CHAPTER II

Theory

2.1 Technetium radiopharmaceuticals

Technetium radiopharmaceuticals are diagnostic imaging agents used in the field of nuclear medicine. They are used to visualize tissues, anatomical structure, and metabolic disorders[12]. The isotope of technetium utilized in nuclear medicine is technetium-99m. The metastable Tc-99m has a short half life of 6.02 hours with dominant single-photon emission having an energy of 140.6 keV. After intravenous administrations, Tc-99m radiopharmaceuticals localize in specific target organs, which can then be imaged using a gamma camera[13].

A wide variety of organs can be visualized with Tc-99m radiopharmaceuticals, including the kidneys, bones, lungs, heart, liver, brain and thyroid gland [14]. Millions of diagnostic imaging procedures are conducted each year. Over 80% involve the use of Tc-99m. Even with the advent of more sophisticated imaging procedures such as Positron Emission Tomography (PET) and Nuclear Magnetic Resonance (NMR) imaging, technetium radiopharmaceuticals remain the work house of nuclear medicine. Technetium radiopharmaceuticals will maintain this position for some time because they are more readily available and consist of less than other imaging procedures.

Technetium is not a naturally abundant element because the halflife of its most stable isotopes are short on geological time scale. It is indicated as element 43 on the periodic chart, directly under manganese, some of its properties were predicted by Mendeleev in 1869. He called this yet undiscovered element ekamanganese and gave it the symbol Em. Noddack and Tacke claimed to have isolated element 43 in 1925. But Perrier and Segre are universally credited with its discovery in 1937. They produced weighable quantities from transimulation reaction of deuterons and protons with molybdenum. After World War II, Perrier and Segre named element 43 technetium (pronunced tek-ne-she-m), from the Greek word technetos (meaning artificial), to commemorate the first artificial element[12].

Among the long-lived isotopes of Tc, the most readily available one is Tc-99 ($t_{1/2}$ = 2.13x10^5 year, β^- max = 292 keV). Since the first gram quantities of Tc-99 were synthesized by Cobble in 1952, the chemistry of technetium has been studied by few investigators. The chemistry of technetium is similar to its neighboring transition elements, manganese and rhenium [15]. Technetium gives rise to multiple oxidation states and forms coordination complexes with a variety of inorganic and organic ligands[16,17]. Many of the thermodynamically stable technetium compounds have oxygen bound to the technetium (e.g., TcO_4^- , $TcO_2 2H_2O$, TcOL, etc.).

Because technetium can form a wide range of stable Tc-99m complexes, its use in nuclear medicine has grown enormously in the past 10 years. Today Tc-99m is conveniently produced in hospitals or radiopharmaceutical laboratories by the β-decay of Mo-99 (Figure 2.1).

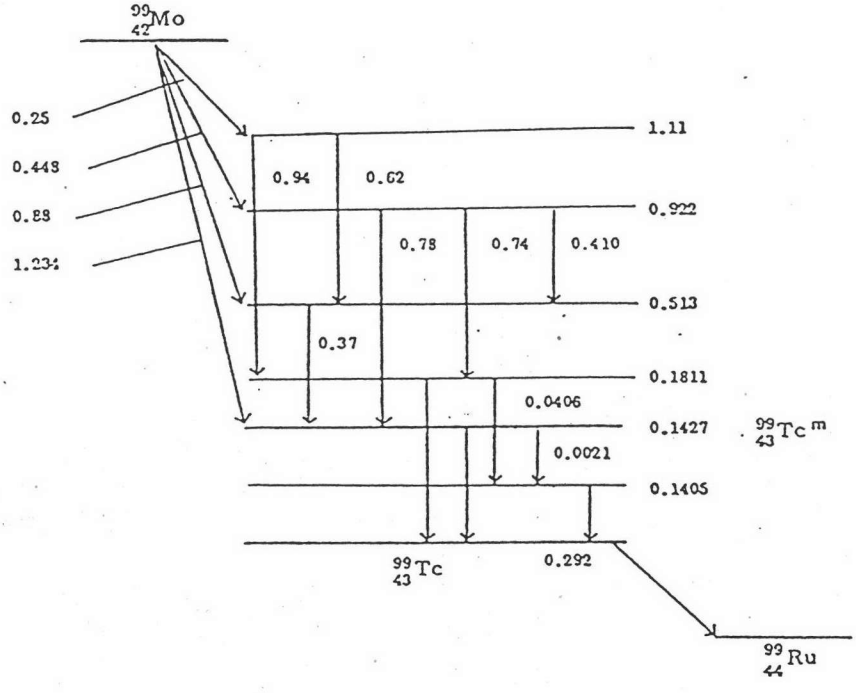


Figure 2.1 Decay scheme and energies (MeV) for molybdenum-99

Molybdate, $^{99}MoO_4^{-2}$, is absorbed on alumina columns encased in lead-shielded containers called generators. The Tc-99m in the form of pertechnetate ion (TcO^-_4) is eluted from the generators with saline solution. In most cases, the solutions of pertechnetate ion are simply injected into a kit. The kit is a sealed vial

containing a lyophilic mixture of a reducing agent (usually stannous chloride), the complexing ligand, and perhaps an antioxidants stabilizer. The reducing reagent reduces the technetium from a Tc(VII) oxidation state to some lower - oxidation states followed by technetium complexing with the ligand. The technetium complexes exhibit a variety of stoichiometries depending on the oxidation states[12]. The technetium(V) and technetium(IV) complexes are believed to have technetium-oxo cores (i.e., $TcOL_n$, TcO_2L_n, $TcO(OH)L_n$, $Tc_2O_2L_n$, etc.); whereas, the Tc(III) and Tc(I) complexes are known to have the ligand coordinated to the technetium alone (i.e., TcL_n, Tc_2L_n etc.). Since technetium can form multiple complex species, it is common that a reaction mixture with only one ligand can form many different complexes. The polynuclear formation of technetium species is usually minimized by keeping the technetium concentration as low as possible. In carrier - free preparation, where Tc-99 is termed the carrier, only the Tc-99m is initially present. In such preparations, the Tc-99m concentration may range from 10^{-7} to 10^{-5} M. Polynuclear complex formation (i.e., dimers, trimers, etc.) is generally unfavorable at such low metal concentrations. To prevent the formation of technetium complexes with more than one oxidation state, efforts are made to control the strength of the reducing agent and reaction conditions.

The biodistribution and accumulation of any substance in body tissue correspond to physical properties of the substance [18,19]. In the case of technetium complexes, the biodistribution depends on, (A) lipid solubility, (B) hydrated volume, (C) ionic charge, (D) protein binding, and (E) target site affinity.

2.2 Kidney Imaging

The kidney is the principle organ responsible for the excretion of water-soluble substances[19-21]. To some degree, all technetium radiopharmaceuticals will be excreted by the kidney and eliminated in the urine. The pattern and amount of such renal excretion can be used to evaluate renal function.

The functional unit of the kidney is called the nephron (Figure 2.2). It is composed of filtering apparatus so called the glomerulus, which is encapsulated in a structure called Bowman's capsule. Following the glomerulus is a multi-segmented tubule system which reabsorbs nutrients and secretes unwanted substances. The arterial blood enters the kidney and passes through the glomerulus, allowing water-soluble, low-molecular-weight species to undergo ultrafiltration. Molecular size, shape, and charge determine glomerular permeability. The basement membrane of the glomerulus, which makes up the filter structure, consists of pores with effective diameters of approximately 2Ø A°. The pores are lined with anionic

charges, thus it is easier for neutral and positively charged substances to pass through. Because plasma proteins are much larger than 20 A° and carried high negative charges, they will not cross the glomerular membrane structure. Dynamic imaging of the glomerular filtration process is normally carried out with Tc^{99m}-DTPA (Technetium-diethylene triamine pentaacetic acid). The Tc^{99m}-DTPA complex readily passes through the glomerular filtration apparatus ; In addition, because it is not reabsorbed into the blood on passing through the tubules, it flows into the urine. The rate of passage of the Tc^{99m}-DTPA through the kidneys becomes a measurement of renal clearance.

Substances which cannot pass through the glomerular filtration membrane are still remain in the blood but it can be eliminated in the urine by active secretion into the renal tubules (Figure.2.2). Uptake of ^{99m}Tc radiopharmaceutical in the renal tubule provides means of statically imaging of the kidneys. The complexes used to image static tubular fixation are indicated in Figure 2.2. The prolonged retention of static imaging agents is due to high binding affinities for sites in either the proximal or distal segments of the renal tubules. Binding sites in the renal tubules include thiol and disulfide moieties of various proteins. One such heavy metal binding protein, metallothionein, contains

50 mercapto groups per mole. Characteristics of the static kidney imagers includes high binding site affinity, plasma protein binding, and low liver uptakes.

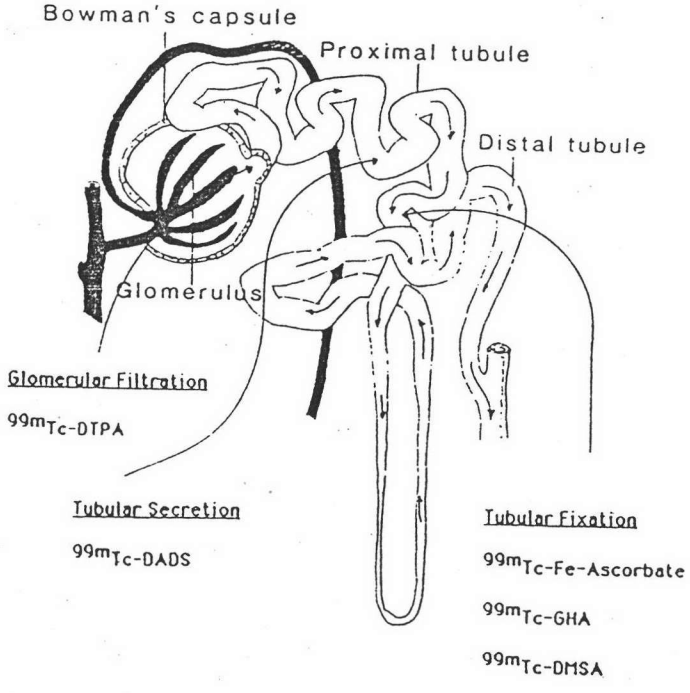


Figure 2.2 Schematic of a kidney nephron

Efforts have been made to design ^{99m}Tc radiopharmaceuticals that will image the dynamic function of renal tubular secretions. These complexes must have particularly high extraction efficiency for some active transport protein but must not be retained for prolonged periods, as are the static imagers. The active transport renal secretion agent was developed by Fritzberg and co-worker[1-3]. They have complexed technetium with MAG_3 (mercaptoacetylglycylglycylglycine) to produce ^{99m}Tc-MAG_3

([N-(N-(N-(mercaptoacetyl)glycyl)glycyl)glycinato(2-)N,N', N",S Oxotechnetate (2-)]), that exhibits active secretion by the renal tubules. This complex is as effective as ^{131}I o-iodohippuric acid, normally used to study renal tubular secretion, it will replace ^{131}I o-iodohippuric acid because the radiation hazard from ^{99m}Tc is less than ^{131}I [22].

2.3 Metal ion in biological system

Metals are essential for the operation of many vital biological processes. Biologically important metals range from the "bulk" metals, sodium, potassium, magnesium and calcium, which together constitute more than 99% of total metal-ion content of a living organism, to ions and the `trace' metals like manganese, cobalt, copper, molybdenum and zinc which are firmly bound within highly complex protein structures like the metalloenzymes and the haemoproteins(11).

The trace metals are associated with protein structures in many molecules fulfilling important biological function. In human body, the most commonly occurring metal is iron (the average amount in a 70 kg is 4.1 g); then come zinc (2.3 g) and copper (0.2 g). The other transition metals are present in amounts below 0.1 g per 70 kg.

In many cases, the exact role of the trace elements is still not completely known. However, the great

majority of these functions as the key components of essential of enzyme systems or of other proteins (for example the haemoprotein haemoglobin) which perform vital biochemical functions. The general roles of metal ions in biological processes are summarized in Table 2.1.

Table 2.1 General roles of metal ions in biological processes

	Na, K	Mg, Ca(Mn)	Zn, Cd(Co)	Cu, Fe Mo(Mn)
Type of complex Biological function	weak charge transfer, nerves	Moderate trigger reactions, hydrolysis phosphate transfer	Strong hydrolysis pH control	Strong oxidation and reduction
Ligand atom prefered	O	O	N and S	N and S

Copper-containing proteins are involved in a variety of biological functions. These functions include electron transport, copper storage and many oxidase activities. Several copper proteins are easily identified by their beautiful blue colour and have been labelled "blue-copper" proteins. The blue copper proteins can be divided into two classes, the oxidases and the electron carriers.

Copper(II) sites in proteins can be classified into three types based on their spectral properties. The blue (Type I) copper proteins are characterized by a visible absorption band near 600 nm with a high molar

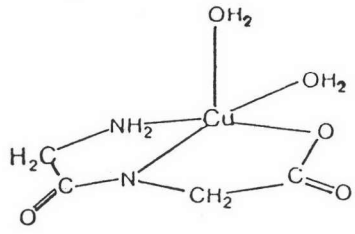
absorptivity. Type I copper protein are often referred to blue-copper proteins because their solutions are blue at typical enzyme concentrations of 10^{-4}-10^{-5} M employed in the laboratory.

Type II, copper(II) proteins, or low blue copper have less intense colours at normal concentrations. However, low blue-copper sites have quite high molar absorptivity when compared with simple copper(II) coordination compounds. Bovine erythrocyte superoxide dismutase (BSOD) is an example of the low blue copper protein with λ_{max}680 nm (ε = 300$M^{-1}cm^{-1}$)

Type III, copper(III) found in Rhus laccase is E.S.R. inactive, i.e. although copper(II) is present, no E.S.R. spectrum can be obtained. Recent magnetic susceptibility measurement on Rhus laccase indicated and antiferromagnetically coupled copper(II) dimer. Galactose oxidase may be the only non-blue protein which contains a single copper(II). The enzyme which catalyses the oxidation of galactose by molecular oxygen has mass of 68,000. The single copper(II) can be removed using diethyl dithiocarbanate ($HSSCNEt_2$) or H_2S. The apoenzyme is quite stable and the enzyme can be totally regenerated by the addition of copper(II).

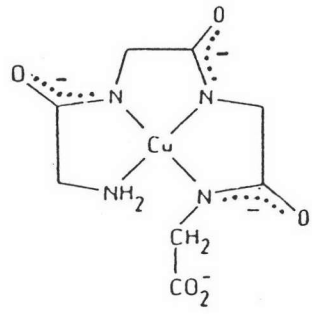
The binding site of copper ions in enzyme had been studied by x-ray crystallography. In blue copper protein, plastocyanin at the binding site are consisted of three

copper-N bonds and one copper-S bond. These observations are constant with tetrahedral distortion at the metal binding sites of blue copper proteins. At the metal binding site of enzyme consisting of peptides chain are the N-terminal or other terminal electron donor atom. When copper(II) is coordinated to oxygen and nitrogen ligands the geometry is usually tetragonal with four short and two long bonds (a result of Jahn - Teller distortion). One or both of the long bonds may be removed completely, or be outside the distance considered as a bond, barely forming a Van der Waals contact. The resulting square-planar and square-pyramidal geometries may be considered as a limiting case of a distorted octahedral. An empirical correlation between the stereochemistry of the copper(II) complexes of peptides and the ligand field effects produced by the four closest donor atoms has been reported. The six-coordinated complexes are blue-green (λ_{max} Ca.730 nm) five-coordinate species are blue (λ_{max} Ca.635 nm) and four-coordinated complexes are violet-pink (λ_{max} Ca.500 nm). Square-pyramidal copper occurs in the copper(II) complexes of glycine[A] and glycylglycylglycine [B] which have λ_{max} 635 nm and 555 nm respectively. In these structure the copper II ion is positioned slightly above the square plans in the direction of the fifth ligand. And tetraglycine with three deprotonated amide groups has λ_{max} 510 nm and also has the square planar structure (figure 2.3)



A.

Cu(glygly)



B.

Cu(GlyGlyGlyGly)

Figure 2.3 The structure of Copper(II) with polyglycine

2.4 The formation and stability of complex in solution

The thermodynamic stability of a complex can be indicated by an equilibrium constant, relating its concentration to the concentration of other species when the system has reach equilibrium [22,23].

If the solution containing metal ions , M and unidentate ligands, L only soluble mononuclear complex are formed, the system at equilibrium may be described by the following equations and equilibrium constants.

$$M + L \rightleftharpoons ML, \quad K_1 = \frac{[ML]}{[M][L]}$$

$$ML + L \rightleftharpoons ML_2, \quad K_2 = \frac{[ML_2]}{[ML][L]}$$

$$ML_2 + L \rightleftharpoons ML_3, \quad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$\vdots \qquad \vdots \qquad \vdots \qquad \vdots$$

$$ML_{n-1} + L \rightleftharpoons ML_n, \quad K_n = \frac{[ML_n]}{[ML_{n-1}][L]}$$

There will be n such equilibria, where n represents the maximum coordination number of the metal ion M for the ligand L and n may vary from one ligand to another. Another way of expressing the equilibrium relations can be shown as follows :

$$M + L \rightleftharpoons ML, \quad \beta_1 = \frac{[ML]}{[M][L]}$$

$$M + 2L \rightleftharpoons ML_2, \quad \beta_2 = \frac{[ML_2]}{[M][L]^2}$$

$$M + 3L \rightleftharpoons ML_3, \quad \beta_3 = \frac{[ML_3]}{[M][L]^3}$$

$$\vdots \qquad \vdots \qquad \vdots$$

$$M + nL \rightleftharpoons ML_n, \quad \beta_n = \frac{[ML_n]}{[M][L]^n}$$

Since there can be only n independent equilibria in such a system, it is clear that the Ki's and βi's must be related.The relationship is indeed rather obvious. Consider, for example, the expression for β_3 let us multiply both numerator and denominator by $[ML][ML_2]$ and then rearrange slightly :

$$\beta_3 = \frac{[ML_3]}{[M][L]^3} \cdot \frac{[ML][ML_2]}{[ML][ML_2]}$$

$$= \frac{[ML]}{[M][L]} \cdot \frac{[ML_2]}{[ML][L]} \cdot \frac{[ML_3]}{[ML_2][L]}$$

It is not difficult to see that this kind of relationship is perfectly general, namely.

$$\beta k = K_1 \cdot K_2 \cdot K_3 \ldots K_k = \prod_{i=1}^{i=k} K_i$$

The K_i's are called the stepwise formation constants and the β_i's are called the overall formation constants (or stepwise stability constant and overall stability constants); each type has its special convenience in certain cases.

Thus, typically, as a ligand is added to the solution of metal ion, ML is first formed more rapidly than any other complex in the series. As addition of the ligand is continued, the ML_2 concentration rises rapidly. While the ML concentration drops, then ML_3 becomes dominant, ML and ML_2 becoming unimportant, and so forth, until the highest complex ML_n is formed, to the nearly complete exclusion of all others at very high ligand concentrations. These relationships are conveniently displayed in diagram such as those shown in Fig.2.4

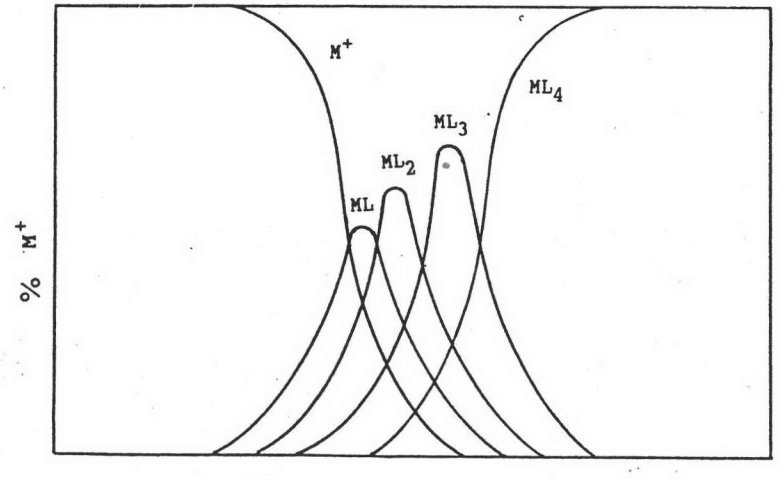


Figure 2.4 Relationships of species distribution

The stability constants has been determined by the various methods such as Spectroscopic, Distribution Electrochemical, Colorimetric and Miscellaneous methods.

Electrochemical, potentiometric titration have been and the most popular method for determination of stability constants because of its high accuracy and precision[26]. The computer programme, SUPERQUAD has been widely used to calculate stability constant of species in solution equilibria from data obtained by potentiometric method[24-27]. The formation constants are determined by minimization of an error-square sum based on measure electrode potentials. The program also permits refinement of any reactant concentration or standard electrode potential.The refinement is incorporated in to new procedure which can be used for model selection. The assumptions for computation of formation constants by SUPERQUAD could be described as follows.

Assumptions: There are a number of assumptions underlying the whole treatment, and each needs to be considered explicitly.

1. For each chemical species $A_aB_b...$ in the solution equilibria there is a chemical constant, the formation constant, which is expressed as a concentration quotient [equation(1)].

$$\beta_{ab\cdots} = \frac{[A_aB_b\cdots]}{[A]^a[B]^b\cdots} \quad (1)$$

A,B... are the reactants (SUPERQUAD allows up to four of them) and [A],[B] are the concentrations of 'free' reactant, electrical charges may be attached to any species, but they are omitted for the sake of simplicity in this discussion. Since the thermodynamic definition of a formation constant is as an activity quotient, it is to be assumed that the quotient of activity coefficients is constant, an assumption usually justified by performing the experiments with a medium of high ionic strength.

2. Each electrode present a pseudo-Nernstian behaviour, equation(2), where [A] is the concentration of the

$$E = E^{\bullet} + S_L \log[A] \quad (2)$$

electro-active ion, E is the measured potential, and $E^{\bullet}$ the standard electrode potential. The ideal value of the slope, S_L, is of course RT/nF, but we assume only that it is a constant for a given electrode. The values of $E^{\bullet}$ and S_L are usually obtained in a separate calibration experiment. If a glass electrode is calibrated in terms of hydrogen-ion concentrations, following Irving et al.[28], the equivalent potential is presented in equation 3.

$$E = 1\emptyset + RT/nF \times pH \quad (3)$$

The alternative calibration method should be measured in volts and E^+ are determined by Gran plot from

a titration of strong acid and base [29].

3. Systematic errors must be minimized by careful experimental work. Sources of systematic error include electrode calibration, sample weighings and dilutions, standardization of reagents (use of carbonate-free alkali in particular), temperature variation and water quality. The last-named factor is more significant today than it was in the past, as water may be contaminated by titratable species which can pass through distillation columns by surface action. All statistical test are based on the assumption that systematic errors are absent from the data.

4. The independent variable is not subject to error. The errors are assumed to have a normal distribution. If these assumptions are true use of the principle of least squares will yield a maximum likelihood result, and computed residuals should not show systematic trends [30].

5. There exists a model of the equilibrium system, which adequately accounts for the experimental observations. The model is specified by a set of coefficients a,b..., one for each species formed. All least-squares refinements are performed in terms of an assumed model. Examination of a sequence of models should yield a 'best' model which is not significantly different from the 'true' model. Choice of the best model is known as 'species selection'.