Illustrative Review on Radiopharmaceuticals

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ABSTRACT
Radiopharmaceutical is a key component involved in the field of nuclear medicine. It serves various purposes diagnostically and also serves with different diagnostic applications. Radioactive agents are employed in nuclear field for demonstration of high and exact localized radioactive effect in a particular target tissue. In recent years various amount of radionuclides and radiopharmaceuticals are utilized for treating cancer and other complex disease like neuroendocrine disorder. This review focuses on the manufacturing, quality control tests and diagnostic applications of radiopharmaceuticals.

KEYWORDS: Radioactivity, Radiopharmaceuticals, Radionuclide

INRODUCTION
Radioactivity: Radioactivity is termed as a spontaneous emission of radiation which occurs in the form of particles or high energy photons resulting from a nuclear reaction. Radioactivity is also known as nuclear decay, radioactive decay, nuclear disintegration, or radioactive disintegration. There are many forms of electromagnetic radiation but they are not always produced by radioactivity. Radioactive is a substance that contains unstable atomic nuclei. Radioactive decay is a random process which occurs at the level of individual atoms. Based on decay constants or half-lives the rate of decay of a group of atoms may be predicted, while it is impossible to predict exactly when a single unstable nucleus will decay. The time required for half of the sample of matter to undergo radioactive decay is called as half-life.

Radiopharmaceuticals: A radiopharmaceutical is regarded as a radioactive drug which is used for diagnosis or therapy in a tracer quantity. It is comprised of two parts; a radionuclide and pharmaceutical. Pharmaceutical drugs with radioactivity property can be used as Diagnostic and therapeutic agents. Radiopharmaceuticals are drugs which contain radioactive materials called radioisotopes. Radiopharmaceuticals can be administered by different routes such as taken by mouth, inserted into a vein, or placed in a body cavity. Radiopharmaceutical materials travel to different parts of the body for treating cancer or relieve its symptoms. In small amounts radiopharmaceuticals are commonly used for imaging tests, but larger doses can be used to deliver radiation. Radiopharmaceuticals are also used in the diagnosis different diseases like infection, abscess and, biliary tract blockage, Blood volume studies and diseases, Blood vessel diseases of the brain, Bone diseases, etc.


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Manufacturing of Radiopharmaceutical Dosage Form

Radiopharmaceutical Dosage forms
A radiopharmaceutical dosage form is termed as a solid, liquid or gaseous formulation comprising of radioisotopes, present in a ready to use form, which are safe for administration in humans for diagnosis or for therapeutic purpose. A radioactive component is present along with a drug component. Inorganic compounds, organic compounds, proteins, peptides, monoclonal antibodies, fragments and oligonucleotides labeled with radionuclide are included under Radiopharmaceutical products. The half-life of a radiopharmaceutical ranges from a few minutes to several days. The provision of static and dynamic images of internal organs in a non-invasive manner along with therapeutic efficacy is provided by Radiopharmaceutical dosage form.

Manufacturing of Radiopharmaceutical Dosage Forms
The manufacturing process for radiopharmaceutical preparations should meet the requirements of Good Manufacturing Practices.

Radiopharmaceuticals are prepared in three steps.
1: Production of Radionuclide (radioactive isotope)
2: Preparation of Radiopharmaceutical
3: Preparation of Radiopharmaceutical kit
1. Production of Radionuclide (radioactive isotopes)
   - Artificial production of Radionuclides by the process of nuclear activation in a nuclear reactor.
   - In such a reactor stable atoms are bombarded with excess neutrons. The resulting addition of neutron to stable atoms produces radionuclide.
   - Methods of producing radionuclide are as follows:
     A. Nuclear fission
     B. Neutron reactions
     C. Cyclotron method

A. Nuclear fission
   - Nuclear Fission is termed as a radioactive process in which a heavy nucleus split into two new nuclei of almost equal size with simultaneous emission of two or three neutrons.
   - Fission is induced by bombardment of the parent nucleus with a neutron.
     \[^{235}_{92}\text{U} + ^{1}_{0}\text{n} \rightarrow X + Y + 2.5n\]
     X and Y are the radioactive isotopes formed.
   - For each neutron consumed, an average of 2.5 new neutrons are produced that may initiate the fission of other nuclei. Such a reaction is called chain reaction.
     \[^{238}_{92}\text{U} + ^{1}_{0}\text{n} \rightarrow ^{131}_{50}\text{Sn} + ^{106}_{42}\text{Mo} + ^{1}_{0}\text{n} + ^{1}_{0}\text{n}\]
   - Sn (Tin) and Mo (Molybdenum) are radioactive and decay further emitting beta particles.
     \[^{131}_{50}\text{Sn} \rightarrow ^{131}_{51}\text{Sb} \rightarrow ^{131}_{52}\text{Te} + ^{131}_{53}\text{I}\]
   - I-131 (Iodine-131) is used for medical applications.
   - In this process, separation of the desired radioactive element from the mixture is difficult and costly.
B. Neutron Reactions (Neutron Bombardment)
- The preparation of radionuclide is done by neutron activation or through transmutation reactions.
- An acceptable target material is bombarded by neutrons in a nuclear reactor.
- Radioactive phosphorus can be made by transmutation.

$$^{32}_{16}S + ^1n \rightarrow ^{32}_{15}P + ^1P$$

The radioactive phosphorus can be separated by chemical process.

C. Cyclotron Method (Charged Particle Bombardment)
- The cyclotron or particle accelerators can be used only with charged particles like electrons, protons and alpha particles, as operation of the machine is depend upon the interaction of magnetic or electrostatic fields with the charge of the particle which is undergoing acceleration.
- Particles when accelerated to a very high velocity, they are caused to strike a target comprising of the atoms to be bombarded.

2. Preparation of Radiopharmaceutical
Radiopharmaceuticals are prepared by following two methods,
A. Radionuclide Generator
B. Radiolabeling Method

A. Radionuclide Generators
It is advantageous to use a nuclide with short half-life to minimize the radiation dose received by the patient, when radioactive isotopes are to be used clinically. Greater problems of supply are caused due to shorter half-life so radionuclide generator is used. It provides a mechanism for separating a clinically useful, short half-life daughter from a long-lived parent nuclide which is used later for the production of radiopharmaceutical dosage form.

**e.g.:** Molybdenum 99/Technetium 99m generator: It consists of an alumina column on which Molybdenum 99 is adsorbed as Ammonium molybdate.

B. Radiolabeling method
Some radionuclide can be used in their elemental or salt form for medical or pharmaceutical purpose. However, most radionuclide must be incorporated into some molecule or compound to form a useful radiotracer or radiopharmaceutical by a process known as radiolabeling. Carrier addition permits ready handling of the radiopharmaceutical. In some conditions it will be important to add carrier to enhance chemical, physical or biological properties of the radiopharmaceutical preparation. The carrier may be in the form of inactive material, either isotopic with the radionuclide, or non-isotopic, but chemically similar to the radionuclide.

Radiolabeling is done by the following methods

**I. Introduction of a foreign label:** A radioactive isotope is chelated with other compounds to use it medically.

**II. Isotope exchange:** Radioactive isotope is substituted for a stable atom of the same element that is already a natural part of the molecule.

**III. Labelling with bifunctional chelates:** A bifunctional chelate is a molecule used to link another molecule with a radionuclide.

IV. Biosynthesis: A radionuclide is incorporated into a molecule through some biosynthetic process.

3. Preparation of Radiopharmaceutical Kit
A radiopharmaceutical kit permits the radio pharmacist for transforming the radioactive isotope obtained from the generator into the desired radiopharmaceutical. It is a vial containing freeze dried and sterile non-radioactive components into which the appropriate radionuclide is added or in which the appropriate radionuclide is diluted before medical use. The nonradioactive ingredients of a normal formulation are ligands, reducing agents, stabilizers, buffers and antioxidants. The freeze dried kit is reconstituted through aseptic transfer of the necessary close of the radioactive isotope using a sterile syringe or needle. The amount of activity withdrawn for the reconstitution of the kit is depends on the number of patient doses to be manufactured. The reconstituted kit is aseptically divided to provide each patient a dose with sufficient activity. Radiopharmaceutical preparations obtain from kits are normally intended for use within 12 hours of preparation.

The radiopharmaceutical kit consists of
- Methylenediphosphonate: a ligand forming a complex with Technetium.
- Stannous ions (Stannous chloride or fluoride): a reducing agent which enhances the complication of ligand with Technetium.
- Other compounds such as stabilizers, buffers and antioxidants.
- Technetium complex obtained from the radiopharmaceutical kit after reconstitution should be used within 8 hours after production.

Radiopharmaceutical Preparations
Following are some radiopharmaceutical preparations used for diagnostic and therapeutic purpose.

1. Cobalt 57 (Co-57) and Cobalt 60 (Co-60)
Cobalt in cyanocuproamine (Vitamin B12) is replaced either by radioactive cobalt 57 or cobalt 60. They are available as capsules and injection.

Cobalt- 57 may be prepared by irradiating Nickel 58(Ni-58) with gamma rays or by bombarding Iron 56 (Fe-56) with protons. Its half-life is 270 days.

Cobalt- 60 is prepared by bombarding stable Cobalt 59 by a neutron. Its half-life is 5.27 years. It emits both beta and gamma particles.

Cyanocobalamin Co-57 or Co-60 is used in diagnosis of pernicious anemia. Cobalt 60 is converted into needles, wires or seeds and implanted in the body cavity or directly into tumor. It is used in the treatment of the advanced stages of cancer of cervix and vagina.

2. Iodine 125 (I-125) and Iodine 131(I-131)
They are available as capsules, solutions and injection. Iodine 131 emits both beta and gamma radiations. Its half-life is 8.08 days it is obtained as a by-product of Uranium fission. It can also be prepared by bombarding Tellurium 130 by neutrons is used in diagnosis of functioning of thyroid gland. The size, position of the gland and possible tumor location can be assessed. Iodine 131 is used therapeutically
to destroy thyroid tissue in cases of thyroid carcinoma, hyperthyroidism, thyrotoxicosis and severe cardiac disease. In heart diseases like angina pectoris and congestive heart disease, it is used to induce a hypothyroid state as a means of reducing the work load on the heart. Iodinated Serum Albumin (1-125II-131) is used to obtain circulating blood volume. It is also used for diagnosis of pulmonary blood flow, lung and bronchial tumors.

3. Iron 59 (Fe-59)
It is prepared by neutron activation of Iron 58. It emits beta and gamma radiations. Its half-life is 45 days. It is used in diagnosis of problems related to iron metabolism and RBC formation. It is administered orally as Sterile Ferrous citrate solution to study absorption of iron from gastrointestinal tract. It is given intravenously to study plasma iron clearance and incorporation of iron into erythrocytes.

4. Gold 198 (Au-198)
It is available in injection form which is a sterile, colloidal suspension of metallic gold. It emits both beta and gamma particles. Its short half-life is 2.7 days. It is prepared by bombarding stable Au-197 by neutrons. It is used for scintillation scanning of liver which helps in determining the shape, position and size of the organ. It helps to assess functioning of Kupffer's cells in the liver. Therapeutically, it is used in the management of pleural effusion (accumulation of serous fluid in the pleural cavity), ascites (accumulation of serous fluid in the peritoneal cavity) and rheumatoid arthritis.

5. Barium sulphate
Barium sulphate is used as a radiopaque contrast medium. Radiopaque contrast media are compounds or elements of high atomic number. They hinder the passage of X-rays and are used as diagnostic aid in radiology or roentgenology. Barium sulphate is available as oral solution or as an enema. If taken by mouth, it makes the oesophagus, the stomach, and/or the small intestine opaque to the X-rays so that they can be photographed. If it is given by enema, the colon and/or the small intestine can be seen and photographed by x-rays.

6. Technetium-99m
Technetium is an artificial element and all of its isotopes are radioactive. It is prepared by neutron bombardment of Molybdenum 99. It emits gamma rays. Its half-life is 6 hours. It is available as sodium pertechnetate and a colloidal preparation of Technetium sulphide. Technetium 99m labelled human serum albumin is used to obtain lung scans. It is also used to measure cerebral blood flow and for scintigraphy of the salivary glands, stomach, heart and joints. Technetium 99m glucopate is used in assessment of kidney shape, size and position. It also identifies kidney lesions.

Quality Control of Radiopharmaceuticals
It includes numerous specific tests that ensure purity, potency, product identity, biological safety, and effectiveness of radiopharmaceuticals.

Physicochemical Tests
These tests are essential for the determination of the purity and integrity of a radiopharmaceutical. Radiopharmaceuticals they contain radionuclides, therefore these tests become unique and ensure specificity for radiopharmaceuticals.

Physical Characteristics
It contain of the colour and state of a radiopharmaceutical. A real solution mustn’t be containing any particulate matter. Any deflection from the original colour and clarity should be viewed with concern as it may replicate changes in the radiopharmaceutical which would change its biological behaviour. Colloidal or aggregate preparations should have an accurate size range of particles for a given intended purpose.

pH and Ionic Strength
All radiopharmaceuticals must comprise of a proper hydrogen ion concentration or pH scale for maintaining their stability and integrity. Ideal pH scale of a radiopharmaceutical should be 7.4. Due to high buffer capacity of the blood pH ranges between 2 and 9. Radiopharmaceuticals should have correct isotonicity, Ionic strength, Osmolality so as to be appropriate for human administration. By adding a correct Acid, Alkali, or Electrolyte the right ionic strength can be achieved.

Radio nuclidic Purity
It can be defined as the fraction of the total radioactivity in the form of the desired radionuclide present in a radiopharmaceutical. Many times extraneous impurities are known to arise during the production of the radionuclides. Radionuclidic purity affects the absorbed radiation dose and image quality. It can be measured by use of gamma spectrometry, half-life measurement and other methods to detect extraneous nuclide.

Radiochemical Purity
It is defined as the fraction of the total radioactivity within the desired chemical form in the radiopharmaceutical. Radiochemical impurities arise from decomposition due to the action of solvent, Presence of oxidizing or reducing agents, Change in pH scale or temperature, light and radiolysis. Absorption of radiations by tagged molecules leads to the formation of free radicals with unpaired electrons. Which in turn leads to further decomposition of other molecules. A secondary method due to radiolysis produces H₂O₂ or HO₂ from decomposition of water (solvent) that reacts with and ultimately decomposes tagged molecules. Numerous analytical methods can be used to discover and confirm the radiochemical impurities in a given radiopharmaceutical. These methods include Precipitation, High-Performance Liquid Chromatography, Paper and Instant Thin-Layer Chromatography, Paper or Polyacrylamide Gel Electrophoresis, Gel Chromatography, etc.

Chemical Purity
The presence of chemical impurities before radiolabeling may lead to undesirable tagged molecules which can or may not interfere with the diagnostic test. The chemical impurities may also cause a toxic effect. Purification of radiopharmaceuticals from Chemical impurities is usually achieved by strategies of chemical separation methods such as Precipitation, Distillation, Solvent extraction, etc.

Radio assay
The amount of radioactivity of a radiopharmaceutical before dispensing as well as before dose of administration to
patients must be determined. These activity determinations are accomplished by means of an isotope dose calibrator. According to the Nuclear Regulatory Commission regulations the following quality control tests should be performed at the frequencies indicated:

1. **Constancy (daily)**: This test indicates the reproducibility of measurements by a dose calibrator.
2. **Accuracy (at installation, annually, and once repairs)**: The accuracy of a dose calibrator is decided by measuring the activities of minimum two long-lasting reference sources and comparing the measured activity with the expressed activity. The measured activity should consider the expressed activity within G10%.
3. **Linearity (at installation, quarterly, and once repairs)**: The linearity test specify the dose calibrator’s capacity to measure the activity accurately over a wide range of value
4. **Geometry (at installation and after repairs)**: Variations in sample volumes or geometric configurations of the instrument.

### Measurement of Radioactivity

Radioactivity of a radiopharmaceutical is measured by putting the sample within the dose calibrator with the acceptable isotope selector setting. The reading is displayed in applicable units (curie or Becquerel) on the dial.

### Biological Tests

This test carried out essentially to examine the sterility, Pyrogenicity and Toxicity of radiopharmaceuticals before human administration.

**Sterility:**

Sterility indicates the absence of viable microorganisms such as bacteria, fungi and yeast in a radiopharmaceutical preparation. The methods of Sterilization are Autoclaving and Membrane Filtration. Special difficulties arise, in carrying out the test for sterility for radiopharmaceutical preparations because of the short half-life of most radionuclides, small size of batches and the radiation hazards. USP 32 indicates sterility tests are performed by incubating the radiopharmaceutical sample in two different ways in order to detect bacterial and fungal contaminations:

1. For bacterial contaminations the radiopharmaceutical sample should be incubated in fluid thioglycollate medium at 30–35°C for fourteen days
2. For fungal contaminations the radiopharmaceutical sample should be incubated in soybean–casein digest medium for incubation at 20–25°C for fourteen days

**Pyrogenicity:**

Radiopharmaceuticals for administration must be pyrogen free. The pyrogens are both polysaccharides and proteins produced with the aid of microorganisms. They are 0.05 to 1 mm in size, soluble and warmth stable. Administration of pyrogens produces signs and symptoms of fever, malaise, chills, headache, pain in joints, leukopenia, flushing, dilation of the pupils and sweating. In order to detect apyrogenicity of radiopharmaceuticals USP Rabbit test and Limulus Amebocyte Lysate (LAL) test can be carried out. For rabbit test, three mature regular rabbits are taken whose weight not less than 1.5 kg, and their temperatures are controlled by maintaining them in a place of uniform temperature. The volume of the test sample must be an equivalent human dosage, on a weight basis, and often three to ten instances the human dosage by volume is used to achieve a greater protection factor. The ear vein of each of the three rabbits the check sample is injected. The rectal temperatures of the rabbits are measured after 1, 2, and 3 h injection of the test material. If the temperature is push upward in man or woman rabbits is less than 0.6°C and if the sum of the temperature rises in all three rabbits is not more than 1.40°C, then the check sample is considered apyrogenic. If any of the above conditions is not fulfilled, the test must be repeated with five more rabbits. If not more than three of the total eight rabbits show a temperature rise of 0.6°C and if the sum of the man or woman temperature rises does not exceed 3.7°C, the material is taken into consideration pyrogen free.

A rapid method for bacterial endotoxin test, also called the LAL test, is used for the detection and quantitation of endotoxin-type pyrogens. This method uses the lysate of Amebocyte from the blood of the horseshoe crab, Limulus Polyphemus. The principle of the test is based on the formation of an opaque gel by pyrogens in the presence of Ca$^{2+}$ upon incubating the sample with the LAL at 37°C. An assay mixture usually includes of 0.1 mL LAL and a test sample at pH 6–8. The reaction takes place within 15–60 min after mixing and depends on the concentration of pyrogens. The formation of a gel shows the presence of pyrogens. The LAL test is carried out on unknown samples as well as on E. coli endotoxin and water samples. Usually 0.1 mL of every sample and LAL are incubated at 37°C for 60 min. If the E. coli endotoxin sample shows gel formation (positive control) and the water sample shows no gel formation (negative control), then unknown samples are taken into consideration positive or negative depending on whether they form gel or not. The US FDA has authorized the LAL test for endotoxin-kind of pyrogens.

### Diagnostic Applications of Radiopharmaceuticals:

For diagnostic purpose, a radiopharmaceutical dosage form is administered to the patient by numerous routes such as oral, inhalation, intravenous or alternative routes in a very specific dose and acts as radioactive tracer. The radioactive isotope select for diagnostic purpose should have minimum half-life, minimum retention within the body and may be detectable in little amounts.

1. **To find carcinoma growths:** I-131 is employed to find carcinoma tumour that is known by a cold space within the liver.
2. **To assess liver functions:** Au-198 injection is employed for scanning of liver to determine its shape, position and size. The isotope gives information about functioning of kupper cells. It doesn’t enter the tumour tissue, abscesses and cysts which then appear as cold space in the liver scan.
3. **To detect bone tumours:** Technetium - 99m is employed to detect bone tumour which is known by a hot space in the bone.
4. **To detect spleen and bone marrow cancer:** Colloidal solution of Technetium sulphide when injected intravenously is taken up by endothelial cells of liver, spleen and bone marrow.
5. **To assess urinary organ function:** Iodophorpic acid tagged with radioactive iodine (I-131) is injected intravenously. Kidney tubules actively secrete this agent in the urine. The rate of accumulation and removal of I-131 verses time is determined to assess the kidney function. An image of kidney is obtained called as renogram. It is very useful to assess kidney functions in patients with transplanted kidney.

6. **To diagnose Alzheimer's disease:** Iodinated amphetamines (radio labelled with 1123) are used to evaluate the brain’s white matter. The compound will penetrate through the blood brain barrier but has no pharmacologic activity.

7. **To assess cerebral blood flow:** Nuclear brain scans are obtained using Technetium-99m that distributes rapidly in the extracellular fluid and concentrates in the choroid plexus of the brain.

8. **To diagnose pernicious anaemia:** Cyanocobalamin Co-57 or Co-60 is used in the diagnosis of pernicious anaemia.

**Conclusion:**
There are different types of radiopharmaceuticals are available and having an important role in diagnosis of disease. In this review we have summarized the concept of radioactivity in consideration with the preparation, manufacturing, quality control test of radiopharmaceuticals. The specific application of radiopharmaceutical in disease diagnosis is also being emphasized. There has been a significant growth of this branch of nuclear medicine with the introduction of a variety of recent radionuclides and radiopharmaceuticals for the treatment of pathological process bone pain, neuroendocrine and other tumours. The growth of radiopharmaceuticals through the form of radionuclide is undergoing through an extremely significant interesting time phase and a considerably greater development is expected in the upcoming years.

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