ______.

References and Suggested Reading

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Physics, Chemistry, and Regulations
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