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***Inorganic Chemistry: Fundamental Principles as
Applied to the Development and Application of
Metalloradiopharmaceuticals***

Continuing Education for Nuclear Pharmacists and Nuclear Medicine Professionals

By

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INORGANIC CHEMISTRY: FUNDAMENTAL PRINCIPALS AS APPLIED TO THE DEVELOPMENT AND APPLICATION OF METALLORADIOPHARMACEUTICALS

STATEMENT OF OBJECTIVES

1. Discuss the basic principles of thermodynamic and kinetic stability of metal complexes.
2. Discuss the fundamentals for labeling ligands and proteins with radiometal ions such as ^{99m}Tc , ^{188}Re , ^{64}Cu , ^{68}Ga , ^{111}In .
3. Discuss aspects that affect specific activity of radiolabeled products and their stability *in vitro* and *in vivo*.

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INORGANIC CHEMISTRY: FUNDAMENTAL PRINCIPALS AS APPLIED TO THE DEVELOPMENT AND APPLICATION OF METALLORADIOPHARMACEUTICALS

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Inorganic chemistry can be broadly defined as the chemistry of elements where the focus is not on carbon-carbon bonds (organic chemistry). It includes the chemistry of the main group elements (e.g., P, S, Cl, Ar) as well as the chemistry of the metals (e.g., K, Cu, Al) and the metalloids (e.g., Si, As, Te).

In general, metals differ from other elements of the periodic table in several important ways. In their elemental state, metals are typically shiny, ductile, malleable, and conduct electricity. They can be oxidized to cations but do not usually exist as anions. For some metals (e.g., sodium) this reaction can be explosive, while other metals (e.g. gold) are inert to all but the strongest oxidizing agents.

Metalloradiopharmaceuticals are by far the most important group of single-photon radiopharmaceuticals with $^{123/131}\text{I}$ -labeled compounds being the only non-metal single-photon radiopharmaceuticals commonly encountered. Technetium-99m compounds, which are discussed in the accompanying monograph, comprise more than 90% of all metalloradiopharmaceuticals. Other important metal radionuclides include ^{67}Ga and ^{111}In for diagnosis and ^{153}Sm and ^{90}Y for therapy. At the present time, there are relatively few positron-emitting metalloradiopharmaceuticals, but interest in these compounds is increasing as the availability of ^{64}Cu and $^{94\text{m}}\text{Tc}$ improves.

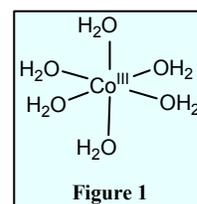
Metals are also important in other types of pharmaceuticals, such as platinum in cisplatin, a potent anti-cancer drug, and gadolinium, used in several MRI contrast agents. From a chemist's point of view, however, the best thing about metal chemistry is that it's fascinating.

METAL COMPOUNDS

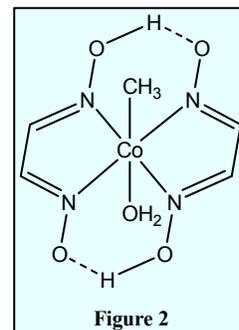
Metals exist in several different ways, as the native metal, as simple salts, (i.e. NaCl) and as compounds with various types of ligands. These compounds may be either coordination compounds or organometallic compounds, in which the ligand forms covalent bonds with the metal.

Coordination compounds are formed when an atom donates an electron pair to a metal atom. This is distinct from a covalent bond, where each atom donates a single electron to form the bond. The molecules that supply the atoms with which to form coordination compounds are called ligands. Ligands may be as simple as a water molecule, which binds to the metal through one of the two free electron pairs on the oxygen atom, or as complex as a hemoglobin, which contains a metalloporphyrin at its active site. The scope of this paper is confined to this subset of inorganic chemistry because this class of compounds comprises the majority of the metalloradiopharmaceuticals, metallopharmaceuticals, as well as most metalloproteins.

An example of a simple coordination compound is $[\text{Co}^{\text{III}}(\text{H}_2\text{O})_6]^{3+}$ (Fig. 1) where the a water molecule is located at each of the six vertices of the octahedron.



Organometallic compounds are formed when covalent bonds between ligands and metal atoms are present. A simple example of an organometallic compound is $[\text{Ni}(\text{CO})_6]^0$ (CO = carbon monoxide), where there are 6 CO molecules in an octahedral arrangement around the Ni^0 core. Both coordinate and covalent bonds can be present in the same compound, as in $[\text{Co}(\text{methyl})(\text{dmg})_2(\text{H}_2\text{O})]$ (dmg = dimethylglyoxime) (Fig. 2). In this octahedral compound there are five coordinate bonds, four N atoms in the equatorial plane and an apical water molecule, as well as a covalently bound methyl group at the other apex. This class of compounds has been used as models for vitamin B₁₂.



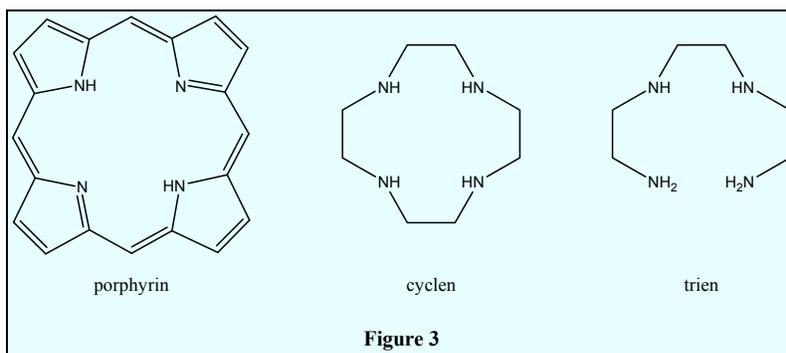
CHELATES

An important aspect of coordination chemistry is the concept of a chelate. A chelate is simply a ligand that coordinates to the metal through more than one binding site. It should not be surprising that the larger the number of binding sites, the more tightly the chelate binds to the metal. A classic example is that of amine ligands binding to Ni^{2+} . The simplest monodentate amine ligand is ammonia (NH_3). The stability constant for $[\text{Ni}(\text{NH}_3)_6]^{2+}$ is $10^{8.6}$. But if the six NH_3 ligands are replaced by three ethylenediamine ligands ($\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$), the stability constant increases to $10^{18.3}$, an increase of almost 10 orders of magnitude. The number of donor atoms on a chelate is its “denticity”. Thus ethylenediamine, which has two N donor atoms, is bidentate.

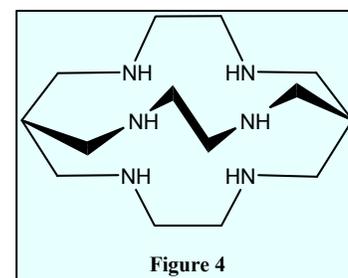
In the development of radiopharmaceuticals, the chelate effect is used to great advantage when selecting chelating agents with which to attach metals to proteins. For example, ^{111}In is tightly retained by DTPA (DTPA=diethylethylenetriaminepentaacetic acid), the octadentate chelator in Octreoscan.

A simple way to understand the reason for the increased stability is to imagine one end of the chelator tethered to the metal while the other end is free. The effect of the tether is to increase the local concentration of the untethered donor atom, increasing the chances that it will bind to the metal. However, the chelate effect is not without constraints. If the length of the linkage between the donor atoms is too long, the local concentration of the second donor atom is not increased as much. Also, as the number of atoms in the chain increases, it becomes increasingly difficult to fit all of them into the space between two binding sites on the metal, creating steric strain.

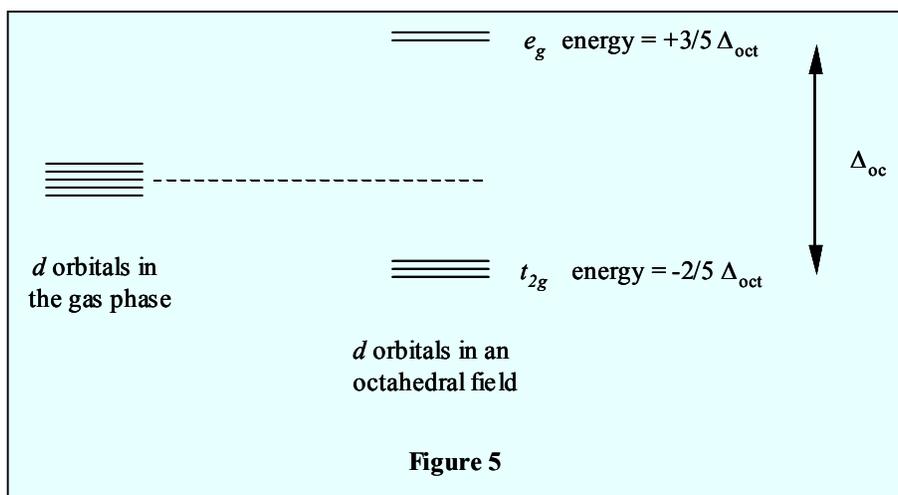
A special case of chelators is macrocycles. A macrocycle is simply a chelate that wraps completely around the metal and closes at the other end. Biologically, the most obvious example of a macrocycle is a porphyrin (Fig. 3), which is found at the core of hemoglobin. Chemically, a common example is cyclen, which is simply the closed version of trien (Fig. 3). The increased stability conveyed by closing the ring can be seen by comparing the stability constants for the Zn^{2+} complexes of trien and cyclen, $10^{11.25}$ and $10^{15.34}$, respectively². The increased stability of macrocycles versus their non-closed analogs can be understood by thinking of the loss of the ligand from the metal as an unwrapping process. If there is no open end to the ligand, it is more difficult for competing ligands to unwrap the ligand from the metal.



Macrocycles are important in drug development because they provide a way to sequester metals that are otherwise too labile to be used *in vivo*. A fascinating example of a macrocyclic ligand is the sarcophanes developed by Sargeson³ in which the metal is completely enclosed by three connected rings of donor atoms (Fig. 4). This ligand has proved particularly useful in complexing metals such as Cu^{2+} that are extremely labile *in vivo*⁴.



TRANSITION METALS



The transition metals include the elements in groups 3 through 11 in the periodic table. The IUPAC defines transition metals as “elements whose atoms have an incomplete d sub-shell or which can give rise to cations with an incomplete d sub-shell”. This definition is

significant because the electronic effects of the incompletely filled *d* orbitals determine the chemical properties of transition metal compounds. A comprehensive discussion of this topic is beyond the scope of this manuscript, but several features are relevant to the chemistry of radiopharmaceuticals.

There are five *d* orbitals, each of which can contain a maximum of two electrons, 10 electrons total. In the gas phase, the energy of these five orbitals is equal. But in an octahedral ligand environment, such as is frequently observed for transition metals, the five *d* orbitals split into three *t_{2g}* and two *e_g* orbitals (Fig. 5). The difference in energy between the two sets of orbitals (Δ_{oct}) is determined both by the metal and by the ligands coordinating the metal. This spacing, in turn, determines the order in which the *d* orbitals are filled with electrons. Ligands that induce large values of Δ_{oct} are called “strong field ligands” while those that induce small values of Δ_{oct} are called “weak field ligands”. Examples of strong field ligands are NO_2^- and CN^- . Examples of weak field ligands are Cl^- , Br^- , and I^- .

With strong field ligands, where the value of Δ_{oct} is large, the electrons fill all the *t_{2g}* orbitals before beginning to fill the *e_g* orbitals. This has several consequences, but the one that is most relevant to drug development is that adding electrons to the *t_{2g}* orbitals increases the kinetic stability of the complexes. Thus metal complexes of strong field ligands with three electrons in the *d* orbitals (d^3 , e.g. Cr^{3+}), where each of the three *t_{2g}* orbitals contains a single electron, are substitution inert. On the other hand, complexes with partially populated *e_g* orbitals are more labile. An example of this is seen with Cu^{2+} , which has nine *d* electrons (d^9), six electrons in the *t_{2g}* orbitals and three in the *e_g* orbitals. As a result, Cu^{2+} complexes are among the most labile of all transition metal complexes, which is a significant problem in the development of new ^{64}Cu or ^{67}Cu -based radiopharmaceuticals.

Transition metals in the second and third row (Y through Ag and La through Au) are more kinetically stable than those in the first row because the values of Δ_{oct} are larger, thus favoring the population of the t_{2g} over the e_g orbitals. For example, in the Ni, Pd, Pt series, Pt^{2+} complexes are typically more stable than Pd^{2+} complexes, which are more stable than Ni^{2+} complexes.

Transition metals are also different from other metals in that they can exist in a wider range of oxidation states. Thus while Ga, a non-transition metal, is almost always present as Ga^{3+} , Mn, which is a transition metal, can exist in oxidation states ranging from II to VII.

The ligands are also important in determining the relative stability of the different oxidation states of transition metals, and changes in the ligand can be used to optimize the biological properties of a radiopharmaceutical. An interesting example of this is the way in which the *in vivo* stability of the $^{64}\text{Cu}^{2+}$ complexes of the ligand PTSM (PTSM = pyruvaldehyde bis(N^4 -methylthiosemicarbazone)) varies with changes in the ligand substituents. The $^{64}\text{Cu}^{2+}$ atom in $[\text{Cu}^{\text{II}}(\text{PTSM})]^{0}$ ($E_{1/2} = -208$ mV) is rapidly reduced to $^{64}\text{Cu}^{1+}$ *in vivo* and lost from the complex, but $[\text{Cu}(\text{ATSM})]^{0}$ ($E_{1/2} = -278$ mV) is stable *in vivo*. $[\text{Cu}(\text{ATSM})]^{0}$ is, however, reduced and subsequently trapped in hypoxic regions of a tumor, thus providing a potential PET agent for hypoxia⁵.

Redox chemistry is the core of the utility of $^{99\text{m}}\text{Tc}$ radiopharmaceuticals. Technetium-99m is eluted from the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator as $\text{Tc}^{\text{VII}}\text{O}_4^-$ and is usually reduced with Sn^{II} to a lower oxidation state in the presence of a ligand to form $^{99\text{m}}\text{Tc}$ radiopharmaceuticals.

KINETICS AND THERMODYNAMICS

It is important to differentiate between the concepts of thermodynamic and kinetic stability.

Thermodynamics (and $\log K$ values) tells you where the equilibrium lies. Kinetics, on the other hand, tells you how quickly you can achieve (or disrupt) the equilibrium. Thermodynamic stability is usually described in terms of the association constant (K) between the ligand and the metal. For example, Cu^{2+} -DOTA ($\text{H}_4\text{DOTA} = 1,4,7,10$ -tetraazacyclododecane-1,4,7,10-tetraacetic acid), which is frequently used to label proteins with ^{64}Cu , has a very high stability constant ($\log K = 22.25$)⁶.



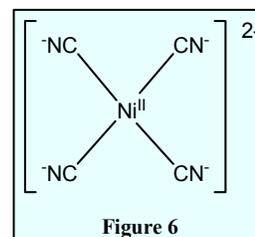
$$K = \frac{[\text{Cu-DOTA}]}{[\text{Cu}][\text{DOTA}]}$$

In vivo, however, a significant amount of ^{64}Cu is lost from the Cu-DOTA complex and accumulates in the liver, more so than might be expected from the high association constant. The primary reason for this is the very high (kinetic) lability of Cu^{2+} . Because the Cu-DOTA complex is in equilibrium with Cu^{2+} , there is always a small, but finite, amount of free Cu^{2+} available to be complexed by endogenous copper carrying proteins. When this free Cu^{2+} is removed from the system by the proteins, the equilibrium is rapidly reestablished, providing yet more free Cu^{2+} to be scavenged by proteins. In contrast, if the Cu^{2+} were to be replaced by a less labile metal (e.g., Co^{3+}), even though endogenous proteins might scavenge the free Co^{3+} , the kinetic inertness of Co^{3+} would mean that the equilibrium would not be quickly reestablished, limiting loss of Co^{3+} from the system.

GEOMETRY

The geometry of non-transition metal complexes is dominated by the relatively simple concept of how to arrange a given number of “objects”, which may be ligands or electron pairs, around a sphere so that their interactions are minimized. For example, the optimal arrangement for four objects around a sphere is a tetrahedron, and this is the geometry that is observed for four ligands around a non-transition metal such as Zn^{2+} (e.g., $[\text{Zn}(\text{NH}_3)_4]^{2+}$).

The geometry of transition metal complexes is considerably more complicated. There are two primary factors that influence the conformation of ligands around a transition metal core: the oxidation state of the metal, and thus its electronic configuration, and the ligands themselves. The electronic configuration of the metal is important because it determines which of the d orbitals are occupied, which in turn determines where the ligands can best fit around the metal center. The ligands themselves are important because, as discussed above, they affect the ligand field (Δ_{oct}) and, therefore, the order in which the d orbitals are filled. For example, $[\text{Ni}^{\text{II}}(\text{CN})_4]^{2-}$ (Fig. 6), which has a d^8 electronic configuration, exhibits square planar rather than tetrahedral geometry because of the way in which the d orbitals are populated in the presence of a strong field ligand such as CN^- .



Even within a given geometry, inorganic complexes can exist in a variety of isomers based on the arrangement of the ligands around the metal. The simplest case is *cis* and *trans* isomers in square planar complexes, as shown in Figure 7, which illustrates the *cis* and *trans* isomers of dichlorodiaminoplatinum(II). The *cis* isomer is “cisplatin”, the anticancer drug, which is extremely effective against testicular cancer, while the *trans* isomer is ineffective.

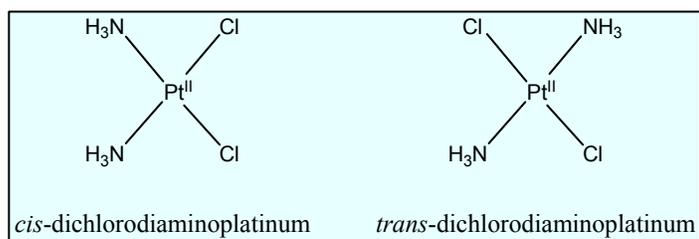


Figure 7.

Octahedral complexes can also exist as geometric isomers. In Figure 9, the two chloro ligands are adjacent to each other along one edge of the octahedron in the *cis* structure but opposite each other, at the two apices of the octahedron, in the *trans* structure, while the four ammine ligands occupy the equatorial positions.



Figure 8.

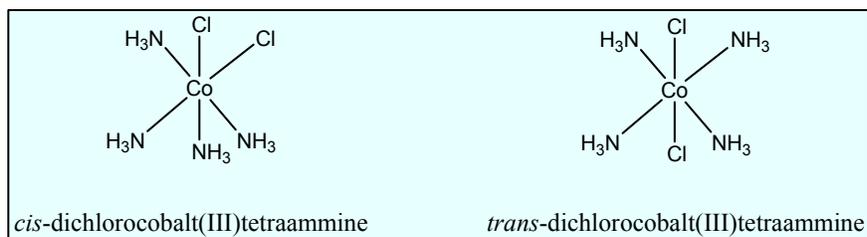


Figure 9.

Another example of geometric differences in octahedral complexes is *mer* (meridonal) and *fac* (facial) isomers (Fig. 8). In the *mer* isomer (right), the three chloro ligands are arranged around the meridian of the octahedron, while in the *fac* isomer (left), they're all on a single face of the octahedron.

Inorganic complexes can also exist as stereoisomers, where the arrangement of the ligands around the metal results in a pair of non-superimposable mirror images (Fig. 10). One way to look at an

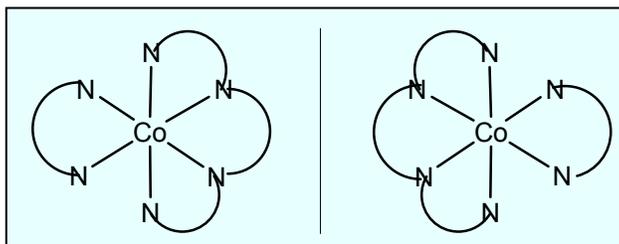


Figure 10.

enantiomeric pair of octahedral complexes is as a pair of screws, one right-handed (on the left in Fig. 10) and the other left-handed (on the right in Fig. 10).

The physical properties of two enantiomers compounds are identical, but the biological properties may be different, since biological processes are frequently stereospecific.

SUMMARY

The material presented here is a very brief summary of a very diverse subject. A 5-6 page monograph (or a 45-minute lecture) cannot begin to cover the diversity of the field. The best one can hope for is to whet a few readers (or listeners) appetites. Those whose appetites are whetted are referred to “Comprehensive Inorganic Chemistry” by Cotton and Wilkinson as a starting point for more in-depth discussion of the topics outlined above.

Aside from the many details of the topics outlined above, perhaps the most important thing to remember is that inorganic chemistry can be endlessly intriguing (and frustrating), with the myriad shapes and colors of transition metal complexes and the flexibility to design drugs ranging from those that cure cancer (cisplatin) to those that can be used to diagnose myocardial ischemia (Cardiolite[®]) or cancer (Octreoscan[®]).

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ASSESSMENT QUESTIONS

1. The most important radiopharmaceuticals are metal radionuclides like technetium Tc-99m. Which of the following radiopharmaceuticals includes a metal radionuclide?
 - a. I-123 sodium iodide
 - b. F-18 FDG
 - c. Y-90 Ibritumomab tiuxetan
 - d. Xe-133 xenon gas
2. An important aspect of coordination chemistry is the concept of chelate. A chelate is a ligand that coordinates to the metal through _____ binding site(s).
 - a. one
 - b. eight
 - c. more than one
 - d. six
3. A transition metal is defined as an element whose atoms have an incomplete *d* sub-shell. The electronic effects of the incompletely filled *d* orbitals determine the chemical properties of transition metal compounds. Adding electrons to the t_{2g} orbitals
 - a. increases the kinetic stability of the complex.
 - b. increases the lability of the complex.
 - c. increases the thermodynamic stability constant (K) of the complex.
 - d. stabilizes the oxidation state of the complex.
4. Transition metals can exist in a wide range of oxidation states. The binding ligands can also determine the relative stability of the different oxidation state of transition metals. Changes in the ligand are used to
 - a. optimize the in vitro stability of metalloradiopharmaceuticals.
 - b. optimize the biological properties of a radiopharmaceutical.
 - c. increase the useful life of compounded radiopharmaceuticals.
 - d. render a radiopharmaceutical substitution inert.
5. The geometry of transition metal complexes is influenced by the ligands themselves and
 - a. the order in which the d orbitals are filled.
 - b. the isomeric state of the transition metal.
 - c. the conformation of the ligand around the metal core.
 - d. the ground state of the transition metal.