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The Chemistry of Tc-99m-Labeled Radiopharmaceuticals

by:

Gopal B. Saha, Ph.D..

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Coordinating Editor

and

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University of New Mexico

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University of New Mexico

THE CHEMISTRY OF ^{99m}Tc-LABELED RADIOPHARMACEUTICALS

STATEMENT OF OBJECTIVES

The primary purpose of this correspondence lesson is to enhance the reader's knowledge of the current concept of technetium chemistry that plays an important role in the preparation of ^{99m}Tc complexes. The principles of general and specific chemistry of technetium in ^{99m}Tc radiopharmaceuticals are discussed.

Upon successful completion of this chapter, the reader should be able to:

1. discuss the significance of various different oxidation states of technetium.

2. recognize the molecular structures of several important ^{99m}Tc radiopharmaceuticals.

3. discuss the operation of a ^{99}Mo-^{99m}Tc generator.

4. discuss the principles of reduction of technetium with a reducing agent to prepare ^{99m}Tc radiopharmaceuticals.

5. describe the principles of preparation of various ^{99m}Tc radiopharmaceuticals.

6. explain various pitfalls encountered in the preparation of ^{99m}Tc radiopharmaceuticals.

7. discuss the techniques of ^{99m}Tc labeling of peptides and proteins.

8. describe the preparation of ^{99m}Tc-labeled colloids and macroaggregated albumin.

9. discuss the principles of ^{99m}Tc labeling of blood cells.

COURSE OUTLINE

THE CHEMISTRY OF Tc-99m-LABELED RADIOPHARMACEUTICALS

By:

Gopal B. Saha, Ph.D.
Director of Nuclear Chemistry and Pharmacy
Department of Nuclear Medicine
CLEVELAND CLINIC FOUNDATION
Cleveland, Ohio 44195

INTRODUCTION

Technetium (Tc), element 43 in the Periodic Table, was discovered by Perrier and Segre in 1937 (1). At present, there are 21 known isotopes and 8 isomers of technetium of which only ^{99m}Tc is commonly used in nuclear medicine(2). All isotopes are artificially produced and hence the name technetium (Greek meaning artificial). Two isotopes, ^{93}Tc ($t_{1/2}$ = 165 min) and ^{94m}Tc ($t_{1/2}$ = 52 min), hold promise for use in positron emission tomography, because they are positron emitters and have short half-lives.

The Brookhaven National Laboratory developed a ^{99}Mo-^{99m}Tc generator (3) from which ^{99m}Tc was eluted. The first human administration of ^{99m}Tc in the form of Na^{99m}TcO$_4$ was made in 1961(4). Since then, the use of ^{99m}Tc radiopharmaceuticals has grown rapidly to the extent that today nearly 85% of nuclear medicine procedures use ^{99m}Tc. The reason for the preeminent position of ^{99m}Tc in clinical use is its favorable physical and radiation characteristics. The 6.0 hr half-life and negligible amount of electron emission permit the administration of millicurie amounts of ^{99m}Tc radioactivity with minimal radiation dose to the patient. Furthermore, the 140 keV γ-radiations are ideal for gamma cameras, providing scintigraphic images with good spatial resolution. In addition, ^{99m}Tc is readily available in a sterile, pyrogen-free and carrier-free state from the ^{99}Mo-^{99m}Tc generator. In the following sections, the chemical properties of technetium and the general and specific chemistry of ^{99m}Tc in ^{99m}Tc radiopharmaceuticals will be presented.

CHEMICAL PROPERTIES OF TECHNETIUM

Technetium is a transition metal with atomic number 43 belonging to Group VIIB (Mn, Tc and Re) in the Periodic Table. Metallic technetium has a silvery grey color and a melting point of 2160°C. Its chemical properties are closer to those of rhenium than those of manganese, which is due to the effect of lanthanide

contraction. Its electronic configuration is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^6 4d^6 5s^1$. Technetium can exist in nine oxidation states, namely -1 to 7+, that result from the loss of 4d and 5s orbital electrons or the gain of an electron to the 5s orbital. The stability of these oxidation states depends on the chemical environment and the type of ligand to which the Tc is chelated. The 7+ and 4+ states are most stable and are found in sulfides, oxides, halides and pertechnetate. The Tc^{1-}, Tc^{1+}, Tc^{2+} and Tc^{3+} states usually oxidize to that of Tc^{4+} and eventually to that of Tc^{7+}; the oxidation states of Tc^{5+} and Tc^{6+} frequently disproportionate into Tc^{4+} and Tc^{7+} according to the following equations:

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

$$3Tc^{6+} \longrightarrow Tc^{4+} + 2Tc^{7+}$$
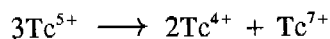
Technetium complexes in all oxidation states, particularly in lower oxidation states of 1+, 2+, and 3+, are stabilized by chelation with various ligands. The coordination number varies from 4 to 9 in these complexes (5). For example, the cationic isonitrile complex of $^{99m}Tc^{1+}$ is stabilized by chelation with six isonitrile ligands and has a coordination number of 6 forming an octahedral structure (6). The $^{99m}TcO_4^-$ ion has the configuration of a tetrahedron with Tc^{7+} located at the center and four oxygen atoms at the base and apex of the pyramid, thus having a coordination number of 4.

Various methods such as polarography, mass spectrometry, electronic and vibration spectroscopy, x-ray crystallography, solvent extraction, electrophoresis, and chromatographic techniques are currently employed in the separation and characterization of ^{99m}Tc complexes. Most of the technetium chemistry has been established by the use of ^{99}Tc which is available in millimolar quantities from the fission products of ^{235}U.

Solvent extraction has been employed for a number of Tc complexes to determine the oxidation state of technetium. The reagent, 8-hydroxyquinoline in chloroform, has been widely used in solvent extraction to establish the presence of a Tc^{5+} state in ^{99m}Tc complexes such as gluconate and citrate. In contrast, Tc-pyrophosphate (PYP), Tc-hydroxymethylene diphosphonate (HDP) and Tc-dimercaptosuccinic acid (DMSA) are extracted by 1,5-diphenylcarbazone in chloroform, but not by hydroxyquinoline in chloroform. In this extraction process, a neutral carbazone complex of Tc^{4+} is formed with unknown composition.

The method of electrophoresis has been applied to solutions of ^{99m}Tc compounds to obtain information about the composition and charge of the compound. Depending on the composition of the compound (i.e., Tc:ligand), various electrophoretic patterns are obtained. It has been shown by this technique that ^{99m}Tc 2,6-

dimethylphenylcarbamoylmethyl iminodiacetic acid (HIDA) complex has two ligands per technetium and an overall charge of 1-(7).

It has been shown that many Tc^{5+} complexes are found to be stabilized in aqueous media by oxo groups such as TcO^{3+}, $trans\text{-}TcO_2^+$ and $Tc_2O_3^{4+}$, which form the central core of the complex(8). Ligands form the coordinate covalent bonds with technetium of the Tc=O core (Fig 1a). In the TcO_2^+ core, there is an oxygen atom in the *trans* position to the Tc=O core (Fig. 1b). The coordination numbers of the complexes in Fig. 1a and Fig. 1b are 5 and 6, respectively. The charge of the complex is determined by adding the charge of ligands to that of the core. Recently, complexes containing a new Tc-amino core such as $(TcNR)^{3+}$ have been reported (9). Some complexes have technetium in a mixture of several oxidation states, e. g. 3+ and 4+ states in ^{99m}Tc-PYP.

The in vivo biological behavior of ^{99m}Tc radiopharmaceuticals is influenced by their stereochemical structure, as is the case with therapeutic drugs. Several factors can influence the stereochemistry of ^{99m}Tc complexes: ligand stereochemistry, the number, type and arrangement of donor atoms, chelate ring size and conformation, the coordination geometry of the metal, and the number of possible ways a ligand can arrange around the metal(10).

Most of the stereochemical studies on ^{99m}Tc radiopharmaceuticals have been made with six-coordinate octahedral complexes containing two or more bidentate ligands and five-coordinate square pyramidal Tc=O complexes. For example, ^{99m}Tc hexamethylpropylene-amine oxime (HMPAO) is an octahedral complex having d, l stereoisomers as well as a meso isomer, and ^{99m}Tc ethyl cysteinate dimer (ECD) has the five-coordinate square pyramidal structure having l, l, d, d and meso isomers. However, only d, l-HMPAO and l, l-ECD isomers are clinically useful.

SOURCE OF TECHNETIUM-99m

$^{99}Mo\text{-}^{99m}Tc$ Generator

Technetium-99m is commonly available from the $^{99}Mo\text{-}^{99m}Tc$ ("Moly") generator. The Moly generator consists of a plastic or glass column loaded with alumina (Al_2O_3) on which ^{99}Mo is adsorbed in the chemical form of MoO_4^-. ^{99}Mo is produced by either the fission of ^{235}U or by the $^{98}Mo(n,\gamma)^{99}Mo$ reaction. Currently, Moly generators used in North America are made with fission-produced ^{99}Mo using activities in amounts ranging from several hundred millicuries to several curies.

Eighty-seven percent of ^{99}Mo ($t_{1/2} = 66$ hr) decays to ^{99m}Tc ($t_{1/2} = 6$ hr) and 13% directly to ^{99}Tc ($t_{1/2} = 2.2$ x 10^5 yr). The ^{99m}Tc activity grows to a maximum in about 4 half-lives of ^{99m}Tc i.e. 24 hr and then establishes a transient equilibrium between the parent and daughter activities at approximately 48 hr, whereupon the ^{99m}Tc

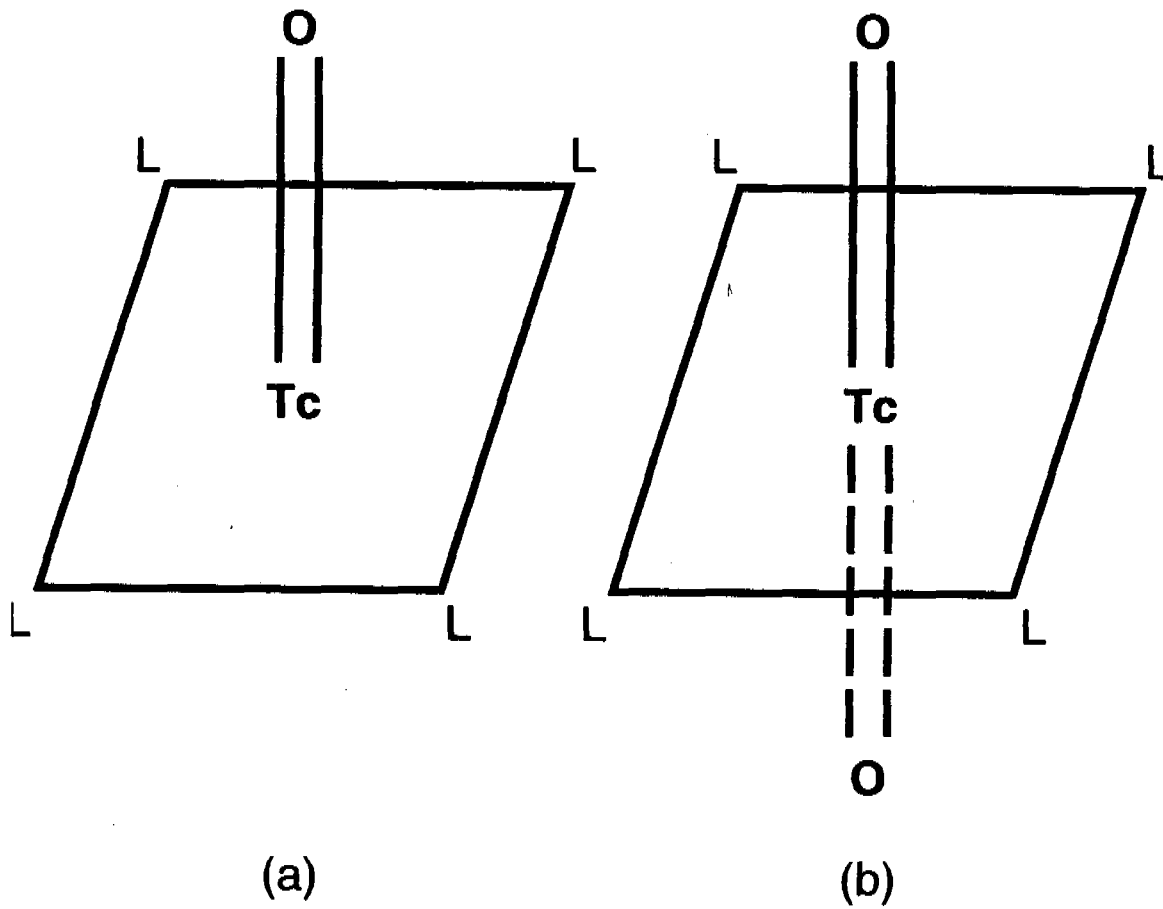**Figure 1.**

Structures of oxotechnetium cores: (a) TcO^{3+} and (b) TcO_2^+ in ^{99m}Tc complexes. "L" represents the ligands that form coordinate covalent bonds with Tc. (Reproduced with permission from Saha GB, *Fundamentals of Nuclear Pharmacy*, 3rd edition, Springer Verlag, New York, 1992)

activity appears to follow the half-life of ^{99}Mo (Fig. 2).

Elution of the Moly generator column with physiologic saline removes both ^{99m}Tc and ^{99}Tc in the form of sodium pertechnetate (NaTcO$_4$). The yield of ^{99m}Tc is about 85% of the theoretical value. The activity $(A_2)_t$ of ^{99m}Tc from the Moly generator at a given time t after the last elution is given by (11)

$$(A_2)_t = 0.87 \frac{\lambda_2}{\lambda_2 - \lambda_1} (A_1)_0 (e^{-\lambda_1 t} - e^{-\lambda_2 t}) + (A_2)_0 e^{-\lambda_2 t}$$

where $(A_1)_0$ is the ^{99}Mo activity at t = 0, $(A_2)_0$ is the activity of ^{99m}Tc at t = 0, and λ_1 and λ_2 are decay constants of ^{99}Mo and ^{99m}Tc, respectively. Since $\lambda_2 > \lambda_1$ and when t is long, then a transient equilibrium is reached and $e^{-\lambda_2 t}$ is almost zero. The above equation then reduces to

$$(A_2)_t = 0.87 \frac{\lambda_2}{\lambda_2 - \lambda_1} (A_1)_t$$

where $(A_1)_t$ is the activity of ^{99}Mo in equilibrium at time t. Inserting the values of decay constants (λ_1 = 0.693/66 = 0.0105 hr^{-1} and λ_2 = 0.693/6 = 0.1155 hr^{-1}) for ^{99}Mo and ^{99m}Tc, respectively, the equation becomes

$$(A_2)_t = 0.957 (A_1)_t$$

^{99}Tc is present in the ^{99m}Tc-eluate to the extent of 13% initially and increases with time due to the decay of ^{99m}Tc. Since ^{99}Tc competes with ^{99m}Tc in chemical reactions, it can reduce the labeling yield of ^{99m}Tc radiopharmaceuticals, particularly if it is present in excess quantity.

Commercially, two types of generators are made, wet column generators and dry column generators. In wet column generators, saline remains in the column after elution, whereas in dry column generators, saline is drawn out by elution using an evacuated vial. In wet column generators, high activity can cause radiolysis of water molecules producing hydrogen peroxide and perhydroxyl molecules (HO$_2$) which are oxidants and can interfere with technetium chemistry(12). However, such problems are rarely encountered in high activity wet column generators such as those distributed by Medi-Physics/Amersham and Mallinckrodt Medical, Inc.

The ^{99}Mo contamination that may originate during the elution of ^{99m}Tc is limited by the Nuclear Regulatory Commission to 0.15 μCi/mCi ^{99m}Tc at the time of injection(13). The assay of ^{99}Mo is most commonly made by detecting its 740 keV photons in a dose calibrator after shielding all 140 keV photons of ^{99m}Tc using a lead container.

The USP limits of other radionuclides that may be present in the ^{99m}Tc eluate include ^{89}Sr (0.0006 μCi/mCi of ^{99m}Tc); ^{90}Sr (0.00006 μCi/mCi of ^{99m}Tc); ^{131}I and ^{103}Ru (0.05 μCi/mCi of ^{99m}Tc); and other radionuclides (not more than 0.01% of all activity at the time of administration)(14).

The presence of aluminum in the ^{99m}Tc eluate originating from the alumina bed interferes with the formation of Tc-sulfur colloid and Tc-red blood cell (RBC) labeling. The USP limit of aluminum concentration is 10 μg/mL of ^{99m}Tc eluate(14). Aluminum breakthrough is assessed by comparing the color intensity of a complex formed between the sample aluminum (in the eluate) and methyl orange on aurin tricarboxylic acid (ATCA) to that of a standard aluminum solution (normally 10 μg/mL) and methyl orange on ATCA.

GENERAL CHEMISTRY OF ^{99m}Tc RADIOPHARMACEUTICALS

Reduction of Technetium-99m

Technetium in pertechnetate ion, ^{99m}TcO$_4^-$, obtained by the elution of the Moly generator, has the most stable Tc^{7+} oxidation state and does not bind to any ligand except in ^{99m}Tc sulfur colloid which is considered to contain the Tc^{7+} state. Therefore, a less stable lower oxidation state of Tc must be formed so that it can readily react with chelating agents. In ^{99m}Tc-labeling of many compounds, the Tc^{7+} state is reduced to lower oxidation states by reducing agents.

Various reducing agents that have been used are stannous salts, concentrated HCl, sodium borohydride, sodium dithionite, ferrous sulfate, ferric chloride plus as-corbic acid, hypophosphorus acid, and hydrazine. Stannous salts include tartrate, citrate, chloride, fluoride and pyrophosphate, of which stannous chloride (SnCl$_2$.2H$_2$O) is the most commonly used reducing agent in ^{99m}Tc radiopharmaceuticals. Another technique of reducing Tc^{7+} involves the electrolysis of a mixture of ^{99m}TcO$_4^-$ and the chelating agent using zirconium and tin electrodes.

The reduction reaction that takes place with stannous chloride in acidic medium is given by

$$2^{99m}TcO_4^- + 16H^+ + 3Sn^{2+} \rightleftharpoons$$
$$2^{99m}Tc^{4+} + 3Sn^{4+} + 8H_2O$$
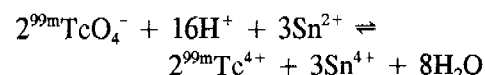
The above reaction shows the formation of Tc^{4+}. Other oxidation states, individually or in a mixture, may be formed under different physicochemical conditions. Experiments with millimolar quantities of ^{99}Tc have shown that Sn^{2+} initially reduces ^{99}Tc to the Tc^{5+} state
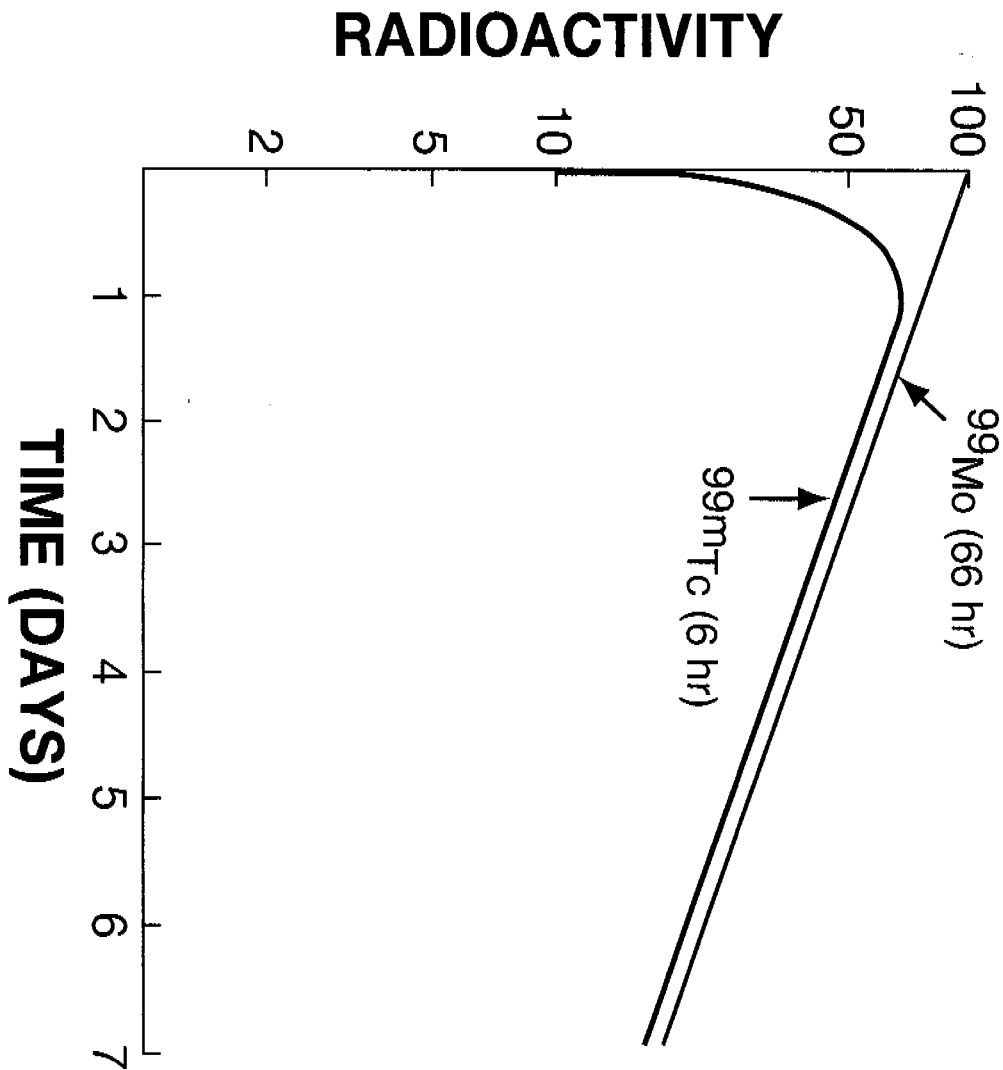
Figure 2.

Plot of logarithm of ^{99}Mo and ^{99m}Tc activities versus time showing transient equilibrium. In about 1 day (4 half-lives of ^{99m}Tc), the ^{99m}Tc activity reaches a maximum, after which a transient equilibrium is reached, and ^{99m}Tc appears to decay with the half-life of ^{99}Mo. Since 87% of ^{99}Mo decays to ^{99m}Tc, the ^{99m}Tc activity is less than that of ^{99}Mo after equilibrium. (Reproduced with permission from Saha GB, *Physics and Radiobiology of Nuclear Medicine*, Springer Verlag, New York, 1993).

and then slowly to the Tc^{4+} state in citrate buffer at pH 7. Sn^{2+} in concentrated HCl reduces the Tc^{7+} state to the Tc^{4+} state of ^{99}Tc.

The above reduction reaction holds in the presence of acidic medium. At neutral pH, stannous ions form colloids which are undesirable in the ^{99m}Tc chelates. This situation is circumvented by forming a stannous complex with the compound to be labeled that is soluble at neutral pH. For example, excess diethylenetriaminepentaacetic acid (DTPA) is added to the reagent kit so that all Sn^{2+} present is chelated to DTPA. A higher ratio of the chelating agent to tin should be used for weaker chelates than for stronger chelates. However, it should be noted that enough tin must be present in the reagent kit to ensure complete reduction of Tc^{7+} and the ratio of Sn^{2+} ions to Tc^{7+} atoms may be as large as 10^6.

Complex Formation with Reduced ^{99m}Tc

The reduced species of ^{99m}Tc are chemically reactive and form complexes with various chelating agents.

$$\text{Reduced }^{99m}Tc + \text{Chelating Agent} \rightleftharpoons {}^{99m}Tc\text{-Chelate}$$

The chelating agent usually donates lone pairs of electrons to form coordinate covalent bonds with ^{99m}Tc. Chelating molecules such as DTPA, macroaggregated albumin (MAA), gluceptate (GH), proteins, and enzymes contain electron donor ligands such as $-COO^-$, $-OH^-$, $-NH_2$ and $-SH$. It has been reported that tin is incorporated in some $^{9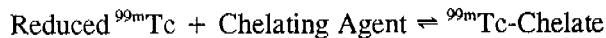9m}Tc$-chelates, e.g. ^{99m}Tc-Sn-dimethylglyoxime(15). However, the presence of tin was not found in the structures of ^{99m}Tc-labeled HIDA, DTPA, PYP and gluconate.

Pertechnetate in ^{99m}Tc Radiopharmaceuticals

From the practical point of view, all pertechnetate ions are not completely reduced and, therefore, a small amount of $^{99m}TcO_4^-$ is typically present in most ^{99m}Tc radiopharmaceuticals. These free pertechnetate ions are considered radiochemical impurities that may cause artifacts in scintigraphic images. The presence of oxygen, free radicals or other oxidizing agents in the reagent kit may oxidize the stannous ions to stannic ions, thus compromising the reduction of Tc^{7+} and ultimately increasing the proportion of free TcO_4^-. In this case, the labeling yield of ^{99m}Tc radiopharmaceuticals decreases.

These problems can be solved by adding a sufficient quantity of Sn^{2+} ions and by avoiding oxygen, air, or oxidizing agents in the reagent kit. Commonly, N_2 is flushed through the kit vial to remove any oxygen and maintain an inert atmosphere. In some kits, such as methylene diphosphonate (MDP) or HDP, antioxidants (e.g.. ascorbic acid or gentisic acid) are added to prevent oxidation.

Hydrolysis of Reduced ^{99m}Tc and Tin

In the absence of sufficient chelating agent, reduced technetium can undergo hydrolysis in aqueous solution at pH 6 or higher forming various species such as TcO_2, TcO^+, and $TcOOH^+$(16). These products, if present in sufficient quantities, can reduce the labeling efficiency of ^{99m}Tc radiopharmaceuticals, and interfere with the diagnostic test. These hydrolyzed species may be present in very small quantities even when enough chelating agent is added to the kit.

The Sn^{2+} ions also readily undergo hydrolysis in aqueous solution at pH 6 to 7 to form insoluble colloids. Reduced technetium can co-precipitate with colloidal tin thus lowering the labeling efficiency of ^{99m}Tc radiopharmaceuticals.

Hydrolysis of both reduced Tc and Sn^{2+} depends on the affinity constants of the ligand molecules and can be largely prevented by the addition of excess chelating agent. In reagent kits, Sn^{2+} ions are complexed with excess chelating agent to form Sn-chelates which are soluble in aqueous solution at pH 6 or higher. The affinity constant of reduced Tc is normally much higher with the chelating agent than for hydrolysis; therefore, almost all of reduced Tc forms the Tc complex.

In a ^{99m}Tc radiopharmaceutical, three major components ("free" ^{99m}Tc, "hydrolyzed" ^{99m}Tc and "bound" ^{99m}Tc-chelate) may be present. Of these, ^{99m}Tc-chelate is the desirable species, while free and hydrolyzed Tc species are undesirable, and their presence must be quantified by radiochemical purity testing prior to administration. These components are assessed by analytical techniques, most commonly by instant thin layer chromatography (ITLC). In most ^{99m}Tc radiopharmaceuticals, a 90% limit of "bound" ^{99m}Tc-chelate is recommended.

Preparation of ^{99m}Tc Radiopharmaceuticals by Ligand Exchange

In the ligand exchange (or transchelation) method, first a ^{99m}Tc complex is formed with a weak chelate in aqueous media and then it is allowed to react with a second stronger chelating agent usually at elevated temperatures (75°C to 100°C). For example,

$$\text{Tc-EDTA} + \text{ECD} \rightarrow \text{Tc-ECD} + \text{EDTA}$$
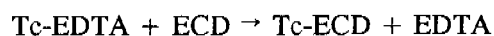
Ethylenediaminetetraacetic acid (EDTA), tartrate, gluconate, and pyrophosphate are all weak chelating agents, whereas ECD (bicisate), isonitrile, and mercaptoacetylglycylglycylglycine(MAG3) are stronger chelating agents. Based on these reactions, reagent kits have been formulated containing both weak and strong chelating agents along with stannous ions. Examples are tartrate and MAG3 for renal imaging, EDTA and ECD

for brain imaging, and citrate and hexakismethoxyisobutyl isonitrile (HEXAMIBI or sestamibi) for myocardial imaging.

Stronger chelating agents such as MAG3, isonitrile and ECD are less soluble in aqueous solution and require heating or longer time to dissolve. In contrast, weaker chelating agents such as tartrate, citrate and EDTA are highly soluble in aqueous solution. In the kits containing both weak and strong ligands, stannous ions primarily remain bound to weaker chelating agents rather than stronger chelating agents because of the ready solubility of the former. After the addition of $^{99m}TcO_4^-$, Tc^{7+} is reduced by Sn^{2+} and the reduced Tc readily forms ^{99m}Tc-chelate with the excess weaker chelating agent. Upon heating or long reaction, stronger chelating agents dissolve, and ligand exchange occurs between the stronger chelating agent and ^{99m}Tc-chelate.

The addition of a weaker chelating agent is necessary to stabilize the reduced Tc, particularly in the lower oxidation states. Because of the lower solubility, the presence of stronger chelating agent alone, in the absence of the weaker chelating agent, would lead to the precipitation of much of the reduced Tc as colloid.

Chemistry of ^{99m}Tc in Dilute Solutions

The principles of dilute solutions play an important role in the chemistry of ^{99m}Tc radiopharmaceuticals. This is because the concentration of ^{99m}Tc in the eluate from the Moly generator is very low. For example, a 2-Ci eluate of ^{99m}Tc in 20 mL would have a ^{99m}Tc concentration of about $1.9 \times 10^{-7}M$. The presence of ^{99}Tc (due to 13% direct decay of ^{99}Mo and also from the decay of ^{99m}Tc) complicates the chemistry of ^{99m}Tc radiopharmaceuticals, because ^{99}Tc competes with ^{99m}Tc compromising the labeling yield. The knowledge of concentrations of both isotopes is essential for estimating the labeling yield of a particular ^{99m}Tc radiopharmaceutical. In preparations in which the Sn^{2+} ion concentration is limited, the total concentration of Tc may be too high for complete reaction with Sn^{2+}, resulting in low labeling yield. The HMPAO (exametazime) kit is one example in which tin content is limited and, therefore, for maximum ^{99m}Tc labeling, freshly eluted ^{99m}Tc is required in order to minimize the amount of ^{99}Tc present (16).

Another problem with the ^{99m}Tc chemistry using Sn^{2+} is the availability of Sn^{2+} used in reagent kits. In the lyophilized form of $SnCl_2$ in the kit, the concentration of Sn^{2+} available for labeling is much lower than expected from the original amount added to the formulation(16). This is attributed to the probable formation of colloidal tin oxide in the later phase of the lyophilization process (17). At present, there is no method for controlling this loss of available Sn^{2+}.

The effects of varying total concentrations of

pertechnetate can be explained by the kinetics of the first order or second order reactions, depending upon one or two pertechnetate ions involved in the chemical reactions, respectively. Compounds formed with one, two or more technetium atoms are called monomers, dimers or polymers, respectively. Evidence of dimer formation involving Tc^{4+} and Tc^{3+} has been found in ^{99}Tc-DTPA by potentiometric titration of $^{99}TcO_4^-$ with $SnCl_2$ in a DTPA solution(18). However, it is not known if such dimerization occurs in clinical preparations at the $10^{-8}M$ level of Tc concentrations. The formation of hydrolyzed reduced technetium in almost all clinical ^{99m}Tc preparations at very low Tc concentration raises the possibility of dimerization or polymerization. However, a thorough analysis of chemical reactions showed that the hydrolyzed technetium is a compound of $^{99m}TcO_2$ complexed with other ingredients (e.g., SnO, MoO_3 or Al)(19).

CHEMISTRY OF SPECIFIC ^{99m}Tc RADIOPHARMACEUTICALS

^{99m}Tc Radiopharmaceuticals for Brain Imaging
^{99m}Tc-Hexamethylpropyleneamine Oxime (HMPAO). Troutner et al.(20) first developed a neutral, lipophilic ^{99m}Tc complex with a chelating agent, propylene amine oxime (PnAO), for brain imaging. This product had a 5-coordinate structure with an oxo group at the apex, four nitrogen atoms at the base corners and a hydrogen bond between the two oxygen atoms of the oxime groups(20). The complex entered the brain efficiently, but washed out rapidly. Subsequently, several methyl-substituted derivatives of PnAO were developed, of which HMPAO showed higher cerebral uptake and a slower clearance(21). HMPAO exists in two stereoisomers: d,l-HMPAO and meso-HMPAO. The d,l isomers of HMPAO each form only one stereoisomeric complex; each has one of the methyl groups α- to the amines syn- and the other anti- to the Tc=O core. The d, l isomer of HMPAO shows cerebral uptake, whereas the meso component does not and, hence, the former is used for brain imaging(21). ^{99m}Tc-HMPAO is a neutral lipophilic complex with a coordination number of 5. Its structure is shown in Fig. 3.

The d,l-HMPAO is available as a reagent kit (exametazime [Ceretec*]; Amersham Corp., Arlington Heights, IL) containing stannous ions as the reducing agent. The kit is reconstituted with $^{99m}TcO_4^-$ whereby ^{99m}Tc-HMPAO is formed. There are some restrictions on the quality of $^{99m}TcO_4^-$ — namely, it must not be older than 2 hours after elution and it must be eluted from a generator that has been previously eluted within 24 hours. ^{99m}Tc-HMPAO is stable for about 30 minutes after formulation, after which time the product tends to break down into a secondary complex that does not diffuse into

the brain. Therefore, it must be administered to the patient within this time. The radiochemical purity of this product must be determined prior to administration and should be at least 80%.

The instability of ^{99m}Tc-HMPAO has been attributed to the high pH (9 to 9.8) that results after reconstitution, and to the presence of radiolytic intermediates such as hydroxy free radicals and excess stannous ions. To offset these effects, phosphate buffer and methylene blue have been added to the new formulation of the Ceretec ® kit. Phosphate buffer lowers the pH to around 6 at which the decomposition of ^{99m}Tc-HMPAO is minimal. Methylene blue acts as a scavenger of free radicals and oxidizes excess stannous ions. Even though decomposition still occurs to some extent in the presence of phosphate buffer, the combination of both phosphate buffer and methylene blue together significantly reduces the decomposition. This new reagent kit is stable for up to 4 hours after reconstitution.

^{99m}Tc-Ethyl Cysteinate Dimer (ECD). ^{99m}Tc-ECD was introduced as a brain perfusion agent by Cheeseman et al.(22) and Walovitch et al.(23) and is now commercially supplied by Du Pont Pharma (bicisate [Neurolite*]). Both l, l-ECD and d, d-ECD isomers diffuse into the brain by crossing the blood brain barrier (BBB), but only l, l-ECD only is metabolized by an enzymatic process to a polar species that is retained within the brain(24). Therefore, only l, l-ECD is used in the reagent kit that contains, besides ECD, stannous chloride and sodium edetate. The latter is added to solubilize stannous ions and thus prevent colloid formation.

Labeling is performed by adding ^{99m}TcO$_4^-$ to the kit vial and incubating for 30 min at room temperature. Initially, ^{99m}Tc-edetate is formed and then ^{99m}Tc-ECD is produced by ligand exchange after incubation at room temperature. ^{99m}Tc-ECD is a neutral lipophilic complex containing a core structure of Tc=ON$_2$S$_2$ with a coordination number of 5 (Fig. 3). The labeling yield is typically more than 90% and the formulation is stable for at least 6 hours after reconstitution.

^{99m}Tc Radiopharmaceuticals for Myocardial Imaging

^{99m}Tc-isonitrile Complexes. Davison, Jones and colleagues(6,25) introduced cationic technetium alkyl isonitrile complexes for myocardial imaging. These lexes were initially synthesized by reducing ^{99m}TcO$_4$ with alkaline sodium dithionite in the presence of isonitrile ligands in an ethanol-water mixture. A later method of preparation of ^{99m}Tc-isonitrile complexes involved the ligand exchange technique by which a weak chelate such as ^{99m}Tc-citrate was first formed, and then the stronger chelate ^{99m}Tc-isonitrile complex formed by transchelation at high temperature in the presence of the isonitrile group(26). This is the method employed in radiolabeling the reagent kit of sestamibi for myocardial imaging. The

kit is supplied by Du Pont Pharma under the brand name Cardiolite*.

After addition of ^{99m}TcO$_4^-$ to the sestamibi kit, initially a ^{99m}Tc^{5+} complex of citrate is formed, which then reacts with excess sestamibi at high temperature to yield ^{99m}Tc-sestamibi. The labeling yield is normally greater than 95%. The ^{99m}Tc-sestamibi has a shelf life of at least 6 hours after formulation.

The oxidation state of Tc in the cationic ^{99m}Tc-sestamibi is Tc^{1+}; the complex has a coordination number of 6 and a net charge of 1+ (Fig. 3). The Tc^{1+} complexes are more stable due to the presence of isonitrile ligands than those of Tc^{3+} or Tc^{5+} which are formed as the metastable intermediates (e.g. citrate) during stannous reduction of Tc^{7+}(26).

^{99m}Tc-Tetrofosmin. Tetrofosmin is an analog of alkyl phosphino ethane complexes. Deutsch et al.(27) first reported that the Tc^{3+} complexes of neutral arsine and phosphine ligands localize in myocardial tissues in animals. The cationic complexes of Tc^{3+} were of the type (TcL$_2$X$_2$) where L is the bidentate ligand of arsine and phosphine [such as O-phenylene-bis(dimethyl)arsine (DIARS) and bis(1,2-dimethylphospine)ethane (DMPE)] and X=Cl, Br or I. Complexes of Tc^{1+} include [Tc(DMPE)$_3$]$^{1+}$, [Tc(TMP)$_6$]$^{1+}$ and [TcPOM-POM)]$^{1+}$ where TMP stands for trimethoxyphosphine and POM-POM for 1,2-bis(dimethoxy phosphino)ethane. All these agents show good localization in myocardium in animals such as rats and dogs but very poor uptake in humans. However, the latest complex of Tc^{1+}, ^{99m}Tc-1,2-bis [bis(2-ethoxymethyl)phosphino] ethane, (^{99m}Tc-tetrofosmin), shows good myocardial uptake in humans.(28,29)

Tetrofosmin is available as a reagent kit (Myoview™; Amersham, UK); the kit also contains stannous chloride and sodium gluconate. Upon adding ^{99m}TcO$_4^-$, ^{99m}Tc-gluconate is formed, which is further reacted with tetrofosmin by ligand exchange at room temperature to form ^{99m}Tc-tetrofosmin. The labeling yield is normally greater than 90%. It is usable for 8 hours after preparation.

^{99m}Tc-tetrofosmin is cationic and has the chemical formula of [^{99m}Tc(tetrofosmin)$_2$O$_2$]$^+$. Its molecular structure has a linear *trans*-oxo core, with the four phosphorus atoms of the two bidentate diphosphine ligands forming a planar array(30).

^{99m}Tc Radiopharmaceuticals for Renal Imaging

Several ^{99m}Tc chelates, such as ^{99m}Tc-DTPA (pentetate), ^{99m}Tc-GH (gluceptate), ^{99m}Tc-DMSA (succimer), and ^{99m}Tc-MAG3 (mertiatide), are commonly used as renal imaging agents. ^{99m}Tc-DTPA is a glomerular filtration agent and ^{99m}Tc-MAG3 is primarily a tubular secretion agent, whereas ^{99m}Tc-DMSA and ^{99m}Tc-GH are excreted by both glomerular filtration and tubular secretion. ^{99m}Tc-GH and ^{99m}Tc-DMSA also

A

C

B

$R = CH_3O - \underset{\underset{CH_3}{|}}{\overset{\overset{CH_3}{|}}{C}} -$
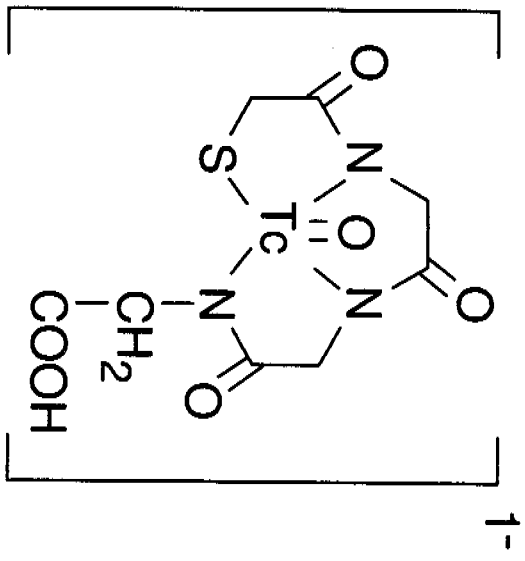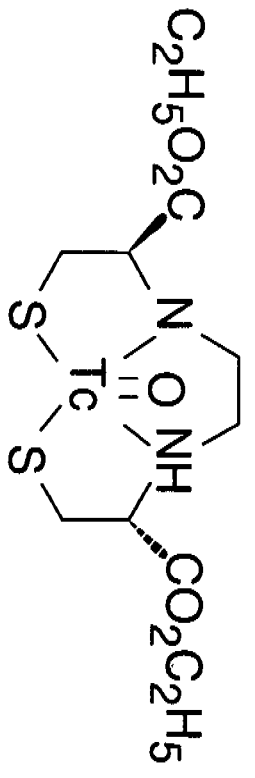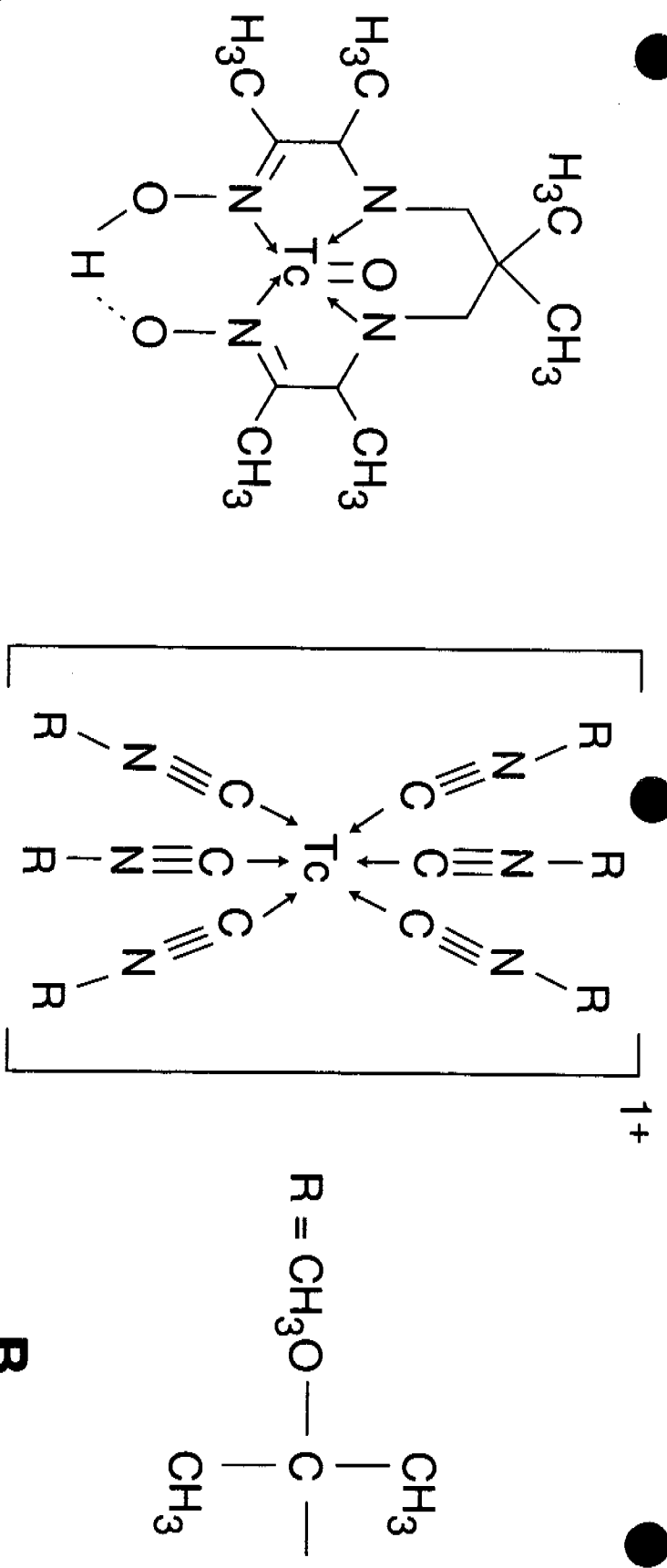
D

Figure 3.

Molecular structures of ^{99m}Tc-labeled complexes. (A) Tc-HMPAO, (B) Tc-sestamibi, (C) Tc-ECD, and (D) Tc-MAG3. (Reproduced and modified with permission from Saha GB, *Fundamentals of Nuclear Pharmacy*, 3rd edition, Springer Verlag, New York, 1992)

localize in the cortex of the kidneys.

The DTPA, DMSA and GH complexes are available in reagent kit form in which the main ingredients are the chelating agent and the stannous ion. Addition of $^{99m}TcO_4^-$ leads to the reduction of Tc^{7+} to lower oxidation states followed by the formation of ^{99m}Tc-chelate. The radiochemical yield is typically more than 95%. While ^{99m}Tc-DTPA and ^{99m}Tc-GH are usable for 6 hours after preparation, ^{99m}Tc-DMSA is stable for only 4 hours.

The structure of these complexes has not been determined experimentally, but the assumption is that 0, N, and S atoms provide lone pairs of electrons to reduced Tc^{3+} or Tc^{4+} to form coordinate covalent bonds producing the stable ^{99m}Tc-chelates. In the case of GH, the presence of an oxo core has been stipulated with a net charge of 1- on the complex. The oxidation state of technetium has been considered to be 4+ in ^{99m}Tc-DTPA complex [with a net charge of 1-] (31), whereas that in ^{99m}Tc-GH was found to be 5+(32). The oxidation state of Tc in ^{99m}Tc-DMSA for renal imaging is suggested to be 3+(33). However, if this complex is formed at higher pH using sodium bicarbonate, Tc exists in the 5+ state, and the ^{99m}Tc-DMSA complex has been found to localize in medullary thyroid carcinoma(34). The structural study shows that pentavalent ^{99m}Tc-DMSA has a structure of $[^{99m}TcO(DMSA)_2]^-$ having a Tc=0 core coordinated by four thiolate groups of two DMSA ligands(35).

^{123}I- or ^{131}I-labeled orthoiodohippurate (OIH) was the agent of choice for the assessment of renal function until the recent introduction of ^{99m}Tc radiopharmaceuticals. The first ^{99m}Tc complex was a N_2S_2 ligand containing ^{99m}Tc-labeled N,N'-bis-(mercaptoacetyl)-ethylenediamine (DADS), the renal clearance of which was higher than the glomerular filtration rate which implied tubular secretion but was slower than that of OIH(36,37). Because of the lower renal extraction (53%) and excretion, this agent could not replace OIH. The next ^{99m}Tc agent was the carboxylate analog of DADS (^{99m}Tc CO_2DADS with two isomers), but it did not give the optimum renal clearance so as to replace OIH. The latest development was the introduction of ^{99m}Tc-MAG3 [mertiatide] (38) which showed plasma clearance values (~340 mL/min) to be less than those of OIH (~600 mL/min) but had urinary excretion similar to OIH(39). Since the clearance ratios of the two agents have been found to remain constant in many clinical situations, ^{99m}Tc-MAG3 is now accepted as a routine radiopharmaceutical for renal function studies.

MAG3 is available in a reagent kit form containing betiatide(N-[N-[N-[(benzoylthio)acetyl]glycyl] glycyl]glycine), stannous ions and sodium tartrate. Addition of $^{99m}TcO_4^-$ to the kit forms ^{99m}Tc-tartrate initially, followed subsequently by the formation of ^{99m}Tc-mertiatide(disodium[N-[N-[N-(mercaptoacetyl)glycyl]glycyl]glycinato(2-

)N,N',N",S]oxotechnetate(2-)) [i.e.,^{99m}Tc-MAG3] by ligand exchange upon heating. The labeling yield is usually found to be greater than 95%. The preparation has the shelf-life of 6 hours after reconstitution.

^{99m}Tc-MAG3 has a core of $Tc=ON_3S$ with the oxygen atom at the apex and three nitrogen atoms and one sulfur atom occupying the corners of the planar square base. ^{99m}Tc-MAG3 has a coordination number of 5 with a negative charge of 1-(38) (Fig. 3).

^{99m}Tc Radiopharmaceuticals for Bone Imaging

^{99m}Tc-labeled diphosphonate complexes are the routinely used radiopharmaceuticals for bone imaging. Although once used for bone imaging, ^{99m}Tc-PYP is now used primarily for myocardial infarct imaging, and cold PYP for red blood cell labeling. The difference between phosphate and phosphonate groups is that in phosphate groups, a P-O-P bond exists, whereas a P-C-P bond forms in phosphonate groups. The latter bond is more stable in vivo than the former, and that is why diphosphonate complexes are commonly used for bone imaging.

Three diphosphonate complexes have been utilized for bone imaging: 1-hydroxyethylidene diphosphonate (HEDP), MDP and HDP. The structures of phosphate and phosphonate complexes are shown in Fig. 4. Of these, MDP and HDP are most commonly used. Kits are available that contain MDP or HDP along with an appropriate amount of stannous ion. Some MDP kits contain ascorbic acid and HDP kits contain gentisic acid as the stabilizer antioxidant. Labeling is carried out by adding $^{99m}TcO_4^-$ to the kit, whereby Tc^{7+} is reduced by stannous ion and then the reduced Tc binds to MDP or HDP. The labeling yield is found to be greater than 90%. The labeled product is good for use for 6-8 hours in the case of ^{99m}Tc-MDP and 8 hours for ^{99m}Tc-HDP.

The reverse phase high performance liquid chromatography (HPLC) analysis of ^{99m}Tc-MDP prepared by sodium borohydride reduction(40) and $SnCl_2$(41) showed multiple peaks depending on the pH of the solution and whether ^{99m}Tc is carrier added or carrier free. With no-carrier-added (NCA) Tc, fewer peaks are obtained suggesting that at carrier level, polymeric species like $(Tc)_x(DP)_y$ are formed and at no carrier level, a monomer is favored. Russel and Cash(42) have shown by the polarographic technique that for ^{99m}Tc-MDP and ^{99m}Tc-PYP, Tc^{7+} is reduced to Tc^{3+} at pH up to 10, above which it is reduced to Tc^{4+}. Sn^{2+} was thought to be a part of Tc-phosphonate complex(43), but it was later shown by HPLC and double isotope labeling that tin was not a part of the Tc complex(41).

^{99m}Tc Radiopharmaceuticals for Hepatobiliary Imaging

The derivatives of iminodiacetic acid (IDA) form complexes with the technetium metal ion; these serve as
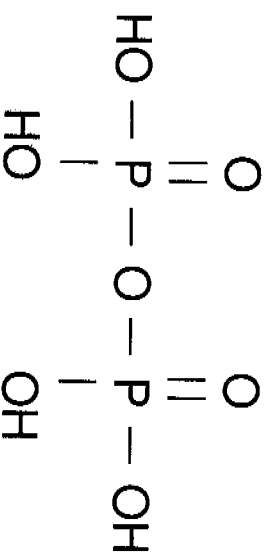
Pyrophosphate
(PYP)
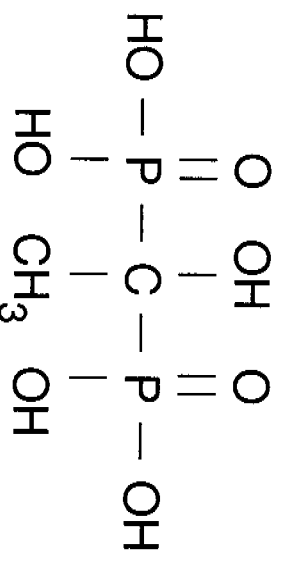
1-Hydroxyethylidene
diphosphonate
(HEDP)
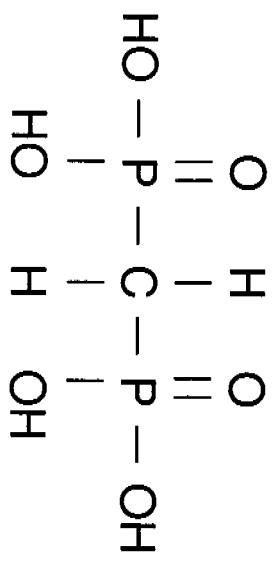
Methylene
diphosphonate
(MDP)
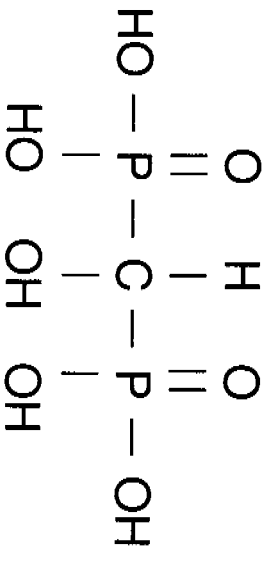
Hydroxymethylene
diphosphonate
(HDP)

Figure 4.

Molecular structures of various phosphate and phosphonate complexes used in bone imaging. (Reproduced with permission from Saha GB, *Fundamentals of Nuclear Pharmacy*, 3rd edition, Springer Verlag, New York, 1992)

agents for hepatobiliary imaging. The first complex was HIDA, introduced by Loberg et al.(44). Being a derivative of lidocaine, it is also termed lidofenin. Several derivatives of IDA have been developed, namely, 2,6-diethyl (DIDA or etilfenin), paraisopropyl (PIPIDA or iprofenin), parabutyl (BIDA or butylfenin), diisopropyl (DISIDA or disofenin) and bromotrimethyl (mebrofenin). These derivatives are obtained by adding various substituents to the benzene ring. Among these derivatives, disofenin and mebrofenin are the two derivatives that are most commonly used in nuclear medicine. The structures of several IDA derivatives are shown in Fig. 5.

Commercial reagent kits are available for lidofenin, disofenin, and mebrofenin containing the respective chelating agent and stannous chloride. ^{99m}Tc-labeled chelate is obtained by adding $^{99m}TcO_4^-$ to the kit. The rate of chelation increases with increasing acidity and ligand concentration and is found to be independent of Sn^{2+} concentration. The substituents on the phenyl ring, mainly their size, polarity, and position (on the ring), determine the hepatic extraction and excretion. For example, the longer the chain of the substituent, the better the hepatic extraction.

The labeling yield of ^{99m}Tc-IDA complexes is typically found to be greater than 95%. ^{99m}Tc-HIDA and ^{99m}Tc-DISIDA preparations are useful for 6 hours, whereas ^{99m}Tc-mebrofenin has a stated shelf-life of 18 hours after preparation.

It has been found by iodometric titration that the oxidation state of Tc in ^{99m}Tc-HIDA complex is 3+ and the structure of $Tc^{3+}(HIDA)_2$ has been suggested to have an octahedral configuration with a net charge of 1-(45). Similar structures may exist for other ^{99m}Tc-IDA complexes.

^{99m}Tc-labeled Colloids and Particles

^{99m}Tc-Sulfur Colloid (SC). Apart from $^{99m}TcO_4^-$, the only other $^{99m}Tc^{7+}$ compound used in nuclear medicine is technetium heptasulfide ($^{99m}Tc_2S_7$) or ^{99m}Tc-SC. An early preparation of ^{99m}Tc-SC was made by passing H_2S gas through hydrochloric acid solution of $^{99m}TcO_4^-$(46). $^{99m}Tc_2S_7$ was precipitated along with colloidal sulfur giving a yellowish color.

The most commonly used ^{99m}Tc-SC colloid is prepared by heating a mixture of $^{99m}TcO_4^-$ and sodium thiosulfate in acidic medium in boiling water for 5 to 10 min(47). The pH of the final solution is adjusted to 6 to 7 by adding a suitable buffer. Hydrochloric or phosphoric acid in the reagent kit reacts with sodium thiosulfate to form H_2S and colloidal sulfur. Hydrogen sulfide reacts with $^{99m}TcO_4^-$ to form $^{99m}Tc_2S_7$ which co-precipitates with colloidal sulfur. The particle size ranges between 0.1 and 1 micrometer (μm) with a mean size of 0.3 μm(48) and varies in different kits. The particle size is checked with a

hemocytometer under a light microsope. The labeling yield is greater than 99%.

In commercial kits, gelatin is added to prevent aggregation of colloidal particles. EDTA is added to chelate with Al^{3+} ion, if present in the $^{99m}TcO_4^-$ eluate, because Al^{3+} tends to form aggregates in some colloid kits — particularly those containing phosphate buffer(49). ^{99m}Tc-SC is clinically useful for 6 hours after preparation.

^{99m}Tc-Albumin Colloid. This reagent kit is made from human serum albumin microcolloid and stannous chloride. Labeling is carried out by adding $^{99m}TcO_4^-$ to the vial and mixing it for a few minutes. The majority of particles have the size of 0.4 to 2 μm. The advantage of this kit is that it does not require any heating. It is useful for 6 hours after preparation. The mechanism of reaction is perhaps the initial reduction of Tc^{7+} to a lower oxidation state which binds to albumin microcolloid.

^{99m}Tc-Antimony Sulfur Colloid. This colloid was introduced by Ege and Warbick(50) and is prepared by adding antimony potassium tartrate to a solution saturated with hydrogen sulfide. The Sb_2S_3 colloid is stabilized with polyvinylpyrolidine and has the particle size of 5 to 15 nanometer, which is much smaller than Tc sulfur colloid. The labeling is carried out by adding $^{99m}TcO_4^-$ to the cold antimony colloid in acidic solution and heating the mixture at 100°C for 30 minutes. Because of the smaller size of the particles, it is used primarily for lymphoscintigraphy at sites of melanoma, internal mammary lymphoscintigraphy, and bone marrow imaging. This colloid is not approved by the FDA and is not available commercially.

^{99m}Tc-MAA. Macroaggregated albumin (MAA) is prepared by heating a mixture of human serum albumin (HSA) and stannous chloride or tartrate in acetate buffer at 80° to 90°C for about 30 min(51). Commercial kits are prepared by using MAA in lyophilized form. The number of particles varies between 1 and 4 million particles per milligram of albumin in different kits from various manufacturers. The size of the particles ranges between 10 and 90 μm with no particles larger than 150 μm and should be checked with a hemocytometer under a light microscope. Most nonradioactive MAA kits are stored at 2 to 8°C, whereas the Du Pont MAA kit is stored at room temperature. Labeling is performed by adding $^{99m}TcO_4^-$ to the kit and the reactions involve the reduction of $^{99m}Tc^{7+}$ by stannous ions followed by the formation of ^{99m}Tc-MAA. ^{99m}Tc-MAA is stored at 2 - 8°C and is good for 6 - 8 hours after formulation. The tagging efficiency normally exceeds 95%.

^{99m}Tc-labeled Peptides and Proteins

There are three methods available for labeling peptides and proteins with ^{99m}Tc: (1) direct labeling, (2) indirect labeling by first forming a ^{99m}Tc complex with a chelating

$$Z \overset{Y}{\underset{X}{\bigcirc}} X \quad NH - \overset{O}{\underset{\|}{C}} - CH_2 - N \overset{CH_2COOH}{\underset{CH_2COOH}{\diagdown}}$$
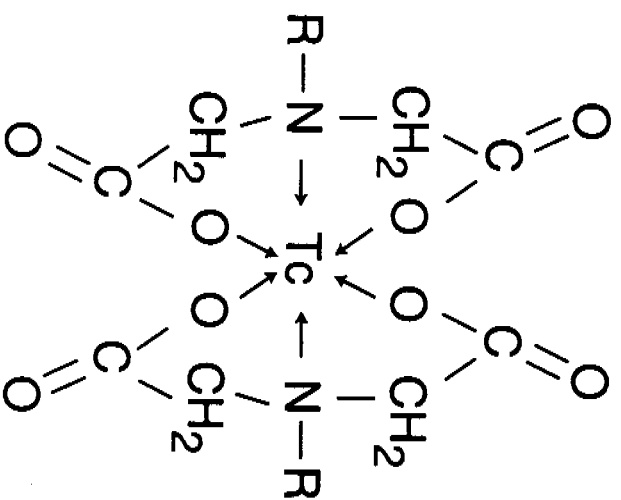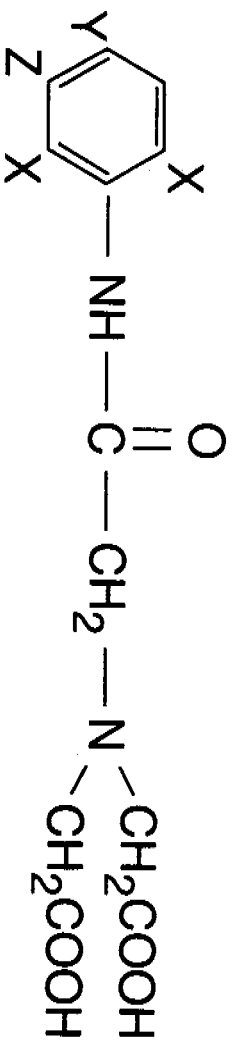
LIDOFENIN: X = CH₃ Y = H Z = H

ETILFENIN: X = CH₃CH₂ Y = H Z = H

MEBROFENIN: X = CH₃ Y = CH₃ Z = Br

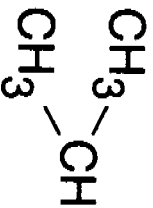DISOFENIN: $X = \overset{CH_3}{\underset{CH_3}{\diagup}} CH$ Y = H Z = H

Figure 5.   Molecular structures of various IDA derivatives and their ^{99m}Tc complexes. (Reproduced with permission from Saha GB, *Fundamentals of Nuclear Pharmacy*, 3rd edition, Springer Verlag, New York, 1992)

agent (preformed chelate) which is then attached to the protein, and (3) the indirect labeling via a chelating group attached to the peptide or protein followed by ^{99m}Tc labeling.

Direct Labeling. Direct labeling of peptides or proteins can occur by two chemical processes or a combination of these processes(52). ^{99m}Tc can be bound to (1) a colloid that is coated with antibody or (2) the sulfhydryl groups in the antibody in proportion to the reducing agent added. The possibility of colloid formation in ^{99m}Tc direct labeling was suggested by Eckelman et al.(53) who found that a colloid was formed during ^{99m}Tc-labeling of albumin using stannous ion as the reducing agent; this colloid was a competitive binder for ^{99m}Tc. Williams and Deegan(54) obtained 90% labeling of albumin with ^{99m}Tc reduced by concentrated HCl and ascorbic acid, in which case a colloid formation was suggested.

The ^{99m}Tc-labeling of proteins via sulfhydryl groups was accomplished by a pretinning method introduced by Rhodes et al.(55). In this method, a solution of stannous chloride, potassium phthalate and sodium potassium tartrate is added to the solution of the antibody and incubated for 21 hours at room temperature. The pretinned antibody is stored frozen at -70°C. To radiolabel the antibody, ^{99m}Tc pertechnetate is added to the pretinned kit and incubated for 1 hour at room temperature. Various transchelation challenge tests were carried out by mixing equal volumes of ^{99m}Tc antibody and EDTA, pretinned antibody or phosphate buffer. High performance liquid chromatography of these mixtures showed that ^{99m}Tc remained bound to IgG.

In the pretinning process, the sulfhydryl groups are freed by the reduction of disulfide bonds of the antibody using stannous ions. Because of the high concentration and strong reducing action of stannous ions, antibody fragments tend to break down into smaller fragments. Other milder reducing agents such as 2-mercaptoethanol and dithiothreitol have been used in the place of stannous ions (56,57). Also, ^{99m}Tc is presented to the antibody not as pertechnetate, but in a reduced state as a weak complex. For example, an anti-CEA antibody was labeled with ^{99m}Tc by using 2-mercaptoethanol as the reducing agent followed by the addition of ^{99m}Tc-phosphonate or pyrophosphate(56). Pak et al.(57) employed dithiothreitol as the reducing agent and glucarate as the weak chelating agent to label an antifibrin antibody. In both studies, a further purification of the labeled antibody was not needed.

Thakur et al.(58) titrated free sulfhydryl groups in IgGs using ninhydrin and found that using a molar ratio of 1000 to 5000 for dithiothreitol and 2-mercaptoethanol to IgG or molar ratios of 500 to 2500 for stannous chloride to IgG, only 1.8 to 2.0% of the available 35 disulfide groups were reduced. Blocking the sulfhydryl

groups with iodoacetate or cysteine decreased ^{99m}Tc binding.

The advantage of direct labeling is the simplicity in developing a kit for routine use. However, disadvantages are the possibility of colloid formation leading to liver accumulation and that some monoclonal antibodies are difficult to label by this method. Because the disulfide bonds are broken, some loss of immunoreactivity is expected, although for antibodies that are readily labeled by this method, no significant loss of immunoreactivity has been found.

Indirect Labeling with Preformed ^{99m}Tc Complex using Bifunctional Chelators. In this technique, ^{99m}Tc-chelates are preformed using bifunctional chelating agents such as diamidodithio(59), boronic acid adduct of technetium dioximes (BATO)(60) and cyclam derivatives (61), which are then used to label proteins by forming bonds between the chelating agent and the protein. Although this method appears to be most desirable, the disadvantage is that several steps are required to prepare ^{99m}Tc-labeled antibody, including modification of structural groups in the chelating agent (e.g., protection or removal of the functional groups). The labeling yield is poor and for these reasons, this method is not favorably accepted.

Indirect Labeling using Bifunctional Chelators. In this approach, a bifunctional chelate (BFC) is conjugated with a macromolecule (protein or peptide) on one side and a metal ion (e.g., Tc) by chelation on the other side. The BFCs contain reactive sites such as carboxycarbonic mixed anhydride, cyclic anhydride, N-hydroxysuccinimide ester and isothiocyanate. The conjugation of macromolecules takes place between the -NH_2 or -SH group of these macromolecules and one of the reactive sites of the BFCs. In practice, the antibody is reacted with the BFC to form a BFC-antibody complex which is separated from the reaction mixture. The BFC-antibody complex is then labeled with ^{99m}Tc by reacting with ^{99m}TcO$_4^-$ in the presence of a reducing agent (e.g. Sn^{2+}) or by ligand exchange with a weak ^{99m}Tc-chelate such as ^{99m}Tc-GH.

In 1974, Sundberg et al.(62) first synthesized 1-(para-benzyl-diazonium)-EDTA to bind protein with ^{111}In. Because of their higher stability, DTPA and its analogs were preferably used replacing EDTA. Later on, cyclic anhydride of DTPA was developed by Hnatowich et al. for use as a BFC in protein labeling with ^{111}In(63).

Several studies reported ^{99m}Tc-labeling of proteins using DTPA as the BFC(64,65), but it was found that this chelator binds to many nonspecific binding sites on the antibody and that it tends to release ^{99m}Tc easily in vivo(64). For these reasons, alternative chelators such as metallothionein(66), dithiosemicarbazone(67) and diamide dimercaptide (N_2S_2)(68) have been employed in ^{99m}Tc-labeling of various proteins. Among these, N_2S_2 chelator

is most popular, providing sufficient immunoreactivity, and it has been used to label antimelanoma antibody. However, nonspecific labeling, resulting in rapid dissociation of ^{99m}Tc complex, prompted the use of prelabeled ^{99m}Tc-chelator to conjugate to the antibody(59).

^{99m}Tc-labeled antibodies are used for detecting a variety of pathophysiological conditions depending on the type and characteristics of the antibody. Many monoclonal antibodies have been developed against various cancers and, at least theoretically, these antibodies labeled with ^{99m}Tc could lead to the detection of these cancers. In clinical situations, the half-life of ^{99m}Tc is somewhat short to allow for sufficient localization in tumors. However, with the use of higher activity, it has been possible to detect these tumors scintigraphically. Further studies to improve rapid background reduction in vivo are warranted so that imaging can be performed in a shorter time after administration of ^{99m}Tc-labeled antibody to the patients.

The difficulty of imaging with radiolabeled antibodies due to slow plasma clearance and tumor uptake is somewhat mitigated by the use of peptides whose molecular size is smaller than those of proteins. The plasma clearance of the peptides is much faster. They exhibit rapid target tissue uptake and rapid excretion, primarily due to the degradation of the peptides by peptidases (69). Various derivatives of native peptides can be readily synthesized to enhance their binding to its biological target.

High specific activity of ^{99m}Tc-labeled peptides can be obtained, although due to the small size of the peptide, ^{99m}Tc labeling is likely to alter the regions of the peptide which interact with the biological target and consequently lose their binding affinity (69). Direct labeling of peptides with ^{99m}Tc using Sn^{2+} ions as the reducing agent may cause substantial damage to the molecule by breaking open the disulfide bonds. Thus, labeling with bifunctional chelators is preferable. ^{99m}Tc-MAG3 is the only approved ^{99m}Tc-labeled peptide for clinical use. Other ^{99m}Tc peptides that have been experimentally used in animals include P280 and P748 peptides which bind to GPIIb/IIIa receptors in thrombi(69), tumor detecting peptides such as P587 and P829(69) and peptides for infection/inflammation imaging(70).

^{99m}Tc-labeled Red Blood Cells

Three methods are employed to label red blood cells (RBCs) with ^{99m}Tc: (1) in vitro method, (2) in vivo method, and (3) modified in vivo method. In the in vitro method, blood is drawn from the patient, RBCs are primed with Sn^{2+} ions, and then $^{99m}TcO_4^-$ is added. The Sn^{2+} ion enters into the RBCs and remains bound to hemoglobin. Subsequently, the $^{99m}Tc^{7+}$ ion enters into the RBCs and is reduced by Sn^{2+}, whereupon almost 80% of

the reduced ^{99m}Tc binds to the beta chain of the globin part of the hemoglobin and the remaining 20% to the heme(71). The labeling efficiency is greater than 97%. Heat-denatured RBCs are used for spleen imaging. A kit for in vitro labeling of RBCs is commercially available(72).

In the in vivo method(73), Sn^{2+} ions in the form of stannous pyrophosphate are administered intravenously to the patient. After a delay of 30 minutes, $^{99m}TcO_4^-$ is administered, whereby the labeling occurs in the same manner as in in vitro labeling. It should be pointed out that the optimum quantity of Sn^{2+} ions required is 10 - 20 $\mu g/kg$ body weight. The tagging efficiency is somewhat lower (80 - 90%), partly due to extravascular distribution such as thyroid trapping, gastric secretion and renal excretion. Certain drugs such as heparin, dextran, doxorubicin, penicillin, hydralazine and iodinated contrast media often inhibit Sn^{2+} transport through the RBC membrane and thus decrease the ^{99m}Tc-labeling of RBCs.

In the modified in vivo method(74), Sn-PYP is administered intravenously to the patient in whom an infusion set fitted with a three-way stopcock has been secured. One port of the stopcock is connected to a syringe containing heparinized saline and the other port to a syringe containing $^{99m}TcO_4^-$. Residual heparin in the infusion line acts as an anticoagulant. Twenty minutes after injection of Sn-PYP, blood is withdrawn into the ^{99m}Tc-syringe and incubated with gentle shaking for 10 minutes. The labeling efficiency is better than 95%. The labeled RBCs are injected back into the patient, followed by flushing with heparinized saline.

^{99m}Tc-labeled Leukocytes and Platelets

Leukocytes and platelets separated from blood by centrifugation and washing are commonly labeled with ^{111}In (using ^{111}In-oxine) for use in detecting infection and thrombi respectively. Attempts have been made to label these cells with ^{99m}Tc with variable success. An early method of ^{99m}Tc-labeling of leukocytes was based on the phagocytosis of ^{99m}Tc-SnF_2 colloid by the cells in the whole blood(75). This method uses a prelabeled ^{99m}Tc-colloid to label leukocytes and depends on the type and size of the colloid. The labeling yield by this method is of the order of 80%. Hirsch et al. (76) employed a slightly modified technique of labeling leukocytes with ^{99m}Tc-SnF_2 colloid and obtained a labeling yield of >95%. In a later study using the technique of Hanna et al.(75), the mechanism of labeling was shown to be surface adherence rather than phagocytosis of the colloids(77). A commercial human serum albumin colloid (Microlite®; Du Pont Pharma) has also been used to label leukocytes, but has not been widely accepted.

The most common and practical method of ^{99m}Tc labeling of leukocytes utilizes ^{99m}Tc-HMPAO, a neutral lipophilic compound that easily enters into the leukocyte

by passive diffusion. Peters et al.(78) first reported this method in which freshly prepared ^{99m}Tc-HMPAO is added to separated leukocytes suspended in a plasma/ACD mixture. After a 15 minute incubation, cells are separated by centrifugation, washed, and finally suspended in plasma for injection. The labeling yield is of the order of 50% to 60%. The advantage of this method is that imaging can be performed within 6 hours rather than 24 hours after the administration of the labeled cells as in the case of ^{111}In-leukocytes. However, in vivo, the labeled cells release ^{99m}Tc-containing intermediates over time and an appreciable amount of ^{99m}Tc activity appears in gastrointestinal areas several hours after administration. For this reason, abdominal infections are difficult to detect with this agent and if used for this indication, the patient should be imaged in a few hours after administration.

Investigators have developed antibodies against granulocytes, labeled them in vitro with ^{99m}Tc and then injected the labeled antibodies into the patient(79). Labeled leukocytes are obtained by a reaction in vivo between the labeled antibody and granulocytes. The kinetics of these ^{99m}Tc-labeled leukocytes appear to be different from those of ^{111}In-leukocytes and ^{99m}Tc-HMPAO-labeled leukocytes. Abscess uptake of ^{99m}Tc-leukocytes is faster than the in vitro labeled cells suggesting upregulation of the antigen on activated cells and more rapid labeling of the sequestered cells than of the circulating cells. In addition to the advantage of not having to handle blood, this method promises faster results.

The labeling of platelets with ^{99m}Tc is still in its infancy. One method involves pretinning of the cells with stannous glucoheptonate as the tinning agent followed by addition of ^{99m}TcO$_4^-$. The yield is almost 90%, when labeled in physiologic saline medium. Recent efforts to label platelets with ^{99m}Tc using ^{99m}Tc-HMPAO appear to be promising(80).

SUMMARY

The majority of ^{99m}Tc radiopharmaceuticals are prepared by reducing Tc^{7+} to a lower oxidation state and allowing the reduced technetium to react with different chelating agents. Stannous ion is the most common reducing agent. The concentrations of reactants, pH, and temperature dictate the optimum yield of ^{99m}Tc radiopharmaceuticals.

^{99m}Tc-SC is the only radiopharmaceutical which does not require any reduction of Tc^{7+}. Antibodies are labeled with ^{99m}Tc by a direct method using the pretinning technique or by the indirect method using a bifunctional chelate that binds to an antibody at one end and to the technetium ion at the other. RBCs, leukocytes and platelets are labeled with reduced ^{99m}Tc with various

labeling efficiencies. A Tc=0 core is found to exist in the structure of many ^{99m}Tc complexes, whereas some complexes contain a *trans*-TcO$_2$ core.

Since the inception of nuclear medicine, much progress has been made in the development of various ^{99m}Tc radiopharmaceuticals for clinical use. Whereas most radiopharmaceuticals were considered "magic bullets" during their infancy period, in-depth chemistry and structural information of these agents are now well established. The chemistry of ^{99m}Tc radiopharmaceuticals has matured to the extent that the feasibility of newer complexes for clinical use can be predicted. It is hoped that a new generation of ^{99m}Tc complexes will be developed in the near future so that scintigraphic images of various organs can be obtained with better resolution and accuracy.

GLOSSARY

Butyl iminodiacetic acid	BIDA
Diethylenetriaminepentaacetic acid	DTPA
Diisopropyl iminodiacetic acid	DISIDA
Dimercaptosuccinic acid	DMSA
Dimethylphenyl carbamoylmethyl iminodiacetic acid	HIDA
Ethyl cysteinate dimer	ECD
Ethylendiaminetetraacetic acid	EDTA
Glucoheptonate (gluceptate)	GH
Hexamethylpropyleneamine oxime	HMPAO
Human serum albumin	HSA
Hydroxymethylene diphosphonate	HDP
Macroaggregated albumin	MAA
Mercaptoacetylglycylglycylglycine	MAG3
Methylene diphosphonate	MDP
Orthoiodohippurate	OIH
Paraisopropyl iminodiacetic acid	PIPIDA
Pyrophosphate	PYP
Red blood cell	RBC
Sulfur Colloid	SC

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QUESTIONS

1. Technetium is an element belonging to which group in the Periodic Table?

   A. Group II
   B. Group V
   C. Group VII
   D. Group III

2. Technetium obtained from the Moly generator is in the form of:

   A. TcO_4^-
   B. $Tc(OH)_2$
   C. TcO_2
   D. none of the above

3. Maximum activity of ^{99m}Tc is available from the Moly generator in:

   A. 4 half-lives of [99m]Tc
   B. 5 - 7 half-lives of [99m]Tc
   C. 66 hr
   D. 6 hr

4. The maximum permissible concentration of [99]Mo in the ^{99m}Tc-eluate is:

   A. 5 μCi per mCi of ^{99m}Tc at the time of injection
   B. 0.15 μCi/mCi of ^{99m}Tc at the time of injection
   C. as much as maximum permissible concentrations in water allowed by NRC
   D. 1 μCi/mCi of [99m]Tc

5. The oxidation state of technetium in [99m]TcO_4^- is:

   A. 3+
   B. 4+
   C. 7+
   D. 7-

6. Technetium in [99m]TcO_4^- must be reduced prior to binding to any chelating agent. Reduction of ^{99m}Tc is accomplished by using:

   A. cold saline
   B. methanol
   C. stannous ions
   D. stannic chloride

7. To prevent the formation of hydrolyzed products in ^{99m}Tc radiopharmaceuticals the following is added:

   A. excess of tin
   B. excess of hydrochloric acid
   C. excess of chelating agent
   D. excess of free ^{99m}Tc

8. Oxygen must be avoided in ^{99m}Tc radiopharmaceuticals primarily to prevent:

   A. reduction of $^{99m}TcO_4^-$
   B. formation of hydrolyzed ^{99m}Tc
   C. formation of ^{99m}Tc complex
   D. oxidation of Sn^{2+}

9. The important chemical reaction in the ^{99m}Tc-labeling of MAG3, sestamibi and HMPAO kits involves:

   A. only chelation
   B. ligand exchange
   C. heat release
   D. vaporization

10. The size of the MAA particles ranges between:

    A. 0 - 10 nanometer
    B. 10 - 90 nanometer
    C. 10 - 90 micrometer
    D. 10 - 90 centimeter

11. The shelf-life of most ^{99m}Tc-labeled radiopharmaceuticals is:

    A. 6 hr
    B. 12 hr
    C. 6 months
    D. 1 yr

12. EDTA is added to the sulfur colloid kit in order to prevent:

    A. formation of larger particles due to Al^{3+}
    B. formation of colloid due to Al^{3+}
    C. precipitation of Sn^{2+}
    D. none of the above

13. Which of the following agents does not affect the in vivo labeling of RBCs with ^{99m}Tc?

    A. doxorubicin
    B. heparin
    C. sodium chloride
    D. iodinated contrast agents

14. The direct labeling of antibodies with ^{99m}Tc involves:

    A. heating of the antibodies
    B. reduction of disulfide bonds by Sn^{2+} freeing sulfhydryl groups
    C. using a bifunctional chelator such as DTPA
    D. condensation of ^{99m}Tc and Sn^{2+}

15. In the indirect labeling of proteins (antibodies) with ^{99m}Tc, the common bifunctional chelator used is:

    A. DTPA
    B. HCl
    C. DMSA
    D. GH

16. The primary reason for using ^{99m}Tc-HMPAO in ^{99m}Tc-labeling of leukocytes is its:

    A. solubility in water
    B. lipophilicity
    C. heat stability
    D. affinity for brain cells

17. Lengthening the alkyl chain on the phenolic group of the iminodiacetic acid (IDA) molecule:

A. increases hepatobiliary extraction of IDA complex
B. decreases hepatobiliary excretion
C. decreases renal excretion
D. both A. and C.

18. ^{99m}Tc-HMPAO preparation requires:

A. ^{99m}Tc in acidic medium
B. ^{99m}Tc-eluate that is no more than 2 hr old
C. ^{99m}Tc obtained from a generator that has been eluted within 24 hr
D. B. and C.

19. ^{99m}Tc-MAG3 has which of the following properties?

A. A coordination number of 6 and a charge of 1+
B. A coordination number of 6 and a charge of 1-
C. A coordination number of 5 and a charge of 1+
D. A coordination number of 5 and a charge of 1-

20. Which of the following stable bonds is found in diphosphonate bone imaging agents?

A. P-O-P
B. P-C-P
C. P-N-P
D. P-S-P

21. In order to prevent oxidation of Sn^{2+} in the reagent kit, the following is performed:

A. vial is filled with oxygen
B. vial is flushed with nitrogen
C. air is introduced into the vial
D. vial is heated

22. Which of the following ^{99m}Tc radiopharmaceuticals does not require reduction of ^{99m}Tc in the preparation?

A. ^{99m}Tc-DTPA
B. ^{99m}Tc-DMSA
C. ^{99m}Tc-SC
D. ^{99m}Tc-sestamibi

23. Which of the following statements is not true?

A. ^{99m}Tc-HMPAO is lipophilic and thus crosses the brain cell membrane
B. Labeling of leukocytes with ^{99m}Tc-HMPAO is carried out in plasma
C. ^{99m}Tc-HMPAO can be used to label many antibodies
D. ^{99m}Tc-HMPAO is stabilized by methylene blue and phosphate buffer.

24. The column of the Moly generator is made of:

A. SnO_2
B. Al_2O_3
C. NaCl
D. DTPA

25. In the in vivo labeling of RBCs with ^{99m}Tc, the optimum amount of Sn^{2+} needed is:

A. 10 - 20 μg/kg
B. 20 - 30 μg/kg
C. 10 - 20 mg/kg
D. 10 - 20 g/kg