General Introduction

Radiopharmaceuticals are medicinal products that are radioactive. They are used for both diagnosis and therapy as reviewed by Vera–Ruiz 1998 in nuclear medicine. Radiopharmaceuticals vary from inorganic salts to large organic molecules and complexes.

The radionuclides incorporated into radiopharmaceuticals are produced either as a result of nuclear fission of heavy nuclides such uranium-235 or in a reactor or in a cyclotron. In choosing a production route, the manufacturer considering factors such as the yield, the radionuclidic purity of the product and whether the outcome of the process is a carrier-free radionuclide. A radionuclide is described as " carrier-free "when every atom of the element is present as the radionuclide and therefore no other isotopes of the element are present.

The carrier-free state can only be achieved when the production process leads to the formation of a new element. By the use of a carrier-free radionuclide only a trace amount of the element is administered to the patient. Free from carrier is particularly important in radiopharmaceuticals, which contain toxic elements such as the ^{201}Tl isotope of thallium and the ^{67}Ga isotope of gallium.

In the synthesis of a radiolabeled compound, the success of labeling reaction may depend upon the radionuclide being in a carrier-free state.

1.1Radionuclide produced in a reactor:

Most of the radionuclides used in nuclear medicine are produced in a nuclear reactor as reviewed by Poggenburg (1974).

In this process, a target element is inserted into the core of the reactor where it is bombarded by neutrons. When a neutron enters the nucleus of a target atom, the nucleus undergoes a rearrangement and a

new isotope of the target element is produced. In the most common reaction of this type the capture of the neutron is accompanied by emission of a γ-ray. This process is known as (n,γ) reaction, is the route by which most reactor–produced radionuclides are prepared and results in the formation of radionuclides with the excess of neutrons. An example of the reaction is the production of chromium-51, the reaction is described as ^{50}Cr (n,γ) ^{51}Cr. As this type of reaction is not 100 % efficient, the product contains both ^{50}Cr and ^{51}Cr.

Chemical separation of these isotopes is not possible and therefore ^{51}Cr can not be obtained as a carrier–free product when prepared by this route. The important radionuclide produced by this reaction are ^{32}P, ^{59}Fe, ^{75}Se, ^{113}Sn and ^{198}Au. In the reactor, two types of interactions with thermal neutrons are of considerable importance in the production of various useful radionuclides:

Neutron capture or (n,γ) reaction and fission of heavy elements

1.1.1 Neutron capture or (n,γ) reaction:

A variation of the (n,γ) reaction occurs when the radionuclides produced decays to a daughter radionuclide.

The reaction is an important means for the production of ^{131}I.

A tellurium target irradiated in the reactor to form ^{131}Te, which disintegrates by β^-emission to form ^{131}I.

$$^{130}Te\ (n,\gamma)\ ^{131}Te \xrightarrow[25\ min]{\beta^-} {}^{131}I$$

Same examples of neutron capture reactions are: -

$^{98}Mo(n,\gamma)\ ^{99}Mo$, $^{196}Hg(n,\gamma)\ ^{197}Hg$ and $^{50}Cr(n,\gamma)\ ^{51}Cr$.

I.1.2 Fission or (n,f) reaction :

As already mentioned, fission is a breakup of heavy nucleus into two fragments of approximately equal mass. When a target of heavy elements is inserted in the reactor core, heavy nuclei absorb thermal neutrons and undergo fission.Fission heavy elements are ^{235}U,^{239}Pu,^{237}Np,^{233}U,^{232}Th, and many others having atomic numbers greater than 92. Nuclides produced by fission may range in atomic number from about 28 to nearly 65. These isotopes of different elements are separated by appropriate chemical procedures that involve precipitation, solvent extraction, ion exchange, chromatography and distillation. The fission radionuclides are normally carrier-free or NCA, therefore isotopes of high specific activity are available from fission. Many chemically useful radionuclides such as ^{131}I,^{99}Mo,^{133}Xe,and ^{137}Cs are produced by fission from ^{235}U.

An example of thermal fission of ^{235}U is presented as follows: -

$$^{235}_{92}U + ^{1}_{0}n \longrightarrow ^{236}_{92}U \longrightarrow ^{131}_{53}I + ^{102}_{39}Y + 3\,^{1}_{0}n$$

$$\longrightarrow ^{99}_{42}Mo + ^{135}_{50}Sn + 2\,^{1}_{0}n$$

$$\longrightarrow ^{133}_{54}Xe + ^{101}_{38}Sr + 2\,^{1}_{0}n$$

$$\longrightarrow ^{137}_{55}Cs + ^{97}_{37}Rb + 2\,^{1}_{0}n$$

$$\longrightarrow ^{155}_{62}Sm + ^{78}_{30}Zn + 3\,^{1}_{0}n$$

Besides, other radionuclides are also produced in the example.

- **Iodine-131:**

For chemical separation of ^{131}I from irradiated ^{235}U target, the latter is dissolved in 18 % NaOH by heating, and hydroxides of many metal ions are precipitated by cooling. The supernatant containing sodium iodide is acidified with sulfuric acid in a distillation system. Iodide is oxidized to iodine by the acid, and iodine is collected in NaOH solution by distillation.

- **Molybdenum-99:**

For ^{99}Mo separation, the irradiated uranium target is dissolved in nitric acid and solution is adsorbed on an alumina (Al_2O_3) column.The column is then washed with nitric acid to remove uranium and other fission product cations. Molybdenum-99 is then eluted with ammonium hydroxide, and ultimately used for ^{99}Mo -^{99m}Tc generator. The ^{99}Mo radionuclide produced by fission is carrier-free or NCA and its most common contaminants are ^{131}I and ^{103}Ru.

I.2. Radionuclides produced in cyclotron: -

In a cyclotron, charged particles such as protons, deuterons, α particles, ^{3}He particles, and so forth are accelerated in circular paths in dees under vacuum by means of an electromagnetic field. These accelerated particles can possess a few kiloelectron volts (KeV) to several billion electron volts (BeV) of energy depending on the design and type of the cyclotron.

When targets of stable elements are irradiated by placing them in the external beam of the accelerated particles or at a given radius in a cyclotron, the accelerated particles irradiate the target nuclei and the nuclear reactions take place. In a nuclear reaction, the incident particle may leave the nucleus after interaction, leaving some of its energy in it, or it may be completely absorbed by the nucleus, depending on the incident particle.

In either case a nucleus with excitation energy is formed and the excitation energy is disposed of by the emission of nucleons(i.e., protons and neutrons). Each nuclear reaction for the production of a nuclide has a definite threshold or Q energy, which is either absorbed or released in the reaction. This energy requirement arises from the difference between the masses of the target nucleus plus the irradiating particle and the masses of the product nuclide plus the emitted particles.

In nuclear reactions requiring the absorption of energy, the irradiating particles must possess energy above the threshold energy; otherwise the nuclear reaction would not take place.

An example of a simple cyclotron – produced radionuclide is ^{111}In, which is produced by irradiating ^{111}Cd, with 12-MeV protons in a cyclotron. The nuclear reaction is written as follows:-

$$^{111}Cd(p,n)\,^{111}In$$

As another example, relatively high energy nuclear reactions induced in ^{89}Y irradiation with 40-MeVprotons are listed below: -

$$^{89}Y+P(40MeV) \longrightarrow {}^{89}Zr + n$$
$$\longrightarrow {}^{89}Y + P$$
$$\longrightarrow {}^{88}Zr + 2n$$
$$\longrightarrow {}^{88}Y + Pn$$
$$\longrightarrow {}^{88}Sr + 2P$$
$$\longrightarrow {}^{87}Zr + 3n$$
$$\longrightarrow {}^{87}Y + P2n$$

Although all reactions mentioned in the above example are feasible, the most probable reactions are (P,3n) and (P,P2n) reactions with 40-MeV protons. The target material for irradiation must be pure and preferably mono isotopic or at least enriched isotope, in order to avoid the production of extraneous radionuclides.

The energy and type of the irradiating particle must be chosen so that contamination with undesirable radionuclides resulting from extraneous nuclear reaction can be avoided. Since various isotopes of different elements may be produced in a particular irradiating system, it is necessary to isolate isotopes of a single element; this can be accomplished by appropriate chemical methods such as solvent extraction, precipitation, ion exchange, and distillation. Cyclotron

produced radionuclides are usually neutron deficient and therefore decay be β^+ emission or electron capture. Methods of preparation of several useful cyclotron-produced radionuclides are described as follows: -

- **Gallium-67:-**

Gallium-67 ($t_{1/2}$-78h) can be produced by several nuclear reactions such as $^{66}Zn(d,n)\,^{67}Ga$, $^{68}Zn(p,2n)^{67}Ga$, $^{64}Zn(\alpha,p)^{67}Ga$. A pure natural zinc target or enriched zinc isotope in the form of oxide is irradiated with 20MeV, 8MeV deuterons or 23MeV α particles in a cyclotron at a certain beam current for a specified time after irradiation the target is dissolved in 7NHCl and carrier-free ^{67}Ga are extracted with isopropyl ether. The organic phase is then evaporated to dryness in water bath and the residue is taken up in dilute HCl for supply as gallium chloride. It may be complexed with citric acid to form gallium citrate, which is most commonly used in nuclear medicine.

- **Iodine-123:-**

Iodine-123 has gained considerable importance in nuclear medicine because it has good radiation characteristics such as decay by electron capture, half-life of 13.2h and γ ray emission of 159 KeV, it is produced directly or indirectly in a cyclotron by several nuclear reactions. Direct nuclear reactions are those reactions whereby ^{123}I is produced directly and likely to be contaminated with other iodine isotopes such as ^{124}I and ^{125}I depending on the type of target and irradiating particle.

Examples of such reactions are $^{121}Sb(\alpha,2n)^{123}I$, $^{123}Te(p,n)^{123}I$ $^{122}Te(d,n)^{123}I$ and $^{124}Te(p,2n)^{123}I$. Depending on the target composition, and energy of the irradiating particles, other side reactions obviously may produce various radioisotopes of iodine.

In the direct methods, after irradiation the target is dissolved in mineral acid and iodine is collected into dilute sodium hydroxide (NaOH). In the indirect method, the nuclear reaction is so chosen that ^{123}Xe is produced initially which then decays with a half-life of 2.1h to produce ^{123}I with a half-life of 13.2h. These reactions allow the production of ^{123}I free of other radioisotopes of iodine. Various reactions include $^{122}Te(\alpha,3n)^{123}Xe$ using 42-46 MeV α particles, $^{122}Te(^{3}He,2n)^{123}Xe$ using 20-30 MeV ^{3}He particles.

- **Indium-111:**

Indium-111 is produced by the $^{111}Cd(P,n)^{111}In$ and $^{109}Ag(\alpha,2n)^{111}In$ reaction. After irradiation with 15-MeV protons, the cadmium target is dissolved in mineral acid, the acidity is made in HCl. The solution is passed through anion-exchange resin (Dowex-I). Indium-111 is removed by elution with 1NHCl, Leaving cadmium on the column.

- **Thallium-201:**

Thallium-201 is primarily produced by the $^{203}Tl(p,3n)^{201}Pb$ reaction, whereby ^{201}Pb decays to ^{201}Tl with a half-life of 9.4h. Thallium-201 obtained in this way is pure and free of other contaminants. After irradiation with 35-45 MeV protons, the natural thallium target is dissolved in a mineral acid, and ^{201}Pb are isolated by the ion-exchange method. The lead radionuclides are then adsorbed on another ion-exchange column, and sufficient time is allowed for decay ^{201}Pb to ^{201}Tl. Thallium-201 is then eluted as thallous chloride in NCA form.

I.2.1. Short-lived radionuclides:

Considerable interest has developed for the production of short-lived radionuclides and their clinical uses because of the availability of the positron emission tomography (PET) imaging system. Among them are the key radionuclides such as ^{11}C, ^{13}N, ^{15}O, and ^{18}F which decay by positron emission. These positron emitters are useful in imaging by

(PET). Because they have very short half-lives, a cyclotron or a medical cyclotron must be located on site in the laboratory.

- **Carbon-11:**

Carbon-11 has a half-life of 20.4 min. and can be produced by $^{10}B(d,n)^{11}C$, $^{11}B(p,n)^{11}C$, and $^{14}N(p,\alpha)^{11}C$ reactions in the cyclotron. In the first two reactions, B_2O_3 is the target, and nitrogen gas in the third, both ^{11}CO and $^{11}CO_2$ are produced in boron targets, which are then flushed out by neutral gases. Both ^{11}CO and $^{11}CO_2$ are commonly used as precursors in the preparation of various clinically useful compounds, such as ^{11}C-Palmitate for myocardial perfusion imaging by (PET).

- **Nitrogen-13:**

Nitrogen-13 has a half-life of 10 min. and is commonly used as NH_3. It is produced by the $^{12}C(d,n)^{13}N$ reaction by bombarding Al_4C_3 or methane with 6-7 MeV deuterons, or by the $^{16}O(p,\alpha)^{13}N$ or $^{13}C(p,n)^{13}N$ reaction. In the latter two reactions, a target of slurried mixture of ^{13}C powder and water is used for irradiation with 11-12 MeV protons. Nitrogen –13 is converted to NH_3 in a aqueous medium.
$^{13}NH_3$ in the form of NH_4^+ ion is used primarily for myocardial perfusion imaging by (PET) $^{13}NH_3$ is also used to label glutamine and asparagine for assessment of viability of tissues.

- **Oxygen-15:**

Oxygen-15 has a half-life of 2 min. and is produced by the $^{14}N(d,n)^{15}O$ reaction by deuterons irradiation of gaseous nitrogen or by the $^{15}N(p,n)^{15}O$ reaction by proton bombardment of enriched ^{15}N target.

$^{15}O_2$ is then passed over activated charcoal heated at 600°C. in order to convert it to $C^{15}O$ and $C^{15}O_2$, which are used for labeling heamoglobin and for clinical investigation of pulmonary and cardiac malfunctions.

- **Fluorine-18:**

Fluorine-18 ($t_{1/2}$ = 110 min.), is produced by the $^{18}O(p,n)^{18}F$ reaction on ^{18}O-water target. ^{18}F is recovered from water by passing the mixture through a column of quaternary ammonium resins, and ^{18}O-water can be reused as the target. Fluorine-18 is used primarily to label glucose to give ^{18}F-labeled fluorodeoxyglucose (FDG) for cerebral metabolic studies.

I.3. Radionuclide generator:

The use of short-lived radionuclides has grown considerably, because larger doses of these radionuclides can be administered to the patient with only minimal radiation dose and excellent image quality. This increasing appreciation of short-lived radionuclides has led to the development of radionuclide generators that serve as convenient sources of their production. A generator is constructed on the principle of decay-growth relationship between a long-lived parent nuclide and its short-lived daughter radionuclide.

The chemical property of the daughter nuclide must be distinctly different from that of the parent nuclide, so that the former can be readily separated. In a generator, basically a long-lived parent nuclide is allowed to decay to its short-lived daughter nuclide and the latter is then chemically separated. The importance of radionuclide generators lies in the fact that they are easily transportable and serve as sources of short-lived radionuclides in institutions far from the site of any cyclotron or reactor facility. A radionuclide generator consists of a glass or plastic

column fritted at the bottom with a fritted disk. The column is filled with adsorbent material such as cation or anion-exchange resin, alumina, and zirconia, on which the parent nuclide is adsorbed.

The daughter radionuclide grows as a result of the decay of the parent until either a transient or a secular equilibrium is reached within several half-lives of the daughter, after which the daughter appears to decay with the same half-life as the parent. Because there are differences in chemical properties, the daughter activity is eluted in a carrier-free state with an appropriate solvent, leaving the parent on the column.

After elution the daughter activity starts to grow again in the column until an equilibrium is reached in the manner mentioned above; the elution of activity can be made repeatedly. The first commercial radionuclide generator was the ^{132}Te (t 1/2 =78h)$\longrightarrow$^{132}I (t 1/2 = 2.3h) system developed at the Brookhaven National Laboratory in the early 1960s. Since, then a number of other generator systems have been developed and tried for use in nuclear medicine. They are the ^{99}Mo-^{99m}Tc, ^{113}Sn-^{113m}In, ^{87}Y- ^{87m}Sr, ^{82}Sr-^{82}Rb, ^{81}Rb-^{81m}Kr, and ^{68}Ge-^{68}Ga system.

1.4. Technetium:

In 1937 the element of the atomic number 43 was discovered by Segre and Perrier who showed that radioactivity obtained by irradiation of molybdenum with deuterons was due to isotopes of missing element eckamanganese.

The metastable isomers ^{95m}Tc and ^{97m}Tc had been produced by **the** following nuclear reactions: -

^{94}Mo(d,n)^{95m}Tc and ^{96}Mo(d,n)^{97m}Tc .....(1)

Perrier & Segre (1947) suggested the name technetium, since it was the first element to be prepared artificially from ^{99}Mo, as reported by Seaborg et al (1939). About twenty isotopes and numerous with half-lives ranging between one second and several million years have been shown

ıpe with the longest half-lives are $^{97}Tc(2.6 \times 10^{6}$ y),

ınd ^{99}Tc (2.1×10^{5} y), In the range of the mass numbers

ıife isotopes, stable isobars of the neighbouring elements

.n and ruthenium are known.

Table(1) : Isotopes and isomers of technetium

Nuclide	Half-Life	Decay	Nuclide	Half-Life	Decay
^{91}Tc	3.2 m	B^+ , γ	^{99m}Tc	6.0 h	γ,IT
^{92}Tc	4.4 m	B^+ , γ	^{99}Tc	2.1x105 y	B^-
^{93m}Tc	43.5 m	EC , γ	^{100}Tc	15.8 s	B^- , γ
^{93}Tc	2.7 h	EC,B^+,γ	^{101}Tc	14 m	B^- , γ
^{94m}Tc	53 m	B^+ , γ	^{102}mTc	4.3 m	B^- , γ
^{94}Tc	4.9 h	EC,B^-,γ	^{102}Tc	6.3 m	B^- , γ
^{95m}Tc	60 d	EC,B^+,γ	^{103}Tc	50 s	B^- , γ
^{95}Tc	20 d	EC , γ	^{104}Tc	18.0 m	B^- , γ
^{96m}Tc	52 m	EC , γ	^{105}Tc	7.6 m	B^- , γ
^{96}Tc	4.3 d	EC , γ	^{106}Tc	36 s	B^- , γ
^{97m}Tc	91 d	γ	^{107}Tc	21 s	B^- , γ
^{97}Tc	2.6x10^6y	EC	^{108}Tc	5.0 s	B^- , γ
^{98}Tc	4.2x10^6y	B^- , γ	^{109}Tc	1.0 s	B^- , γ
			^{110}Tc	0.83 s	B^- , γ

1.4.1. Properties of Technetium: -

Technetium was the first element to be produced artificially. Minute amounts have been isolated by Perrier & Segre (1937), Technetium is a metallic element belonging to the transition elements of group VIIB of the periodic table. The chemistry of the element lies between that of manganese and rhenium. Its electronic configuration is $4s^2,4p^6,4d^6,5s^1$ or $4s^2,4p^6,4d^5,5s^2$. It forms compounds in all states of oxidation from 1- to 7+ but the most stable being those 4+ and 7+ states as reviewed by Deutsch(1983), It has different oxidation states between 1-and 7+ but the oxidation states of 5+ and 6+ are important in some

analytical applications and in the chelate compounds. The Tc^{5+} and Tc^{6+} species frequently disproportionate into Tc^{4+} and Tc^{7+} states:-

$$3Tc^{5+} \longrightarrow 2Tc^{4+} + Tc^{7+}$$

$$3Tc^{6+} \longrightarrow Tc^{4+} + 2Tc^{7+}$$

Technetium of oxidation state 7+ is present as the pertechnetate ($^{99m}TcO^-_4$) which is the most stable. Chemical form of technetium followed by the tetravalent state (TcO_2) which is the other oxidation states gains stability through complex formation. $^{99m}TcO^-_4$ is the the starting material of the preparation of ^{99m}Tc radiopharmaceuticals. Technetium is a silver gray metal it has a melting point of 2200°C, and a boiling point of 4973°C. Metallic technetium dissolves in acids that are oxidants such as nitric acid, aquaregia, and concentrated sulfuric acid.

Metallic technetium dissolves in bromine water and also in neutral and alkaline solutions of hydrogen peroxide. First type of technetium oxide which is the volatile Tc_2O_7 was prepared by burning the technetium in excess of oxygen at 500°C. Other type of technetium oxide is the relatively TcO_2 which can be obtained by reduction of aqueous solutions of pertechnetate with zinc and hydrochloric acid.

As reviewed by Boyd (1959), Technetium forms two sulfides the first one was technetium heptasulfides Tc_2S_7 which is prepared by passing hydrogen sulfide through acid solution of pertechnetate and the other one was technetium disulfides TcS_2 which can be obtained by heating Tc_2S_7 with elemental sulfur in an autoclave for 24 h. at 1000°C.

Technetium forms compounds with halogens at different oxidation states. When technetium metal was reacted with excess of fluorine in a closed nickel vessel for 2h at 400°C, technetium hexaflouride(TcF_6) was formed.

When technetium metal is fluorinated directly technetium pentaflouride(TcF_5) is formed also, when gaseous chlorine is passed over

technetium metal at 200°C, a reaction begins at 400°C which takes place rapidly, with the formation of $TcCl_6$ which is very unstable, it decomposes even at room temperature to $TcCl_4$.

$TcCl_4$ can also be obtained by reacting technetium heptaoxide with carbon tetrachloride in autoclave at 400°C. Some chemical relationships of technetium are presented in Fig (1)

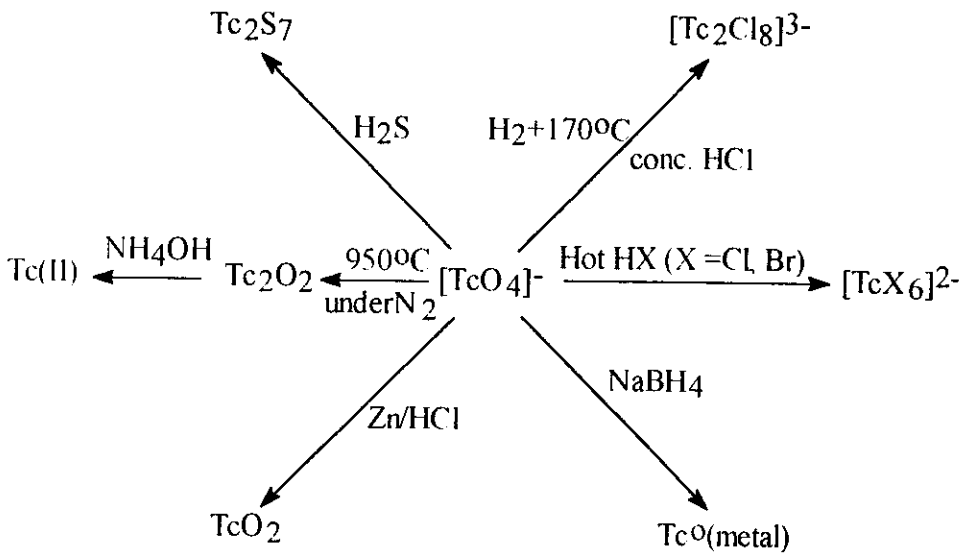


Fig.1: Selected relationships of technetium chemistry.

The radionuclide ^{99m}Tc has a half-life of 6h and decays to ^{99}Tc by isomeric transition or γ-transition of 140 keV. Approximately 10% of these transitions are via internal conversion. The ground state ^{99}Tc has a half-life of 2×10^5 years and decays to stable ^{99}Ru by β^--emission (Fig.2). These excellent physical and nuclear properties of ^{99}Tc are nearly ideal for a current generation of imaging devices such as single photon emission computed tomography.

(SPECT) or gamma camera, which applied in nuclear medicine as, reported by Dechiara (1987). Technetium–99m is considered as one of the most useful radionuclides used in diagnostic nuclear medicine as

suggested by Richards (1960). It is not expensive and ready availability to all hospitals via $^{99}Mo/^{99m}Tc$ generators. The ^{99}Mo radionuclide has a half-life of 67h and decays by β^-emission (87%) to the metastable ^{99m}Tc and has the remaining (13%) to the ground state ^{99}Tc (Fig 3). Molybdenum 99 has photon transition of 740KeV and 780 KeV.

^{99m}Tc–Radiopharmaceuticals are currently used for imaging brain, liver, kidneys, skeleton and blood pool as reported by Eckelman et al (1977), Recently, many substances of interesting behaviour labeled with ^{99m}Tc are used for characterizing the morphology and function of different human organs as presents by Johannsen (1991).

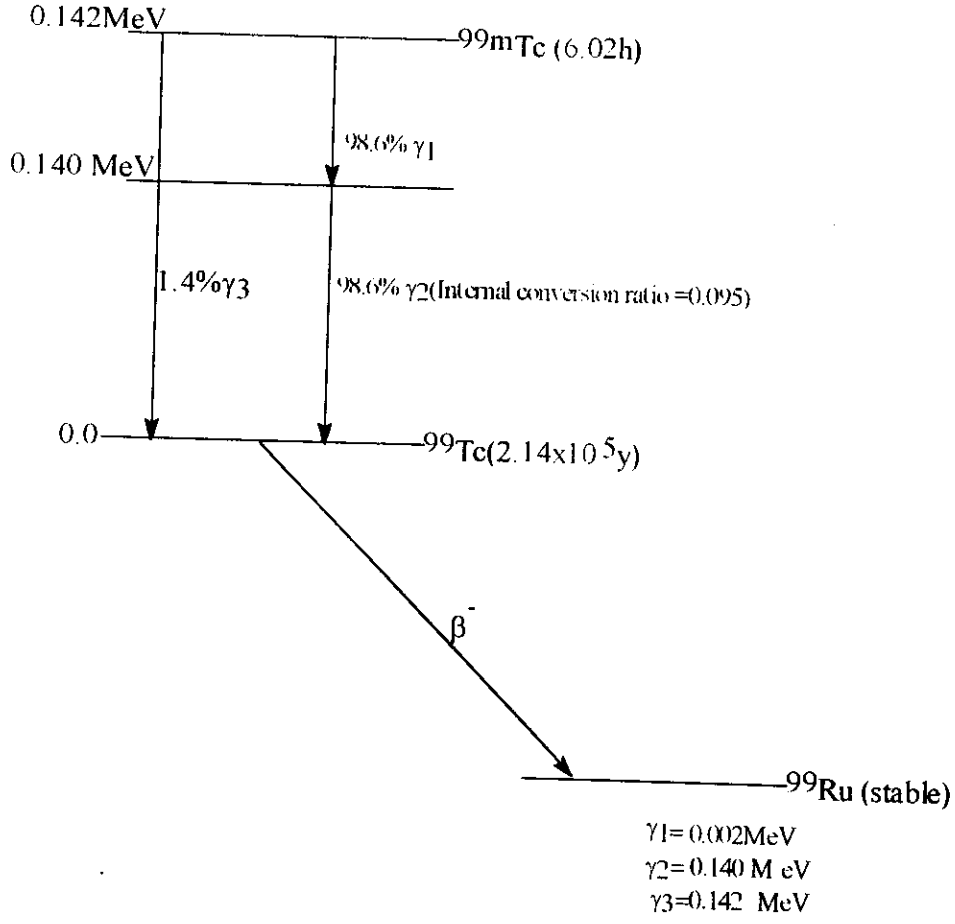


Fig.2: Decay scheme of technetium-99m

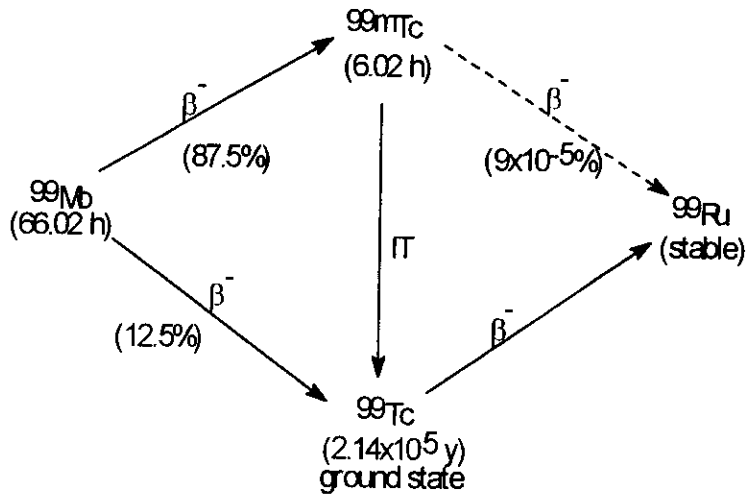


Fig.(3): Decay scheme of ^{99}Mo - ^{99m}Tc generator

I.4.2. Source of technetium: -

The isotope ^{99m}Tc is produced by the β^--decay of ^{99}Mo which is obtained either from uranium fission products or from neutron irradiated $^{98}MoO_3$ according to the reaction.

$$^{235}U \xrightarrow{(n,f)} {}^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc \quad \text{.....(3)}$$

$$^{98}Mo \xrightarrow{(n,\gamma)} {}^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc \quad \text{.....(4)}$$

Technetium-99m is the radioactive daughter nuclide of ^{99}Mo as shown from the Fig (3)

I.4.3. Methods of ^{99m} Tc separation:

Various Methods for the separation of ^{99m}Tc from ^{99}Mo are available, and the most important one is the alumina column chromatography on the $^{99}Mo/^{99m}Tc$ generators are based as reported by Boyed (1982), Marques et al (1987).

Three methods of technetium separation are in current use. These are: -

(i) Elution from ^{99}Mo adsorbed on an aluminium oxide ion-exchange column.

(ii) Extraction with methyl-ethyl-ketone from ^{99}Mo-sodium molybdate in sodium hydroxide solution.

(iii) Distillation (sublimation) from ^{99}Mo-molybdenum trioxide.

I.5. ^{99}Mo / ^{99m}Tc generator systems:

I.5.1. Chromatographic generator:

This system is the most widely used as pointed by Tucker et al(1958). The technique is based on the relative difference in distribution coefficients between the two radionuclides on aluminum oxide for both the anion molybdate and pertechnetate.

The molybdate anion is strongly adsorbed on alumina while the pertechnetate is easily eluted by saline. The passage of physiological saline through the alumina bed containing adsorbed molybdate /pertechnetate will result in the elution of the pertechnetate component leaving the molybdate on the bed. Two kinds of chromatographic generators namely, The wet generator and the dry one were developed. Regarding to the wet generator, the radiation emitted from ^{99}Mo on the column will induce radiolysis of water with the formation of hydrogen peroxide (H_2O_2) and other free (OH) radicals these species are highly oxidant and in presence of ^{99m}Tc eluate, they will interact with technetium, on the other hand in a dry column generator, after routine elution, the left over saline in the column drawn out by using an evacuation vial without adding any more saline.

The suggestion for a dry column generator a high elution efficiency of ^{99m}Tc.(Fig (4)).

- **Advantages of AL_2O_3 column chromatography:**

1- Ability to incorporate the column in a closed system for maintenance of sterility.

2- Simple operation.

3- Less radiation dose to the operator.

4- Less time consuming and hence less decay of ^{99m}Tc.

5- ^{99m}Tc can be obtained more than one in a day resulting in more efficient use.

I.5.2. Solvent extraction generator: -

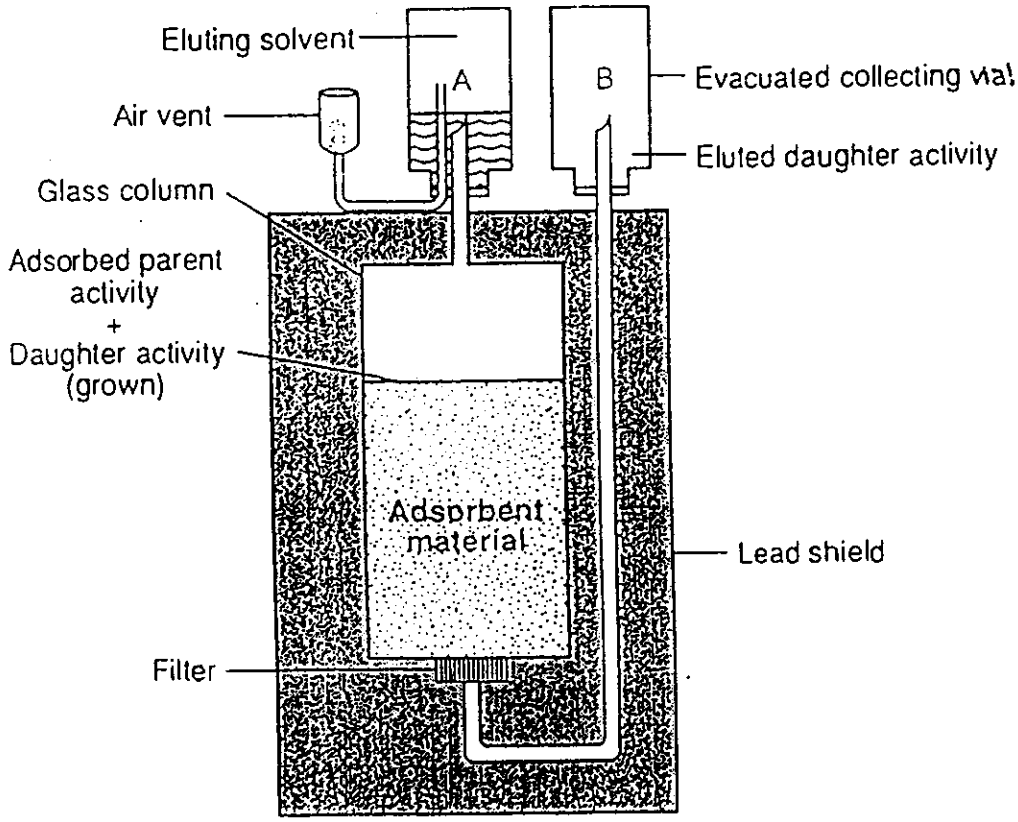
The ^{99}Mo–^{99m}Tc generator was first introduced at brook haven National Laboratory by Tucker et al (1958). Before the development of this generator, the ^{99m}Tc radioactivity is used to be extracted with methyl–ethyl ketone (MEK) from 20% NaOH solution (pH~10–12) of ^{99}Mo.

After extraction, the organic phase was evaporated and the $^{99m}TcO_4^-$ dissolved in isotonic saline for clinical use. This method of solvent extraction has been employed to construct the liquid–liquid extractor type of generator. ^{99}Mo radioactive alkaline solution was kept in a glass column and then letting MEK flow through the column from the bottom, MEK will extract $^{99m}TcO_4^-$ leaving ^{99}Mo in the aqueous solution, Repeated elutions of the column can be made after or before the transient equilibrium between ^{99}Mo and ^{99m}Tc system.

The advantage of this generator is that the cost of ^{99m}Tc is low, but the disadvantage is that it needs a lot of manipulations in the overall method, It is rarely used in nuclear medicine (Fig (5)).

I.5.3. Sublimation generator:

In this type of generators, ^{99m}Tc are separated at increased temperature from suitable ^{99}Mo compounds by sublimation with carrier gas or air. The starting material, neutron–activated $^{99}MoO_3$ is placed into an electrically heated quartz furnace, $^{99m}TcO_7$ is volatilized at some hundreds C^0 transferred by carrier gas, usually by carrier air, into cooling



Fig(4):Column chromatographic generator.

trap. ^{99m}Tc is dissolved with isotonic NaCl solution and sterilized by membrane filtration to obtain a ready–to–inject solution.
Sublimation generators have not found wide applications due to their insufficient yield.

1.5.4. Gel generator:

The gel generator system for ^{99m}Tc combines the advantages of chromatographic column generator and using non polluting $(n,\gamma)^{99}Mo$. This generator is based on eluting the ^{99m}Tc from a column of ^{99}Mo–MoO_4^{2-} obtained either by converting ^{99}Mo to an insoluble molybdate or by irradiation of an insoluble molybdate by neutrons. In view of its close functional similarity with the alumina column generator, considerable efforts have been made towards preparation of gel generators and under standing the various factors critical for obtaining ^{99m}Tc with good yield and purity. Since the gel generator is only recently introduced as a source of ^{99m}Tc, experience in its patient use is limited as reported by El-Kollaly (1996).

(a)Gel generators based on converting ^{99}Mo into gel matrix:

^{99m}Mo produced by(n,γ) is precipitated as zirconium or zirconium molybdophosphate (molybdate) under carefully controlled conditions and separated by filtration. The precipitate is dried carefully, powdered and packed into a column over an inactive bed. The column is eluted with 0.9% NaCl to get ^{99m}Tc. The drying conditions and the water content of the dried precipitate are critical to get good ^{99m}Tc yield. The same purity of ^{99m}Tc obtained from generators is comparable to ^{99m}Tc from other generators.

(b)Gel generators based on neutron activation of metallic molybdates. A metallic molybdate, prepared under carefully controlled conditions, is irradiated in the nuclear reactor and directly used in a

column for eluting ^{99m}Tc. This method would probably offer the simplest method for making column type ^{99m}Tc generators.

A variety of metallic (Sn, Ti, Ce) molybdate can be evaluated as suitable target for irradiation.

The ^{99m}Tc yield depends mostly on the conditions of preparation of the metallic molybdate gel, and its final water content.

The ^{99m}Tc yield were also found to depend on irradiation conditions such as neutron flux and the temperature of the target during irradiation.

1.6. ^{99m}Tc – Radiopharmaceuticals: -

1.6.1. Labeling with ^{99m}Tc:

Labeling a molecule by ^{99m}Tc is a complex formation, where the pertechnetate in oxidation state VII, is reduced to reactive forms of Tc(V),Tc(IV) or Tc(III). After reduction of pertechnetate, the radionuclide can be complexed by suitable chelating ligands. Radio pharmaceutically used complexing agents (chelating ligands) are compounds, which contain oxygen, nitrogen, phosphorus and sulphur in donor sites as shown in Table (2).

Table (2): Essential groups present in many chelating agent used for ^{99m}Tc-complexation

Ligands	Function groups (donor groups)
Cirate	OH,COOH
Glucoheptonate	OH,COOH
Gluconate	OH,COOH
DTPA (diethylenetriaminepentaacetic acid)	COOH
DMSA (dimercaptosuccenic acid)	SH,COOH
Penicillamine	SH,NH2,COOH
HEDP(hydroxyethylene diphosphonate)	OH,PO3H
MDP (methylene diphosphonate)	PO3H
HIDA {N-(2,6-dimethylephenyl carpamoyl methyl) iminodiacetic acid}	NH,COOH
DIARS (phenyldimethyl arsine	As
TMP (trimethyl phosphine)	P
Mercapto acetyl triglycine	SH/NH
Ethyl cysteinat dimer	SH/NH
Ethyl cysteinate	SH/NH
2-Methoxy isobutylisonitrile	CN

Recent developments in ^{99m}Tc labeled myocardial radiopharmaceuticals rely on substituted arsine and phosphine chelating sites as mentioned by katti et al (1992).

The ligands currently used contain hydroxyl, carboxyl, phosphate isonitrile, amino or mercapto sites as shown in the above Table(2).

These obviously essential groups occur in many chelating agents such as carbohydrates, proteins, hydrophil drugs, mercapto acids and

substances with phosphate sites. The complex formation leads to new molecules and thus drastically alters the original reactivity and other important properties of the ligand.

The following are the possible changes of the ligand properties as a result of ^{99m}Tc–complex formation: -

1- Partially or completely blocked functional sites.

2- Inevitably increased molecular weight.

3- Altered size form and shape.

4- More or less changed electrical charge.

Thus the change of biological behaviour can be expected to be caused by considerably different properties of the free ligand and labeled complex. At least small ^{99m}Tc–labeled molecules cannot be considered representative for unlabelled substances as it is partially possible for compounds labeled by ^{3}H, ^{14}C or radioiodine they deviate essentially from one another. A halogen atom is a covalent bond atom and thus forms a molecular analogue of similar size, shape and electron configuration, whereas the complex formation yields a substantially altered molecule that mainly concerns. Of course, low molecular substances but not high-molecular substances which can advantageously be labeled by ^{99m}Tc. Generally the following substance can be labelled with ^{99m}Tc as reviewed by Dewanjee et al(1990).

Chelating agents such as methylene diphosphonate (MDP), colloids (include proteins and particles,monoclonal antibodies and receptor binding agents, blood elements, and diethylene triamine pentaacetic acid.

1.6.2 Reduction of $^{99m}TcO_4^-$

The chemical form of ^{99m}Tc available from the ^{99}Mo/^{99m} Tc generator is sodium perrhenate (Na ^{99m} TcO_4^-).

The pertechnetat ion, $^{99m}TcO^-_4$ having the oxidation state 7+ for ^{99m}Tc, resembles the permanganate ion, MnO^-_4, and the pertechenat ion, ReO_4^-.

It has a configuration of a pyramidal tetrahedron with Tc^{7+} located at the center and four oxygen atoms at the apex and corners of the pyramid.

Chemically, $^{99m}TcO_4^-$ is a rather non-reactive species and does not label any compound by direct addition.

In ^{99m}Tc labeling of many compounds, prior reduction of ^{99m}Tc from the 7+ state to a lower oxidation state is required. Various reducing systems that have been used are stannous chloride ($SnCl_2 2H_2O$), stannous citrate, stannous tartrate, concentrated HCl, sodium borohydride ($NaBH_4$), dithionite, and ferrous sulfate as reviewed by Saha (1992). Among these, stannous chloride is the most commonly used reducing agent in acidic medium in most preparations of ^{99m}Tc–labeled compounds. Another method of reduction of $^{99m}Tc^{7+}$, involves the electrolysis of a mixture of sodium pertechnetate and the compound to be labeled using an anode of zirconium .

The chemical reactions that occur in the reduction of technetium by stannous chloride in acidic medium can be stated as follows:-

$$3Sn^{2+} \rightleftharpoons 3Sn^{4+} + 6e^- \quad \ldots\ldots (5)$$

$$2\ ^{99m}TcO4^- + 16\ H^+ + 6e^- \rightleftharpoons 2\ ^{99m}Tc^{4+} + 8\ H_2O \quad \ldots\ldots (6)$$

adding the two equations, one has

$$^{99m}TcO_4^- + 16\ H^+ + 3Sn^{2+} \rightleftharpoons 2\ ^{99m}Tc^{4+} + 3Sn^{4+} + 8\ H_2O \ldots\ldots (7)$$

Equation (6) indicates that $^{99m}Tc^{7+}$ has been reduced to $^{99m}Tc^{4+}$. Other reduced states such as $^{99m}Tc^{3+}$ and $^{99m}Tc^{5+}$ may be formed under different physicochemical conditions.

It may also be possible for a mixture of these species to be present in a given preparation. Experiments with milimolar quantities of ^{99}Tc have shown that Sn^{2+} reduces ^{99m}Tc to the 5+ state and then slowly to the

4+ state in citrate buffer at pH 7 as investigated by Steigman et al (1975). Technetium –99m is reduced to the 4+ state by Sn^{2+} in acidic media.

The amount of ^{99m}Tc atoms in the ^{99m}Tc– eluate is very small (~ 10^{-9} M), and therefore only a minimal amount of Sn^{2+} is required for reduction of such a small quantity of ^{99m}Tc. However, enough Sn^{2+} is added to ensure complete reduction. The ratio of Sn^{2+} ions to ^{99m}Tc atoms may be as large as 10^6.

I.6.3. Labeling methods:

There are three methods for labeling compounds with ^{99m}Tc:

I.6.3.1. Direct labeling: -

The reduced ^{99m}Tc species are chemically reactive and combine with a wide variety of chelating compounds. A schematic reaction would be represented as follows:

Reduced ^{99m}Tc + chelating agent $\rightleftharpoons$ ^{99m}Tc-chelate

The chelating agent which contain donar atoms such as S, P, N, As, O, usually donates lone pairs of electron to form coordinate covalent bonds with ^{99m}Tc. Chemical groups such as – COO, - OH ,- NH_2 and – SH are electron donors in compounds such as DTPA, gluceptate and various protein which mentioned before in Table (2).

However, Eckelman et al (1977), reported several compounds labeled with ^{99m}Tc by this method such as DTPA , N [N^- (2,6 – dimethylphenyl carbamoyl methyl)imminodiaacetic acid (HIDA), methylene diphosphonate (MDP), pyrophosphate (PYP), hydroxy ethylidene diphosphonate (HEDP) and gluconate, ethyl cysteine (EC), 2-methoxy isobutyl isonitril (MIBI).

I.6.3.2. Ligand exchange method:

The ligand exchange method, also termed the transchelation, involves first forming a ^{99m}Tc–complex with a weak ligand in aqueous media and then allowing the complex to react with a second ligand that is forming more relatively stable complex. Because of the difference

In stability of the two ligands, a ligand exchange occurs, forming a more stable ^{99m}Tc–complex with the second ligand. For example, in the preparation of ^{99m}Tc–labeled mercaptoacetylglycylglyclglycine(MAG_3) by Fritzberg et al (1986), ^{99m}Tc–tartrate, ^{99m}Tc–gluconate is first formed by reduction of $^{99m}TcO_4^-$ with stannous ion in the presence of sodium tartrate or gluconate. Subsequent heating with MAG_3 results in ^{99m}Tc–MAG_3.

The following are the sequences of reactions for ^{99m}Tc MAG_3:

$$^{99m}Tc\ O^-_4 + Sn^{+2} \rightleftharpoons \text{reduced } ^{99m}Tc + Sn^{+4} \quad \ldots\ldots(8)$$

$$\text{Reduced } ^{99m}Tc + \text{tartrate} \rightleftharpoons ^{99m}Tc - \text{tartrate} \quad \ldots\ldots(9)$$

$$^{99m}Tc - \text{tartrate} + MAG_3 \rightleftharpoons ^{99m}Tc - MAG_3 + \text{tartrate} \quad \ldots\ldots(10)$$

These reactions normally occur when the solubility of the stronger chelate and the stability of the Sn(II)complex are lower in aqueous media gluconate, tartarate, citrate, and EDTA are weaker ligands.

I.6.3.3. Bifunctional chelation method:

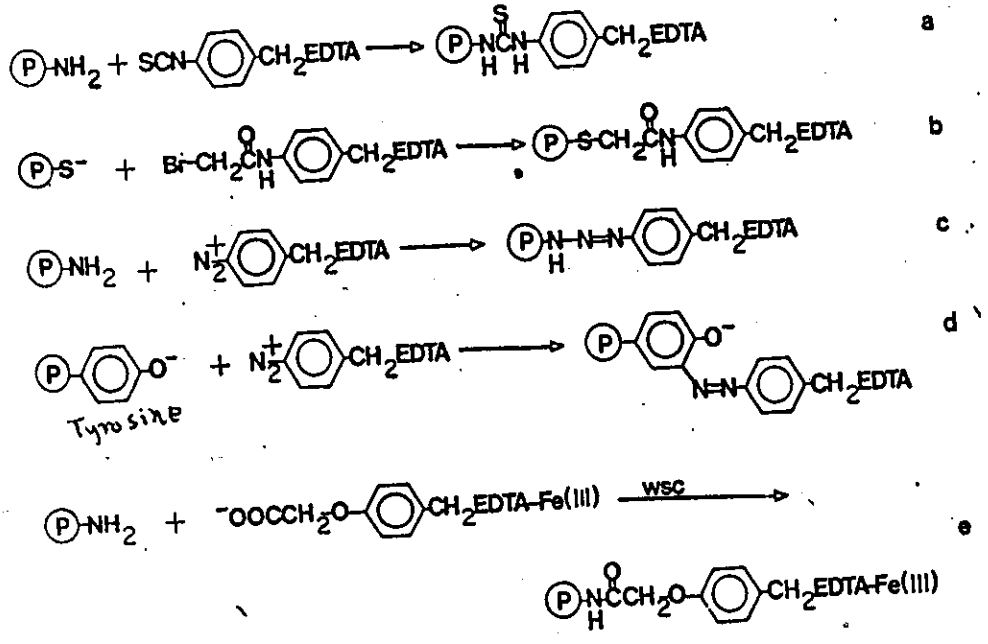
In general, chelating agents are compounds that comprise both powerful metal chelating group and a second functional group that is usually chemically reactive in nature.

The metal chelators are often derived from polyamino carboxylic acids such as ethylene diamine tetraacetic acid (EDTA), diethylene tetra amine penta acetic acid (DTPA), or imino diacetic acid (IDA), because of there large formation constants with a variety of metal ions and their

Many of the most interesting applications of this technology are found in medicine Radioactive metal ions attached by chelation to small molecules, peptides, or proteins such as monoclonal antibodies have been used clinically for diagnosis of cancer as reviewed by Vera–Ruiz (1998). and for study various organs. With the availability of monoclonal antibodies which had been developed by Kohier and Milslein in the (1975), which bind with great selectivity to biological molecules.

These antibodies can be used for in-vitro for analysis of hormones and other biological compounds, or they can be used for diagnosis and treatment of disease. One of the principal reasons for the development of bifunctional chelating agents for radiolabeling is the availability of radionuclides with convenient half–life and useful radiation, for example ^{99m}Tc. Fig.(6) Shows some examples of bifunctional, chelating agents that commonly used.

Linking Radiometals to Proteins



Bifunctional chelator is first conjugated to the antibody and then reduced ^{99m}Tc is allowed to couple to the chelator DTPA, EDTA, dithiosemicarbazone and N_2S_2 which have been used as chelating agents.

I.7. Groups of ^{99m}Tc–radiopharmaceuticals:

These are compounds, which contain chelating group to bind the reduced technetium, and are concentrated in the organs of choice depending on the ability of that organs to remove foreign substance from the blood circulation as reviewed by Eckelman et al(1977). Recently, the field of nuclear medicine imaging has viewed as the portrayal of regional physiology and biochemistry as presented by Narasimhan, Johannsen (1992).

The following are the different classes of ^{99m}Tc– radiofarmaceuticals:

I.7.1. Pertechnetate ion ($^{99m}TcO_4^-$):

Technetium-99m eluted directly from $^{99}Mo/^{99m}Tc$generator as the $^{99m}TcO_4^-$ ion, which was first evaluated by Harper et al (1964) as possible biological tracer. This work led to the present wide spread use for brain tumor localization and for thyroid imaging.

I.7.2. ^{99m}Tc–Labelled colloids and particulates:

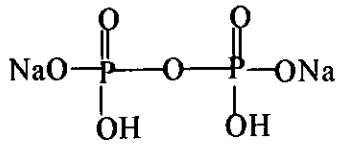
^{99m}Tc–sulfur colloid is prepared by heating a mixture of $^{99m}Tc\ O_4^-$ and sodium thiosulphate in acidic medium for 5 to 10 min in boiling water bath. Gelatin is added before the reaction with the acid in order to stabilize sulphur in the colloid state.

It is used for scintigraphy of the recticuloendothelial lymph system as tried by Harper et al (1964). Other ^{99m}Tc–labeled colloids e.g. $^{99m}Tc(OH)_4$, $Sn(OH)_2$, ^{99m}Tc antimony sulphide ^{99m}Tc–Sb_2S_3 colloid, and ^{99m}Tc

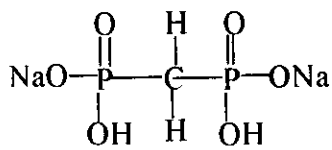
microparticulates of denatured albumin are used for imaging the resident pool of macrophage in the recticuloendothelial system as reviewed by Eckelman et al(1977).The ^{99m}Tc–antimony sulphide colloids have a narrow particles size distribution (5 to 15 μm) than ^{99m}Tc sulphide colloid (300 to 900 μm) and the former migrate much faster after interstitial administration facilitating regional lympho–scintrography less erythema at the sites of infection. Some of these colloids were also used for labeling polymorphonuclear neutrophilic (PMN) granulocytes, which are used for diagnosis of infection as carried by Mock et al (1987).

I.7.3. ^{99m}Tc–chelates for skeletal imaging: -

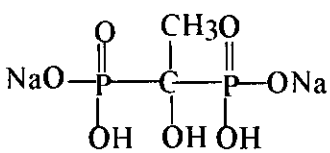
These ^{99m}Tc– chelates are used for skeleton scintigraphy such as MDP, HEDP, HMDP, pyrophosphates and polyphosphates as tried by Subramanian (1971). The structure of these chelating agents are presented in Fig.(7). Diphosphonates chelating agents are analogue to pyrophosphate whose P–O–P structure is replaced by P–C–P which is more stable against enzymatic decomposition by phosphatase enzyme. The labeling of ^{99m}Tc–phosphonate and pyrophosphate complexes by addition of pertechnetate to a freeze kit has been reported by Subramanian et al (1971). This revolutionized bone scanning by providing the most sensitive method for surveying skeletal abnormality.



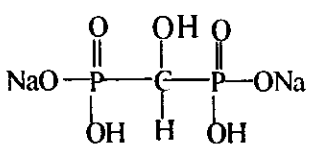
Disodium pyrophosphate(PYP)



Disodium methylenediphosphonate (MDP)



Disodium hydroxyethylenediphosphonate(HEDP)



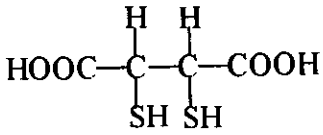
Disodium hydroxymethylenediphosphonate (HMDP)

Fig.(7): Structures of some pharmaceuticals used for skeletal imaging

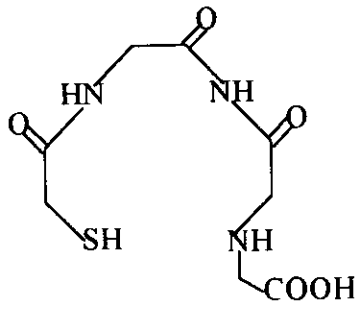
I.7.4. ^{99m}Tc–Chelates for renal imaging: -

Dewenjee et al (1990) mentioned that several ^{99m}Tc–chelates, like a variety of organic acids and bases are filtered by the glomerulus. A few of them may be partially secreted by the proximal tubular reabsorption passively depending on their pk_a's lipid solubility, and pH of the tubular fluid. The tubular cells retain a variety of metal ions by chelations with thiol groups present in these proteins. Numerous ^{99m}Tc–complexes are frequently used for kidney function study such as ^{99m}Tc iron – ascorbate, which reported, by Harper et al (1966), diethylene triamine pentaacetic acid glucoheptonate and dimercaptosuccenic acid reviewed by El Asrag et al(1988). These ^{99m}Tc–complexes are prepared by reduction of pertechnetate in presence of Sn(II) salts.

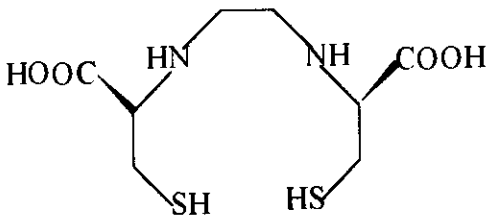
Several ^{99m}Tc–labelled compounds had been prepared and tested for the measurement of effective renal plasma flow (ERPF) and to find a replacement for radioiodohippuran. Fritzberg et al (1981, 1986) synthesized,N,N–bis(mercaptoacetyl)2,3-diaminopropanoate(CO_2DADS) N_2S_2 and mercaptoacetylglycylglycylglycin (MAG_3)N_3S. ^{99m}Tc MAG_3 was prepared by ligand exchange method in which the technetium is reduced in the presence of pro–ligand to form ^{99m}Tc–weak complex and in the presence of MAG_3 a ligand exchange occurs and a more stable ^{99m}Tc– MAG_3 complex was formed. Some ^{99m}Tc–complexes for renal imaging are in Fig.(8).



Dimercaptosuccinic acid (DMSA)



Mercaptoacetyltriglycine (MAG :



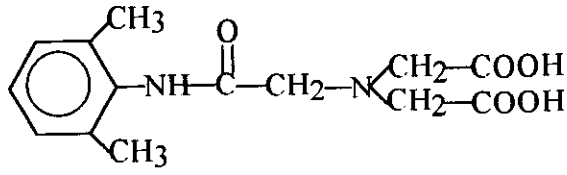
Ethylenedicysteine diacid (EC)

Fig.(8):Some radiopharmaceuticals for kidney imaging and renal function study.

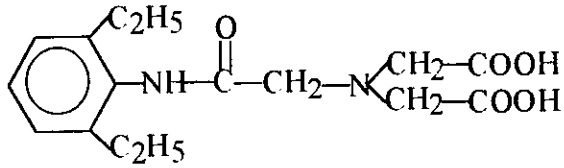
I.7.5. ^{99m}Tc–Chelates for hepatobillary imaging :-

The derivatives of iminodiacetates (IDA) are excellent chelating agents for ^{99m}Tc. The first complex was developed by Loberg et al (1976) and involved the ligand 2,6–dimethylacetanilidoiminodiacetate. ^{99m}Tc–IDA derivatives are prepared by the direct reduction of pertechnetate with Sn(II) in presence of the ligand (IDA) where a ^{99m}Tc–IDA complex is formed. A variety of ^{99m}Tc–chelate of IDA derivatives were evaluated clinically, the trimethyl bromo, iodo and tertiary butyl derivatives of IDA were found to be excellent ligands as reported by Mitta et al (1982) and

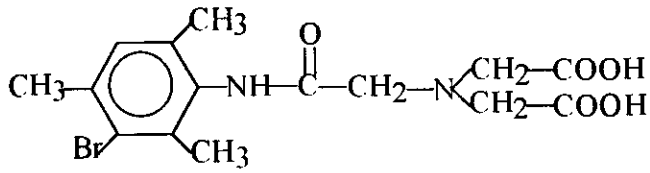
Arguelles et al (1988). The structure of some ^{99m}Tc–IDA derivatives are presented in Fig.(9).



N(2,6–dimethylacetanilido) iminodiacetic acid (HIDA)



N(2,6–diethylacetanilido) iminodiacetic acid



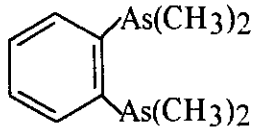
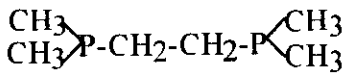
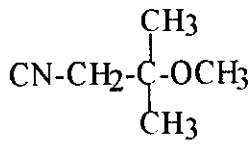
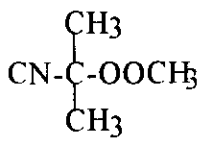
N(3-bromo, 2,4,6–trimethylacetanilido) iminodiacetic acid

Fig(9): Structures of some ^{99m}Tc– IDA complexes suggested as hepatobillary imaging agents.

I.7.5.1. ^{99m}Tc– Chelates for myocardial imaging:

Deutsch et al(1981) first demonstrated that cationic complexes of ^{99m}Tc(III) with ligands of arsine and phosphine, like the monovalent alkali metal ions, localize in the muscle cells. The ligands used for myocardial imaging reviewed by Dewaujee et al (1990). Some of ^{99m}Tc–cationic complexes used for myocardial perfusion imaging are shown in Table(3).

Table (3): The structures of some ligands used for myocardial imaging

Ligand	Abbreviation	Structure
Phenyldimethyldiarsine	DIARS	
Dimethyl Phosphinoethane	DMPE	
2-Methoxyisobutylisonitril	MIBI	
Carbomethoxyisopropyl-isonitril	CPI	

I.7.6. ^{99m}Tc–Complexes for brain perfusion imaging:

^{99m}Tc-Propylene amine oxime (PnAO) was reported by Trounter et al (1984), as brain SPECT imaging agent. Several derivatives of PnAO were synthesized by different methyl substitutions on the amine oxime backbone. One of these derivatives is d,l–hexamethyl propylene amine oxime (d,l-HMPAO). ^{99m}Tc–HMPAO is neutral lipophilic chelate used for measurement of cerebral perfusion of brain by SPECT technique as developed by Ballinger et al(1988). Walovitch et al(1987), demonstrated that oxo-^{99m}Tc(v) complexes of amine derivatized diamine dithiol (DADT) ligands cross the blood brain barrier, permitting measurement of cerebral perfusion. The ^{99m}Tc complex of N, N′-1,2–ethylenediyl–bis–L–cysteine ester (L,L–ECD) demonstrated cerebral uptake and longer retention time in brain as reported by Harris et al(1992). The structure of some ^{99m}Tc chelates used for brain imaging are presented in Fig.(10).

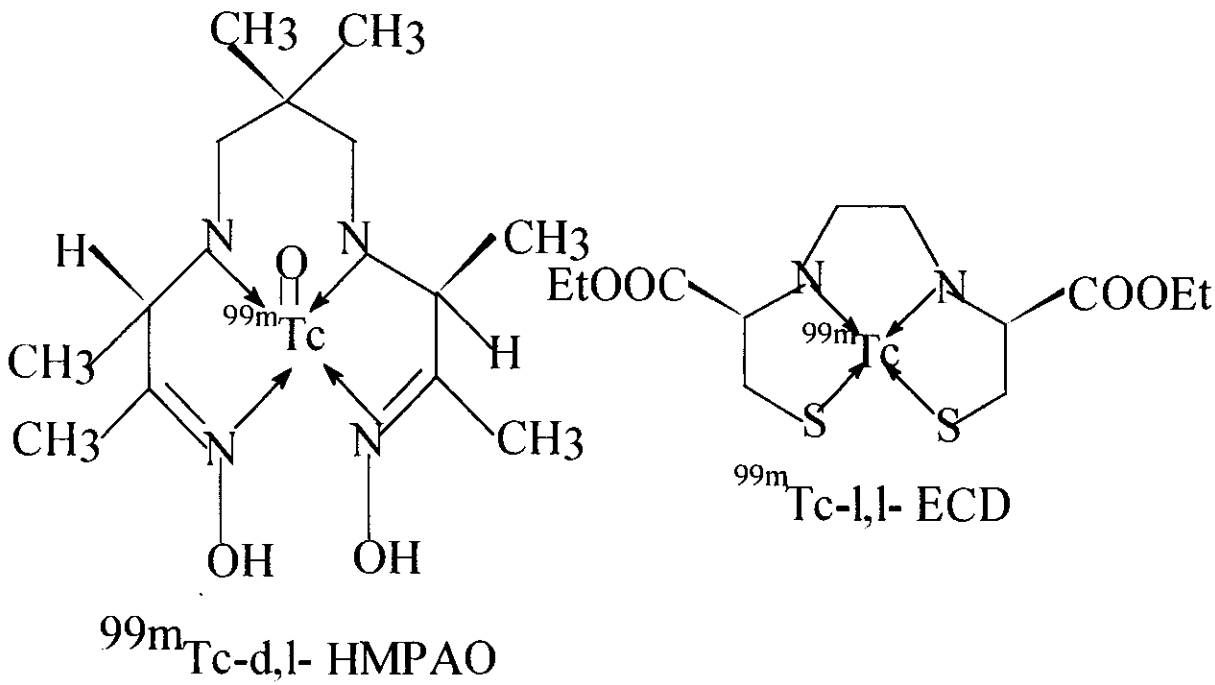


Fig (10): Some ^{99m}Tc-chelates for brain imaging

I.7.7. ^{99m}Tc–Complexes of proteins:

Several proteins (human albumin, neoga–lactoalbumin, fibrmigen, monoclonal and polyclonal antibodies) have been labeled with ^{99m}Tc radionuclide as reported by Hanatawich et al (1987).

The labeling was accomplished by direct chelation of macromolecules (which contain large number of binding sites) with reduced ^{99m}Tc (pertining method) as reported by Eckelman (1990) or by indirect conjugation of ^{99m}Tc complex (pre-complexing agent method) to protein via bifunctional chelating agent as developed by Hnatwisch et al(1987). ^{99m}Tc–Human serum albumin has been used as blood pool imaging agent. Monoclonal antibody fragments $(Fab)_2$ and Fab have been labeled with ^{99m}Tc for radioimmun scintigraphy as reported by Rods (1991). The labeling of these fragments (MoAb) is performed with a variety of bifunctional chelating agents.

^{99m}Tc is coordinated to N_2S_2 (diaminodithiol), EDTA (ethylen diaminetetracetic acid) or (DTPA) which in turn conjugated with MoAb fragments as reported by Baidoo et al (1990) and Rods et al(1991). ^{99m}Tc–MoAb is used for diagnosis of tumers and infraction and thrombosis as reviewed by Baum (1989).

I.7.8. ^{99m}Tc–Blood elements: -

^{99m}Tc–Red blood cells are frequently labeled in vivo by pretining method as reported by Eckelman (1991) method. It is used for measurements of cardiac output, ejection fraction and regional motion. White blood cells and platelets are labeled by ^{99m}Tc using lipid soluble ^{99m}Tc-HMPAO and evaluated in the diagnosis of thrombosis and injection by Dewanjee et al (1989).

I.8. Kit preparation: -

The kits are prepacked sets of sterile pyrogen free reagents (organic chelates of divalent tin) in the lyophilized form, upon addition of

$^{99m}TcO^{-}_{4}$ to the kit, labeled complex is formed. These ^{99m}Tc-complexes have been used as diagnostic agents for the different organs of the body such as DTPA for kidney function, phytate for liver scanning and phosphate for bone scanning as reported by Eckelman et al (1977).

The production of kits for ^{99m}Tc–radiopharmaceuticals is a highly sophisticated process. The composition of such kits has to provide a complete conversion of pertechnetate into the desired ^{99m}Tc–radiopharmaceuticals which must be stable for several hours.

To protect the highly diluted Sn(II) solution from oxidation, the whole process is carried out under purified nitrogen gas. The obtained solution of Sn.(II) chelating agent is immediately dispensed, quickly frozen by liquid nitrogen and instantly lyophilized. After lyophilization the lyophilizator is floaded by oxygen–free nitrogen and the vials are closed under protective gas. The sealed vials can be stored at 2–8°C

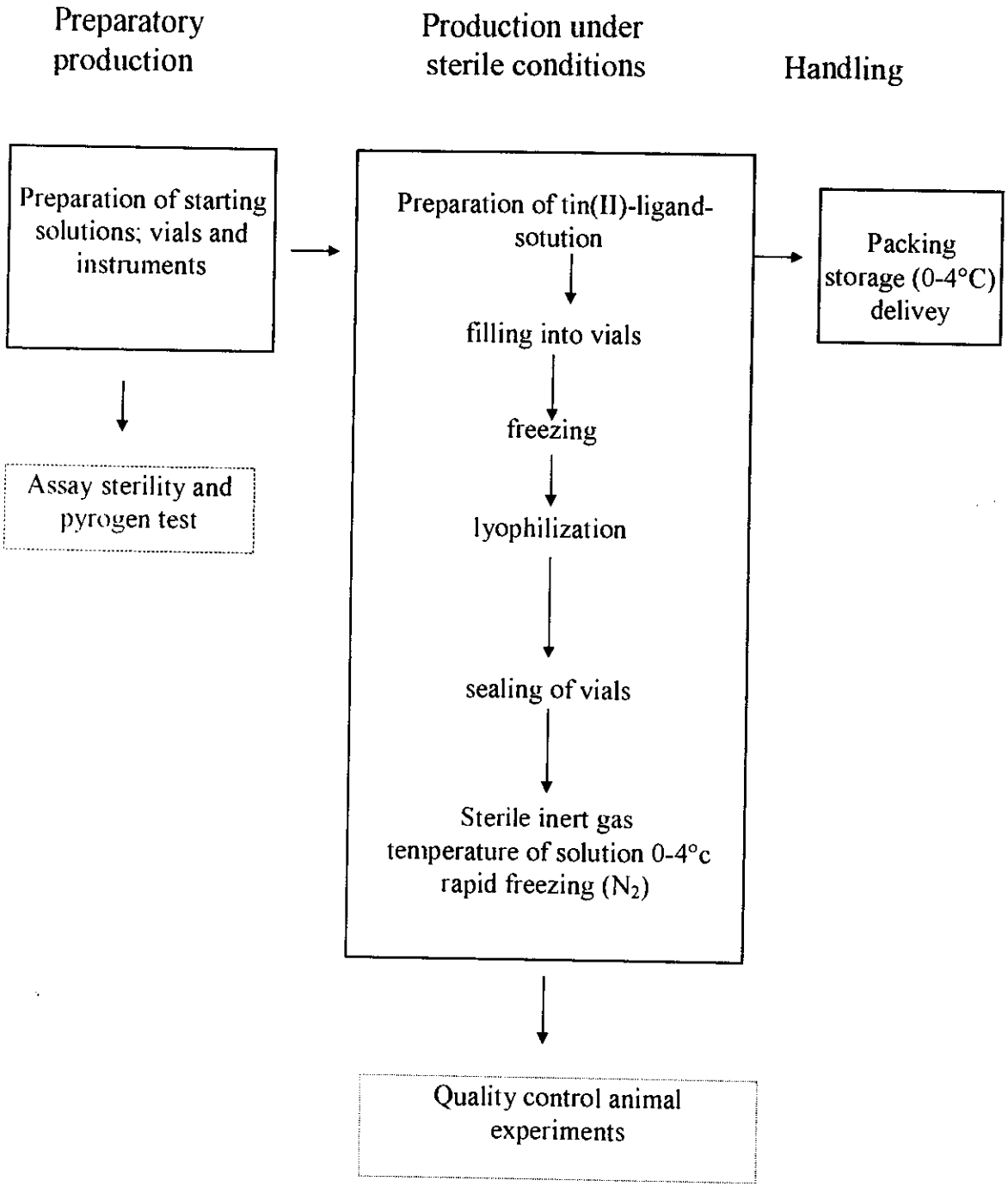


Fig. 12:Scheme for the production of kits for the preparation of ^{99m}Tc - radiopharmaceuticals

For each kit the following points must be involved in its production using the freeze drying process.

- Choice of the suitable parameters that affects the formulation of the kit such as pH, Sn^{2+} content, active ingredients and addition of stabilizing agent.
- Labeling with ^{99m}Tc to determine the labeling yield and biological distribution.
- Quality control has to be performed, stability of kit upon storage and application in nuclear medicine has to be studied to find its eligability for human application.

I.9. Freeze drying:

Freeze-drying is a very important technological process of great relevance to the drug, pharmaceutical industry and food preservation industry. There have been other novel applications also in the preparation of very fine metal powders, preservation of old manuscripts, biological specimens. This technique is primarily used for preserving over a long period of time under ambient temperature conditions of material which otherwise will bio–degrade. Practically the technique involves freezing the specimen below its eutectic point and then drying it by removal of moisture from the condensed phase to vapour phase by sublimation as reported by Goldblith et al (1975).

The removal of water vapour from frozen solution by sublimation forms the basis of freeze-drying technique.

The first consideration in freeze drying of any solution is the temperature at which it must be held for sublimation to occur from the solid state in the absence of dissolved solid, the solution must be cooled below the triple point temperature and the pressure is reduced in the drying chamber to a value below the pressure equivalent to the triple–

point. But when the solution of solids are dried, the depression of freezing point of water by solutes must be considered.

It is essential that the temperature is brought below the eutectic point so that no liquid phase is present. When the eutectic point is not known, freezing the product to about –40°C is usually sufficient.

The production of freeze dried material may be considered in three stages.

I.9.1. Preliminary freezing:

A definite quantity of solution are introduced into the final container and cooled below its eutectic point or to about -40°C as follows:

(1) Freezing by contact with cooled surface: -

This is a static freezing technique where the refrigerating device must be capable of adjusting the freezing rate between 1°C and 4°C per minute. A final temperature of -50°C will normally be sufficient to meet all requirements.

(2) High speeds vertical spin freezing:

This is a dynamic freezing technique which is used wherever larger quantities of a liquid product are to be frozen and dried, e.g. plasma or serum, the bottles are spun on either vertical axes at between 750 and 1000 rpm. The liquid is there by distributed uniformly inside the bottle round the periphery, leaving a conical air space in the center reaching to the bottom of the bottle. The liquid after super–cooling freezes suddenly in this position.

I.9.2. Vacuum evaporation:

The maintenance of the contents in the solid frozen condition during application of the vacuum is virtually important. If a liquid were formed as a result of partial thawing, a considerable amount of degassing

would occur with probable loss of the material, which would float over the sides of the container. Air is exhausted by double–stage, high–vacuum until a suitable vacuum is obtained (a vacuum of 0.01 mm Hg is possible). A condensing coil at the top or the bottom of the chamber is maintained at -50°C. When the pressure is sufficiently low, ice evaporates.

I.9.3. Heat requirement for evaporation:

Latent heat being required for evaporating the residual moisture of frozen material. The solution would cool still further a small source of radiant heat is therefore placed in the chamber head and evaporation takes place rapidly with the frozen material remaining at -20°C until all the ice has evaporated.

Freeze dried powder has many properties:

1. No concentration occurs and the evaporated solid occupies practically the same volume as that of the original frozen solution.
2. No denaturation occurs
3. Easily soluble.
4. Low moisture content and so increased shelf–life.

I.10. Quality assurance of ^{99m}Tc–radiopharmaceuticals: -

^{99m}Tc–Radiopharmacenticals are subjected to quality control tests, and measurements to assure safety, sterility, apyrogenicity, radiochemical purity and suitability for the purpose intended, These criteria are applied to those newly developed products for clinical application.

1.10.I. Moisture content: -

The moisture content in the freeze dried kits intended for ^{99m}Tc–radiopharmaceuticals was determined by heating the freeze dried solid in vacuum oven at 50°C for 7 days. All the kits have ≤ 2% water content of the dry residue as reported by El–Sayed (1989).

I.10.2. Sn (II) Content:

The Sn(II) of the freeze-dried kits intended for reduction of $^{99m}TcO^-_4$ was determined by iodometric titration using standard KIO_3 solution. This method is a modification of iodometic titration previously reported by El-Kolaly(1983) in using indefinitely stable iodate solution instead of the iodine solution. The accuracy and reproducibility of KIO_3 method gives the actual Sn(II) content used in most of ^{99m}Tc-radiopharmaceuticals as reported by El-Asrag et al(1988).

I.10.3. Radiochemical purity:

It is the proportion of total activity in the desired form, ^{99m}Tc-radiofarmaceuticals contains ^{99m}Tc in the following states: -

(1) ^{99m}Tc-chelates : ^{99m}Tc (+4) bound to the chelate .

(2) ^{99m}Tc-reduced and hydrolyzed(^{99m}Tc-RH), ^{99m}Tc(+4) unbound to the chelate .

(3) Tin colloid (Tc): stannous hydroxide $Sn(OH)_2$ formed by hydrolysis of stannous chloride binds for reduced ^{99m}Tc(+4) to form insoluble stannous hydroxide complex .

(4) Sodium pertechnetate (Na $^{99m}TcO_4^-$): state of ^{99m}Tc before reduction or produced after reoxidation by air.

Different chromatographic methods are used to determine the radiochemical purity of ^{99m}Tc-radiopharmaceuticals as reviewed by Robbins (1984).

Chromatography with paper or thin layer (silica gel) with aqueous solution of 0.9% NaCl is used for checking the presence of non-migrating ^{99m}Tc-labeled particulate materials ^{99m}Tc-chelates and RH ^{99m}Tc. Organic solvents (e.g. methyl alchol, methyl ethyl ketone (MEK) and acetone) used to check the presence of free ^{99m}Tc pertechnetate where the water-soluble complex is retained at the origin and pertechnetate

migrates with the solvent front. For lipid soluble complexes such as ^{99m}Tc–HMPAO ethyl alchole with paper media is used for chromatography, where lipid soluble ^{99m}Tc–HMPAO migrates with solvent front. HPLC is used for separation of labeled stereoisomers from pertechnetate e.g. d,l–HMPAO as described by Ballinger et al (1988). It was reported by Phan et al(1981), that the radiopharmaceuticals preparation should contain more than 95% in the ^{99m}Tc–chelate and less than 5% in the form of tin colloid and free $^{99m}TcO_4^-$.

I.10.4. Sterility, apyrogenicity and undue toxicity:

^{99m}Tc–Radiopharmaceuticals are used for human diagnosis by intravenous injection. These products must be sterile–pyrogen free Sterilization of base kits is done by millipore filtration.

Distribution of the filtrate is done in a laminar flow clean bench. All the base kits for ^{99m}Tc-labeling are tested for sterility with thioglycolate medium and for pyrogen in rabbits according to the U-S-Pharmacopoeia (1985) procedures. The undue toxicity test is performed by injection of ^{99m}Tc– complex in the tail vein of mice. No death or abnormal reaction was observed after injection. This test is a quality control to assure that the product is safe for human application and non-toxic.