Technetium Radiopharmaceutical Chemistry

Continuing Education for Nuclear Pharmacists and Nuclear Medicine Professionals

By

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TECHNETIUM RADIOPHARMACEUTICAL CHEMISTRY

STATEMENT OF OBJECTIVES

Upon completion of this course the reader will be able to understand and discuss the chemistry of technetium radiopharmaceuticals.

Specifically, the recipient should be able to:

1. Describe the electron configuration of technetium oxidation states.
2. List the general chemical composition of technetium radiopharmaceutical kits.
3. Explain the function of kit ingredients.
4. Explain the chemical processes involved with the preparation of technetium compounds.
5. Describe the significance of stereochemistry related to radiopharmaceuticals.
7. Distinguish between first-generation and second-generation technetium-tagged radiopharmaceuticals.
8. Describe the radiolabeling approaches for bifunctional chelates.
9. Explain the kit and coordination chemistry involved with the preparation of technetium compounds of specific oxidation states.
10. Discuss the chemistry involved with technetium labeling of proteins, antibodies, and blood cells.
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OVERVIEW

Technetium, as element 43, was discovered in 1937 by Perrier and Segrè in a sample of molybdenum which was irradiated by deuterons. The new element received its name from the Greek word *technetos*, meaning artificial, because technetium was the first element previously unknown on earth to be made artificially. In 1939 Seaborg and Segrè observed that molybdenum-98 irradiated with slow neutrons gave rise to \(^{99}\text{Tc}\) through decay of the metastable isomer, \(^{99m}\text{Tc}\). Eventually 21 isotopes of technetium were discovered ranging from \(^{90}\text{Tc}\) to \(^{110}\text{Tc}\), with technetium-110 having the shortest half-life (0.86 sec) and \(^{97}\text{Tc}\) the longest (2.6 x 10\(^6\) y). All technetium isotopes are radioactive.

\(^{99m}\text{Tc}\) has achieved widespread application in diagnostic nuclear medicine. This metastable isomer decays with a half-life of 6.01 hr to \(^{99}\text{Tc}\), shown in the decay scheme in Figure 1.

Technetium-99 has a half-life of 2.13 x 10\(^5\) years, so it is essentially stable. Technetium-99 has been useful in elucidating the precise chemistry of technetium in its radiopharmaceutical compounds. The metastable state of \(^{99m}\text{Tc}\) is 0.1427 MeV above the ground state of \(^{99}\text{Tc}\). Three gamma photons (\(\gamma_1\), \(\gamma_2\), and \(\gamma_3\)), of 0.0022, 0.1405, and 0.1427 MeV, respectively, are released in the decay of \(^{99m}\text{Tc}\). The most abundant of these is \(\gamma_2\) (0.1405 MeV), being produced in 89.1% of all nuclear transitions. It is the principal photon detected in nuclear medicine imaging studies.

In the 1950s, a purification work on the tellurium-132 / iodine-132 generator at Brookhaven National Laboratory (BNL) turned up a contaminant which was proved to be technetium. The technetium contaminant was due to the presence of \(^{99}\text{Mo}\) in the chemical separation, which was also present, because it had followed tellurium in the chemical separation process. This discovery eventually led to
the production of the $^{99}$Mo/$^{99m}$Tc generator in 1957 at BNL. Final improvements were made by Powell Richards. A simplified production and decay scheme for the $^{99m}$Tc generator is shown in Figure 2.

The molybdenum-99 used in present-day generators is obtained as a fission by-product of $^{235}$U. Radiochemical methods are used to separate $^{99}$Mo from the other radionuclides in the reactor product. The purified $^{99}$Mo is used to prepare the generator. In general, $^{99}$Mo is adjusted to an acidic pH, forming various anionic species such as molybdate ($\text{MoO}_4^{2-}$) and paramolybdate ($\text{Mo}_7\text{O}_{24}^{6-}$). The anionic molybdate solution is then loaded onto a generator column containing alumina ($\text{Al}_2\text{O}_3$) which was previously washed in pH 5 saline. The positively charged alumina firmly adsorbs the negatively charged molybdate ions. Generators are then autoclaved, assembled under aseptic conditions, and eluted with normal saline (0.9 percent sodium chloride injection). Quality control tests by the manufacturer include generator elution efficiency, eluate volume, radiochemical and radionuclidic purity, pH, aluminum ion concentration, sterility and pryogen tests.

When generators are used in nuclear pharmacy practice the $^{99m}$Tc activity is eluted with sterile normal saline. The eluate consists of normal saline and sodium pertechnetate. The pertechnetate ion ($\text{TcO}_4^{-}$) is readily displaced from the alumina column by chloride ion (Cl$^-$) in the saline solution. The $^{99}$Mo activity remains firmly bound to the alumina, since it is more negatively charged compared to pertechnetate. Between 70 to 90 percent of the available $^{99m}$Tc activity is removed during the elution. At least 5 mL of saline is required to remove the $^{99m}$Tc activity, and typically between 5 and 20 mL are eluted depending on the activity concentration desired in the final eluate. $^{99m}$Tc activity in the generator
builds up rapidly after generator elution. Maximum buildup of $^{99m}$Tc activity is achieved in about 23 hours following elution, however about 50 percent of the maximum activity is reached about 5 to 6 hours after elution. Thus, the generator may be eluted at other times during the day to obtain more pertechnetate. The $^{99}$Mo activity remaining on the column continues to decay, generating more $^{99m}$Tc activity. A generator has a useful life of two weeks, however weekly replacement is the norm to supply the required amount of technetium activity for daily needs. The generator eluate consists of both $^{99m}$Tc and $^{99}$Tc as pertechnetate. The chemical amount of technetium in the eluate is important for the radiolabeling of several technetium compounds and will be addressed later in the discussion on $^{99m}$Tc sodium pertechnetate.

The first technetium generator investigated for clinical use was purchased from BNL by Harper at the University of Chicago. The generator yielded technetium as the pertechnetate ion $^{99m}$TcO$_4^-$. After intravenous injection of $^{99m}$TcO$_4^-$ into mice, activity was observed to localize in the thyroid gland, salivary glands, stomach, and urinary bladder, similar to iodide ion (Figure 3). Subsequent clinical studies in humans confirmed the biodistribution pattern in animals, leading to the widespread investigation of $^{99m}$Tc-sodium pertechnetate for diagnostic studies (Figure 4).

Eventually studies with other $^{99m}$Tc-labeled compounds demonstrated the vast potential of $^{99m}$Tc for the diagnosis of disease.

These early investigational studies identified the following advantages of $^{99m}$Tc for imaging:

1. Low radiation dose due to short half-life and the absence of beta radiation.
2. High photon yield (89%) of 140 keV gamma; good tissue penetration; easily collimated and efficiently detected by the gamma camera.
3. Availability from a generator for local use.
4. Capable of being compounded into a variety of chemical forms.
TECHNETIUM CHEMISTRY

The chemical forms of the earliest technetium compounds prepared for clinical use were $^{99m}$Tc-sodium pertechnetate (for brain and thyroid imaging) and $^{99m}$Tc-sulfur colloid (for liver, spleen and bone marrow imaging). Different compounds were soon developed for imaging other organs. An essential requirement for preparing technetium-labeled compounds was changing technetium’s oxidation state. This was achieved by employing a reducing agent in the radiolabeling procedure. Early technetium compounds were prepared extemporaneously following a step-wise admixture of ligand, reducing agent, pertechnetate, and adjuvants and any necessary pH or temperature adjustments. Labeling yields were often not quantitative requiring a final purification step to remove unbound pertechnetate. The final product was sterilized by membrane filtration or autoclaving. Eventually sterile radiopharmaceutical kits were developed that simplified the compounding procedure and yielded high purity technetium compounds without the need to remove unreacted pertechnetate. This methodology continues to be the standard of practice today.

TECHNETIUM OXIDATION STATES

Technetium is positioned in the periodic table along with manganese and rhenium, but its chemistry is more similar to rhenium. Table 1 lists technetium’s electronic configuration compared to other elements.

<table>
<thead>
<tr>
<th>Z</th>
<th>Element</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>s</td>
<td>p</td>
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<tr>
<td>4</td>
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<td>2</td>
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<td>6</td>
<td>2</td>
<td>6</td>
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</table>

Table 1: Electron Shell Configuration of Selected Elements

$Z =$ element atomic number
As a transition metal in group VII B, technetium has seven electrons beyond krypton’s noble gas configuration and these electrons reside in the 4d and 5s subshells (d⁷ configuration) (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Core *</th>
<th>Principal Level &amp; Sublevel</th>
<th>Oxidation State</th>
<th>Configuration Descriptor</th>
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<tbody>
<tr>
<td>[Kr] 6</td>
<td>4 d f 5 s p d f</td>
<td>Te⁰ (metal)</td>
<td>d⁷</td>
</tr>
<tr>
<td>[Kr] 6</td>
<td></td>
<td>Te⁺¹</td>
<td>d⁶</td>
</tr>
<tr>
<td>[Kr] 5</td>
<td></td>
<td>Te⁺²</td>
<td>d⁵</td>
</tr>
<tr>
<td>[Kr] 4</td>
<td></td>
<td>Te⁺³</td>
<td>d⁴</td>
</tr>
<tr>
<td>[Kr] 3</td>
<td></td>
<td>Te⁺⁴</td>
<td>d³</td>
</tr>
<tr>
<td>[Kr] 2</td>
<td></td>
<td>Te⁺⁵</td>
<td>d²</td>
</tr>
<tr>
<td>[Kr] 1</td>
<td></td>
<td>Te⁺⁶</td>
<td>d¹</td>
</tr>
<tr>
<td>[Kr] 0</td>
<td></td>
<td>Te⁺⁷</td>
<td>d⁰</td>
</tr>
</tbody>
</table>

* [Kr] core = 1s²2s²2p⁶3s²3p⁶3d¹⁰4s²4p⁶
† This level represents pertechnetate [Tc⁺⁷O₄]⁻ with technetium at its highest oxidation state

Technetium readily loses these 7 electrons to yield the 7+ oxidation state (d⁰ configuration), which exists in pertechnetate, TeO₄⁻. While pertechnetate is the most stable state in aqueous solution, technetium compounds have been prepared with oxidation states from 1- to 7+. Radiochemical studies have shown that technetium is reduced to Tc(V) as [TcOCl₄]⁻ in cold concentrated HCl and to Tc(IV) as (TcCl₆)₂⁻ in hot HCl. Hydrolysis to insoluble TeO₂ is prevented under these conditions but can occur in solutions less concentrated than 2 M HCl unless a coordinating ligand is present. Many coordination complexes of technetium have been made by displacing the halide ligands in these reduced chlorocomplexes with coordinating ligands that confer chemical stability and favorable biological properties for diagnostic imaging.

Ascorbic acid and ferrous iron were reducing agents used in early radiolabeling studies, but they often led to incomplete reduction, requiring removal of unreacted pertechnetate. Reducing agents capable of more complete reduction of technetium have since been introduced. Sodium borohydride (NaBH₄) and sodium dithionite (Na₂S₂O₄) are effective in alkaline pH while stannous chloride (SnCl₂) is typically used in acidic pH. Stannous chloride is capable of producing high yields of technetium-labeled compounds, eliminating the need to remove free pertechnetate. This led to the introduction of "instant kits" for the preparation of ⁹⁹ᵐTc-radiopharmaceuticals. Other stannous salts, such as stannous fluoride and stannous tartrate, are also used in kit formulations.
The oxidation state of technetium and the stability of technetium-labeled compounds are controlled by several factors including pH, the type of reducing system, the chemical properties of the coordinating ligand, and adjunctive ingredients in the kit. The most stable states of technetium in water are Tc(VII) as TcO₄⁻ and Tc(IV) as the insoluble hydrolyzed reduction product, TcO₂·H₂O. Reduction/titration experiments have shown that some ligands can produce complexes with technetium in a specific oxidation state while other ligands yield complexes with technetium in more than one oxidation state, determined by the number of electrons, n, acquired by pertechnetate. Thus, when n = 2, 3, 4, and 6, technetium is reduced to the (V), (IV), (III), and (I) oxidation states, respectively. For example, ligands such as diethylenetriaminepentaacetic acid (DTPA), pyrophosphate, tripolyphosphate, and others can form Tc(III) complexes initially which then oxidize to Tc(IV). Technetium (V) and (VI) oxidation states can disproportionate, as follows, to (IV) as TcO₂ and (VII) as TcO₄⁻, unless adequate concentrations of complexing ligand are present:

\[
3 \text{Tc (VI)} \rightarrow \text{Tc (IV)} + 2 \text{Tc (VII)} \\
3 \text{Tc (V)} \rightarrow 2 \text{Tc (IV)} + \text{Tc (VII)}
\]

These unwanted reactions may compromise labeling yields of the desired technetium complex if the radiopharmaceutical kit formulation is not optimized.

Some complexes are quite stable to oxidation (e.g. ⁹⁹ᵐTc-DTPA, ⁹⁹ᵐTc-HIDA derivatives, ⁹⁹ᵐTc-glueptate) while others are more labile (e.g. ⁹⁹ᵐTc-DMSA, ⁹⁹ᵐTc bone agents) and may require addition of an antioxidant to the formulation. The oxidation states of technetium in several compounds are listed in Table 3.

<table>
<thead>
<tr>
<th>Oxidation State</th>
<th>Chemical Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc(VII)</td>
<td>Pertechnetate, Sulfur Colloid</td>
</tr>
<tr>
<td>Tc(V)</td>
<td>Citrate, DMSA (high pH), ECD, Glucpeptate, Gluconate, HMPAO, MAG3, Tetrofosmin</td>
</tr>
<tr>
<td>Tc(IV)</td>
<td>DTPA, EHDP, HDP, MDP, PPI (PYP), TcO₂·H₂O</td>
</tr>
<tr>
<td>Tc(III)</td>
<td>DMSA (low pH), HIDA analogs, Furifosmin, Teboroxime</td>
</tr>
<tr>
<td>Tc(I)</td>
<td>Sestamibi</td>
</tr>
</tbody>
</table>

It should be noted, however, that electron transfer studies to identify the oxidation state of technetium in first-generation complexes, such as ⁹⁹ᵐTc-succimer (DMSA), ⁹⁹ᵐTc-pentetate (DTPA), ⁹⁹ᵐTc-pyrophosphate (PPI or PYP), and ⁹⁹ᵐTc-diphosphonates, have not been conclusive and depend on the reducing conditions. On the other hand, technetium's oxidation state in many second-generation...
complexes have been well characterized. Representative examples of electronic configurations and oxidation states of technetium in several radiopharmaceutical compounds are as follows: in $^{99m}$Tc-sestamibi (Cardiolite), the $d^6$ configuration exists where technetium attains the Tc(I) oxidation state by gaining six electrons; in $^{99m}$Tc-mebrofenin (Choletec), the $d^4$ configuration exists where technetium gains four electrons to attain the Tc(III) oxidation state; and in $^{99m}$Tc-mertiatide (TechneScan MAG3), the $d^2$ configuration exists where technetium gains two electrons to attain the Tc(V) oxidation state. An exception to the requirement for chemical reduction is $^{99m}$Tc-sulfur colloid, where technetium retains the Tc(VII) oxidation state by virtue of its stability as insoluble technetium hepta-sulfide, Tc$_2$S$_7$. Thus, technetium exhibits a diverse chemistry allowing it to be incorporated into a variety of chemical forms for diagnostic use in nuclear medicine. The oxidation states listed in Table 3 are considered to be the usual state present in technetium radiopharmaceuticals prepared from kits. Further discussion of of particular technetium compounds will be addressed later in this article.

**KIT CHEMISTRY**

Technetium kits are typically formulated with a reducing agent, a coordinating ligand, and adjuvants such as antioxidants, buffers, and ancillary chelating agents. The most common reducing agent in $^{99m}$Tc kits is Sn(II) as stannous chloride dihydrate, SnCl$_2$·2H$_2$O. Typically, very little of the Sn(II) in kits is present as free ions in radiopharmaceutical solutions, most of it being complexed with ligand and some of it as colloidal tin aggregates. Being a powerful reducing agent, stannous chloride is easily oxidized to Sn(IV) by oxygen dissolved in solution or in air. Usually, a large excess of stannous chloride is present with respect to pertechnetate in radiopharmaceutical solutions, with the ratio of SnCl$_2$-to-$^{99m}$TcO$_4^-$ being as high as $10^8$ to $10^9$. Thus, very little of the Sn(II) present is oxidized by pertechnetate, per se, and most of its reducing power is lost due to oxidation by oxygen and free radicals. Thus, except for a few special situations, it is important to exclude air from most technetium radiopharmaceuticals during and after preparation. To preserve Sn(II) in the kit, ingredients are lyophilized and sealed under an oxygen-free atmosphere such as nitrogen or argon.

Most technetium radiolabeling reactions occur near neutrality and sufficient complexing agent must be available to keep all metal ions soluble, including stannous ion, stannic ion, and reduced $^{99m}$Tc and $^{99}$Tc. An excess of coordinating ligand (Figure 5) is present in kits to ensure technetium complex formation and to minimize hydrolysis reactions (formation of tin hydroxides and technetium dioxide colloid).
A chelating agent may be added to the kit to enhance chelation of metal ions. The optimal formulation has to be determined for each type of kit.\textsuperscript{10,11} The pH of the radiolabeling mixture is important for reactions to proceed appropriately and buffers are used to adjust pH. Once the technetium complex is formed it may be subject to degradation by oxidation from air inadvertently introduced into the kit. Degradation can also be caused by autoradiolysis mediated by free-radicals. Oxidative degradation is mediated by OH$^-$ and reductive degradation by H$^+$ or e$^{-}_{aq}$. Kits susceptible to autoradiolysis may limit the technetium concentration during radiolabeling and may need free-radical scavengers, such as ascorbic acid, gentisic acid, or para-aminobenzoic acid (PABA).\textsuperscript{12} In some circumstances air may be deliberately added to kits to minimize the formation of radiochemical impurities. For example, air is added to the $^{99m}$Tc-MAG3 kit to oxidize excess stannous ion, which might lower the oxidation state of technetium in the desired complex. The addition of air to a $^{99m}$Tc-tetrofosmin kit minimizes autoradiolysis of the technetium complex by reducing free radicals.\textsuperscript{13}

Ancillary chelating agents may be included in a kit. These agents help to keep tin soluble when the coordinating ligand may not be effective, they enhance the reduction potential of the system through
complexation of Sn(II) and Sn(IV) species\(^7\), and they may function as transfer ligands. A transfer ligand, otherwise known as a donor ligand or exchange ligand, forms a weak complex with reduced technetium. This stabilizes technetium against disproportionation when its reaction rate with the coordinating ligand is slow. During the radiolabeling reaction, the stronger coordinating ligand displaces the weaker ligand. Some examples of transfer ligands are sodium tartrate in the mertiatide kit, sodium gluconate in the tetrofosmin kit, sodium glucoheptonate in the apcitide kit, and sodium citrate in the sestamibi kit.

TECHNETIUM RADIOPHARMACEUTICALS

DEVELOPMENTAL HISTORY

A primary goal in nuclear medicine has been the development of target-specific radiopharmaceuticals. With first-generation agents technetium was labeled or “tagged” to a variety of molecular species that delivered technetium to organs of interest based on non-substrate specific localization mechanisms i.e., there was no specific reaction between the technetium compound and a substrate located in the target organ. Localization occurred, for example, by simple diffusion, phagocytosis, entrapment, or cell sequestration mechanisms. In essence, technetium was a passenger atom not essential for localization. The early technetium complexes were not well characterized chemically because their technetium concentration (ca \(10^{-8}\) to \(10^{-9}\) M, e.g.10 mCi \(^{99m}\)Tc = 1.9 ng) was below that required for conventional chemical methods of analysis. Identifying the properties of technetium compounds was limited to analysis of tracer concentrations of technetium solutions. Properties such as oxidation state, formation constant, and electrical charge were characterized primarily by polarography, various types of chromatography, solvent extraction, and electrophoresis. As investigative studies progressed, however, \(^{99}\)Tc as ammonium pertechnetate was used to prepare carrier added (CA) technetium compounds, which permitted structural characterization by conventional methods such as infrared spectroscopy, mass spectroscopy, nuclear magnetic resonance spectroscopy, and x-ray crystallography. These studies demonstrated the equivalence of many second-generation technetium complexes in CA (macroscopic) and no carrier added (NCA) (tracer) quantities. Over time, all the lower oxidation states of technetium were examined. An important development in the design of second-generation technetium radiopharmaceuticals was the creation of ligands that could not only stabilize technetium in lower oxidation states but could also be modified to influence site-specific localization in vivo. In the 1970’s Loberg and Fields,\(^{14}\) in their quest to develop a technetium heart imaging agent, serendipidously discovered the substituted iminodiacetic acid analogs for hepatobiliary imaging (HIDA compounds)
Their elegant work in characterizing the structure of the first complex (99mTc-lidofenin or 99mTc-HIDA) led to two important contributions to the future development of technetium radiopharmaceuticals. The first was the finding that, in the 99mTc-HIDA complex, the technetium atom was essential for its uptake and excretion by the liver.14,15 Without technetium, the IDA analog ligands exhibited renal excretion after intravenous injection. However, when technetium was coordinated with two IDA ligands, the principal excretion pathway was hepatobiliary.16 The second important contribution from this work was introduction of the concept of bifunctional chelators, i.e. ligands that not only chelate technetium, but can be modified with functional groups to control biodistribution of the technetium complex. This concept was developed further in the 1990’s whereby a reactive site on the bifunctional chelator was introduced to enable binding of the technetium complex to another molecule for targeting purposes.

STEREOCHEMICAL CONSIDERATIONS

The biological localization of any drug molecule is determined by many factors including lipid solubility, molecular size, charge, structure, and stereochemical configuration. During the design and testing of second-generation technetium radiopharmaceuticals it became evident that the stereoisomeric form of the complex was also important for its distribution and localization. Some new
Technetium-essential complexes are peptides. Peptides are short-chain amino acid sequences and these complexes may contain the D- or L-enantiomer of a particular amino acid. Natural amino acids are of the L-form and may undergo enzymatic degradation in vivo. Therefore the corresponding D-enantiomer may be substituted in a peptide to gain in vivo stability. Enantiomers are mirror-image stereoisomers whose three-dimensional configuration cannot be arranged so that one enantiomer can be overlaid upon the other. This occurs because the molecule is chiral and lacks symmetry. A common reason for chirality in organic molecules is the presence of one or more asymmetric carbon atoms. Thus, the D-enantiomer has a configuration that prevents it from interacting with the enzyme that metabolizes the L-enantiomer, resulting in less chance of in vivo degradation.

In some instances, when an organic molecule contains several different functional groups bonded to stereogenic centers, such as asymmetric carbon atoms, it is desirable to describe the spatial configuration of one functional group relative to another. If ligands on the stereogenic centers are on the opposite sides of the plane, the relative configuration is anti (antiperiplanar) and if they are on the same side of the plane, they are syn (synperiplanar) (Figure 7).

Another type of isomerism found in octahedral complexes having three identical groups coordinated to a metal atom is meridional / facial isomerism. The meridional (mer) isomer has the three identical groups bound to the metal in the same plane, whereas the facial (fac) isomer has the three groups occupying the same face (Figure 7). An example of this type of isomerism in technetium chemistry is the technetium tricarbonyl compounds which have three carbonyl groups coordinated in a facial configuration.
Figure 8. Chemical structures of technetium-labeled DADS, CO₂-DADS (syn and anti forms), and ⁹⁹ᵐTc-MAG3. In the syn-⁹⁹ᵐTc-CO₂DADS isomer the carboxyl group is oriented in the same direction as the technetium atom, out of the plane of paper, whereas in the anti-isomer the carboxyl group is oriented into the plane of paper, opposite to the technetium atom. The spatial orientation is due to the asymmetry of the carbon atom that bonds the carboxyl group. This asymmetry is not present with the carbon atom that bonds the carboxyl group in the MAG3 ligand.

The effect of stereochemical configuration of the technetium complex on its in vivo behavior became evident during the development of second-generation complexes. For example, during the development of a technetium complex to replace ¹³¹I-o-iodohippuric acid (¹³¹I-OIH) for renal imaging, the N₂S₂ diamidedithiol ligands, N,N'-bis(mercaptoacetyl)-ethylenediamine (DADS) and N,N'-bis(mercaptoacetyl)-2,3-diaminopropanoate (CO₂-DADS), were investigated (Figure 8). The initial technetium complex (⁹⁹ᵐTc-DADS) had good renal excretion but was inferior to ¹³¹I-OIH. This led to the structural modification of adding a carboxylate group to the ethylene bridge of the DADS ligand to produce ⁹⁹ᵐTc-CO₂DADS.¹⁷,¹⁸ This modification, however, created an asymmetric carbon atom and resulted in two chelate ring stereoisomers. Renal handling was affected by the orientation of the carboxyl group relative to the technetium oxo core, with the syn isomer having better renal excretion than the anti isomer.¹⁸,¹⁹

This result led to changing the core donor ligand from N₂S₂ to N₃S and placement of the carboxyl group on the third amido nitrogen, producing a radiochemically pure product without an asymmetric carbon. The simplest ligand having the necessary groups for renal excretion was MAG3 (Figure 8). Thus, experience has shown that technetium can be coordinated in a stable, substitution-inert complex that can be modified to influence in vivo distribution and localization.

**Technetium-Essential Radiopharmaceuticals**

A number of contributions were made in the 1970's and 80's to the development of technetium compounds in use today. With the ability to structurally characterize technetium compounds, efforts were directed toward examining ligands that could stabilize technetium in lower oxidation states, previously thought to be unstable. Davison, Jones, and colleagues²⁰ at MIT demonstrated that Tc(V) oxo complexes with bisdithiolate (S₄) and diamidedithiolate (N₂S₂) ligands could produce oxidation-
Work with these ligands laid the foundation for introducing ligand backbone substitutions with non-coordinating functional groups that could direct in vivo localization. This permitted the development of a new generation of technetium-labeled radiopharmaceuticals that were technetium-essential. Deutsch and co-workers at the University of Cincinnati contributed significantly to an understanding of technetium’s basic chemistry through their efforts to develop a technetium-labeled myocardial imaging agent. Their design and characterization of CA Tc(III) monocationic complexes with the diars, or o-phenylenedibis(dimethylarsine), ligand, namely $[^{99m}\text{Tc(diars)}_2\text{Cl}_2]^{+}$ and $[^{99m}\text{Tc(diars)}_2\text{Br}_2]^{+}$, developed further the concept of technetium-essential compounds designed around a technetium core. Many other investigators made significant contributions to the development of these second-generation technetium compounds, several of which are used in nuclear medicine today. Important among these technetium-essential compounds are complexes of Tc(I), (e.g., $^{99m}\text{Tc-sestamibi}$) and Tc(V) (e.g., $^{99m}\text{Tc-bicisate}$, $^{99m}\text{Tc-exametazime}$, $^{99m}\text{Tc-mertiatide}$, and $^{99m}\text{Tc-tetrofosmin}$). Through the development of these and other agents a number of technetium cores were identified (Figure 10). Extensive experience was gained regarding the chemistry of bifunctional chelating agents (BFCA), many of which are now being developed further in the design of second-generation technetium-tagged compounds that localize by substrate-specific localization mechanisms.

As a consequence of this developmental history, technetium-labeled compounds are considered to be of two types: technetium-essential and technetium-tagged. Technetium-essential compounds have technetium as a necessary core atom around which other components are arranged. Neither of the separated components (coordinating ligands or technetium) localize the same way that the integrated molecule does. The ligands that coordinate with the core may be monodentate or multidentate and are designed to stabilize technetium in its oxidation state and provide desirable pharmacokinetic properties to the final complex. Technetium’s coordination number may be satisfied by multiple monodentate
ligands, such as the six individual isonitrile ligands in $^{99m}$Tc-sestamibi, one or two multidentate ligands, such as those in $^{99m}$Tc-bicisate and $^{99m}$Tc-tetrofosmin, respectively, or a combination of a multidentate ligand and individual monodentate ligands, such as that found in $^{99m}$Tc-furifosmin. The functional groups on the ligands are chosen to confer certain properties to the final complex, such as lipophilicity, ionic charge, and molecular size. Such modifications alter the pharmacokinetic properties of the technetium complex to enhance its localization and excretion.\(^{29}\) For example, the design of $^{99m}$Tc-mertiatide or $^{99m}$Tc-MAG3 involved, not only careful selection of the N\(_3\)S coordinating ligand, but required the strategic location of a carboxylate substituent in the peptide sequence to give it the necessary renal excretion properties that mimic \(\text{o-iodohippurate (OIH)}\).\(^{26}\) Another example is the rearrangement of two methyl groups on the propyleneamine oxime (PnAO) ligand in $^{99m}$Tc-PnAO in order to increase its brain retention properties, producing the configuration found in $^{99m}$Tc-HMPAO.\(^{25}\) Still, another example is the technetium-based folate chelation peptide $^{99m}$Tc-EC20, which is being investigated for targeting folate receptor-positive ovarian tumors.\(^{30}\)

![Figure 10. Technetium cores and the general structures of technetium complexes with ligands. L = neutral donor ligand.](image)

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<thead>
<tr>
<th>Core</th>
<th>General Structure</th>
<th>Ligands</th>
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<tr>
<td>$^{1+}$, $^{3+}$, $^{4+}$</td>
<td>$\text{Tc}^{1+}$, $\text{Tc}^{3+}$, $\text{Tc}^{4+}$</td>
<td>Neutral donor ligand</td>
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<td>$\text{Tc}^{1+}$ (L)</td>
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TECHNETIUM-TAGGED RADIOPHARMACEUTICALS

Technetium-tagged compounds have technetium bound to a localizing moiety (transporter) that routes technetium to a specific site in the body determined by the properties of the transporter. First-generation technetium-tagged compounds have transporters that are relatively simple. For example, they include complexing agents (e.g. DTPA), particles (e.g. sulfur colloid), blood cellular elements (e.g. leukocytes), and proteins (e.g. human serum albumin). Second-generation technetium-tagged compounds have receptor-specific transporters (targeting molecules), such as peptides and antibodies, that are covalently linked to technetium via a BFCA. The design and labeling methods of these compounds are more sophisticated than first-generation technetium-tagged radiopharmaceuticals. Two approaches have been used to design second-generation technetium-tagged radiopharmaceuticals: the integrated approach and the bifunctional chelate approach.31-34

The integrated approach incorporates technetium into a binding site built into the molecule so that technetium becomes an integral part of the molecule, affecting its conformation and localization in vivo. This approach has been applied, for example, to the design of radiotracers that mimic the 3-dimensional configuration of biologically important molecules such as steroids (testosterone, progesterone and estradiol) with limited success, but may prove more successful at a future time.31,34

A more widely explored direction is the bifunctional chelate approach where the key component in the design of technetium-tagged radiopharmaceuticals is a BFCA. The extensive experience gained from the development of technetium-essential radiopharmaceuticals with BFCAs, particularly with the tripeptide MAG3, made the BFCA approach to peptide labeling a natural extension of that work. Furthermore, it instilled the idea of incorporating a technetium-binding amino acid sequence into an active peptide biomolecule. The peptide sequence allows the introduction of coordinating donor groups to facilitate the formation of a stable complex with a technetium-oxo core.

Technetium-tagged compounds have the following general components: Targeting Molecule – Linker – BFCA – Tc-99m.32 The targeting molecule is typically a peptide, antibody, or some other small molecule designed to target a specific receptor in vivo. The linker is usually a simple hydrocarbon chain of variable length for modifying pharmacokinetics or to distance the technetium chelate region from the receptor-binding region of the molecule. The BFCA serves two main purposes: (1) to coordinate technetium; and (2) to provide a molecular backbone that can be modified with functional groups for attachment to the targeting molecule. Some examples of BFCAs are the N₂S₂
ligands diaminedithiol, diamidedithiol, and monoaminemonoamide dithiol; triamidethiol (N₃S); and
tetramine (N₄), which form five-coordinate square pyramidal technetium complexes; and hydrazine
nicotinamide (HYNIC). The functional group on the BFCA is the conjugation site where it covalently
attaches to the targeting molecule, either directly or through the linker molecule. With this design the
technetium chelate is often far removed from the receptor binding motif to minimize possible
interference with binding at the biological receptor site. In most instances, technetium is a pendant or
“passenger” nuclide to be transported to the receptor site. However, in some complexes, such as with
small peptides, the biodistribution and target uptake will be influenced by the metal chelate because the
technetium atom may contribute greatly to the overall size and molecular weight of the
radiopharmaceutical. In such cases the technetium is not entirely passive and such radiopharmaceuticals
could also be considered to be technetium-essential.

The principal targeting molecules employed as transporters in technetium-tagged radiopharmaceuticals
are antibodies and peptides. They differ primarily in molecular weight and structure. Antibodies are
analogous to large and small proteins in size. Whole antibodies have molecular weights on the order of
150 kDa and antibody fragments about 50 to 100 kDa. By contrast, peptides usually contain less than
100 amino acids and have molecular weights of about 10 kDa or less. A small peptide is considered to
consist of less than 30 amino acids or a molecular weight less than 3.5 kDa. While antibodies exhibit
high receptor binding affinity and specificity, their limited effectiveness has been attributed to their
inaccessibility to tumor cells in solid masses and to the heterogeneous distribution of tumor-associated
antigens on the tumor surface. By contrast, the affinities of many peptides for their receptors are
significantly greater than that of antibodies or their fragments. Also, they can tolerate harsher chemical
conditions for modification or radiolabeling. Peptides are relatively easy to synthesize, exhibit rapid
blood clearance, and are less likely to be immunogenic. In most cases, the receptors for peptides are
readily accessible on the external surface of cell membranes. One disadvantage of peptides is that they
are prone to enzymatic degradation by plasma proteases and peptidases. Therefore, to mitigate this
problem, structural modifications may be required. Modification has been accomplished by use of a D-
amino acid in place of the L-form and use of alternative amino acids. Another confounding problem is
the potential loss of receptor-binding affinity when the peptide is conjugated to the BFCA and labeled
with a radionuclide. Small peptides with only four to six amino acid residues are particularly
vulnerable in this regard.
RADIOLABELING APPROACHES FOR BIFUNCTIONAL CHELATES

Conjugation of the peptide, protein, or antibody targeting molecule (TM) with the BFCA often occurs through a reaction between a primary amino group on the TM and an activated ester group or an isothiocyanate group on the BFCA, or between a sulfhydryl group on the TM and a maleimide group on the BFCA (Figure 11).\(^{32}\) Conjugation can occur either after coordination with technetium (prelabeling approach) or before coordination with technetium (postlabeling approach) (Figure 12). The prelabeling approach involves technetium chelation with the BFCA, activation of the BFCA, and conjugation with the TM. With this approach, the \(^{99m}\text{Tc}\)-chelate is formed before conjugation with the TM. The advantage of the prelabeling approach is that the TM is not subjected to the sometimes harsh labeling conditions (e.g., low pH, high temperature) necessary for coordination of technetium with the BFCA. The disadvantage of this approach is that it is not particularly amenable to simple kit formulation. The postlabeling approach involves activation of the BFCA, conjugation with the TM, and chelation with technetium. Advantages of this approach is that it permits a carefully worked out chemistry for conjugation of the TM with the BFCA and it has particular appeal for kit formulation if the chelation reaction conditions with technetium are not detrimental to the TM. Radiolabeling can be accomplished with either approach by direct reduction of pertechnetate in the presence of the BFCA-TM conjugate or via ligand exchange with a technetium donor complex such as \(^{99m}\text{Tc}\)-glucoheptonate. The postlabeling approach is used with the preparation of \(^{99m}\text{Tc}\)-Apicitide (AcuTect) and \(^{99m}\text{Tc}\)-Depreotide (NeoTect).

A similar labeling approach is used for some non-technetium radiopharmaceuticals labeled with radioactive metals. For example, capromab pendetide (ProstaScint) labeled with \(^{111}\text{In}\) is an antibody covalently conjugated with a short peptide-DTPA linker (glycyl-tyrosyl-lysyl-DTPA or GYK-DTPA), to complex indium by the postlabeling approach. Similarly, ibritumomab tiuxetan (Zevalin) is an antibody conjugated with tiuxetan, an isothiocyanatobenzyl-derivatized DTPA linker, bound covalently by a thiourea bond to the antibody to form a site for chelation with indium or yttrium by the postlabeling approach.
Technetium Compounds of Specific Oxidation States

Technetium compounds have a core where the technetium atom exists in a specific oxidation state determined by the number of electrons in its d orbitals. In some cores the technetium atom exists alone ("naked" technetium atom) while in other cores it is associated with another functional group such as

Figure 11. Typical methods of conjugating bifunctional chelating agents (BFCAs) with targeting molecules.

Figure 12. Prelabeling and postlabeling approaches for coordinating technetium to a targeting molecule (peptide). See text for details.

Technetium Compounds of Specific Oxidation States

Technetium compounds have a core where the technetium atom exists in a specific oxidation state determined by the number of electrons in its d orbitals. In some cores the technetium atom exists alone ("naked" technetium atom) while in other cores it is associated with another functional group such as
oxygen or nitrogen. The different oxidation states of technetium are stabilized in these cores by a variety of coordinating ligands.\textsuperscript{21} Technetium cores found in a number of diagnostic radiopharmaceuticals are shown in Figure 10. Cores associated with other technetium complexes have also been identified.\textsuperscript{29,32}

**TC(I) COMPOUNDS**

Reduction of Tc(VII), as pertechnetate, to Tc(I) creates a technetium atom with six additional electrons in a d\textsuperscript{6} configuration that must be stabilized by pi-acceptor ligands.\textsuperscript{29} Some of the coordinating groups that will stabilize Tc(I) are the phosphines (P), diphosphines (P-P), isonitrides (CNR), and carbonyl (CO). Technetium is stabilized by these ligands because they have pi-bonding orbitals devoid of electrons that can be filled by the d-orbital electrons from the Tc(I) atom. The cores most frequently explored are the naked Tc\textsuperscript{+} core and the Tc(CO)\textsubscript{3}\textsuperscript{+} tricarbonyl core. Tc(I) complexes are very stable when six coordination sites are occupied, forming an octahedral complex. This is readily accomplished under no-carrier-added labeling conditions in radiopharmaceutical kits because the excess ligand available forces complete coordination around the metal to maintain reducing conditions. Additionally, the ligands in Tc(I) complexes can be functionalized with groups that alter chemical and biological properties, such as lipophilicity, without affecting complex stability.

**Tc\textsuperscript{+} Core**

\textit{\textsuperscript{99m}Tc-Sestamibi}

A prime example of a radiopharmaceutical with this core is the lipophilic heart imaging agent \textsuperscript{99m}Tc-sestamibi, where the Tc(I) atom is coordinated by six monodentate 2-methoxy-isobutyl isonitrile (MIBI) ligands, forming a stable octahedral complex (Figure 13). Since the MIBI ligands are neutral the sestamibi complex retains the single positive charge of the Tc\textsuperscript{+} core. In the Cardiolite kit, the MIBI ligands are complexed into a copper/boron fluoride complex to facilitate lyophilization since MIBI alone is a volatile liquid.\textsuperscript{13} Cysteine and stannous chloride are reducing agents.\textsuperscript{35} Citrate forms complexes with Tc(V) and Tc(IV) and with Sn(II) and Sn(IV). Tin citrate complexes can increase the reducing power of the mixture because Sn(IV)-citrate is a much more stable complex than Sn(II)-citrate.\textsuperscript{7} Mannitol is a bulking agent in lyophilized samples but can also form a weak complex with reduced technetium.\textsuperscript{7} In the heating step of the radiolabeling process, the copper-MIBI complex is broken releasing the MIBI ligands. The MIBI ligands displace citrate from the preformed \textsuperscript{99m}Tc-citrate intermediate to form \textsuperscript{99m}Tc-sestamibi.
After intravenous injection, the lipophilic $^{99m}$Tc-sestamibi complex is taken up into the heart by passive diffusion in proportion to myocardial blood flow. Although $^{99m}$Tc-sestamibi is a cation it is not extracted by the Na-K ATPase membrane pump. However, uptake is associated with intact myocyte sarcolemmal and mitochondrial membrane potentials. It is bound in the heart muscle in association with myocyte mitochondria. Heart retention is long having a biologic half-life of 6 hours. $^{99m}$Tc-sestamibi is used to assess myocardial perfusion in ischemia and infarction.

**Tc(CO)$_3$**$^+$ Core

A variety of technetium complexes can be made starting with the tricarbonyl core. This core can be coordinated with a bifunctional chelating agent having residual functional groups to couple technetium to receptor-avid molecules. At this time no technetium radiopharmaceuticals have been approved by the FDA for routine use with this core but it offers a unique method of creating target-specific radiotracers.

Radiopharmaceuticals with the Tc(CO)$_3$$^+$ core form stable octahedral organometallic complexes of two subtypes: (1) $^{fac}$-Tc(CO)$_3$$^+$ in which Tc$^+$ can accommodate a variety of ligands besides the three carbonyls, to complete the octahedral sphere, and (2) CpTc(CO)$_3$$^+$, where, in addition to the three carbonyls, Tc$^+$ is coordinated to a functionalized cyclopentadiene ligand that can attach the complex to a targeting molecule. A novel synthon of $^{fac}$-Tc(CO)$_3$$^+$ is the water and air stable organometallic aqua complex $^{[\text{Tc(H}_2\text{O)}_3\text{(CO)}_3]^+}$ (Figure 13). (Note: a synthon is a molecular unit designed to facilitate the synthesis of a desired complex). The $^{[\text{Tc(H}_2\text{O)}_3\text{(CO)}_3]^+}$ synthon can be produced directly from pertechnetate by reduction with sodium borohydride in saline at pH 11 under 1 atm of CO at 75°C. It has also been prepared from a lyophilized kit, containing the reducing agent sodium boranocarbonate, Na$_2$[H$_3$BCO$_2$], to which is added sodium pertechnetate followed by heating at 98 °C for 20 minutes. The aqua complex is stable from pH 1 to pH 13. The labile water ligands can be readily substituted with donor ligands provided by a BFCA, which can be derivatized to attach the complex to an appropriate targeting molecule. The synthon $^{[\text{Tc-Cl(H}_2\text{O)}_2\text{(CO)}_3]^+}$ has been used to prepare a neutral lipophilic complex, $^{99m}$Tc- TROTEC-1 (Figure 13), wherein two water ligands are displaced by the sulfurs in a dithioether-derivatized tropane analogue. The complex is neutral due to
the chloride ion. $^{99m}$Tc-TROTEC-1 has been shown to have high affinity for the dopamine transporter (DAT) in the brain, however brain uptake is low.

![Chemical structures of various Tc(I) coordination compounds.](image)

The CpTc(CO)$_3^{+}$ core is interesting because it is highly stable, lipophilic, and can be readily derivatized for conjugation to bioactive molecules. This core has been used to prepare diagnostic technetium or therapeutic rhenium compounds. The method was originally designed to accomplish the reduction, carbonylation, and cyclopentadienylation of pertechnetate in a relatively mild, one-pot reaction termed a “double ligand transfer” reaction because two ligands (Cp and CO) are transferred together from two different metals (Fe and Mn) to a third metal, Tc.\textsuperscript{42, 43} For example, this method was applied to produce a Cp$^{99m}$Tc(CO)$_3$-octreotide conjugate (Figure 13) which demonstrated receptor-mediated uptake in the adrenal glands and pancreas.\textsuperscript{44}

**TC(III) AND TC(IV) COMPOUNDS**

The “naked” technetium atom with intermediate oxidation states, Tc(III) (Tc$^{3+}$ core) and Tc(IV) (Tc$^{4+}$ core), typically are six-coordinate but may have coordination numbers of five, six, or seven. Additionally, complexes of intermediate oxidation states may undergo redox reactions exhibiting different oxidation states with a coordinating ligand.\textsuperscript{29} Evidence for this has been demonstrated in
technetium complexes with DTPA, citrate, and other ligands.\textsuperscript{7} Mixed ligands are sometimes necessary to stabilize an oxidation state as exemplified by the boronic acid adducts of technetium oxime (BATO compounds).

**Tc\textsuperscript{3+} Core**

Reduction of Tc(VII) as pertechnetate to Tc(III) creates a technetium atom with four additional electrons (d\textsuperscript{4} configuration). The Tc(III) state is sometimes reached in multiple reduction steps, first to Tc(V) and then to Tc(III).\textsuperscript{29} Several technetium compounds with a Tc\textsuperscript{3+} core have been developed for use in nuclear medicine. These include the 99m\textsuperscript{Tc}-iminodiacetic acid (IDA) analogs, 99m\textsuperscript{Tc}-succimer, 99m\textsuperscript{Tc}-teboroxime, and 99m\textsuperscript{Tc}-furifosmin.

**99m\textsuperscript{Tc}-IDA Analogs**

One of the first Tc(III) compounds to achieve clinical usefulness in nuclear medicine is 99m\textsuperscript{Tc}-lidofenin (99m\textsuperscript{Tc}-HIDA). Although 99m\textsuperscript{Tc}-lidofenin is no longer available, the analogs, 99m\textsuperscript{Tc}-disofenin and 99m\textsuperscript{Tc}-mebrofenin (Figure 6), are currently used for hepatobiliary imaging. They are more effectively extracted by hepatocytes at high plasma bilirubin compared to 99m\textsuperscript{Tc}-lidofenin.\textsuperscript{45} These hexa-coordinate complexes have the general form $[\text{Tc-(IDA)}\text{$_{2}$}]^-$ with the Tc(III) atom stabilized by two nitrogens and four oxygens from two IDA ligands. The four negatively charged oxygens neutralize the 3+ charge on the Tc(III) atom to give the complex a net charge of 1-. The complex is kinetically inert. Ligand exchange experiments between 99m\textsuperscript{Tc}-HIDA and ethylenediaminetetraacetic acid (EDTA) have shown that 99m\textsuperscript{Tc}-EDTA does form in such incubation mixtures, but the rate of technetium release from HIDA is pH dependent and is extremely slow at physiologic pH. Thus, although 99m\textsuperscript{Tc}-HIDA is not as stable as 99m\textsuperscript{Tc}-EDTA thermodynamically, it is kinetically inert in vivo.\textsuperscript{15} The stability in vivo is supported by a study where re-injection of urinary and gall bladder contents from dogs previously injected with 99m\textsuperscript{Tc}-HIDA showed an excretory pattern similar to the original compound.\textsuperscript{46} 99m\textsuperscript{Tc}-disofenin and 99m\textsuperscript{Tc}-mebrofenin are expected to have similar stability in vivo.

After intravenous injection, 99m\textsuperscript{Tc}-mebrofenin and 99m\textsuperscript{Tc}-disofenin are rapidly extracted from blood into bile by active transport via the anionic site on the hepatocyte membrane, which is the same site for transport of bilirubin. These complexes are used to assess hepatobiliary function in acute and chronic cholecystitis.
Ikeda et al found that technetium can form four different complexes with 2,3-dimercaptosuccinic acid (DMSA or Succimer). The formation of these complexes depends on pH, the concentration of pertechnetate, and the Sn(II) / Sn(IV) ratio. Complexes I and II form at low pH and complexes III and IV at high pH. Spectrophotometric measurement and stoichiometric titration studies of acidic pertechnetate-DMSA solution with stannous chloride demonstrated that Complex I was formed spontaneously and was determined to be Tc(IV)-DMSA. Biologic localization studies revealed that Complex I localized in bone and was excreted in urine with little kidney retention. Further studies demonstrated that Complex I was converted to Complex II in the presence of excess stannous ion and that complex II localized primarily in the kidney. It was deduced from these studies that Complex II was Tc(III)-DMSA, and was formed as a reduction product from Tc(IV)-DMSA in the presence of excess stannous ion. Complexes III and IV are formed by titration of Complexes I and II, respectively, to an alkaline pH. Complex III, similar to complex I localizes primarily in bone and Complex IV localizes in kidney. Primarily, complexes I and II should be present in the DMSA radiopharmaceutical kit because of the low pH. The maximum yield of Complex II was found to be dependent on the pH, oxygen concentration of the reaction mixture and incubation time. Kidney localization of complexes prepared at one pH is not appreciably altered if the pH is later changed. The lyophilized kit currently on the market can be labeled with up to 6 mL pertechnetate, is stable for 4 hours following preparation, and has a final pH between 2 and 3. The labeling reaction of $^{99m}$Tc-DMSA proceeds in two steps: rapid formation of Complex I followed by a slower, rate-determining step from Complex I to Complex II, the latter being greatly affected by oxygen. This is the reason for a 10-min incubation period. Once Complex II is formed it may revert back to Complex I by oxidation. This is promoted by the oxidation of Sn(II) to Sn(IV) which lowers the reduction potential of the system. Diminished kidney uptake will occur because Complex I is readily excreted. The inclusion of ascorbic acid in present-day kits retards this oxidation.

$^{99m}$Tc-succimer is a dimeric complex of Tc(III)(DMSA)$_2$ having the proposed structure shown in Figure 14. After intravenous injection, the complex accumulates slowly in the renal cortex, where it becomes fixed, primarily in the cells of the proximal convoluted tubule. It is indicated for kidney imaging for the evaluation of renal parenchymal disorders.
**99mTc-Teboroxime**

99mTc-teboroxime, [bis[1,2-cyclohexanedione dioximato(1-)-O]-[1,2-cyclohexanedione-ioximato(2-)O]methylborato(-)-N,N',N'',N''',N''''',N''''']-chlorotechnetium, is a neutral lipophilic complex for myocardial perfusion imaging of the general class of compounds known as boronic acid adducts of technetium dioxime (BATOs). This complex is prepared by the general method of template synthesis wherein the technetium metal ion serves as a template to organize the course of complex multi-step reactions. 99mTc-teboroxime is unique from other technetium complexes in that the ligand is not present in the reaction vial before addition of pertechnetate, but is formed around technetium as the template atom. The essential reactants in the kit are cyclohexanedione dioxime, chloride as the axial ligand, methyl boronic acid, and stannous chloride. After heating (15 min at 100°C) the final complex contains a heptacoordinate Tc(III) atom bound to a chlorine atom and to the six nitrogens of the three dioximes (Figure 14). One end of the molecule is capped by a boron atom covalently bound to one oxygen atom from each of the dioximes. The BATO complexes are of interest because they illustrate technetium template synthesis chemistry and the use of multiple ligands to stabilize technetium in a particular oxidation state.

![Chemical structures](image)

**Figure 14.** Chemical structures of various Tc(III) coordination compounds.

After intravenous injection, 99mTc-teboroxime exhibits very high first-pass extraction by the myocardium and rapid back-diffusion into the vascular space. The rapid in vivo myocardial kinetics made it difficult to image the heart following injection, which led to its eventual removal from the market.

**99mTc-Furifosmin**

99mTc-furifosmin (or Q-12) is a phosphine compound for myocardial perfusion imaging with a Tc(III) core coordinated by a bidentate SWL ligand via N and O atoms and two monodentate TMPP ligands,
The SWL ligand is 1,2-bis[dihydro-2,2,5,5-tetramethyl-3(2H)-furanone-4-methyleneamino]ethane, a Schiff base, and the TMPP ligand is tris(3-methoxy-1-propyl)phosphine. The overall charge on the technetium complex is $1^+$. A one-step kit preparation has been developed (TechneScan Q-12, Mallinckrodt Medical) wherein 2 to 3 mL of pertechnetate is added to the kit and heated on a boiling water bath for 15 minutes. Radiochemical purity is assessed by loading the product onto an ethanol-wetted SepPak Alumina A cartridge eluted first with saline and then ethanol to recover the $^{99m}$Tc-furifosmin. The labeled product is stable for 6 hours.

After intravenous injection, $^{99m}$Tc-furifosmin is extracted by the myocardium in proportion to regional blood flow for the evaluation of myocardial ischemia. The furifosmin kit has been used in Europe but is not available in the United States.

**Tc$^{4+}$ Core**

Reduction of Tc(VII) to Tc(IV) creates a technetium atom with three additional electrons ($d^3$ configuration). Several first-generation technetium compounds have technetium in the 4+ oxidation state. Tc(IV) radiopharmaceuticals prepared from kits are formed by reduction of pertechnetate with stannous chloride in the presence of a coordinating ligand. These include technetium complexes with pentetate (DTPA), the phosphonates (MDP, HDP), and pyrophosphate (PPi). It should be noted that although these agents are included under the Tc$^{4+}$ oxidation state, studies have not demonstrated this conclusively in every case. The same can be said for several of the technetium agents discussed under the Tc$^{3+}$ oxidation state. In this group of Tc$^{4+}$ agents, only the insoluble hydrolyzed TcO$_2$ is known to be in the Tc(IV) state. TcO$_2$ is not useful in nuclear medicine, per se, but is problematic because it acts as a thermodynamic trap of reduced technetium in radiopharmaceutical kits when it forms as a radiochemical impurity.

**$^{99m}$Tc-Pentetate**

One of the first radiopharmaceutical kits developed with stannous chloride as reducing agent was for the preparation of $^{99m}$Tc-diethylenetriamine pentaacetic acid (DTPA). Technetium’s oxidation state in the DTPA complex prepared in kits was initially believed to be Tc(IV). Other investigations have suggested a mixture of Tc(III) and Tc(IV) in $^{99m}$Tc-DTPA depending on the reaction conditions. When mixtures of pertechnetate and 0.4 M DTPA are titrated with stannous chloride at pH 4 and millimolar amounts of $^{99m}$Tc-pertechnetate in excess of Sn(II), the electron transfer number is 3.5, indicating that an equimolar mixture of $^{99m}$Tc(III)-DTPA and $^{99m}$Tc(IV)-DTPA are formed. However,
when the complex is formed with Sn(II) in excess of pertechnetate, at a 6:1 molar ratio of stannous chloride-to-pertechnetate, the electron transfer number is 4, indicating that $^{99m}$Tc(III)-DTPA is formed. Reduction of technetium is proposed to occur in a two-step rapid complementary reaction, first from Tc(VII) to Tc(V) and then from Tc(V) to Tc(III). Since the preparation of $^{99m}$Tc-DTPA in radiopharmaceutical kits occurs with a Sn(II) molar excess over nanomolar amounts of pertechnetate, a deductive conclusion is that primarily $^{99m}$Tc(III)-DTPA is formed as the radiopharmaceutical product. The experimental data, however, do not substantiate one particular oxidation state of technetium in $^{99m}$Tc-DTPA.

The chemical structure of this polyamine carboxylate complex has not been characterized, however a proposed structure for $^{99m}$Tc(IV)-DTPA is shown in Figure 15. $^{99m}$Tc-DTPA is likely a six-coordinate complex with three nitrogen and three oxygen donor atoms for coordination.

Following addition of sodium pertechnetate to a DTPA radiopharmaceutical kit, reduction and complexation occurs within one to fifteen minutes depending on the kit manufacturer.

After intravenous injection, $^{99m}$Tc-DTPA is removed rapidly from the bloodstream by glomerular filtration and is excreted unchanged into the urine. In nuclear medicine, $^{99m}$Tc-DTPA is used to assess kidney function in a variety of conditions and to measure the glomerular filtration rate.

$^{99m}$Tc-Diphosphonate and Pyrophosphate

Various titration experiments have been conducted to determine the oxidation state of technetium in bone complexes with pyrophosphate (PPi), etidronate (EDHP), and methylene disphosphonate (MDP) ligands. Russell and Cash found through titration studies with millimolar $^{99}$Tc-pertechnetate and pyrophosphate, etidronate, and medronate ligands that the oxidation state of technetium varied with

Figure 15. Chemical structures of Tc(IV) coordination compounds.
ligand type and reaction pH. With each of these ligands below pH 6, Tc(VII) was reduced to Tc(III), which reoxidized to Tc(IV). Between pH 6 and 10 with MDP and PPI, Tc(VII) is reduced in two steps to Tc(IV) first and then to Tc(III) with reoxidation of Tc(III) back to Tc(IV) and Tc(VII); with EHDP, Tc(VII) is reduced stepwise to Tc(V) and Tc(III) and reoxidized to Tc(IV) and Tc(VII). Thus, it appears from these studies that the dominant reduced species of technetium in radiopharmaceutical bone kits is likely to be Tc(IV).

The chemical structure of $^{99m}$Tc-MDP has been characterized by Deutsch as being a 1:1 polymer of technetium and MDP, and more likely a polymeric mixture, with the smallest complex being a dimer of one reduced technetium atom and two MDP ligands (Figure 15) in order to satisfy the coordination requirements of reduced technetium.

The technetium phosphonate complexes are readily formed by adding $^{99m}$Tc-sodium pertechnetate to the kit. The complexation reaction occurs within a few minutes at room temperature. After intravenous injection, $^{99m}$Tc-MDP or $^{99m}$Tc-HDP are localized in bone by chemisorption to calcium, with greater amounts bound to amorphous calcium phosphate than hydroxyapatite.

**TC(V) COMPOUNDS**

Somewhat opposite the Tc(I) oxidation state is Tc(V), d$^2$ configuration, which is two electrons reduced from Tc(VII) in pertechnetate. As such, Tc(V) has a high electron deficiency (5-) and requires good electron-donating ligands to confer stability to its complexes. Typical donor atoms coordinating with Tc(V) are N, O, S, and P. Two common cores present in Tc(V) radiopharmaceuticals are Tc=O$^{3+}$ and O=Tc=O$^+$. The net charge on the core is determined by balancing the total charge of the oxo groups with that of technetium. For example, the 3+ charge on the Tc=O$^{3+}$ core results from the sum of 5+ on technetium and 2- on oxygen. Likewise, the net charge on a technetium complex is a balance of the total charge of the ligating groups and that of the technetium core.

**Tc=O$^{3+}$ Core**

Technetium compounds containing this core are five-coordinate forming square pyramidal complexes (Figure 9). Important ligands that have produced stable in vivo complexes with the Tc=O$^{3+}$ core are propylene amine oxime (PnAO) and its hexamethyl-functionalized derivative HMPAO, the diaminedithiol N$_2$S$_2$ ligand [N,N′-1,2-ethenediylbis-L-cysteine diethylester, otherwise know as ethylcysteinate dimer [ECD] found in $^{99m}$Tc-bicisate, and the triamidethiol (N$_3$S) ligand (N-
(mercaptoacetyl)glycylglycylgalycline) found in $^{99m}$Tc-mertiatiade ($^{99m}$Tc-MAG3). These types of ligands are rich in the electron-donor atoms N, O, and S. The $\text{N}_2\text{S}_2$ and $\text{N}_3\text{S}$ ligands form very stable technetium complexes and can be fitted with functional groups to alter biodistribution. The PnAO ligand is more difficult to functionalize and only the lipophilicity of the complex has been varied.\textsuperscript{29}

$^{99m}$Tc-Exametazime

$^{99m}$Tc-PnAO was developed as a potential brain imaging agent. It proved to be stable in aqueous solution and is neutral and lipophilic (Figure 16).\textsuperscript{62}

![Figure 16. Chemical structures of brain imaging ligands and their technetium coordination compounds: $^{99m}$Tc-PnAO, $^{99m}$Tc-HMPAO, $^{99m}$Tc-ECD.](image)

An advantage of the PnAO type of BFCAs is that radiolabeling can be performed at ambient temperature and this ligand has been used to develop several radiopharmaceuticals. Some interesting findings surfaced in the development of the Tc(V) complexes related to stereoreactivity. While the $^{99m}$Tc-PnAO complex demonstrated rapid brain uptake following intravenous administration, its rapid washout precluded its use for SPECT imaging.\textsuperscript{62} Consequently, several derivatives of PnAO were synthesized with methyl groups on the amineoxime backbone with hopes of finding an agent that remained bound in the brain. One of these was the hexamethyl derivative, $^{99m}$Tc-
hexamethylpropyleneamine oxime (\(^{99m}\text{Tc}\)-exametazime or \(^{99m}\text{Tc}\)-HMPAO), which exists in two diastereomeric forms, \(\text{D,}\text{L}\-) and \(\text{meso}\-) (Figure 17).\(^{63}\)

Diastereomers are stereoisomers that are not mirror images as enantiomers, and have completely different properties. The \(^{99m}\text{Tc}\)-HMPAO complex ends up neutral because when the nitrogen groups coordinate with technetium, the two amine nitrogens and one oxime nitrogen ionize by loss of hydrogen ions. An intermolecular hydrogen bond forms with the oxygens and the three negative charges on the nitrogens cancel the three positive charges on the technetium-oxo group. Because \(^{99m}\text{Tc}\)-HMPAO has two chiral carbons it can form up to four stereoisomers, however only three forms exist since the \(\text{meso}\) isomers are identical and the \(\text{D,}\text{L}\) isomers are enantiomers. The commercial kit contains the \(\text{D,}\text{L}\-) racemate. In the \(^{99m}\text{Tc}\)-HMPAO complex, each enantiomer has one of the methyl groups \(\text{syn}\-) and the other methyl group \(\text{anti}\-) to the \(\text{Tc}=\text{O}\) moiety. Studies with this complex demonstrated that it was neutral and lipophilic, but unstable in aqueous solution. The instability was found to be a conversion from the primary lipophilic complex to a secondary hydrophilic complex, which was mediated by reducing agents.\(^{64,65}\) Studies in animals and humans demonstrated that the \(\text{meso}\) form had greater in vitro stability but little brain retention, while the \(\text{D,}\text{L}\) form had poor in vitro stability but high brain retention. It was then surmised that brain uptake was due to the lipophilic complex and brain retention was due to its intracellular conversion to the non-diffusible hydrophilic complex. The brain conversion was shown to be due to the intracellular reducing agent glutathione, with much faster conversion of the \(\text{D,}\text{L}\) form than the \(\text{meso}\) form.\(^{64}\) The slow conversion of the \(\text{meso}\) form was believed responsible for its low brain retention which necessitated its separation from the \(\text{D,}\text{L}\) form prior to labeling with technetium. In other studies, Neirinckx et al.\(^{63}\) demonstrated that the \(^{14}\text{C}\)-labeled \(\text{D,}\text{L}\-)HMPAO isomer without technetium did not cross the blood-brain barrier, contrary to the identical compound labeled with technetium. Thus, the technetium complex with \(\text{D,}\text{L}\-)HMPAO is considered to be a technetium-essential radiopharmaceutical (Figure 16).
Instability of the lipophilic $^{99m}$Tc-HMPAO complex in vitro is mediated by reducing agent, requiring small amounts of stannous ion in the kit. This limits the amount of $^{99m}$Tc-sodium pertechnetate that can be added to the kit. Shelf-life of the reconstituted kit without stabilizer is 30 minutes. The shelf-life was extended to 4 hours when the U.S. kit was modified by incorporating a stabilizing buffer and an antioxidant/radical scavenger (methylene blue). European kits contain cobaltous chloride ($\text{CoCl}_2$) as the stabilizer.

$^{99m}$Tc-Bicisate

Two N$_2$S$_2$ ligands, diaminedithiol, found in $^{99m}$Tc-ECD, and diamidedithiol, found in $^{99m}$Tc-MAG3, produce very stable complexes with the Tc=O$_{3+}$ core. In the radiolabeling of $^{99m}$Tc-ECD, the diaminedithiol ligand loses three ionizable hydrogens from one nitrogen and two sulfur atoms during complexation with Tc=O$_{3+}$. The resulting three negative charges on these donor atoms neutralize the three positive charges on the core to yield a neutral complex, which is lipophilic and stable in aqueous solution (Figure 16).

The ECD ligand exists as the L,L and D,D isomers; both isomers demonstrate brain uptake but only the L,L isomer exhibits brain retention. Brain retention is not only stereospecific but species specific; $^{99m}$Tc-ECD localizes only in the brains of primates (monkeys and humans). While the carbon backbone of the ligand system is quite stable, substitution on this backbone with two ester functionalities make it labile to enzymatic hydrolysis. After intravenous injection, $^{99m}$Tc-ECD localizes in the brain by passive diffusion of the unionized, lipid-soluble complex. Slow hydrolysis in blood and rapid hydrolysis in brain tissue to the more hydrophilic metabolite results in high brain uptake and retention.

Radiolabeling requires adding 2 mL (100 mCi) of $^{99m}$Tc-sodium pertechnetate to a phosphate buffer for pH adjustment. Using less than 50 mCi may cause incomplete labeling. The bicisate kit is reconstituted with 3 mL of 0.9% sodium chloride injection. Within 30 seconds 1 mL of this solution is admixed with the pertechnetate/buffer solution and incubated for 30 minutes to achieve labeling. The labeled product is stable for 6 hours at room temperature.
$^{99m}$Tc-Mertiatide

The development of $^{99m}$Tc-MAG3, as a $^{99m}$Tc replacement for $^{131}$I-orthiodihippurate ($^{131}$I-OIH), followed a long and patient course (see previous discussion under Stereochemical Considerations section). It required changing the core donor ligand from $N_2S_2$ to $N_3S$ and addition of a carboxyl group to produce a radiochemically pure product which had renal clearance properties similar to $^{131}$I-OIH.\textsuperscript{68,69} The kit formulation for preparing $^{99m}$Tc-MAG3 contains an S-benzoyl mercaptoacetyltrimegic acid coordinating ligand (betiatide), stannous chloride as the reducing agent, and sodium tartrate as transfer ligand.\textsuperscript{70} The reactive thiol (SH) in betiatide is protected with a benzoyl group. Addition of $^{99m}$Tc-sodium pertechnetate and heating releases the protective group and Tc=O$^{3+}$ transfers from tartrate to mercaptoacetyltrimegic acid in quantitative yield to form $^{99m}$Tc-MAG3 (Figure 18).

The $^{99m}$Tc-MAG3 complex acquires a single negative charge by neutralization of the 3+ charge in the Tc=O$^{3+}$ core with four negative charges created on the coordinating donor atoms; three on nitrogen atoms through loss of hydrogen ions, and one on the sulfur atom by release from its protective group. Factors that may cause radiochemical impurities during $^{99m}$Tc-MAG3 preparation are, using more than 100 mCi (3700 MBq) and less than 4 mL to reconstitute the kit, waiting longer than 5 minutes to place the vial into the boiling water bath, and not adding air to the reaction vial during radiolabeling.\textsuperscript{70} The main impurities are pertechnetate, $^{99m}$Tc-tartrate, and reduced hydrolyzed technetium. Air is required...
to oxidize excess Sn(II) which could possibly reduce Tc(V) to Tc(IV).\textsuperscript{13} After intravenous injection, \textsuperscript{99m}Tc-MAG3 localizes in the kidney primarily by tubular secretion whereupon it is excreted unchanged into the urine.\textsuperscript{99m}Tc-MAG3 is used for the assessment of renal function.

\textbf{\textsuperscript{99m}Tc(V)-Succimer}

When technetium pertechnetate is reduced by dithionite in the presence of DMSA at alkaline pH, a stable complex of \textsuperscript{99m}Tc(V)-DMSA is formed having the formula [\textsuperscript{99m}TcO(DMSA)\textsubscript{2}]\textsuperscript{-}.\textsuperscript{71} The complex contains the Tc=O\textsuperscript{3+} core coordinated by four thiol groups of two DMSA ligands. Three geometric isomers of this complex have been characterized (Figure 19).\textsuperscript{72} The isomers shown have a 1- charge, however, the four carboxylate groups are nearly ionized giving the complex a 5- charge at physiologic pH. A method for preparing high-purity \textsuperscript{99m}Tc(V)-DMSA using the Amersham DMSA kit requires reconstituting the kit with 1.0 mL of 4.2% sodium bicarbonate followed by rapid addition of 20 to 40 mCi (740 – 1480 MBq) of \textsuperscript{99m}Tc-pertechnetate diluted to 3 mL with 0.9% sodium chloride injection.\textsuperscript{73} Following incubation for 10 minutes at room temperature, sterile oxygen is bubbled through the solution for 10 minutes followed by sterile filtration. The oxygen oxidizes excess Sn(II) which would reduce \textsuperscript{99m}Tc(V)-DMSA to \textsuperscript{99m}Tc(III)-DMSA resulting in kidney localization. Compared to the \textsuperscript{99m}Tc(III)-DMSA kidney imaging agent, \textsuperscript{99m}Tc(V)-DMSA localizes differently, with little kidney uptake. It has been used for tumor imaging, in particular medullary thyroid carcinoma.\textsuperscript{74,75}
**99mTc-Citrate**

The oxidation state of 99mTc-citrate has been examined by Steigman, et al.56 The reduction of 99Tc-pertechnetate in millimolar concentrations by excess stannous chloride in the presence of a citrate buffer at pH 7 resulted in a rapid reduction to Tc(V) (5 minutes) followed by a slow reduction (2 to 3 hrs) to Tc(IV). The same reduction is expected to occur with nanomolar 99mTc-pertechnetate in radiopharmaceutical kits. Citrate is used in some kits as a transfer ligand to facilitate the radiolabeling process. On the basis of the above investigation, noting the rapid reduction of Tc(VII) to Tc(V) in citrate buffer, the donor complex in radiopharmaceutical kits is likely to be Tc(V)-citrate.

**99mTc-Gluceptate**

99mTc-gluceptate (or glucoheptonate) is a Tc(V) complex with the seven-carbon carboxylic acid sugar glucoheptonate. Studies on the formation of Tc-glucoheptonate with millimolar 99Tc and nanomolar 99mTc pertechnetate demonstrate that the complexes formed rapidly in high yield when the ligand to technetium molar ratio is 25 or greater.76 The chemical stability of the complex is high over a long period in the absence of other ligands. Electrophoretic measurements determined the net charge on the complex as 1-. Titration of 99TcO4− in a 100-fold excess of glucoheptonate with stannous chloride, while monitoring complex formation by UV-vis spectrophotometry, yielded a 1:1 molar reaction between Sn(II) and pertechnetate. Titration with stannous chloride in the presence of excess of pertechnetate did not yield additional complex, indicating a reduction of technetium from Tc(VII) to Tc(V) during complex formation. No significant in vitro and in vivo differences were found between the 99mTc and 99Tc glucoheptonate complexes.76 Similar results were found when a NaBH4 reductant was used in place of stannous chloride. Further analysis characterized the complex as an oxo-bis(glucoheptonato)technetate(V) anion composed of a TcO3+ core and two glucoheptonate ligands (Figure 19).76 The stoichiometry of the complexation reaction is described as follows:

\[
TcO_4^- + 2(C_7H_{12}O_8)^- + Sn^{2+} + H_2O \rightarrow [TcO(C_7H_{12}O_8)_2]^- + 2H^+ + [SnO_2(OH)_2]^2-
\]

The 2:1 gluceptate:Tc complex proposed by de Kiviet was questioned by Hwang et al.77 on the basis that there is a shift to a lower wavelength in the visual spectrum in the analysis of Tc(V)-gluceptate and a slowed electrophoretic migration toward the anode in acidic pH. They attribute this to protonation of the carboxylate group and suggested that gluceptate does not complex with technetium as de Kiviet proposed. Hwang et al. suggested a 1:1 Tc:gluceptate complex and proposed that a dimer
with a Tc-O-Tc linear bond and one gluceptate ligand bound to each technetium. No structural characterization was done.

\(^{99m}\text{Tc}-\text{gluceptate}\) is prepared by adding \(^{99m}\text{Tc}-\text{pertechnetate}\) to the radiopharmaceutical kit followed by incubation for 15 min. Its expiration following preparation is 6 hours when stored at 2 to 8 °C. After intravenous administration, Tc(V)-gluceptate is rapidly excreted into urine by glomerular filtration. Approximately 12% of the injected dose is bound in the kidney cortex.\(^{58}\) The complex is approved for brain and kidney imaging. Glucoheptonate is also used as a transfer ligand in radiopharmaceutical kits (e.g. Apcitide kit).

\(^{99m}\text{Tc}-\text{Gluconate}\)

Complexation of gluconic acid with technetium has been investigated.\(^{77-79}\) The titration of gluconate and stannous chloride with standard \(^{99}\text{Tc}-\text{pertechnetate}\) revealed an electron number of 2 at pH 12 and 2.14 to 2.4 at pH 5. This indicates a reduction of Tc(VII) to Tc(V) with some tendency to a lower oxidation state at pH 5.\(^{77}\) Other investigations support the formation of Tc(V)-gluconate as well.\(^{78,79}\) The Tc(V)-gluconate complex has been shown to localize in kidneys but no radiopharmaceutical kit is approved for this application. Gluconate, however, is used as a transfer ligand in radiopharmaceutical kits (e.g. Tetrofosmin kit).

\(^{99m}\text{Tc}-\text{Apcitide}\)

Synthetic peptide ligands have been designed to complex with the Tc=O\(^{3+}\) core. Two technetium-labeled biochemical markers in this group that have achieved FDA approval for use are \(^{99m}\text{Tc}-\text{apcitide}\), a platelet receptor-binding peptide for imaging acute venous thrombosis and \(^{99m}\text{Tc}-\text{depreotide}\), a somatostatin receptor marker for imaging malignant lung tumors.\(^{80,81}\) However, \(^{99m}\text{Tc}-\text{depreotide}\) is no longer on the market.

Platelets express the glycoprotein (GP) IIb / IIIa receptor which binds fibrinogen when the platelet is activated resulting in platelet aggregation in the blood-clotting process. The peptide arginine-glycine-aspartate (–Arg-Gly-Asp or RGD in single-letter amino acid code) is the amino acid sequence identified as the platelet attachment site within fibrinogen and platelet adhesion proteins.\(^{82}\) Synthetic peptides containing the RGD sequence can effectively compete with endogenous fibrinogen during the clotting process, and therapeutic drugs have been developed to control clotting by this mechanism.\(^{83}\) \(^{99m}\text{Tc}-\text{apcitide}\) is a thrombus-localizing radiopharmaceutical designed to mimic the RGD peptide.
sequence. Apcitide contains the mimetic sequence -Apc-Gly-Asp (-ApcGD). The synthetic amino acid Apc (S-aminopropyl-L-cysteine) is an arginine surrogate that not only replaces arginine in the receptor-binding sequence but also confers additional selectivity on the molecule.\textsuperscript{82} The chemical structures of apcitide and \textsuperscript{99m}Tc-apcitide have been characterized as shown in Figure 20.\textsuperscript{82} Apcitide contains a platelet receptor-binding region consisting of the peptide (-Apc-Gly-Asp-) and the technetium-binding region consisting of the peptide (-Gly-Gly-Cys-NH\textsubscript{2}-). \textsuperscript{99m}Tc-apcitide is prepared by adding 1 to 3 mL of \textsuperscript{99m}Tc-sodium pertechnetate (\(\leq\) 50 mCi/mL) to the apcitide kit and heating on a boiling water bath for 15 minutes. The precursor ligand (bibapcitide) is split into two apcitide monomers that displace Tc=O\textsuperscript{3+} from a \textsuperscript{99m}Tc-glucoheptonate donor complex as follows:

\[
\text{TcO}_4^- + \text{Sn – Glucoheptonate} \rightarrow \text{Tc – Glucoheptonate}
\]

\[
\text{Bibapcitide} + \text{Tc – Glucoheptonate} \xrightarrow{\text{Heat}} \text{Tc – Apcitide}
\]

The \textsuperscript{99m}Tc-apcitide complex has a net charge of 1- due to four negative charges formed on three nitrogens and one sulfur during coordination (Figure 20).

After intravenous administration \textsuperscript{99m}Tc-apcitide binds to GP IIb / IIIa receptors on activated platelets involved in thrombus formation. It is indicated for the detection of acute venous thrombosis in the lower extremities.\textsuperscript{84,85}
A significant amount of work is being devoted to developing peptide-based radiopharmaceuticals using BFCAs. Comprehensive reviews of the chemistry involved have been presented.29-34, 82, 83

**Novel Tc(V) Complexes**

Several novel Tc(V) compounds with interesting ligands have been developed that may hold promise for future use in nuclear medicine. A compound that targets the dopamine transporter (DAT) is $^{99m}$Tc-TRODAT-1 (Figure 21). This is a diaminedithiol complex of the TcO$_3^{3+}$ core with a tropane analog derivatized from one nitrogen. The complex is prepared by reacting the preformed TRODAT-1 ligand dissolved in ethanolic HCl with sodium pertechnetate in the presence of stannous glucoheptonate and sodium EDTA. The mixture is sterilized by autoclaving, phosphate-buffered to pH 6 to 7, and purified by solvent extraction and HPLC separation.86 The two diastereomers that are formed bind to DAT receptors in rat striatum. Imaging studies in humans have demonstrated localization in the basal ganglia consistent with DAT receptor binding.87,88

![Figure 21. Chemical structures of various investigational TcO$_3^{3+}$ coordination compounds.](image)

Another novel ligand approach to technetium complex design for receptor-specific imaging is the 3 + 1 concept.89 A complex formed by coordination of the Tc=O$_3^{3+}$ core with a tridentate ligand and a monodentate ligand constitutes a 3 + 1 mixed-ligand complex. The technetium complex is of the type TcO-[(HS-(C$_2$H$_5$)-X-(C$_2$H$_5$)-SH) + (R-SH)], where X = S or NCH$_3$ and R is the receptor-binding molecule. In principle the method consists of saturating three of the four available coordination sites on the Tc=O$_3^{3+}$ core with a small tridentate ligand and filling the fourth site with a monodentate co-ligand that is the receptor-targeting molecule. The manner in which these complexes are constructed puts
them into the category of the integrated approach to labeling with technetium. The general labeling sequence is shown below which begins with a $^{99m}$Tc(V)-gluconate donor complex.\textsuperscript{90}

\[
\begin{align*}
99m\text{Tc(V)}\text{-Gluconate} & \xrightarrow{R\text{-SH} \atop 1\text{-min} @ \text{RT}} 99m\text{TcO-(SR)}_x \\
\text{X} & \xrightarrow{\text{SH}} \text{S} \quad \text{or NCH}_3
\end{align*}
\]

One requirement of the $3 + 1$ approach is that the co-ligand must contain a thiol group to coordinate with technetium. The method has been used to prepare technetium-labeled dopamine, serotonin, estrogen and androgen receptor complexes. Johannsen and colleagues\textsuperscript{89} have chosen this approach to design a brain receptor imaging agent to mimic ketanserin, a prototype serotonin receptor (5-HT) antagonist. By preparing different fragments of ketanserin as the monodentate co-ligand, this group has produced a series of technetium complexes that target the serotonin receptor in the brain.\textsuperscript{89,90} The method lends itself to controlling biodistribution by altering the structural elements of the co-ligand. This same group has demonstrated that by modification of the co-ligand to change pKa, brain uptake of the complex can be increased significantly with the particular 5-HT$_{2A}$ ($3 + 1$) complex shown in Figure 21.\textsuperscript{91} One concern that has arisen with these compounds is the lability of the thiol co-ligand group to displacement in vivo by glutathione.\textsuperscript{92} This effect was found to be dependent on small structural variations. For example, in the S-X-S tridentate ligand, complexes with X = N-CH$_3$ were more stable than complexes with X = S.

$^{99m}$Tc-ethylenedicysteine ($^{99m}$Tc-EC) is a member of the diaminedithiol family of chelation compounds. It is the diacid metabolite produced from de-esterification of $^{99m}$Tc-ethylenecysteinate dimer ($^{99m}$Tc-ECD). The serendipitous observation of high renal excretion of $^{99m}$Tc-ECD metabolites led to an investigation that identified $^{99m}$Tc-EC as a possible renal imaging agent.\textsuperscript{93} This complex contains the Tc=O$_3^+$ core and exists in different isomeric forms as shown in Figure 21. When the D,D, L,L and D,L isomers were measured in human subjects their respective clearances, were 82\%, 70\%, and 40\%, relative to $^{131}$I-OIH. The pharmacokinetics of $^{99m}$Tc-D,D-EC appears to be closer to $^{131}$I-OIH than $^{99m}$Tc-L,L-EC.\textsuperscript{94} Further work will need to be done to determine if any of these complexes will become useful renal imaging agents.

\textbf{O=Tc=O$^+$ Core}

The dioxo core of Tc(V) has two trans-oxygen atoms that neutralize four of the five positive charges on the technetium atom, creating an overall core charge of 1+. The O=Tc=O$^+$ core forms six-coordinate
octahedral complexes with technetium. Cyclam (N-N-N-N) and diphosphine (P-P) ligands have been successfully used to produce stable complexes with the O=Tc=O$^+$ core.$^{29}$

**$^{99m}$Tc-Tetrofosmin**

The most important technetium dioxo compound to date is the cationic complex \( [^{99m}\text{Tc-(tetrofosmin)}_2\text{O}_2]^+ \) where tetrofosmin is the ether functionalized diphosphine ligand 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane. Structural characterization of this dimeric complex has shown that the $^{99m}\text{Tc}$ and $^{99}\text{Tc}$ complexes are identical and possess the O=Tc=O$^+$ core (Figure 22).$^{27,95}$

Because the donor ligands are neutral, the technetium complex has a net charge of 1+. The complex is labeled at room temperature employing a gluconate transfer ligand.

![Figure 22. Chemical structures of Tc(V) coordination compounds: $^{99m}\text{Tc-(tetrofosmin)}_2\text{O}_2$ and $^{99m}\text{Tc-N(NOEt)}_2$.](image)

The original formulation of $^{99m}\text{Tc}$-tetrofosmin had a pH range of 8.3 to 9.1 and was prepared without admission of air to the reaction vial. It had a shelf life of 8 hours. Stability work at Amersham revealed that the complex was sensitive to autoradiolytic decomposition and that admission of 2 mL of air at the time of pertechnetate addition and a final pH range of 7.5 to 9.0 would result in a product that was
stable for 12 hours. The increased stability is attributed to the ability of oxygen to scavenge reducing species (the hydrated electron e\textsuperscript{−}\textsubscript{aq} and the hydrogen radical H*) shown by the reactions below:\textsuperscript{13}

\[
\begin{align*}
H \cdot + O_2 & \rightarrow \bullet HO_2 \\
e^-\textsubscript{aq} + O_2 & \rightarrow \bullet O_2^- 
\end{align*}
\]

After intravenous administration \(^{99m}\text{Tc}\)-tetrofosmin is taken up into the heart in proportion to myocardial blood flow. Uptake does not involve cation channel transport but occurs by by potential-driven diffusion of the lipophilic cationic complex across the sarcolemmal and mitochondrial membranes.\textsuperscript{96} \(^{99m}\text{Tc}\)-tetrofosmin is bound in the intracellular cytosol of myocytes.\textsuperscript{97} Washout from the heart is slow being 4% per hour after exercise and 0.6% per hour at rest.\textsuperscript{98} \(^{99m}\text{Tc}\)-tetrofosmin is used in nuclear medicine to assess myocardial perfusion in ischemia and infarction.

**Tc≡N\textsuperscript{2+} Core**

The Tc≡N\textsuperscript{2+} core can be produced in stable form at the no carrier-added level.\textsuperscript{29} The nitrido atom N\textsuperscript{3−} was developed by Baldas to complex with Tc(V).\textsuperscript{99,100} This led to the development of a \(^{99m}\text{Tc}\)-nitrido compound for myocardial perfusion imaging, [bis (N-ethyl-N-ethoxydithiocarbamato)nitrido \(^{99m}\text{Tc}\)(V)].\textsuperscript{101} It is a neutral lipophilic complex with a Tc≡N\textsuperscript{2+} core where the Tc(V) atom is triple-bonded to a strong pi-electron donor nitride atom (N\textsuperscript{3−}) and four donor sulfur atoms. The complex is prepared in a two-step procedure.\textsuperscript{101} The first step involves reduction of \(^{99m}\text{Tc}\)-pertechnetate in acidic conditions by trisodium tri(\textit{m}-sulfophenyl) phosphine in the presence of S-methyl N-methyl dithiocarbazate, H\textsubscript{2}NN(CH\textsubscript{3})C(=S)SCH\textsubscript{3}, as the nitrido nitrogen donating agent. This mixture is then heated at 100 °C for 20 minutes, producing an intermediate species bearing the Tc≡N\textsuperscript{2+} core. After cooling and neutralizing with buffer, the dithiocarbamate ligand is added to the mixture, whereupon ligand exchange occurs immediately to form the final \(^{99m}\text{Tc}\)-nitrido dithiocarbamate complex, \(^{99m}\text{Tc}\)-N-(NOEt)\textsubscript{2}. A lyophilized kit has been developed (\(^{99m}\text{Tc}-\text{N}-(\text{NOEt})\textsubscript{2}\), CIS Bio-International, France) using stannous chloride that permits labeling at neutral pH.\textsuperscript{101}

After intravenous injection, \(^{99m}\text{Tc}\)-N-(NOEt)\textsubscript{2} localizes in the myocardium proportional to blood flow. Because \(^{99m}\text{Tc}\)-N-(NOEt)\textsubscript{2} redistributes from the heart it has been compared with \(^{201}\text{Tl}\) for myocardial perfusion imaging.\textsuperscript{102,103}
**Tc-HYNIC Core**

HYNIC (6-hydrazinonicotinamide) in the Tc-HYNIC core was first utilized by Abrams to label polyclonal IgG.\(^{104}\) Since then, it has been used to label chemotactic peptides, somatostatin analogs, and other interesting biomolecules.\(^{32}\) This core forms complexes of the general form \([^{99m}\text{Tc}(\text{HYNIC-Peptide})(L)_2]\), where the HYNIC group satisfies one coordination site on technetium and the remaining sites are completed by various co-ligands. A ternary ligand system with the general form \([^{99m}\text{Tc}(\text{HYNIC-Peptide})(\text{tricine})(L)]\) has been used to prepare technetium complexes. The system contains three different ligands: the bifunctional coupling group (HYNIC), a monodentate triphosphine co-ligand (L), which is trisodium triphenylphosphine-3,3’,3”-trisulfonate (TPPTS), and a tetradeutate co-ligand, tris(hydroxymethyl)methylglycine (tricine). Following this idea, a technetium complex that targets the GP IIb / IIIa platelet receptor (Figure 23) was developed employing the postlabeling approach, where the peptide-HYNIC conjugate is formed prior to coordination with technetium and the co-ligands.\(^{105}\) The components in the GP IIb / IIIa ternary ligand complex exist in a 1:1:1:1 ratio of Tc:HYNIC-Peptide:tricine:phosphine. This complex demonstrated arterial and venous thrombi in a canine model with thrombus-to-muscle ratio of \(\sim 10:1\) at 2 hours post injection.\(^{106}\)
The oxidation state of technetium in HYNIC complexes is not clear and may depend on the type of co-ligand, taking into account that some ligands, e.g. phosphines, have reducing capability. \(^{32,105}\)

**TC(VII) COMPOUNDS**

The Tc(VII) oxidation state is characterized by technetium in the d\(^0\) configuration; Tc(VII) is the highest and the most stable oxidation state where it has lost all seven valence electrons. There are two principal compounds used in nuclear medicine with technetium in the 7+ oxidation state: \(^{99m}\)Tc-sodium pertechnetate and \(^{99m}\)Tc-sulfur colloid.

**\(^{99m}\)Tc-Sodium Pertechnetate**

\(^{99m}\)Tc-sodium pertechnetate is obtained from the \(^{99}\)Mo/\(^{99m}\)Tc generator as described earlier. Briefly, technetium exists in the generator as pertechnetate ion (TcO\(_4\)^-) which is readily eluted by 0.9 % sodium chloride (saline). In general, > 90 % of the technetium activity in the generator can be eluted with about 6 mL of saline. The maximum buildup of \(^{99m}\)Tc activity will occur about 24 hours after an elution. About 6 hours after generator elution, approximately 50% of the activity that will be available in 24 hours can be eluted, because the buildup of activity is more rapid early after elution than at later times (Figure 24). The generator eluate will contain both \(^{99m}\)Tc and \(^{99}\)Tc isomers of technetium. Up to about 10 hours following elution the amount of the \(^{99m}\)Tc isomer in the generator will be greater than the amount of \(^{99}\)Tc isomer. However, because the \(^{99}\)Tc isomer is longer-lived than the metastable isomer, the amount of \(^{99}\)Tc isomer will be greater

![Figure 24](image)

*Figure 24.* Graph of the radioactivity over time of \(^{99}\)Mo and \(^{99m}\)Tc in a \(^{99m}\)Tc generator. A\(_1\) = \(^{99}\)Mo activity over time; A\(_2\) = ingrowth of \(^{99m}\)Tc activity following generator elution; X = point in time when \(^{99}\)Mo and \(^{99m}\)Tc activities are equal in the generator and \(^{99m}\)Tc activity is at a maximum following generator elution; Y\(_1\) (solid line) = theoretical \(^{99m}\)Tc activity in the generator in transient equilibrium with \(^{99}\)Mo if 100% of \(^{99}\)Mo decays to the \(^{99m}\)Tc isomer; Y\(_2\) (dashed line) = actual \(^{99m}\)Tc activity in the generator in transient equilibrium with \(^{99}\)Mo when 86% of \(^{99}\)Mo decays to the \(^{99m}\)Tc isomer; B = decay of \(^{99m}\)Tc activity alone after elution from the generator.
than the amount of $^{99m}\text{Tc}$ isomer after this time point. For example, twelve hours after generator elution, the $^{99}\text{Tc}$ isomer is 1.2 times greater than the $^{99m}\text{Tc}$ isomer (Table 4 and Figure 25).

**Table 4**

<table>
<thead>
<tr>
<th>Time Since Elution</th>
<th>Mole Fraction</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{99m}\text{Tc} / \text{Tc}_{\text{total}}$</td>
<td>$^{99}\text{Tc} / \text{Tc}_{\text{total}}$</td>
</tr>
<tr>
<td>3 hr</td>
<td>0.73</td>
<td>0.27</td>
</tr>
<tr>
<td>6 hr</td>
<td>0.62</td>
<td>0.38</td>
</tr>
<tr>
<td>12 hr</td>
<td>0.46</td>
<td>0.54</td>
</tr>
<tr>
<td>24 hr</td>
<td>0.28</td>
<td>0.72</td>
</tr>
<tr>
<td>48 hr</td>
<td>0.13</td>
<td>0.87</td>
</tr>
<tr>
<td>72 hr</td>
<td>0.077</td>
<td>0.923</td>
</tr>
</tbody>
</table>

At all times after generator elution, the mole ratio of the metastable isomer is less than unity, and its specific activity continually declines with time. It is never “carrier-free”. The decline in specific activity over time can be important in technetium radiopharmaceutical chemistry because a number of radiopharmaceutical kits are formulated with low amounts of reducing agent, which limits the amount

![Figure 25. Relative number of $^{99}\text{Mo}$, $^{99m}\text{Tc}$, and $^{99}\text{Tc}$ atoms in a $^{99m}\text{Tc}$ generator over time.](image)
of pertechnetate that can be added to the kit. Kits with limited reducing power (e.g. Ceretec®, Ultra-Tag®, MAG₃®) that are reconstituted with pertechnetate from generators with long ingrowth, will be most vulnerable to reduced labeling yields. In such situations, labeling problems can be minimized by eluting the generator twice, with the second elution made 1 to 2 hours after the first elution. Pertechnetate from the second elution will have a more favorable ratio of ⁹⁹ᵐTc-to-⁹⁹Tc and have less likelihood of causing inefficient labeling.

On rare occasions much of the available technetium activity remains bound to the generator column, which results in poor elution efficiency. The cause of this problem has been attributed to a change in oxidation state of technetium on the generator due to the effects of radiolysis.¹⁰⁸,¹⁰⁹ This was a more frequent problem with “wet system” generators in the past, however the problem occasionally occurs with “dry system” generators today. The cause of the problem is associated with residual saline remaining on the column after elution and can be corrected by drawing sufficient air through the generator column to remove all saline. If a reduced yield occurs following generator elution, re-elution in 1 to 2 hours will usually remove the bound technetium, because the first elution removes radiolytic contaminants on the column and reintroduces fresh oxygen to restore technetium to its ⁷⁺ valence state.

**⁹⁹ᵐTc-Sulfur Colloid**

Of the technetium-labeled radiopharmaceuticals currently used in nuclear medicine, only ⁹⁹ᵐTc-sulfur colloid has technetium in the non-reduced ⁷⁺ oxidation state. Technetium is able to maintain this state because of its stability as insoluble technetium hepta-sulfide, Tc₂S₇.⁷ The chemistry of ⁹⁹ᵐTc-sulfur colloid has been extensively reviewed.⁷,⁹

⁹⁹ᵐTc-sulfur colloid injection is a colloidal dispersion of sulfur particles. It is prepared from a kit which consists of three components: (1) a Reaction vial containing a lyophilized mixture of anhydrous sodium thiosulfate (the source of sulfur), disodium edetate (EDTA) (Al³⁺ ion chelator), and gelatin (protective colloid); (2) a Solution A vial of 0.148 M hydrochloric acid; and (3) a Solution B vial of buffer consisting of anhydrous sodium biphosphate and sodium hydroxide.

⁹⁹ᵐTc-sulfur colloid injection is prepared by adding 1 to 3 mL (500 to 1500 mCi) of ⁹⁹ᵐTc-sodium pertechnetate to the reaction vial to dissolve the powder. After addition of 1.5 mL of Solution A (acid), the vial is placed into a boiling water bath for 5 minutes. The vial is cooled and 1.5 mL of Solution B (buffer) is
added. The pH of the final mixture is between 4.5 and 7.5. During the boiling step thiosulfate is hydrolyzed, releasing elemental sulfur. Hydrolysis is accelerated in the acidic pH. The sulfur atoms aggregate forming particles ranging in size from 0.1 to 1.0 µm. Gelatin controls particle size and aggregation by forming a negatively charged protein coat on the sulfur particles, causing them to repel each other. Technetium heptasulfide is formed during the reaction and becomes incorporated into the sulfur particles. These reactions are summarized below:

\[
S_2O_3^{2-} + H^+ \xrightleftharpoons{\text{Heat}} S + HSO_3^-
\]

\[
2 \text{TcO}_4^- + 7 S_2O_3^{2-} \xrightarrow{\text{Heat}} \text{Tc}_2\text{S}_7 + 7 \text{SO}_4^{2-} + \text{H}_2\text{O}
\]

EDTA in the formulation chelates any aluminum ion that may be present in the sodium pertechnetate solution, which prevents the possible precipitation of aluminum phosphate and the co-precipitation of \(^{99m}\)Tc-sulfur colloid. This would result in excessively large particles causing technetium to localize in the lungs.

\(^{99m}\)Tc-sulfur colloid has had a wide variety of uses in nuclear medicine, but it is often used in lymphoscintigraphy to identify sentinel lymph nodes in melanoma patients. It can also be incorporated into a solid meal such as scrambled egg for gastric emptying studies. Other applications include liver-spleen imaging, bone marrow studies and shunt patency evaluation.

**TECHNETIUM-LABELED PROTEINS**

A variety of proteins have been labeled with technetium, including enzymes, fibrinogen and albumin. The labeling mechanism has been studied to some degree but has not been well characterized. The oxidation state of technetium within the protein complex is not known. The most significant work regarding technetium protein chemistry has been conducted with human serum albumin and monoclonal antibodies.

**\(^{99m}\)Tc-Human Serum Albumin**

A protein’s conformation is complicated and can be affected by a variety of factors such as pH. The unfolding of a protein’s structure can make available active binding sites within the protein. These sites may include disulfide groups and various amino acids that can form weak complexes with technetium. Methods for preparing technetium-labeled human serum albumin (HSA) for nuclear medicine applications have been reviewed. \(^{99m}\)Tc-HSA was first produced using ascorbic acid and ferric chloride as reductants for technetium. Using this method, Steigman et al, found that if the
SH groups were blocked, technetium labeling efficiency would be reduced significantly, suggesting that SH groups were the binding sites. However, other studies using stannous chloride or ferrous sulfate as reductants demonstrated no effect on labeling efficiency when SH groups were blocked. While several methods have been developed for preparing $^{99m}$Tc-albumin and labeling mechanisms have been proposed, no technique has been found to characterize the exact oxidation state of technetium conjugated with albumin. The most universal method adopted for labeling albumin employed stannous tin as the reducing agent. A radiopharmaceutical kit for imaging the cardiac blood pool was eventually marketed using stannous tartrate as reductant, but unfortunately it is no longer available in the market. The need for $^{99m}$Tc-albumin occurs occasionally for various applications and a more recent method for its extemporaneous preparation has been reported. A method for preparing $^{99m}$Tc-HSA, similar to the formulation previously on the market using stannous tartrate as reductant, can be found at the following patent website (http://www.freepatentsonline.com/4042677.html). A study of the various methods for preparing $^{99m}$Tc-HSA has shown that all methods produce variable amounts of pertechnetate and insoluble technetium impurities. In this analysis, $^{99m}$Tc-HSA prepared by the stannous chloride method was compared to other methods of preparation. By in vitro analysis it showed higher amounts of insoluble technetium and lower amounts of pertechnetate impurities compared to other methods of preparation, and by in vivo analysis it showed a smaller percentage of the injected dose in the blood at 30 min (~35%) compared to other methods (45 to 58%).

$^{99m}$Tc- Human Serum Albumin Aggregated

When stannous chloride and human serum albumin are heated under controlled conditions, typically in an acetate buffer at pH 5, the albumin denatures into aggregate particles in the general size ranging from 10 to 90 microns. Commercial kits of stannous macroaggregated albumin (MAA) are marketed for the preparation of $^{99m}$Tc-MAA, which is primarily used for perfusion lung imaging. When $^{99m}$Tc-sodium pertechnetate is added to a stannous-MAA kit, pertechnetate is reduced and conjugated to the MAA particles. No studies have identified the nature of the complexation or the oxidation state of technetium bound to MAA particles. Labeling studies suggest that stannous chloride reduces disulfide bonds in the albumin molecule and reduced technetium is conjugated to SH groups in the protein.
TECHNETIUM-LABELED ANTIBODIES

Two basic methods, namely direct and indirect, have been used to label antibodies with technetium. The direct method relies on the reduction of disulfide bridges in the antibody to generate sulfhydryl groups. Although the exact labeling mechanism is not known, studies have indicated that technetium binds to SH groups in the antibody (Figure 26).\textsuperscript{113,118} There are various techniques for direct labeling of antibodies. Pertechnetate can be reduced simultaneously with the antibody or it can be added afterward to “pre-tinned” antibody in kits. Rhodes et al\textsuperscript{119} prepared monovalent Fab’ antibody fragments by incubating divalent F(\text{ab’})\textsubscript{2} fragments with stannous chloride in a phthalate / tartrate mixture, then labeling them with \textsuperscript{99m}Tc-pertechnetate. The labeling mixture was purified through a Sephadex column. In this investigation the binding affinity of technetium with antibody was shown to be both strong and weak. The amount of the strong affinity bond was a function of exposure time of antibody to stannous ion. Stronger binding was presumably related to the generation of more sulfhydryl groups. Labeling efficiency was high (> 90\%) but immunoreactivity was low (~55\%).\textsuperscript{119} In another study, Paik et al\textsuperscript{118} labeled reduced antibody with technetium in the presence of DTPA as a competitive chelator to minimize technetium binding to weak sites in the antibody. The findings of this study suggested that technetium is bound by both high affinity, low capacity sites and by low affinity, high capacity sites, with binding to high affinity sites being related to the presence of sulfhydryl groups. They recommended that labeling in the presence of excess DTPA reduced the binding of technetium to non-specific low affinity sites. While this technique lowers the radiochemical yield of labeled antibody, it assures an inert chemical bond to high affinity sites with direct antibody labeling.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure26}
\caption{Model describing the generation of antibody fragments from whole antibody, reduction of their disulfide bonds, and direct labeling of antibody fragment at with technetium.}
\end{figure}
The indirect method of labeling of antibodies involves conjugation of a BFCA such as DTPA to the antibody and then adding reduced technetium to the conjugate. Technetium is bound to the antibody through complexation with the BFCA. The bifunctional chelate approach involves competition for technetium between weak- and strong-affinity direct binding sites in the antibody and indirect binding via the covalently bound chelating agent. In a comparison of direct and indirect labeled antibody, it was shown that a $^{99m}$Tc-DTPA conjugated antibody labeled indirectly, cleared more slowly from the blood than the direct labeled antibody, suggesting that the $^{99m}$Tc-DTPA conjugate provides a more stable bond with the antibody than the direct labeled antibody. However, when the $^{99m}$Tc-DTPA conjugated antibody was compared to an $^{111}$In-DTPA conjugated antibody, the $^{99m}$Tc-DTPA conjugate cleared faster from the blood than the $^{111}$In-DTPA conjugate. The investigators concluded that this was possibly due to a higher level of protein degradation in the antibody conjugated with $^{99m}$Tc-DTPA compared to antibody conjugated with $^{111}$In-DTPA.

Another indirect technique that can be used for labeling antibodies with technetium is to modify the antibody with a hydrazine nicotinamide (HYNIC) group and labeling it with $^{99m}$Tc through the glucoheptonate trans chelation method. With this approach, a $^{99m}$Tc-labeled IgG polyclonal antibody showed similar biodistribution parameters in rats compared with $^{111}$In-labeled IgG. When this same method was compared with a direct labeling method for preparing $^{99m}$Tc-IgG, greater instability and faster blood clearance was observed with the direct-labeled antibody.

The results from multiple studies indicate that antibodies can be labeled with technetium successfully by both direct and indirect methods, but there are differences in the properties of antibodies produced by both methods. Some of these differences may depend on the type of antibody labeled. A fair amount of evidence, however, seems to indicate that direct labeling of antibodies is less satisfactory than indirect labeling that employs a BFCA attached to the antibody.

A third approach to labeling antibodies with technetium is to employ a pre-labeled ligand (prelabeling approach). In this method, dithionite-reduced technetium is complexed to an $\text{N}_2\text{S}_2$ ligand functionalized with a carboxylate group (Figure 27). The carboxylate is then activated with an ester group through which it is conjugated to the antibody via acylation with lysine amine residues. This labeling approach offers the advantage of obviating non-specific binding of technetium to the antibody that occurs with direct labeling or the postlabeling DTPA-antibody conjugate approaches. While this
method produces a stable antibody label without non-specific binding, the method of preparation is somewhat cumbersome and less adaptable to simple kit formulation.

Several technetium-labeled antibodies have either been approved for use in nuclear medicine or are under investigation. Those that have been approved are $^{99m}\text{Tc}$-arcitumomab (CEA-Scan, Immunomedics) for the detection of recurrent or metastatic colorectal cancer, $^{99m}\text{Tc}$-nofetumomab merpentan (Verluma, Dupont-Pharma) for detection of small-cell lung cancer, and $^{99m}\text{Tc}$-fanolesomab (NeutroSpec, Tyco Healthcare) for the detection of appendicitis. For various reasons, each of these agents has been removed from the market. Other technetium-labeled antibodies have been investigated. One of these is $^{99m}\text{Tc}$-sulesomab (LeucoScan-Immunomedics), an Fab’antigranulocyte monoclonal antibody fragment designed to label white cells in vivo for localizing bone infection in patients with suspected osteomyelitis. Another is $^{99m}\text{Tc}$-bectumomab (LymphoScan-Immunomedics) an Fab’ fragment of the anti-CD-22 antibody LL-2 for localizing B-cell non-Hodgkin’s lymphoma.\textsuperscript{125}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure27.png}
\caption{Method of labeling antibodies with technetium using the prelabeling approach (i.e., technetium reduction and chelation to a BFCA, activation of the BFCA at the carboxyl group, and conjugation of the BFCA-Tc complex to the antibody).}
\end{figure}
TECHNETIUM-LABELED RED BLOOD CELLS

Erythrocytes and leukocytes are the principal blood cellular elements labeled with technetium for nuclear medicine studies. Labeling procedures for preparing technetium-labeled blood cells vary and may require whole blood separation and isolation of the cell type to be labeled. Technetium-labeled red blood cells are used primarily for cardiac blood pool studies, gastrointestinal bleeding studies, and spleen imaging with heat-denatured red cells. Technetium-labeled white blood cells are used for localization of infections.

The three principal methods for labeling red blood cells with technetium are the in vitro method, the in vivo method, and the modified in vivo method.

In Vitro Method

The in vitro method of labeling red blood cells can be done in whole blood using the Ultratag RBC kit (Tyco Healthcare). The kit consists of three components:

1. 10-mL Reaction Vial: Lyophilized mixture of stannous chloride dihydrate, sodium citrate dihydrate, and dextrose anhydrous at pH 7.1 to 7.2.

2. Syringe I: sodium hypochlorite in 0.6 mL at pH 11 to 13.

3. Syringe II: citric acid monohydrate, sodium citrate dihydrate, and dextrose anhydrous in 1.0 mL at pH 4.5 to 5.5.

One to three mL of whole blood, anticoagulated with ACD or heparin, is incubated for 5 minutes with the stannous citrate/dextrose mixture in the reaction vial. The contents of syringes I and II are then added sequentially followed by 10 to 100 mCi (370 to 3,700 MBq) of sodium pertechnetate and the reaction mixture is incubated for 20 minutes. During the labeling procedure, stannous citrate enters the red cells and becomes associated with intracellular hemoglobin. Sodium hypochlorite is added to oxidize the extracellular stannous ion to stannic ion, which prevents extracellular reduction of pertechnetate when it is added to the cells. Extracellular reduced technetium does not penetrate the red cell membrane and results in poor labeling efficiency. Stannous ion within red cells is not oxidized by hypochlorite because it cannot cross the red cell membrane. The citrate solution, which is added in syringe II, sequesters extracellular stannous ion, enhancing its oxidation by hypochlorite. After its addition to the pre-tinned cells, 99mTc-sodium pertechnetate diffuses into the cells and becomes reduced and bound in the cell. The labeling efficiency is typically > 95 %.127
**In Vivo Method**

The in vivo method requires intravenous injection of stannous pyrophosphate (Sn-PPi) (optimally between 10 and 20 μg Sn (II)/kg body weight) 20 to 30 minutes prior to intravenous administration of 15 to 25 mCi (555 to 925 MBq) of $^{99m}$Tc-sodium pertechnetate. Labeling yield of the in vivo “tinned” red cells is variable, ranging between 60 and 90 percent,\textsuperscript{126} and is not quantitative because of competition for pertechnetate between the red cells, extracellular fluid, excretory processes, and pertechnetate-avid tissues such as thyroid, stomach, and GI tract. Variable amounts of gastric and urinary activity may be evident on scans due to free pertechnetate in the blood stream. The in vivo clearance half-life of $^{99m}$Tc-RBCs is 29 hr.\textsuperscript{128} A caveat with this method is that in vivo labeling of RBCs with pertechnetate can occur for several weeks following administration of Sn-PPi. Twenty-five percent labeling of RBCs 42 days after intravenous administration of stannous pyrophosphate has been reported.\textsuperscript{126,129}

**Modified In Vivo Method**

The modified in vivo method, also known as the “in-vivitro” or “in vivo / in vitro” method, was developed to increase labeling efficiency of $^{99m}$Tc-RBCs.\textsuperscript{130} With this technique the patient receives ~500 μg of stannous ion as Sn-PPi intravenously. Twenty minutes later, 3 mL of “tinned” red blood cells are withdrawn through a heparinized infusion set into a shielded syringe containing 20 mCi (740 MBq) of $^{99m}$Tc-sodium pertechnetate. The mixture is incubated for 10 minutes with gentle agitation and then reinjected into the patient. More than 90% labeling yield is achieved because red cells compete only with plasma for pertechnetate in the syringe.

**Labeling Mechanism of $^{99m}$Tc-Red Blood Cells**

After incubation of red blood cells with stannous pyrophosphate or stannous citrate, stannous ion is transported across the cell membrane\textsuperscript{131} where it is believed to be associated with an intracellular protein.\textsuperscript{132} RBCs are labeled with technetium through the binding of reduced Tc to hemoglobin after pertechnetate diffuses into the cells and is reduced by stannous ion.\textsuperscript{132} Approximately 20 % of $^{99m}$Tc is associated with heme and 80 % with globin.\textsuperscript{133} Pertechnetate anion is believed to be transported across the red cell membrane via the band-3 protein transport system in exchange for chloride or bicarbonate ion.\textsuperscript{134}
TECHNETIUM-LABELED WHITE BLOOD CELLS

Radiolabeling leukocytes with technetium requires a lipophilic technetium complex. This is typically done by incubating isolated leukocytes with $^{99m}$Tc-exametazime ($^{99m}$Tc-HMPAO). Peters et al.\textsuperscript{135} demonstrated that leukocytes labeled with $^{99m}$Tc-HMPAO compared favorably with $^{111}$In-tropolone-labeled leukocytes. Labeling efficiency with the method is about 50%, with 78% of the activity associated with granulocytes.\textsuperscript{136,137} In addition to convenience and the ideal imaging properties of $^{99m}$Tc, a significant advantage of the $^{99m}$Tc-HMPAO method over $^{111}$In-labeled leukocytes is that leukocytes can be labeled in the presence of up to 20% plasma, an important factor for maintaining leukocyte viability.\textsuperscript{136}

The routine method for labeling leukocytes with technetium requires the separation of leukocytes from whole blood. Technique is important for cell viability. In general low speed centrifugation is used to obtain the leukocyte button. A routine method, illustrated in Figure 28, involves collection of ADC-anticoagulated whole blood which is incubated for about 45 minutes mixed with 10 mL of 6% Hetastarch to enhance settling of RBCs. The supernatant leukocyte-rich plasma (LRP) is removed and

![Figure 28](image_url). General method for the isolation and radiolabeling of white blood cells (WBC) with $^{99m}$Tc-HMPAO.
Centrifuged at 450g to pellet the leukocytes. The supernatant leukocyte-poor plasma (LPP) is removed and saved and the leukocyte button is incubated with 30 mCi of freshly prepared $^{99m}$Tc-HMPAO in 5 mL for 15 to 20 minutes to label the cells. The mixture is then centrifuged at 450g and the supernatant containing unbound technetium is removed. The labeled leukocytes are re-suspended in leukocyte-poor plasma for reinjection into the patient.

During the incubation period, after $^{99m}$Tc-HMPAO is added to the leukocyte button, the lipophilic $^{99m}$Tc-HMPAO complex diffuses into the leukocytes and is converted to a non-diffusible hydrophilic complex that becomes trapped within the cell.\textsuperscript{138} Subcellular binding studies have shown that $^{99m}$Tc-HMPAO preferentially labels eosinophils and is predominately stored in the secretory granules.\textsuperscript{139} The plasma half-life of $^{99m}$Tc-HMPAO-labeled leukocytes is approximately 4 hours compared to 6 hours for $^{111}$In-labeled leukocytes.\textsuperscript{138} The shorter half-life in vivo is attributed to elution of the technetium label from leukocytes.
REFERENCES


127. Package Insert, UltraTag RBC, Mallinckrodt Inc, St. Louis, MO; 2000.


QUESTIONS

1. The chemical state of technetium as it is obtained from the $^{99m}$Tc generator is:
   
   A. TcO$_3^{3+}$
   B. TcO$_4^-$
   C. TcO$_2$
   D. TcO$_2^{+}$

2. The oxidation state of technetium as it is obtained from the $^{99m}$Tc generator is:
   
   A. Tc(I)
   B. Tc(III)
   C. Tc(V)
   D. Tc(VII)

3. The number of electrons technetium pertechnetate must gain if it is reduced to the ground state of technetium metal is:
   
   A. 7
   B. 5
   C. 2
   D. 1

4. In the $^{99m}$Tc- mertiatide ($^{99m}$Tc-MAG3) complex, technetium’s oxidation state is changed and it acquires the following electronic configuration in the complex.
   
   A. d$^2$
   B. d$^3$
   C. d$^4$
   D. d$^5$

5. Which one of the following technetium compounds does not require reducing conditions in its preparation?
   
   A. $^{99m}$Tc-exametazime
   B. $^{99m}$Tc-medronate
   C. $^{99m}$Tc-sulfur colloid
   D. $^{99m}$Tc-mebrofenin
6. Which one of the following technetium oxidation states is not typically found in technetium radiopharmaceuticals?

A. Tc(IV)
B. Tc(III)
C. Tc(II)
D. Tc(I)

7. Intermediate oxidation states of technetium are found in certain technetium complexes, such as $^{99m}$Tc-pentetate ($^{99m}$Tc-DTPA), $^{99m}$Tc-medronate ($^{99m}$Tc-MDP), and $^{99m}$Tc-oxidronate ($^{99m}$Tc-HDP), where studies show that Tc(III) is formed initially in the complex and then changes to Tc(IV). Which one of the following statements best describes what occurs with technetium’s oxidation state in the formation of these complexes?

A. Technetium initially gains 3 electrons and then loses 1 electron.
B. Technetium initially loses 4 electrons and then gains 1 electron.
C. Technetium initially gains 4 electrons and then loses 1 electron.
D. Technetium initially is oxidized and then reduced.

8. Technetium coordination compounds that contain the TcO$_3^{3+}$ core form:

A. octahedral complexes.
B. square-planar complexes.
C. square-pyramidal complexes.
D. linear complexes.

9. Which one of the following reducing agents is typically used when labeling technetium compounds in alkaline pH?

A. Iron-ascorbate.
B. Stannous chloride.
C. Stannous fluoride.
D. Sodium borohydride.

10. An undesirable stable radiochemical reduction product that may form during technetium radiolabeling reactions when insufficient complexing agent is present is:

A. TcO$_4^-$.
B. TcO$_2$H$_2$O.
C. TcO$_2^{+}$.
D. TcO$^{3+}$. 
11. Which one of the following is not used as a transfer ligand in technetium kits?

A. Sodium tartrate.
B. Sodium citrate.
C. Sodium thiosulfate.
D. Sodium gluconate.

12. Typically oxygen is excluded from technetium kits to minimize the generation of radiochemical impurities. However, one kit that requires the presence of air during radiolabeling to minimize the formation of technetium radiochemical impurities is the:

A. exametazime kit.
B. medronate kit.
C. sulfur colloid kit.
D. mertiatide kit.

13. One cause of radiochemical impurity formation in technetium kits is autoradiolysis. All of the following adjuvants are used in radiopharmaceutical kits to retard radiolytic degradation except:

A. citric acid.
B. ascorbic acid.
C. para-amino benzoic acid.
D. gentisic acid.

14. Addition of air to the $^{99m}$Tc-tetrofosmin kit during its preparation will result in all of the following except:

A. a decrease in the amount of H•.
B. a decrease in the amount of OH•.
C. an extension of the shelf-life to 12 hours.
D. a decrease in the amount of $e_{aq}$.

15. Which one of the following is not a technetium-essential radiopharmaceutical?

A. $^{99m}$Tc-exametazime
B. $^{99m}$Tc-mertiatide
C. $^{99m}$Tc-pentetate
D. $^{99m}$Tc-mebrofenin
16. All of the following technetium complexes are first-generation technetium-tagged radiopharmaceuticals except:

A. $^{99m}$Tc-human serum albumin.
B. $^{99m}$Tc-methylene diphosphonate.
C. $^{99m}$Tc-glucoheptonate.
D. $^{99m}$Tc-apcitide.

17. All of the following technetium cores are best stabilized by pi-electron donor ligands except:

A. Tc(CO)$_3$\(^{+}\).
B. TcO$_3^{3+}$.
C. TcO$_2^{+}$.
D. TcN$_2^{2+}$.

18. Which one of the following $^{99m}$Tc-radiopharmaceuticals has a technetium core that is stabilized by pi-electron acceptor ligands?

A. $^{99m}$Tc-apcitide.
B. $^{99m}$Tc-sestamibi.
C. $^{99m}$Tc-tetrofosmin.
D. $^{99m}$Tc-(V)succimer.

19. Which one of the following technetium electron configurations is best stabilized by electron-donor ligands, such as S and O?

A. d$^2$
B. d$^3$
C. d$^4$
D. d$^6$

20. Which one of the following compounds was the first to demonstrate that technetium was essential for in vivo localization?

A. $^{99m}$Tc-bicisate
B. $^{99m}$Tc-mertiatide
C. $^{99m}$Tc-pentetate
D. $^{99m}$Tc-lidofenin

21. In which one of the following complexes is technetium’s oxidation state Tc(V)?

A. $^{99m}$Tc-exametazime
B. $^{99m}$Tc-sestamibi
C. $^{99m}$Tc-pentetate
D. $^{99m}$Tc-lidofenin
22. Which one of the following is an example of a first-generation technetium-tagged radiopharmaceutical?

   A. $^{99m}$Tc-tetrofosmin  
   B. $^{99m}$Tc-mertiatide  
   C. $^{99m}$Tc-pentetate  
   D. $^{99m}$Tc-apcitide

23. Which one of the following is not a characteristic property of second-generation technetium-tagged radiopharmaceuticals?

   A. A receptor-avid peptide may be present in the radiopharmaceutical.  
   B. Technetium is essential for in vivo localization.  
   C. A receptor-avid antibody may be present in the radiopharmaceutical.  
   D. The radiopharmaceutical contains a BFCA.

24. Which one of the following is an example of a second-generation technetium-tagged radiopharmaceutical?

   A. $^{99m}$Tc-sestamibi  
   B. $^{99m}$Tc-mertiatide  
   C. $^{99m}$Tc-apcitide  
   D. $^{99m}$Tc-pentetate

25. Which one of the following modifications to a technetium compound is least likely to alter its vivo localization or metabolism?

   A. Use of a D-enantiomer amino acid in place of the L-enantiomer.  
   B. Use of the syn-isomer instead of the anti-isomer.  
   C. Introduction of a chiral carbon atom into the compound’s structure.  
   D. Use of the Tc-99 isomer in place of the Tc-99m isomer.

26. $^{99m}$Tc-sodium pertechnetate is reduced in concentrated HCl to form the $[\text{TcOCl}_4]$ core. The oxidation state of technetium in this core is:

   A. 1-  
   B. 3+  
   C. 4+  
   D. 5+
27. A \([\text{TcOCl}_4]\) core is reacted with 2 neutral ligands (L) and 2 negatively charged ligands (L\textsuperscript{-}) to form the complex \([\text{TcOL}_4]\textsuperscript{\text{\text{-}}}\). The charge on this complex would be:

A. 0  
B. 1+  
C. 2+  
D. 3+

28. Technetium coordination compounds with a TcO\textsuperscript{3+} core are:

A. four coordinate.  
B. five coordinate.  
C. six coordinate.  
D. seven coordinate.

29. The general chemical structure of second-generation technetium-tagged compounds is:

A. Targeting Molecule – BFCA – Linker – Tc-99m.  
B. BFCA - Targeting Molecule – Linker - Tc-99m.  
D. Targeting Molecule – Tc-99m – Linker – BFCA.

30. Which one of the following does not describe a property of the \(^{99m}\text{Tc}\)-sestamibi radiolabeling process?

A. A heating step is required to release MIBI ligands.  
B. A displacement reaction occurs between MIBI ligands and \(^{99m}\text{Tc}\)-gluconate.  
C. The reducing agent is stannous citrate.  
D. The \(^{99m}\text{Tc}\)-MIBI complex contains a Tc\textsuperscript{+} core.

31. Which one of the following is not descriptive of \(^{99m}\text{Tc}\)-IDA analogues?

A. They are technetium-essential.  
B. They contain a Tc\textsuperscript{4+} core.  
C. They are kinetically inert.  
D. They are hexa-coordinate complexes.
32. Which one of the following statements is false regarding $^{99m}$Tc-DMSA preparation and use?

A. Tc(III)-DMSA is formed preferentially in alkaline pH.
B. Tc(V)-DMSA is used for localizing specific tumor types.
C. The kidney-localizing complex of $^{99m}$Tc(III)-DMSA is formed after a 10-minute incubation.
D. Ascorbic acid in the DMSA kit retards the oxidation of $^{99m}$Tc(III)-DMSA Complex II to Complex I.

33. All of the following are dimeric technetium complexes except:

A. $^{99m}$Tc-succimer.
B. $^{99m}$Tc-mebrofenin.
C. $^{99m}$Tc-mertiatiode.
D. $^{99m}$Tc-bicisate.

34. All of the following are neutral, lipophilic technetium complexes except:

A. $^{99m}$Tc-exametazime.
B. $^{99m}$Tc-sestamibi.
C. $^{99m}$Tc-bicisate.
D. $^{99m}$Tc-N-(NOEt)$_2$.

35. Which one of the following statements about $^{99m}$Tc-exametazime is false?

A. It’s retention in brain tissue is mediated by glutathione.
B. The kit contains a mixture of the D, L, and meso isomers.
C. Instability of the brain-localizing complex in vitro is mediated by stannous ion.
D. The complex is stabilized by methylene blue or cobaltous chloride.

36. Which one of the following statements about $^{99m}$Tc-bicisate is false?

A. Up to 50 mCi of $^{99m}$Tc-sodium pertechnetate can be added to the kit.
B. The kit requires 30 minutes to effect labeling.
C. The complex undergoes in vivo hydrolysis to $^{99m}$Tc-ethylenedicysteine.
D. Brain localization is species specific.

37. Which one of the following statements about $^{99m}$Tc-mertiatiode is false?

A. The complex is a tripeptide.
B. Oxygen is required in the kit to reduce the amount of radiochemical impurities.
C. Technetium is reduced by stannous ion and coordinated directly with the MAG3 ligand.
D. The complex is excreted unchanged by active tubular secretion.
38. All of the following complexes require technetium transfer ligands in their preparation except:

A. $^{99m}$Tc-apcitide.
B. $^{99m}$Tc-tetrofosmin
C. $^{99m}$Tc-mebrofenin.
D. $^{99m}$Tc-sestamibi.

39. Which one of the following statements does not apply to $^{99m}$Tc(V)-DMSA?

A. It forms three geometric isomers.
B. It is prepared at pH 2.
C. It requires oxygen during preparation.
D. Sodium bicarbonate is added to the kit during preparation.

40. During the labeling of $^{99m}$Tc-DMSA for kidney imaging which one of the following labeling steps is required to form the desired complex?

A. Addition of 2 mL of air following pertechnetate addition.
B. Adjustment of the pH to 8 with sodium bicarbonate.
C. Incubation for 10 minutes at room temperature.
D. Heating in a boiling water bath for 5 min.

41. $^{99m}$Tc-ethylenedicysteine is the diacid metabolite of which one of the following complexes?

A. $^{99m}$Tc-exametazime
B. $^{99m}$Tc-mertiatiide
C. $^{99m}$Tc-pentetate
D. $^{99m}$Tc-bicisate

42. All of the following require various co-ligands to satisfy technetium’s coordination sphere except:

A. Tc-HYNIC core complexes.
B. Tc(CO)$_3^+$ core complexes.
C. IDA complexes.
D. BATO complexes
43. Which one of the following statements is false regarding technetium chemistry in the $^{99m}$Tc-generator?

A. The amount of $^{99}$Tc isomer in the generator is always greater than the amount of $^{99m}$Tc isomer.
B. When incomplete elution of $^{99m}$Tc activity occurs, the activity retained in the generator may be recovered by re-elution of the generator within 1 to 2 hours.
C. Six hours after generator elution approximately 50% of the activity that will be available in 24 hours can be eluted.
D. Technetium-99m in the generator eluate is never “carrier-free”.

44. A generator received on Friday gives low $^{99m}$Tc labeling yields with a particular kit on Monday mornings. Other days of the week the labeling efficiency with this kit is acceptable. The most likely cause for low binding efficiency on Monday is:

A. low levels of stannous ion in the kit.
B. high levels of $^{99}$Tc in the generator eluate.
C. low levels of ligand in the kit.
D. high levels of free-radicals in the eluate.

45. Technetium in $^{99m}$Tc-sulfur colloid retains the oxidation state of pertechnetate because:

A. it is protected by being imbedded in elemental sulfur particles.
B. the colloid has a protective coat of negatively charged gelatin.
C. of its stability as an insoluble heptasulfide.
D. thiosulfate is able to maintain the oxidation potential of the system.

46. The presence of EDTA in the sulfur colloid kit:

A. acts as a buffer.
B. acts as a transfer ligand in the formation of $^{99m}$Tc-sulfur colloid.
C. prevents formation of aluminum phosphate.
D. controls particle size of the sulfur particles.

47. Which one of the following statements about labeling proteins/antibodies with technetium is false?

A. Direct labeling of antibody is superior to indirect labeling methods.
B. Technetium binding in antibodies is associated with high-affinity, low capacity sites and and low-affinity, high capacity sites.
C. Technetium binding sites in proteins are SH groups.
D. Use of unconjugated DTPA in the labeling mixture improves strength of the technetium bond but lowers radiochemical yield.
48. Which one of the following antibody labeling methods has been shown to reduce non-specific binding of technetium within the antibody?

A. Direct labeling using pre-tinned antibody.
B. Direct labeling mixing technetium with tin and antibody together.
C. Indirect labeling via a pre-labeled ligand.
D. Indirect labeling via an antibody-BFCA conjugate.

49. With the in vitro method of labeling red blood cells with technetium if the sodium hypochlorite step is left out, which one of the following effects would likely occur?

A. Red blood cells would hemolyze.
B. RBC labeling yield would decline.
C. Excess extracellular stannic ion would be present.
D. Oxidized technetium impurities would form in the extracellular fluid.

50. Which one of the following statements is false?

A. The efficiency of methods to label erythrocytes with technetium is, in vitro > modified in vivo > in vivo.
B. Technetium activity in labeled erythrocytes is bound to hemoglobin.
C. Leukocyte labeling with $^{99m}$Tc-HMPAO can be performed in 10 to 20% plasma.
D. Technetium activity in labeled leukocytes is bound to intracellular protein.