Chapter (1)

1. GENERAL INTRODUCTION

1.1 Nuclear medicine and radioactivity:

Application of radioactive tracers will remain or even increase because of their sensitivity combined with the ease of detection from outside a closed system, such as the human body. Nuclear medicine is a medical modality that utilizes radioactivity (radiopharmaceutical) to diagnose and treat a disease [(1)]. The speciality of nuclear meadicine can be divided into two main categories ***: diagnostic,*** which is used to determine noninvasively the absence, presence and extent of disease; and ***therapeutic,*** which takes advantage of hight-LET (Linear Energy Transfer) radiation emitted by various radionuclides to deposit large amounts of energy in specific tissues.

In nuclear medicine nearly 95% of the radiopharmaceuticals are used for diagnostic purposes, while the rest are used for therapeutic treatment.

Radiopharmaceuticals usually have no pharmacological effect, because in most cases they are used in tracer quantities. In these cases, they do not show any dose-respond relationship and thus differ from conventional drugs. Because they are administered to human, they should be sterile and pyrogyn free, and they should undergo all quality control measures required for a conventional drug.

Radiopharmaceutical may be radioactive element such as ^{133}Xe, or a labelled compound such as ^{131}I-iodinated compounds and ^{99m}Tc-labelled compounds.

A radiopharmaceutical has two components: a radionuclide and a pharmaceutical. A pharmaceutical is chosen on the basis of its preferential localization in a given organ or its participation in the physiologic function of the organ. Then, a suitable radionuclide is tagged onto the chosen

pharmaceutical such that after administration of the radiopharmaceutical, radiations emitted from it are detected by a radiation detector [2].

Radionuclides used in nuclear medicine are mostly artificial ones. They are primarily produced in a cyclotron or a reactor. The type of a radionuclide produced in a cyclotron or a reactor depends on the irradiating particle, its energy and the target nuclei. Since they are extremely expensive, these facilities are available only in a limited number of institutions. For this reason, very short radionuclides are only available institutions having cyclotron facilities. However, they can not be transferred to remote institutions or hospitals because they decay rapidly.

For remote institutions, there is anothore some of radionuclides, particularly, short-lived ones, which is called a radionuclide generator.

A radionuclide generator is constructed on the principle of the decay-growth relationship between a long-lived parent radionuclide and its short-lived daughter radionuclide. The chemical properties of the daughter nuclide must be distinctly different from those of the parent nuclide so that the former can be readily separated. In a radionuclide generator, basically a long-lived parent nuclide is allowed to decay to its short-lived daughter nuclide and the latter is then chemically separated [2].

The selection of a suitable radionuclide for nuclear medicine depends on whether it will be used in diagnostic or therapeutic purposes. Thus, the morphologic structure or the physiologic function of the organ can be assessed. A radionuclide used in nuclear medicine should be in a carrier free state and give radiation easily detected by nuclear instrument. 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 carrier-free radionuclide only a trace amount of the element is administered to the patient. Freedom from carrier is particularly important in radiopharmaceuticals, which may contain toxic elements such as the ^{201}Ti isotope of the thallium and the ^{67}Ga isotope of gallium.

In the formation of a radiolabelled compound, the success of labelling reaction may depend upon the radionuclide being in a carrier-free state. The selection of a suitable radionuclide for nuclear medicine depends on whether it will be used in diagnostic or therapeutic purposes.

1.1.1 Radionuclides used for diagnostic nuclear medicine:

The radionuclides used for diagnostic nuclear medicine should have the following characteristics :

The first one is that, they should have short half-lives ranging from 10 second to 80 h. This is preferred because higher amounts can be given to the patients without significantly increasing the radiation dose. The second is that, they should emit only γ-rays, no particles emission (the best example is ^{99m}Tc radioisotope). This is preferred bcause a high local irradiation would be eliminated [(1)]. The last one is that, the energies of the emitted γ-rays should be in the range of 100 - 300 KeV to be easily detected by modern gamma cameras.

Some commonly used γ-ray emitting radionuclides and their routine production methods are summarized in Table (1) [(3)].

Gallium-67 is used in diagnostic nuclear medicine as ^{67}Ga-citrate complex, which is employed to detect chronic occult abscesses, lung cancer, hepatoma and melonoma. The complex was localized in these tissues utilizing iron-binding proteins [(1)]. The importance of this nuclide is not only to identify the lesion but also to determine the response to therapeutic intervention.

Table (1): Routine methods of production of some commonly used γ-ray emitter [3]

Radio-isotope	$T_{1/2}$	Mode of decay (%)	Main γ-ray energy in KeV (%)	Production data	
				Nnclear Process	Energy range Mev
^{67}Ga	3.26 d	EC(100)	93 (37) 185 (20)	$^{68}Zn(p,2n)$	26 → 18
^{99}Mo ↓ (generator) ^{99m}Tc	2.75 d 6.02 h	β^-(100) EC(100)	181(6) 740(12) 141(87)	$^{235}U(n,f)$ $^{98}Mo(n,\gamma)$	
^{111}In	2.8 d	EC(100)	173(91) 247(94)	$^{112}Cd(p,2n)$	25 → 18
^{123}I	13.2 h	EC(100)	159(83)	$^{123}Te(p,n)$ $^{124}Te(p,2n)$ $^{127}I(p,5n)^{123}Xe^a$ $^{124}Xe(p,pn)^{123}X^a$ $^{124}Xe(p,2n)^{123}Cs^b$	14.5 → 10 26 → 23 65 → 45 29 → 23 29 → 23
^{201}Tl	3.06 d	EC(100)	69-82 (X-rays) 166(10.2)	$^{203}Tl(p,3n)^{201}Pb^c$	28 → 23

Note: a) ^{123}Xe decays by EC (87%) and β^+ emission (13%) to I^{123}

b) ^{123}Cs decays by B^+ emission and EC to ^{123}Xe.

c) ^{201}Pb decays by EC (100%) to ^{201}Tl.

Indium-111 is extensively used as indium-111 labelled polyclonal human immunoglobulin G (^{111}In*IgG). This agent was designed to image infection. Preliminary studies have been published which indicate that ^{111}In*IgG is an inflammation detecting agent that could be considered a replacement for the present agents, ^{67}Ga or ^{111}In white blood cells

(WBC)[4]. It has shown utility for detection of both chronic and acute infections.

Iodine-123 has gained considerable importance in nuclear medicine because it has good radiation characteristics such as decay by electrons capture, half-life of 13.2 h and γ-ray emission of 159 KeV. Iodine-123 radiopharmaceuticals find applications in functional diagnostics.

For examples, 3-[^{123}I]Iodo-α-methyl-L-tyrosine found application in brain tumor diagnosis [5] and [^{123}I]-monoclonal antibodies used for radio-immunoscintigraphy.

Thalium-201 used potentially in nuclear medicine as thallous-201 chloride. This nuclide is widely used throughout the world in the management and diagnosis of coronary artery disease [1].

Imaging with ^{201}Tl facilitates a functional assessment of the myocardium that directly reflects the blood flow to myocardial tissue.

This nuclide provides a physiological assessment of the heart segments which are no longer functioning after a heart infraction.

Technetium-99m is considered as one of the most useful radionuclides used in diagnostic nuclear medicine [6]. It became the backbone of routine imaging. The reason for such a permanent position of technetium-99m in clinical use is its extremely favorable physical and radiation characteristics [7]. The monoenergetic gamma emission of 140 KeV is well suited for use with the modern gamma cameras. A total lack of both alpha and beta paricles results in the absence of any unnecessary radiation dose to the patient. Its physical half-life 6.02 h so high amounts of radioactivity can be administrated to the patient, thus providing good resolution and increased information content in the collected images without significant radiation dose to the patient. Technetium-99m is readily available as sterile, pyrogen-free and no-carrier-added radionuclide from

$^{99}Mo/^{99m}Tc$ generators. Due to the importance of ^{99m}Tc in today nuclear medicine, it was found necessary to mention it in more details.

1.2 Chemistry of technetium:

Technetium does not occur naturally and it was the first man-made element. Technetium-99m, is today the most widely used radionuclide in modern diagnostic medicine. The chemistry of technetium has been reviewed in various text books [7-11]. Technetium is a silver gray metal. It is a transition metel belonging to group VIIA (Mn,Tc, Re) [10]. Some of the fundamental data of technetium are summarizedin table (2).

Table (2): Some of the fundamental data of technetium

Property	Tc - element
Atomic number	43
Number of naturally occuring isotopes	0
Atomic weight	98.906[(a)]
Melting point	2200 ^{o}C
Electronic configuration	[Kr] $4d^6$ $5s^1$
Electronegativity	1.9
Atomic radius (A^{o})	1.358
Oxidation state	(-1) to (+7)

(a) This refers to ^{99}Tc ($T_{1/2}$ 2.13×10^5 y)

Technetium metal is less reactive than manganese. It does not react with H_2O, or non-oxidizing acids and it does not dissolve in HCl and HF, but it reacts with oxidizing acids such as concentrated HNO_3 and H_2SO_4, forming pertechnetic acid, ($HTcO_4$).

It has different oxidation states between -1 and +7. Technetium of oxidation state +7 is present as the pertechnetate anion ($^{99m}TcO_4^-$) which is the most stable chemical form of technetium, followed by the tetravalent

state (TcO_2); which gains stability through complex formation. Its electronic configuration of technetium is $4S^2,4P^6,4d^6,5S^1$ or $4S^2,4p^6,4d^5,5S^2$.

It forms compounds of all oxidation states, from –1 to +7 but the most stable being those 4+ and 7+ states. The oxidation state of 5+ and 6+ are important in some analytical applications and in the chelate compounds.

Oxides of technetium in the VII and IV oxidation states (Tc_2O_7 and TcO_2) have been studied by many authors [12,13]. The volatile Tc_2O_7 is obtained on burning the technetium metal in excess of oxygen at 500°C.

Nelson et al.[14] studied the relatively nonvolatile TcO_2 obtained by the reduction of the aqueous solution of pertechnetate with zinc in HCl. TcO_2 can also be produced by thermal decomposition of NH_4TcO_4 at 950°C in nitrogen atmosphere [15].

Technetium can be present in two sulfides forms, namely Tc_2S_7 and TcS_2.

The black insoluble heptasulfide is volatile and can be obtained by the addition of H_2S to TcO_2^- in the presence of 2-4 M HCl. On the other hand, TcS_2 can be prepared by heating Tc_2S_7 with sulfur at 1000°C for 24 h.

Technetium reacts with halogens forming halides with different oxidation state. For example, the hexafluoride is readily formed on fluorination of technetium at 400°C, however TcCl is produced as the only product of the reaction of chlorine with technetium. $TcCl_4$ can be obtained by the addition of CCl_4 to Tc_2O_7 at 400°C and 100 atmosphere.

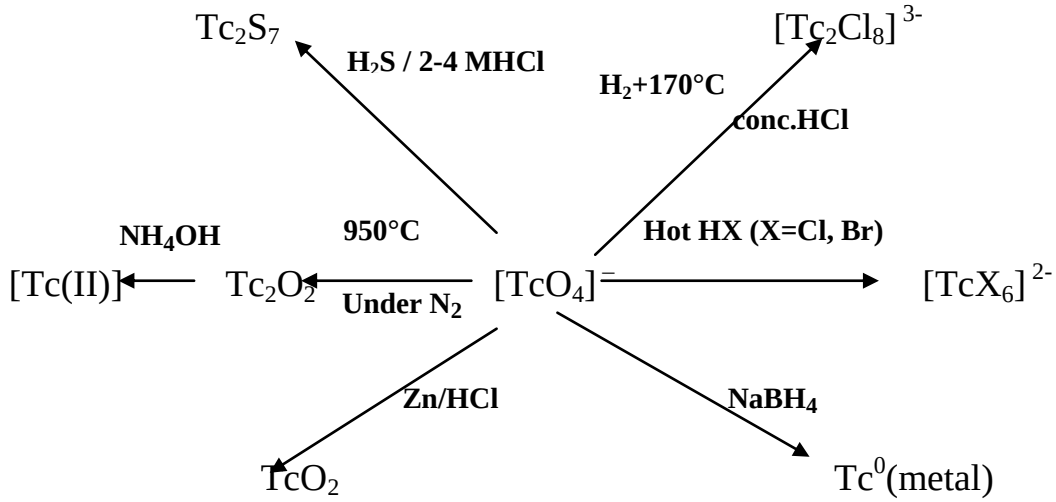


Fig. (1) Selected relationships of technetium chemistry.

The Tc^{5+} and Tc^{6+} species frequently disproportionate into Tc^{4+} and Tc^{7+} states as follow**:-**

$$3Tc^{5+} \longrightarrow 2Tc^{4+} + Tc^{7+} \quad (1)$$

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

Technetium-99m of oxidation state 7+ is present as the pertechnetate anion ($^{99m}TcO_4^-$) which is the most stable chemical form of technetium and it is the starting material of the preparation of nearly all ^{99m}Tc-radiopharmaceuticals followed by the tetravalent state ($^{99m}TcO_2$) which is the other oxidation state gains stability through complex formation.

Technetium metal, has a melting point 2200^{o}C and a boiling point 4973^{o}C. Metallic technetium dissolves in acids that are oxidants such as nitric acid, aqua regia, and concentrated sulfuric acid. It dissolves in bromine water and also in neutral and alkaline solutions of hydrogen peroxide.

1.3 Nuclear and Radiochemistry of Technetium:

All nuclides of technetium are radioactive and the half-lives range from 0.77 msec (technetium-101m) to 2.6 x 10^6 year (technetium-97).

A summary of the most important radionuclides of technetium and their properties is given in Table (3) [5]. Most of the biomedical applications of technetium so far have utilized ^{99m}Tc because it has the best nuclear properties for imaging devices, as mentioned previously.

The radionuclide ^{99m}Tc has a half-life of 6.02 h 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 2.1 x 10^5 years and decays to stable ^{99}Ru by β^--emission as shown in Figure (2).

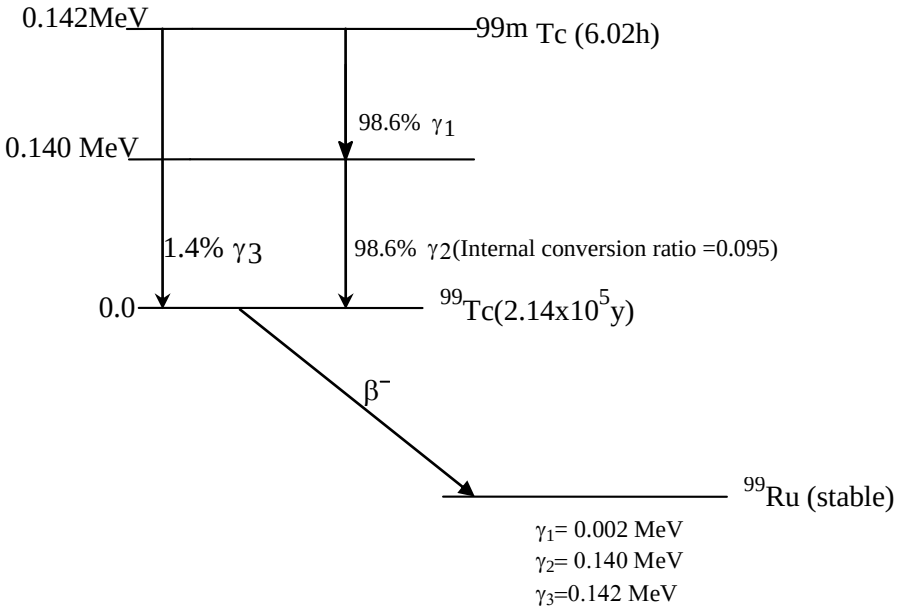


Fig. (2): Decay scheme of technetium-99m

The ^{99}Mo radionuclide has a half-life of 66.02 h and decays by β^--emission; (87.5%) to the metastable state ^{99m}Tc and the remaining 12.5% to the ground state ^{99}Tc, as shown in Fig. (3).

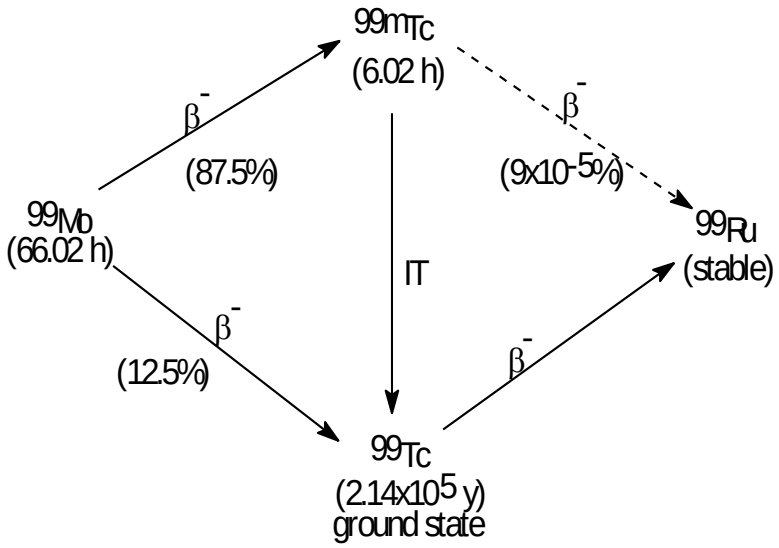


Fig.(3): Decay scheme of ^{99}Mo and ^{99m}Tc radionuclide

1.4 Production of technetium-99m:

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

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

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

Technetium–99m is the radioactive daughter nuclide of ^{99}Mo as shown from Figure (2). The criteria of choosing the method of ^{99}Mo production must involve the economic resourses as well as the mode of utilization [16].

1.4.1 Methods of technetium–99m separation:

Various methods for the separation of the short-lived daughter radionuclide (^{99m}Tc) from the long-lived parent radionuclide (^{99}Mo) are known [17].

The most important techniques, extensively used for this purpose, are:

1- Alumina column chromatography [17-20].

2- Solvent extraction [19,20-23].

3- Sublimation [19].

4- Insoluble ^{99}Mo-compound formation (gel) [24-26].

If the daughter product can be separated simply, reliably and fairly complete from the parent isotope, the system can serve as a source for the former. Systems devised for such separations are known as radioisotope generator.

Table(3): Principal nuclides of technetium [5]

Nuclide	Half-life	Preparation	Decay mode	Principal Radiations (MeV)
^{92}Tc	4.4 mim	-	β^+ γ	4.2 1.51, 0.77, 0.33, 0.15
^{93}Tc	2.73 h	-	E.C., β^+ γ	0.80, 0.64 1.363, 1.522, 1.478, 0.171
^{93m}Tc	43 min	-	I.T. E.C.,ν	0.390 2.65
^{94}Tc	4.8 h	-	E.C., β^+ γ	0.815 0.871, 0.703, 0.85, others
^{94m}Tc	52 min	-	β^+ γ	2.47 0.871, others
^{95}Tc	20.0 h	^{95}Mo (p,n)	E.C., γ	0.766, others
^{95m}Tc	61 day	^{95}Mo (p,n)	E.C., β^+ I.T., γ	0.70, 0.49 0.039, 0.204, 0.582, others
^{96}Tc	4.3 day	^{96}Mo (p,n)	E.C., γ	0.778, 0.850, 0.813, others
^{96m}Tc	52 min	^{96}Mo (p,n)	I.T., γ E.C., γ	0.034 0.778, 1.200, 0.850, others
^{97}Tc	2.6×10^6 y	Daughter, ^{97}Ru	E.C., no γ	-
^{97m}Tc	90 day	^{97}Mo (p,n)	I.T., γ	0.097
^{98}Tc	$\sim1.5\times10^6$ y	^{98}Mo (p,n)	β^- γ	~ 0.3 0.67, 0.76

^{99}Tc	$2.13x10^5$ y	Fission of U(~6%); $^{98}Mo(n,\gamma)^{99}M$, ^{99}Tc	β^-, no γ	0.292
^{99m}Tc	6.02 h	Daughter,^{99}M; $^{98}Mo(n,\gamma)^{99}Mo$ $^{235}U(n,f)^{99}Mo$	I.T., γ no β^- negligible	0.1405
^{100}Tc	16 sec	^{100}Mo (p,n) ^{99}Tc (n,γ)	β^+ γ	3.4, 2.8, others 0.540, 0.591, others
^{101}Tc	14.2 min	^{100}Mo (d,n)	β^- γ	1.32, 1.07, others
^{101m}Tc	0.77 msec	-	γ	0.192
^{102}Tc	5.3 sec	-	β^- γ	4.2, 3.4, 2.2 0.475, 1.10, 0.628
^{102}Tc	4.3 min	-	β^- γ	1.6, others 0.475, others
^{103}Tc	50 sec	-	β^- γ	2.2, 2.0 0.135, 0.35, 0.211
^{104}Tc	18.0 min	-	β^- γ	2.4, 3.2, 4.3 0.358, 0.530, 0.884
^{105}Tc	8.0 min	-	β^- γ	3.4, others 0.143, 0.159, 0.108, others

Note: msec = milliseconds; sec = seconds; min = minutes; h = hours;

β^+ = positron; β^- = beta particles; γ = gamma emission;

E.C.= electron capture; I.T.= isomeric transition; p = protons;

n = neutrons; d = deurtons

1.5 Labelling with technetium-99m:

Labelling a molecule with ^{99m}Tc is a complex formation, where the chemical forms of ^{99m}Tc available from $^{99}Mo/^{99m}Tc$ generator is sodium pertechnetate ($Na^{99m}TcO_4^-$). The pertechnetate ion, $^{99m}TcO_4^-$, has the oxidation state 7+ for ^{99m}Tc. Chemically, $^{99m}TcO_4^-$ is a rather nonreactive species and does not label any compound by direct addition. In ^{99m}Tc-labelling of many compounds, prior reduction of ^{99m}Tc from the 7+ state to a lower oxidation state (5+, 4+ or 3+), which is more chemically reactive, is required. The most important reducing agents used are in the descending order: stannous ion, ferric chloride with ascorbic acid, sodium borohydride ($NaBH_4$) and electrolysis with zirconium electrodes [27]. Stannous chloride is the most commonly used reducing agent in acidic medium in preparations of most ^{99m}Tc-labelled compounds. The chemical reactions that occur in the reduction of technetium by stannous chloride in acidic medium can be stated as follows:

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

$$2^{99m}TcO_4^- + 16H^+ + 6e^- \rightleftharpoons 2^{99m}Tc^{4+} + 8H_2O \qquad (4)$$

by adding the two equations, it gives

$$2^{99m}TcO_4^- + 16H^+ + 3Sn^{2+} \rightleftharpoons 2^{99m}Tc^{4+} + 3Sn^{4+} + H_2O \qquad (5)$$

The last equation indicates that $^{99m}Tc^{7+}$ has been reduced to $^{99m}Tc^{4+}$, and other oxidation states such as $^{99m}Tc^{3+}$ and $^{99m}Tc^{5+}$ may be formed under different physicochemical conditions. The reaction is reversible and not stable unless stabilized by the addition of the complexing agent (pharmaceutical compound).

Compounds, which form complexes with metals may have one donor atom (monodentate), two donor atoms (bidentate) or more (polydentate).

Some of these compounds which have functional groups are shown in Table (4), such as hydroxyl, carbonyl, amino, mercapto, phosphonate, cyanide, and phosphate have been developed [28].

Table (4): Essential groups present in many chelating agents used for ^{99m}Tc-complexation [29]

Ligands	Function groups (donor groups)
Citrate	OH, COOH
Glucoheptonate	OH, COOH
Gluconate	OH, COOH
DTPA(diethylenetriaminepentaacetic acid)	COOH, NH
DMSA (dimercaptosuccenic acid)	SH, COOH
Penicillamine	SH, NH_2, COOH
HEDP (hydroxyethylene diphosphonate)	OH, PO_3H
MDP (methylene diphoshonate)	PO_3H
HIDA{N-(2,6-dimethylphenylcarbamoyl methyl) iminodiacetic acid}	NH, COOH
DIARS (phenyldimethyl arsine)	As
TMP (trimethyl phosphine)	P
Mercaptoacetyltriglycine (MAG_3)	SH, NH
L,L-ethylcystinate dimer (ECD)	SH, NH
Hexamethylpropyleneaminoxime(HMPAO)	OH, NH
2-methoxy-2-isobutyl isonitrile (MIBI)	CN
Ethylenedicystiene (EC)	COOH, SH, NH

These obviously essential groups occur in many chelating agents such as carbohydrates, proteins, hydrophilic drugs, mercaptoacids 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 behavior can be expected to be caused by considerably different properties of the free ligand and labelled complex.

1.5.1 Labelling Methods with ^{99m}Tc:

The labelling methods with ^{99m}Tc can be divided into three main methods:

1.5.1.1 Direct Labelling Method [2]:

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

Reduced ^{99m}Tc + chelating agent $\rightleftharpoons$ ^{99m}Tc-chelate (6)

The chelating agent containing atoms like O, N, S or P usually can donate lone pairs of electrons to form coordinate (covalent) bonds with ^{99m}Tc.

Functional groups such as -COOH, -OH, -NH_2, -PO_3H_2 and -SH are electron donors and found in compounds such as DTPA, glucoheptonate, DMSA, HIDA, MDP, HEDP, HIDA, citrate and various proteins as mentioned before in Table (3).

1.5.1.2 Transchelation or ligand exchange labelling method (Indirect labelling method):

The ligand exchange method, also termed the transchelation [2], involves first forming a ^{99m}Tc-complex with a weak ligand in aqueous media and then allowed to react with a second ligand that is forming relatively more stable complex with ^{99m}Tc. 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-labelled mercaptoacetyltriglycine (MAG_3) [2], ^{99m}Tc-tartrate or ^{99m}Tc-gluconate complex 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 the formation of ^{99m}Tc-MAG_3 complex. The following are the sequence of reactions of the formation of ^{99m}Tc-MAG_3 complex:

$$^{99m}TcO_4^- + Sn^{2+} \rightleftharpoons \text{Reduced } ^{99m}Tc + Sn^{4+} \quad (7)$$

$$\text{Reduced } ^{99m}Tc + \text{tartrate} \rightleftharpoons ^{99m}\text{Tc-tartrate} \quad (8)$$

$$^{99m}\text{Tc-tartrate} + MAG_3 \rightleftharpoons ^{99m}\text{Tc-}MAG_3 + \text{tartrate} \quad (9)$$

El-kolaly et al. (1996) [7] reported that MAG_3 can also be labelled with ^{99m}Tc by the transchelation method by using gluconate, glucoheptonate, pyrophosphate, or citrate as a weak ligand, giving a high radiochemical yield of ^{99m}Tc-MAG_3 (> 99 %). This method is used when the stability and solubility of the stonger chelate is low in aqueous media. The ligand exchange reactions usually required heating at 75°C to 100°C for a relative longer period. Based on these principles, several kits for ^{99m}Tc-labelling have been formulated containing both weak and stronger ligands along with stannous ions.

Examples are tartrate and MAG_3 for renal imaging, EDTA and

ethylcysteine dimer for brain perfusion imaging, and hexakis-methoxyisobutyl isonitrile and sodium citrate for myocardial perfusion imaging.

1.5.1.3 Labelling with bifunctional chelating agents:

In general, bifunctional chelating agents are compounds that comprise both a powerful metal chelating group and a second functional group that is usually chemically reactive in nature. The metal chelators are most often derived from polyaminocarboxylic acids such as EDTA, DTPA, or iminodiacetic acid (IDA) [30] because of their large formation constants with a variety of metal ions and their relative ease of synthetic manipulation. One functional group is always a multidentate metal chelating ligand, but the other functional group can be of various types. It can be a reactive moiety capable of forming covalent bonds with biological molecules, or a hydrophobic aliphatic chain likely to incorporate into a biological membrane, a haptenic molecule with affinity for an antibody, etc. The role of the second functional group is therefore, to direct the stable metal chelate to a region of interest in a biological system. Radioactive metal ions attached by chelation to small molecules, peptides, or proteins such as monoclonal antibodies have been clinically used for diagnosis of cancer [31].

The advantage of this technique is that the chemical modification of the protein is separated from the addition of radionuclide. Labelled proteins may be prepared, purified, characterized, and stored in non-radioactive form. Just before the modified proteins are used for imaging, the radioactive metal ion may be added in a simple and rapid step.

It was reported that, the protein-metal conjugate prepared using bifunctional chelating agents are generally quite stable [32]. One of the principal reasons for the development of bifunctional chelating agents for radiolabeling is the availability of radionuclides with convenient half-lives

and useful radiation, for example, ^{99m}Tc, ^{111}In, ^{67}Ga, and ^{212}Pb. Figure (4) shows structures of some common bifunctional chelating agents.

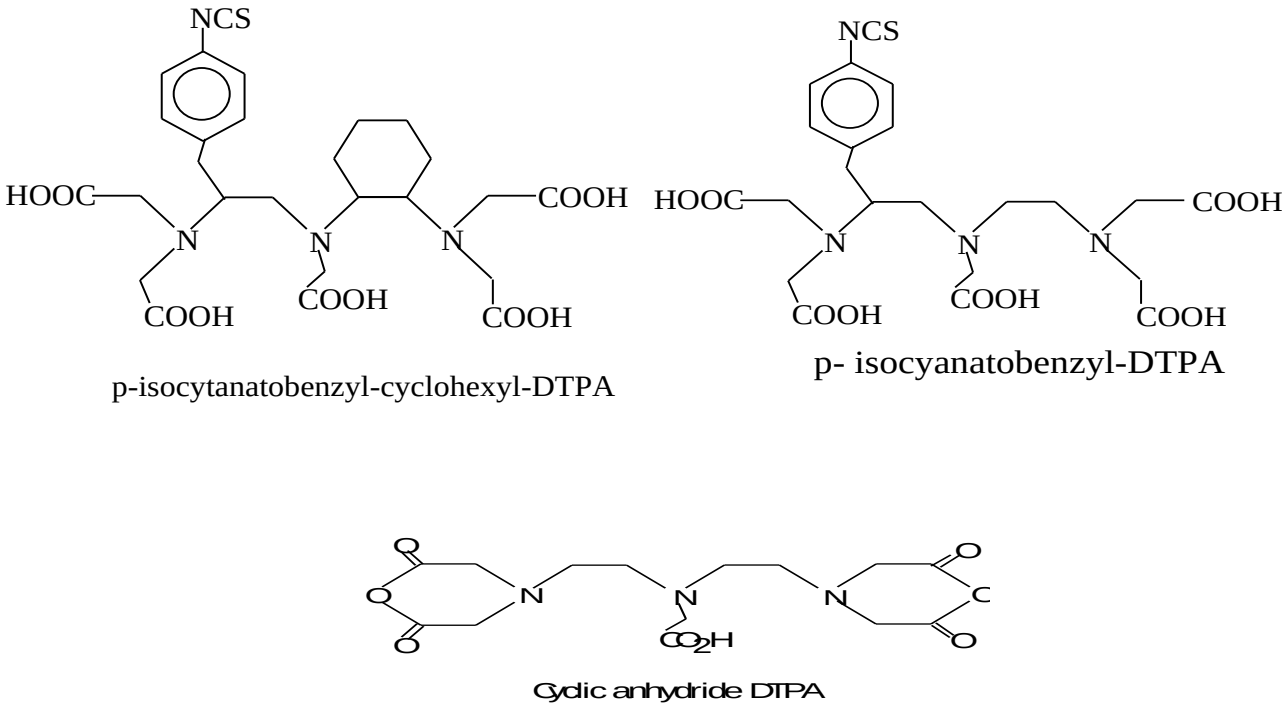


Fig. (4): Structures of some common bifunctinal chelating agents.

1.6 Groups of ^{99m}Tc-radiopharmaceuticals:

^{99m}Tc-radiopharmaceuticals are compounds, which contain chelating groups can bind the reduced technetium, and are concentrated in the organs of choice depending on the ability of that organs to remove foreign substances from the blood circulation [33].

Recently, the field of nuclear medicine imaging has been viewed as the portrayal of regional physiology and biochemistry.

1.6.1 Classes of ^{99m}Tc-radiopharmaceuticals

1.6.1.1 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 as possible biological tracer by Harper et al. [34,35] It may be administrated orally or intravenously in human patient, in the blood stream it is bound to transferrin , then it is concentrated in the thyroid, choroid plexus mucosa of stomach and colon, then extracted via salivary glands, kidneys and retained in the bladder [36].

1.6.1.2 ^{99m}Tc-Labelled colloids and particulates:

^{99m}Tc-sulfur colloid is prepared by heating mixture of $^{99m}TcO_4^-$ and sodium thiosulphate in acidic medium for 5 to 10 min in boiling water bath.

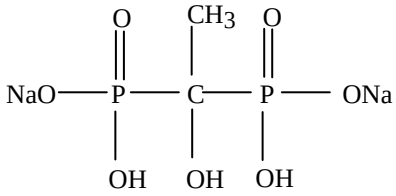
Gelatin is added before the reaction with the acid in order to stabilize sulfur in colloid state. It is used for scintigraphy of the recticuloendothelial system. Other ^{99m}Tc-labelled colloids such as $^{99m}Tc(OH)_4$, $Sn(OH)_2$, ^{99m}Tc antimony sulfide (^{99m}Tc-Sb_2S_3) colloid, and ^{99m}Tc microparticulates of denaturated albumin are used for imaging the resident pool of macrophage in the recticuloendothelial system. The ^{99m}Tc-antimony sulfide colloids have a narrow particles size distribution (5 to 15 μm) than ^{99m}Tc sulfide colloid (300 to 400 μm) and the former migrate much faster after administration facilitating regional lymphoscintrography and less erythema at the sites of injection. Some of these colloids were also used for labeling polymorphonuclear neutrophilic (PMN) granulocytes, which are used for diagnosis of infection.

1.6.1.3 ^{99m}Tc-Chelates for skeletal imaging:

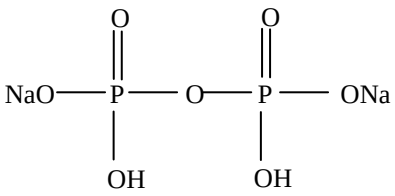
These ^{99m}Tc-chelates are used for skeleton scintigraphy such as MDP, HEDP, HMDP, pyrophosphates and polyphosphates [29].

Diphosphonates chelating agents are analogue to pyrophosphate whose P–O–P structure is replaced by P–C–P which function as regulator of bone metabolism and is more stable against enzymatic decomposition by phosphatase enzyme. The organic phosphonates are more stable than the inorganic phosphates against *in-vivo* metabolis [37].

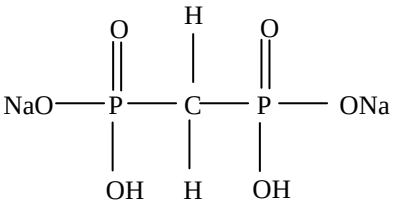
The structure of some pharmaceuticals compounds used for skeletal imaging are presented in Figure (5).



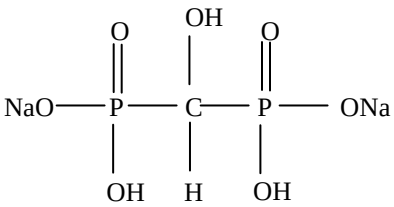
Disodiumhydroxyethylenediphosphonate, (HEDP)



Disodiumpyrophosphate, (PYP)



Disodiummethyleneduphosohonate, (MDP)



Disodiumhydroxymethylenediphosphonate, (HMDP)

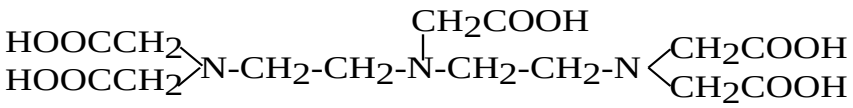
Fig. (5): Structures of some pharmaceuticals used for skeletal imaging after labelling with ^{99m}Tc.

1.6.1.4 ^{99m}Tc-Chelates for renal imaging:

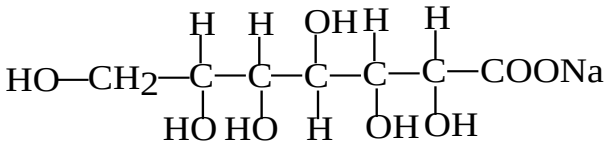
Several ^{99m}Tc-chelates, like a variety of organic acids and bases are filtered by the glomerulus. A few of them may be partially secreted by the proximal tubular cells and may undergo partial tubular reabsorption passively depending on their Pk_a,S, lipid solubility, and pH of the tubular fluid. The tubular cells retain a variety of metal ions by chelation with thiol groups present in these proteins. Numerous ^{99m}Tc-complexes have been prepared examining the kidney function study such as diethylenetriaminepentaacetic acid (DTPA) [38], glucoheptonate (GHA) [19] and dimercaptosuccinic acid (DMSA) [39].

Recently, the triamide mercaptide (N_3S) technetium complex, ^{99m}Tc-mercaptoacetyltriglycine (MAG_3), was proposed by Fritzberg et al [40].

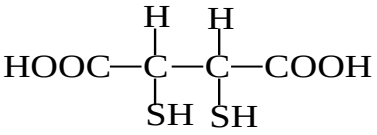
On the other hand, ^{99m}Tc-ethylenedicysteine (EC), showed characteristics comparable to MAG_3 [41]. The structure of some pharmaceuticals compounds for renal imaging are presented in Fig.(6).



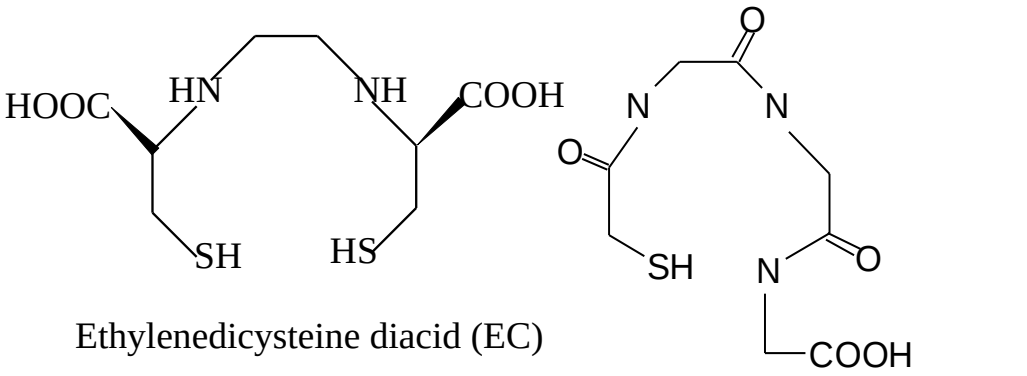
Diethylenetriaminepentaacetic acid (DTPA)



Glucoheptonate (GHA)



Dimercaptosuccinic acid (DMSA



Ethylenedicysteine diacid (EC)

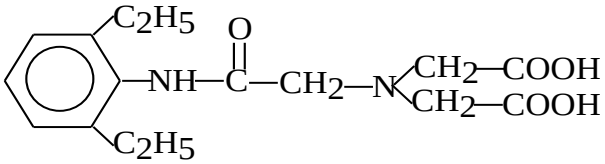
Mercaptoacetyltriglycine (MAG_3)

Fig. (6): Structures of some radiopharmacenticals for kidney imaging and renal function study after labelling with ^{99m}Tc.

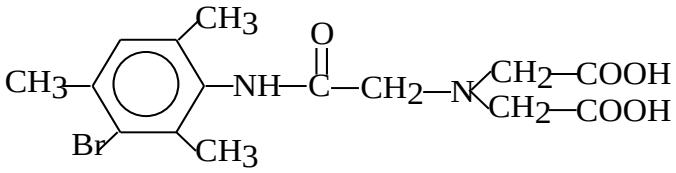
1.6.1.5 ^{99m}Tc-Chelates for hepatobiliary imaging:

The derivatives of iminodiacetates (IDA) are excellent chelating agents for ^{99m}Tc. The first complex involved is 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.

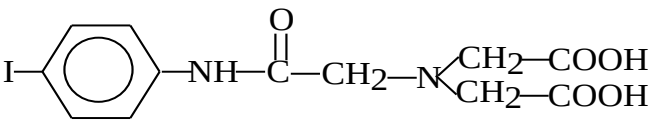
A variety of ^{99m}Tc-chelate of IDA derivatives were evaluated clinically, the 3-bromo-2,4,6-trimethyl, iodo, diethyl derivatives of IDA and tertiary butyl derivatives of IDA were found to be excellent ligands [42,43] where are subsequently used in hepatobiliary imaging. The structure of some IDA derivatives are presented in Figure (7).



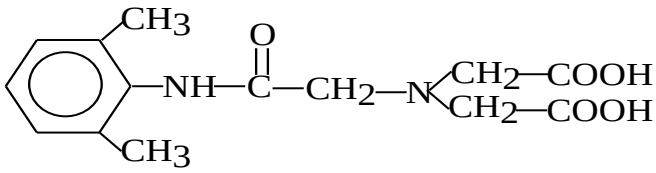
N(2,6–diethylacetanilido) iminodiacetic acid (DIDA)



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



N(p-iodoacetanilido) iminodiacetic acid



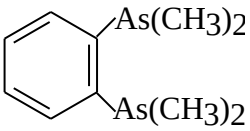
N(2,6–dimethylacetanilido) iminodiacetic acid (HIDA)

Fig. (7): Structures of some IDA complexes suggested as hepatobillary imaging agents after labelling with ^{99m}Tc.

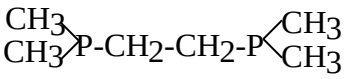
1.6.1.6 ^{99m}Tc-Chelates for myocardial imaging:

The cationic complexes of ^{99m}Tc (III) with the neutral ligands of arsine and phosphine like the monovalent alkali metal ions localize in the muscle cells [44].

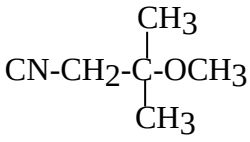
The cationic technetium-99m complexes, alkyl isonitriles complexes, where the alkyl groups are methyl, ethyl, tertairy butyl or methoxy isobutyl were synthesized by Holman et al [45] and Proulx et al [46]. The structures of some ligands used for myocardail imaging are presented in Fig. (8).



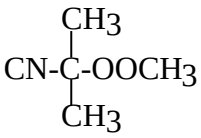
Phenyldimethyldiarsine,(DIARS)



Dimethyl Phosphinoethane, (DMPE)



2-Methoxyisobutylisonitrile, (MIBI)



Carbomethoxyisopropyl-isonitrile, (CPI)

Fig. (8): Structure of some pharmaceuticals used for myocardial perfusion imaging

1.6.1.7 ^{99m}Tc-Complexes for brain imaging:

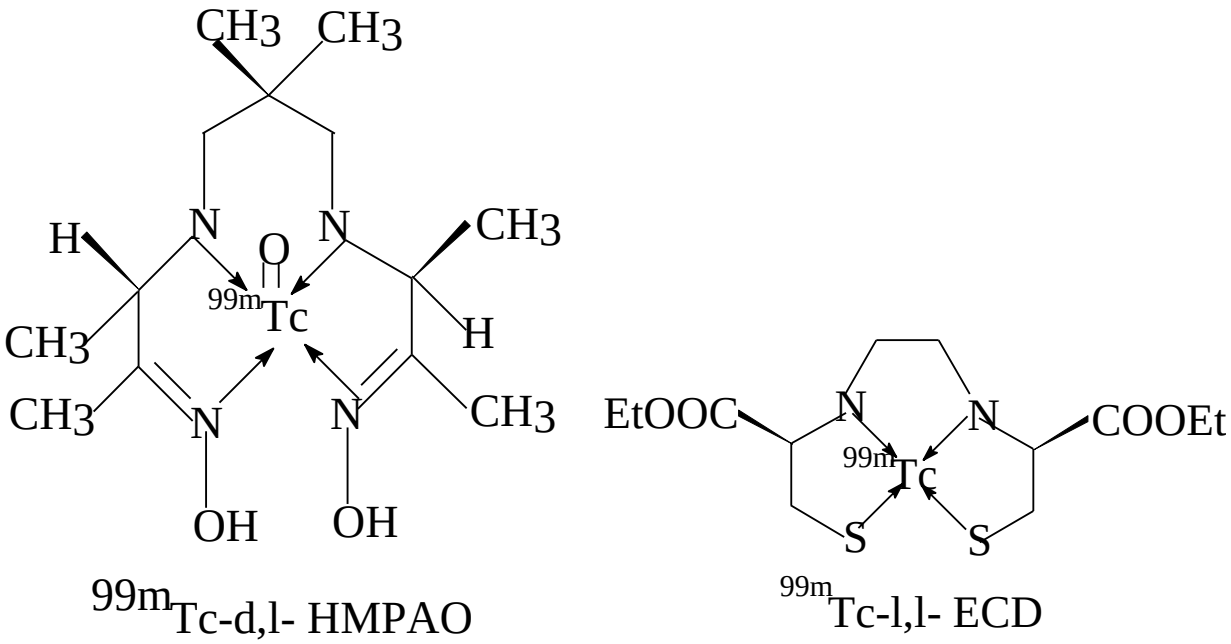
The principle of brain imaging is governed by a mechanism called blood brain barrier (BBB), which excludes many substances from entering the brain from the blood. The BBB is probably a function mixture of anatomic, physiologic, and metabolic phenomena, and which of these are effective in a particular instance depends on the physicochemical properties of the substance in question. Recently, two groups of ligands have been studied extensively for their ability to form neutral lipid soluble complexes with reduced technetium capable of penetrating the blood brain barrier. The first group comprises diaminodioxime derivatives, while the second comprises diaminodithiol derivatives. Several derivatives of propylene amino oxime (PnAO) were synthesized by different methyl substitutions on the amino oxime backbone.

The most important one of these derivatives is d,l–hexamethylpropylene aminoxime (d,l-HMPAO).

^{99m}Tc-d,l-HMPAO is neutral lipid soluble ^{99m}Tc complex used for measurement of cerebral perfusion of brain [47].

The ^{99m}Tc complex of L,L-ethylcysteinate dimer (ECD) demonstrated cerebral uptake and longer retention time in brain [48].

The structures of some ligands used for brain imaging are given in Fig. (9).



^{99m}Tc-d,l- HMPAO ^{99m}Tc-l,l- ECD

Fig. (9): Structures of some ^{99m}Tc-radiopharmaceuticals used for brain perfusion imaging

1.6.1.8 ^{99m}Tc-Complexes for lung imaging:

Lung perfusion imaging is based on the trapping of large particles in the capillary bed of the lungs. Particles larger than 10 mm are lodged in the capillary in the first pass of circulation through the pulmonary artery following intravenous administration. The most widely used ^{99m}Tc-labelled particles for lung study are:

1- Microspheres of denatured human serum albumin.

2- Macroaggregated albumin (MAA).

3- ^{99m}Tc-labeled Aerosol.

1.7 Radionuclides used for therapeutic nuclear medicine:

In recent years, attention has been focused on radiopharmaceuticals therapy. In order to optimize the therapeutic effect, selection of the radionuclides is an important factor. The physical properties of the radioisotope itself (mainly half-life, and the type and energy of the radiation emitted) should be taken into account in the selection [49-51].

The strategy in radiopharmaceutical used as therapeutic agent (frequently known as RPT) is to deposit the greatest amount of energy in shortest time to the malignant target cells, while sparing the healthy ones from unwanted radiation.

The deposition of energy is measured by what is known as Linear Energy Transfer (LET). The type and energy of the emitted particle radiation determine the LET of the radiation in tissues [52]. It is noteworthy that gamma radiation exhibits low values of LET, as it penetrates relatively deeply, on the order of several centimeters and does not deposit much energy along its track [49].

Consequently, pure gamma-emitting radionuclides are not usually used for therapeutic purposes. Beta particle-emitting radionuclide , on the other hand, deposit greater energy near the site of tumor localization, and hence are more suitable for therapeutic applications.

In practice, radionuclides that emit beta particles, as well as those capturing electrons that emit what is known as Auger electrons, are the only ones that have been used in therapeutic nuclear medicine [49], this is due to various reasons, firstly, beta particles have penetration ranges in tissue on the order of millimeters to a few centimeters. Secondly, some of the most promising beta-emitting radionuclides have desirable half-lives (between some hours and about 70 days) [3].

Lastly, many of these radionuclides are easily produced, and this

facilitates their availability. Production routes and physical properties of some suitable radionuclides for therapeutic purposes in the filed of nuclear medicine are listed in Table(5) [3].

Table (5): Physical properties of some therapeutic radionuclides and their production methods

Radio isotope	$T_{1/2}$	$E_{\beta^-,max}$ in MeV	Main γ-emission in KeV	Production route
^{32}P	14.3 d	1.7	-	$^{31}P(n,\gamma)$, $^{32}S(n,p)$
^{67}Cu	2.6 d	0.6	185 (49)	$^{67}Zn(n,p)$, Ga(p,spall)
^{89}Sr	50.5 d	1.5	-	$^{88}Sr(n, \gamma)$
^{90}Y	2.7 d	2.3	-	$^{90}Sr/^{90}Y$ generator
^{124}I	4.2 d	2.0 (β^+)	603 (61)	$^{124}Te(p,n)$, $^{124}Te(d,2n)$
^{125}I	60.2 d	Auger electrons	35 (7)	$^{124}Xe(n, \gamma)\ ^{125}Xe \xrightarrow{EC}$
^{131}I	8.0 d	0.6	364 (81)	$^{130}Te(n,\gamma)\ ^{131}Te \xrightarrow{\beta}$ $^{235}U(n,f)$
^{153}Sm	1.9 d	0.8	103 (30)	$^{153}Sm(n,\gamma)$
^{186}Re	3.7 d	1.1	137 (9)	$^{185}Re(n,\gamma)$, $^{186}W(p,n)$
^{188}Re	17 h	2.0	155 (15)	$^{186}W(n,\gamma)\ ^{187}W(n,\gamma)$ $^{188}W \xrightarrow{\beta}$

The usefullness of these β-emitting radionuclides has also been summarized in Table (6) [3].

Table (6): Some of therapeutic radionuclides and their uses in nuclear medicine [1]

Isotope	Therapeutical	Indication
^{32}P	Phosphate, microspheres	Bone metastases, Liver tumors
^{67}Ga	Mab	Various tumors
^{89}Sr	Chloride	Bone metastases / osteosarcom
^{90}Y	Citrate, glass, Mab, coloids	Bone metastases, Liver tumors, Malignant infusion, Synovectomy
^{124}I	MIBG	Neutral crest tumors
^{125}I	MIBG	Neutral crest tumors
^{131}I	Iodide, MIBG, Lipidol	Differential thyroid Carcinoma, Neutral crest tumors, Liver malignancy
^{153}Sm	EDTMP	Bone metastases / osteosarcom
^{186}Re	Sulfide, Colloid, DMSA, HEDP	Synovectomy / astrocytoma/ cystic craniopharyngiom, Medullary thyroid , Bone metastases
^{188}Re	HEDP	Bone metastases

Among them, radioactive rhenium isotopes, ^{186}Re and ^{188}Re, are especially useful because of their energetic beta particles which have a large penetrability and irrageable gamma rays.

Theoretically, α-particles are suitable for radiotherapy. Some of α-emitters are presented in Table (7) [49].

Table (7): Physical properties of some α-emitters [49]

Radionuclide	Half-life	Mean energy (MeV)
Bismuth-212	60.5 min	7.8
Astatine-211	7.2 h	6.7
Fermium-225	20.1 h	7.0

Among α-particle emitters, only two radionuclides have been considered and studied as potential therapeutic agents: astatine-211 and bismuth-212. This is attributed to the extremely high radiotoxicity and short half-lives of alpha particle-emitters.

Therefore, the radiopharmaceuticals suggested for use in therapeutic nuclear medicine require a good deal of laboratory research to be developed.

1.8 Freeze drying

Freeze-drying is a very important technological process of great relevance to the drug, pharmaceutical industry and food preservation industry.

This technique is primarily used for preserving over a long period of time under ambient temperature conditions of materials which otherwise will biodegrade. The removal of water vapour from frozen solution by sublimation is the basis of freeze-drying.

Practically, the technique involves freezing the specimen below its eutectic point then drying it by removal of moisture content from the solid phase to vapour phase through sublimation as studied by Goldbith et al. [53].

1.9 Kit preparation

^{a}M-radiopharmaceuticals (^{a}M = ^{188}Re ,^{186}Re ,or ^{99m}Tc), contain a lyophilized powder of the material to be labelled (the ligand and Sn(II) salt) in a closed vials filled with inert gas. Labelling of these kits was achieved by the addition of sterile, pyrogen free and isotonic eluate of ^{a}M to kit vials.

Knowledge of the course of all partial and possible side reactions, for a kit preparation, is necessary. Consequently, before a kit can be produced, optimization of the parameters influencing the preparations, stability and efficiency of the desired ^{a}M-radiophamaceutical have to be investigated. Also, the concentrations of ligand as well as Sn(II) content, molar ratios of reactants, pH, addition of stabilizer, presence of atmospheric oxygen, temperature and reaction time have to be optimized.

The kit production requires very special precautions for the preservation of the Sn(II) from oxidation, the whole process is carried out under purified nitrogen gas. The obtained solution of Sn(II)-chelating agent is immediately dispensed, qnickly frozen by liqnid nitrogen and instantly lyophilized. After lyophilization, the lyophilizator is flooded by oxygen-free nitrogen gas and the vials are closed under protective nitrogen gas. The sealed vials can be stored at 2-8°C. The production of kits is illustrated in Fig.(10).

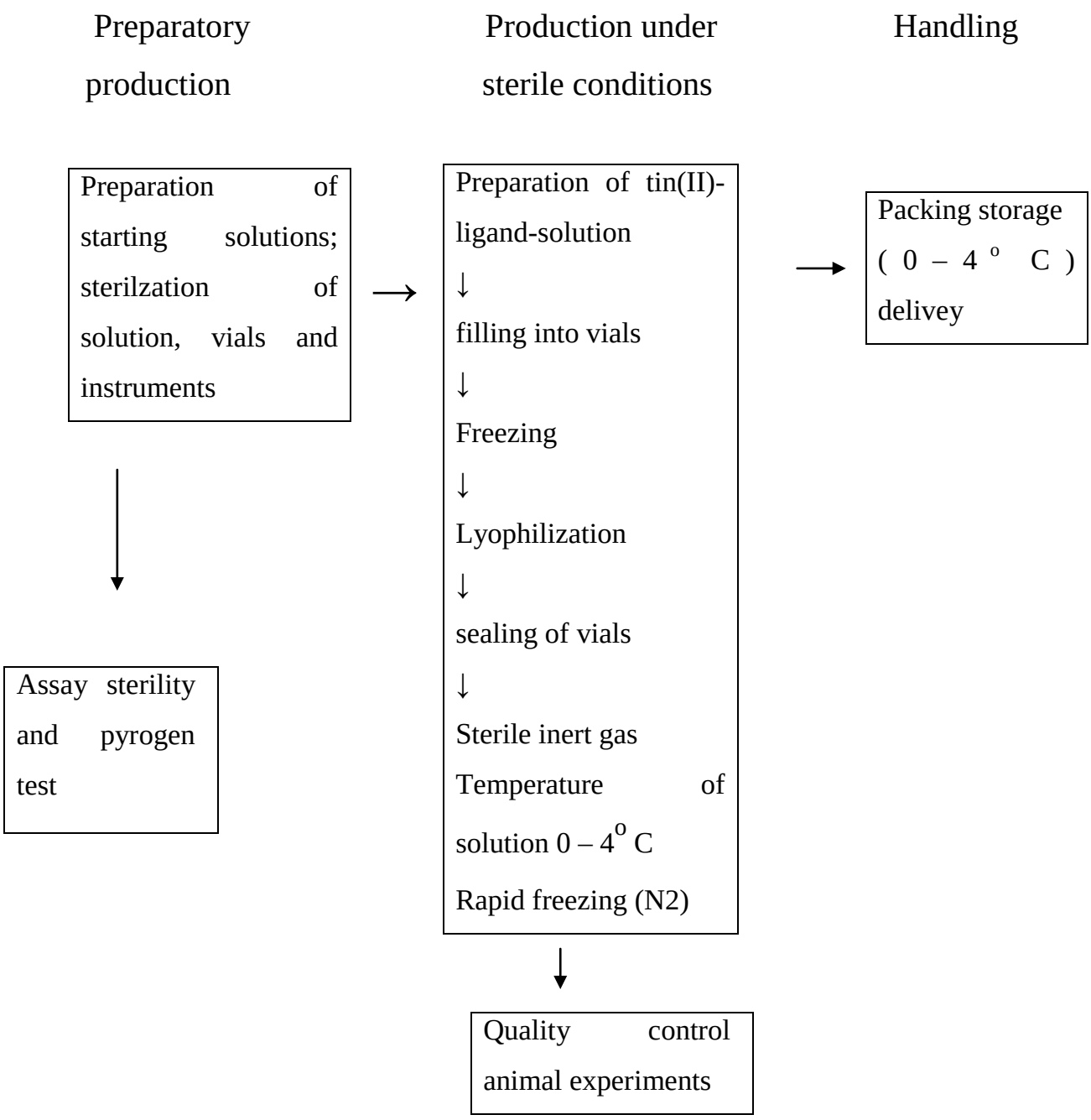


Fig. (10): Scheme for the production of kits for the preparation of nuclear medicine [(1)]

1.10 Quality control of radiopharmaceuticals [2]:

Since ^{a}M-radiophannacenticals (^{a}M= ^{99m}Tc, ^{186}Re, or ^{188}Re) are intended for human administeration, it is imperative that they undergo strict quality control measures. The quality control tests carried out are the following:

The determination of moisture content, Sn(II) content, physicochemical tests and biological tests. The physicochemical tests indicate the level of radionuclidic and radiochemical impurities.

However, the biological tests are carried out essentially to establish the biodistribution, sterility, a pyrogenicity, and toxicity of the radiopharmaceuticals before human administration .