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Metal Speciation in Health and Medicine Represented by Iron and Vanadium

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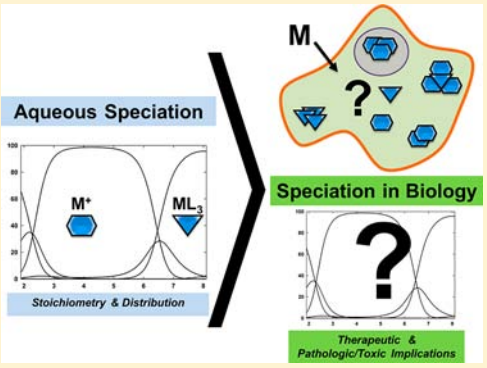
ABSTRACT: The influence of metals in biology has become more and more apparent within the past century. Metal ions perform essential roles as critical scaffolds for structure and as catalysts in reactions. Speciation is a key concept that assists researchers in investigating processes that involve metal ions. However, translation of the essential area across scientific fields has been plagued by language discrepancies. To rectify this, the IUPAC Commission provided a framework in which speciation is defined as the distribution of species. Despite these attempts, contributions from inorganic chemists to the area of speciation have not fully materialized in part because the past decade's contributions focused on technological advances, which are not yet to the stage of measuring speciation distribution in biological solutions. In the following, we describe how speciation influences the area of metals in medicine and how speciation distribution has been characterized so far. We provide two case studies as an illustration, namely, vanadium and iron. Vanadium both has therapeutic importance and is known as a cofactor for metalloenzymes. In addition to being a cation, vanadium(V) has analogy with phosphorus and as such is a potent inhibitor for phosphorylases. Because speciation can change the metal's existence in cationic or anionic forms, speciation has profound effects on biological systems. We also highlight how speciation impacts iron metabolism, focusing on the rather low abundance of biologically relevant iron cation that actually exists in biological fluids. fluids. Furthermore, we point to recent investigations into the mechanism of Fenton chemistry, and that the emerging results show pH dependence. The studies suggest formation of Fe^{IV}-intermediates and that the generally accepted mechanism may only apply at low pH. With broader recognition toward biological speciation, we are confident that future investigations on metal-based systems will progress faster and with significant results. Studying metal complexes to explore the properties of a potential "active species" and further uncovering the details associated with their specific composition and geometry are likely to be important to the action.



INTRODUCTION

Because the influence of metal ions and metal complexes in biology has become more and more apparent within the past century,[1−8] further consideration of the detailed structure and composition of these systems is paramount. Through coordination chemistry, metal ions can form various complexes in biology with proteins or cellular components of high molecular mass (hmm) or small-molecule metabolites referred to as low molecular mass (lmm). Researchers often focus on identifying the potential complexes that are critical to activity, known as the "active species", and the details associated with the specific composition and geometry of this state. Pharmacologists investigate the metabolism a drug undergoes upon administration, a significant consideration during drug design. Commonly the metabolism of organic drugs involves the generation of CO_2 and/or predictable carbon-based decomposition products. In contrast, the metabolism of metal-based therapeutics will generate multiple and variable metal compounds resulting from hydrolysis and redox reactions with the original metal complex. This difference is profound because the metabolism of the original metal complex may result in new active metal-containing materials. These may demonstrate high persistence and differences in cellular and organ localization as well as the potential to substantially impact other off-target pathways. These examples illustrate the uniqueness of metal speciation and its central role in biology. In this manuscript, we will review the definitions for speciation by describing the biological activities of two representative metal ions that illustrate the different responses that metal coordination complexes can exert.

Guidelines by IUPAC defining the term speciation and the processes involved in chemical speciation and the fractionation of elements were created in 2000.[9] In the IUPAC report, they

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recommended that the term "speciation" be used to indicate species distribution and "speciation analysis" be used to indicate the analytical activity of identifying and/or measuring the quantities of one or more chemical species in a sample. Both of these definitions require analytical identification and measurement of the different species within the samples. Some methods of analysis can cause fractionation of an analyte or a cluster of analytes and are distinct from speciation.[9−14] Analytical chemists have undertaken the difficult task of biological metal speciation and in the process have initiated the development of the *metallome*.[10,11,15] The *metallome* requires characterization of all forms of metal ions and their particular species within a certain tissue, cell type, or organism. Because metal coordination complexes have unique reactivity, these studies would benefit from an in-depth understanding of the properties of these compounds. The inorganic and coordination chemist's expertise in characterizing the composition, reactivity, and stereochemistry of metal compounds will enhance speciation studies by further interpretation of the data now achievable because of the state-of-the-art novel analytical methods.

The existing definitions for speciation attest to the emerging, widespread recognition that the specific form of a biologically active metal complex will significantly affect bioavailability, transport, activity, and excretion.[9−11,13,16−19] Classical solution chemists define speciation based on strict composition in terms of the molecular formula.[17,20,21] This definition is based on potentiometric measurements of reactions observing changes in H^+. Recent applications, however, have included the incorporation of other spectroscopic methods.[21−24] More broadly, the chemical speciation definition has recently been extended to include the composition, concentration, and oxidation state of the element forming a compound in a sample.[17,25] The broad and often unspoken definition of speciation generally used in coordination chemistry includes complexes that can form in solution reactions resulting from hydrolysis, redox reactions, ligand coordination, and geometric isomerization reactions.

Coordination complexes are subject to solvent exchange, which in biological systems is water exchange.[26−33] Biologically relevant metal−ligand equilibria are governed by the reaction exchange mechanism and the relevant ligand (often water) exchange rate constants. Briefly stated, for d^0 and d^{10} ions, first-order water-exchange rate constants are proportional to ionic radii and approach 10^9−10^{11} s^{-1} for the largest ions. For other divalent d^n first-row transition-metal ions, the rate constants vary from 10^2 to 10^9 s^{-1}. General mechanistic considerations exist for these exchange processes: dissociative, associative, or interchange. For many of these ions, exchange occurs via a dissociative (D) substitution mechanism with an exchange rate constant of the water ligand that is independent of the nature of the incoming ligand. However, for some ions, substitution reactions are dependent on the nature of the incoming ligand, and these are classified as associative (A) substitution mechanisms. The aforementioned mechanistic designations represent mechanistic "extremes" and only occur in a few exchange processes. It is now well recognized that most exchange processes occur via intermediate (I_a or I_d) pathways, which are a combination of both A and D character. The subscripts indicate which mechanistic feature predominates. For several small or highly charged ions, base hydrolytic pathways exist for ligand substitution and can become rate-limiting with non-base-catalyzed substitution reactions.[30] The metal−ligand equilibria and the redox properties are unique to each metal complex and can be fine-tuned by the formation of coordination complexes.

Classical solution speciation studies demand conditions with constant ionic strength and are devoid of interference from CO_2. As a result, such speciation studies do not represent the conditions of the biological environment well.[17,20,21,25] Recent advances in this field have been carried out under conditions at an ionic strength approaching that of biological fluids. In addition, efforts have developed methods using a combination of titration studies and spectroscopic observations, leading to holistic characterizations of the systems at hand.[17,25,34,35] The increasing sensitivity of instrumentation and availability of analytical characterization have permitted dramatic progress in the past decade,[9,10,12−15,20,36,37] leading to in-depth characterization of the molecular structure of many systems.[38,39] These new technologies are mainly focused on the detection of stable entities. Advances in labile species detection of dynamic systems have not increased at a similar rate. In the absence of such data, the focus and importance attributed to the role of coordination chemistry have been lacking. When evaluating the speciation of labile coordination complexes in biological systems, researchers must resort to predictions and computer modeling.[10,17,25] Considering the complexity of the systems and because computer modeling requires assumptions and simplifications, such studies lose credibility unless some experimental validation accompanies the modeling.

Our objective is for this review to reach inorganic chemists and other life-science researchers and to promote further discussion for future characterization of transition-metal-containing biological systems. In this manuscript, we chose two fundamentally different metals, vanadium and iron, as case studies to describe two examples on transition-metal speciation in biology. These metals were selected because they provide information with regard to the vital role that the hmm and lmm ligands play in distribution, metabolism, uptake, and interactions with the target. Furthermore, these two metals represent the trace metal with druglike activity (vanadium), as well as the essential and exceedingly complex metal ion (iron) involved in multiple aspects of life. The speciation of dynamic systems is and will continue to be critical to the in-depth understanding of drugs and metal metabolism and the increased understanding of compartmentalization in biological responses.

DEFINITION OF CHEMICAL AND STRUCTURAL SPECIATION

Classical solution speciation chemists measure speciation using titrations and potentiometry resulting in species with defined stoichiometry.[21,34,35,40−42] Equation 1 shows H^+, a metal ion (M), and a ligand (L) forming a complex with the stoichiometry defined by p, q, and r in an equilibrium reaction, respectively. The resulting formation constant $\beta(p,q,r)$ is shown in eq 2, where the concentrations of H^+, M, and L are multiplied with each other and divided by the concentration of the complex. The values for p, q, and r will be determined by a titration followed by evaluation using computations in an iterative process. A limitation on such studies is that only reactions with changes in H^+ can be observed and that hydration (i.e., the number of water molecules) cannot be measured. Because hydrolysis reactions do not involve L, these complexes will be described as (p, q, 0), where p is the coefficient for H^+, q is the coefficient for the metal, and the formation constant is defined by the concentration of the

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respective precursors. The complexes are given the notation (p, q, r). Such speciation studies will result in a series of constants that represent the system and allow for prediction of species distribution at defined parameters.

$$pH^+ + qM + rL \rightleftharpoons (H^+)_p(M)_p(L)_r \quad (1)$$

$$\beta(p, q, r) = \frac{(H^+)_p(M)_p(L)_r}{(H^+)^p(M)^p(L)^r} \quad (2)$$

In 2000, three IUPAC Divisions (the Commission on Microchemical Techniques and Trace Analysis, the Commission on Fundamental Environmental Chemistry, and the Commission on Toxicology) collaborated and created a document outlining standard terminology important to interdisciplinary communication and communication to nonscientists such as legislators and consumer groups.[9] One of the goals of this work was to help bring an end to the confusion regarding the usage of the term speciation. The report described some of the history prior to 2000 and, as such, provided background information for understanding some of the important contributions leading to the document. In particular, a 1984 workshop on The Importance of Chemical Speciation in Environmental Processes led to a report by Bernhard, Brinckman, and Sadler describing four possible meanings of speciation. Briefly they defined speciation as describing (i) reaction specificity, (ii) organism evolution, (iii) cycling of an element within geological and environmental chemistry, and (iv) component or ligand distribution within a system of a defined chemical species (inorganic chemistry).[16] Two of these (i and ii) are less commonly used by chemists. For example, speciation is rarely used to mean "reaction specificity".[9,16] In biology, speciation is used to describe the evolutionary process through which new species arise and includes the field of evolutionary biology. In particular, the concept of ecological speciation has been gaining momentum where environmental factors influence the onset of genetic expression and, over time, divergence of species.[43] Importantly, this early report concluded that authors should either avoid the term speciation or clearly define its use.[16]

Within the past decade, analytical chemists have undertaken the difficult task of developing what is now known as the *metallome*, the array of biological metal speciation.[10,11,15,44,45] The goal of the *metallome* is to include all biological metals and their species within certain tissues, cell types, or bodies. As highlighted in the IUPAC report, the previous use of the term "speciation" in these and other studies is used to indicate the analytical activity of identifying chemical species and measuring their distribution.[9] Considering the major advances in the past decade and improving methods for identifying and measuring decreasing amounts of material, one common term in addition to speciation is "fractionation".[9] "Fractionation" has been used to describe the cases when analysis decomposes "an analyte or a cluster of analytes".[10,11,13,14,46] In summary, the IUPAC report recommended that the term "speciation" be used to indicate the distribution of species in a sample and thus is synonymous with "species distribution". For global use, the IUPAC report also suggested using the term "speciation analysis" when referring to the identification and measurement of metal species. These IUPAC guidelines are outlined in Table 1.[9]

Identical stoichiometry but geometrical differences can be observed for organic and inorganic compounds. For example, *cis*- and *trans*-2-butene are different compounds even though their molecular formulas are identical. Similarly, structural isomers exist in inorganic chemistry and particularly in coordination complexes. Specifically, facial or meridional isomers are distinct geometrically but have identical stoichiometry. Therefore, structural diversity goes far beyond detectable differences in composition such as that observed by, for example, complexes with isotopic variation. Indeed, complexes can be different because of their redox states, geometrical isomers, coordination isomers, or conformers, and often these different isomers may not be distinguishable because they have the same stoichiometry. Several processes are involved; the complexes undergo hydrolysis, ligand-exchange reactions, and redox chemistry. Because many metabolites are excellent ligands for the metal ion, reactions range from substitution in the first coordination sphere to formation of complexes associated through weak van der Waals or hydrophobic forces. The inorganic chemist is accustomed to the fact that coordination complexes are readily subject to structural diversity in ligand-rich systems and as a result generally embrace a broader definition of "speciation" to include both stoichiometry and geometrical differences. Considering the problems described above, it is clear that this field needs further contributions from the inorganic and coordination chemist. However, following the recommendations of IUPAC with regard to the terminology associated with speciation are beneficial for providing clear reference definitions to facilitate interdisciplinary collaborations.

Table 1. Recommended IUPAC Speciation Definitions[9]

term	IUPAC definition
chemical species	chemical elements: specific form or an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
speciation analysis	analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample
speciation of an element (speciation)	distribution of a component among defined chemical species in a system

ILLUSTRATION OF THE IMPORTANCE OF SPECIATION IN BIOLOGICAL ACTIVITY: VANADIUM

Background. Named after the Nordic love and beauty goddess, Vanadis,[47] vanadium is a trace element in living organisms with a potentially essential role.[48] Vanadium in oxidation state 5 is an analogue of phosphorus[47] and thus an inhibitor against phosphorylases such as the ATPase,[49,50] protein tyrosine phosphatases,[51,52] and ribonucleases;[53] in other oxidation states, vanadium is in the form of cations. Vanadium demonstrates promising antidiabetic properties and thus has been applied as a potential therapeutic.[54−58] As is common with many essential trace metals, vanadium shows toxicity in large amounts but is considered by some to be an essential element for humans.[47,48,54,59,60] As such, the metal can be found within the vitamin aisle at local grocery stores presented as a nutritional supplement. Vanadium is a cofactor for several enzymes including haloperoxidases[47,61−67] and nitrogenases[47,62,68−70] and interacts strongly with the vanadium-binding enzymes that are found in ascidians (also referred to as tunicates or sea squirts).[47,71−73] The two latter proteins exist in very reducing environments, and the coordination chemistry of these systems involves sulfur coordination

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chemistry. A few studies have also documented that several forms of vanadium, particularly the peroxovanadium species, can cleave DNA.[66,74−77] Finally, the interaction of vanadium coordination complexes with membrane interfaces has shown that some complexes can associate with and, in some cases, penetrate these interfaces.[56,78−82] This is very important because the properties of the coordination complex can rapidly and dramatically change as a result of interaction with the membrane. As more information becomes available on the localization of vanadium in the cell, we predict the appreciation for compartmentalization and speciation with vanadium and other biometals will increase our understanding of these phenomena in the coming decade.

Vanadium represents one of the few elements for which significant work has been carried out to examine speciation with traditional solution aqueous chemistry methods both *in vitro* and *in vivo*. As a result, researchers have shown what happens to vanadium coordination complexes or salts in biological fluids.[17,25,83−86] Such studies have been accompanied by spectroscopic studies in animal cells.[54,87,88] Therefore, describing work carried out with vanadium effectively illustrates the importance of speciation distribution and how speciation distribution impacts the effects of metal compounds in biological systems.

Aqueous Chemistry. *Hydrolysis.* Vanadium exists in many oxidation states, two of which are most commonly encountered in biological systems: the 4+ (V^{IV}) state and the 5+ (V^{V}) state. Both V^{IV} and V^{V} undergo very complex chemistry in aqueous solution, forming a range of species. Figure 1 shows the

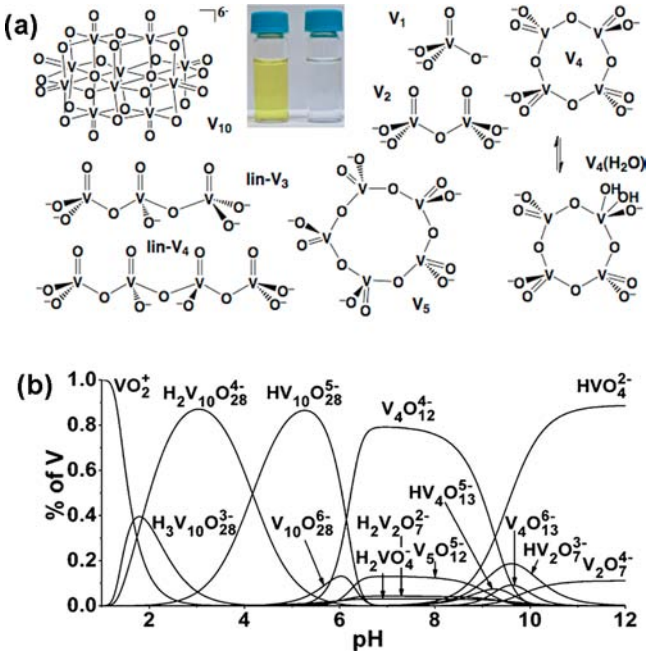

Figure 1. Schematic illustration of the anionic V^{V} ions (a) and the species distribution in a solution of 200 mM V^{V} (b). Adapted with permission from ref 72. Copyright 2003 Elsevier.

structures of and the distribution of anionic forms of V^{V}: V_1, vanadate monomer; V_2, vanadate dimer; V_4, vanadate tetramer; V_5, vanadate pentamer; lin-V_3, linear trivanadate; lin-V_4, linear tetravanadate; V_{10}, decavanadate. The sample of a colorless solution of vanadate contains V_1, V_2, V_4, and V_5, and the yellow solution contains V_{10}. Vanadyl sulfate ($VOSO_4$) is a common source of aqueous solutions of the vanadyl cation (V^{IV}). However, upon dissolution, very low concentrations of VO^{+} cations form[23,47,89−91] because of its propensity to dimerize and oligomerize at neutral pH. The dimerization reaction is shown in eq 3. This reaction involves removal of the monomeric form from solution and can be detected by electron paramagnetic resonance (EPR) spectroscopy. At a pH below 3, the $VO(H_2O)_5^{2+}$ ion is a very stable cation and can readily be observed using this form of spectroscopy.[23,90] However, between pH 3 and 10, the concentration of monomeric V^{IV} is very low. Therefore, only in the presence of ligands can this vanadium species be observed. At a pH above 10, a different signal emerges, and this signal is attributed to $VO(OH)_3^{-}$. In summary, depending on the pH, V^{IV} can be a cationic or an anionic species in solution.

A solution of sodium metavanadate ($NaVO_3$) forms a vanadate monomer at high pH, as shown in eq 4 and in Figure 1a.[21,92,93] At lower pH values, $NaVO_3$ forms several anionic vanadate oligomers including vanadate monomer, dimer, tetramer, and pentamer depending on the pH and concentration, as illustrated in eq 5.[21,47,93,94] The specific protonation state and ratio of oligomers is intimately tied to the specific conditions of the solution. Generally these species are referred to as the colorless oligomeric species and readily interconvert at neutral and basic pH values.[21,93−95] In Figure 1b, the speciation distributions of the species shown in Figure 1a in a solution of 200 mM V^{V} are shown, illustrating the presence of the labile colorless oxovanadates at neutral and alkaline pH.[78]

At a pH below neutral, a more complex mixture arises, primarily composed of decavanadate forms that can exist in six different protonation states depending on the pH.[93] Decavanadate, readily observable because of a characteristic yellow-orange color, forms from vanadate in the presence of acid, as shown in eq 6.[21,94] This equation demonstrates that the formation of decavanadate from metavanadate uses up protons, while hydrolysis of decavanadate to oligomeric vanadates generates protons. This reaction can be qualitatively observed easily by the reversible change in the solution color from the yellow decavanadate-enriched solution to the clear oligomeric vanadates. However, while decavanadate converts rapidly at acidic pH, it is not the thermodynamically stable form of the oxovanadate at neutral pH values. Because this species is kinetically stable for 15−48 h at neutral pH, depending on the conditions, this yellow form will exist for a limited period of time.[94] Often decavanadate solutions are prepared from V_2O_5, and in this case, the reaction produces protons, as shown in eq 7.[93] Acidic solutions of vanadate at pH 3 or less form a cationic ion of V^{V}, VO_2^{+}, which was recently characterized as containing a six-coordinate vanadium in the form of $VO_2(H_2O)_4^{+}$ in solutions.[96] This cationic species is in rapid equilibrium with either or both decavanadate and $H_2VO_4^{-}$ depending on the specific solution conditions.[47,93,97] These V^{V} species are all readily observed using ^{51}V NMR spectroscopy.[47,97] The aqueous solutions of V^{V} in general form anionic species. At acidic pH, however, cationic forms also exist.

$$2VO(H_2O)_5^{2+} \rightleftharpoons (VOOH)_2^{2+} + H_2O + 2H_3O^{+} \quad (3)$$

$$uVO_3^{-} + H_2O \rightleftharpoons H_2VO_4^{-} \quad (4)$$

$$VO_3^{-} + xH_2O \rightleftharpoons yH_2VO_3^{-} + zH_2V_2O_7^{2-} + vV_4O_{12}^{4-} + wV_5O_{15}^{5-} \quad (5)$$

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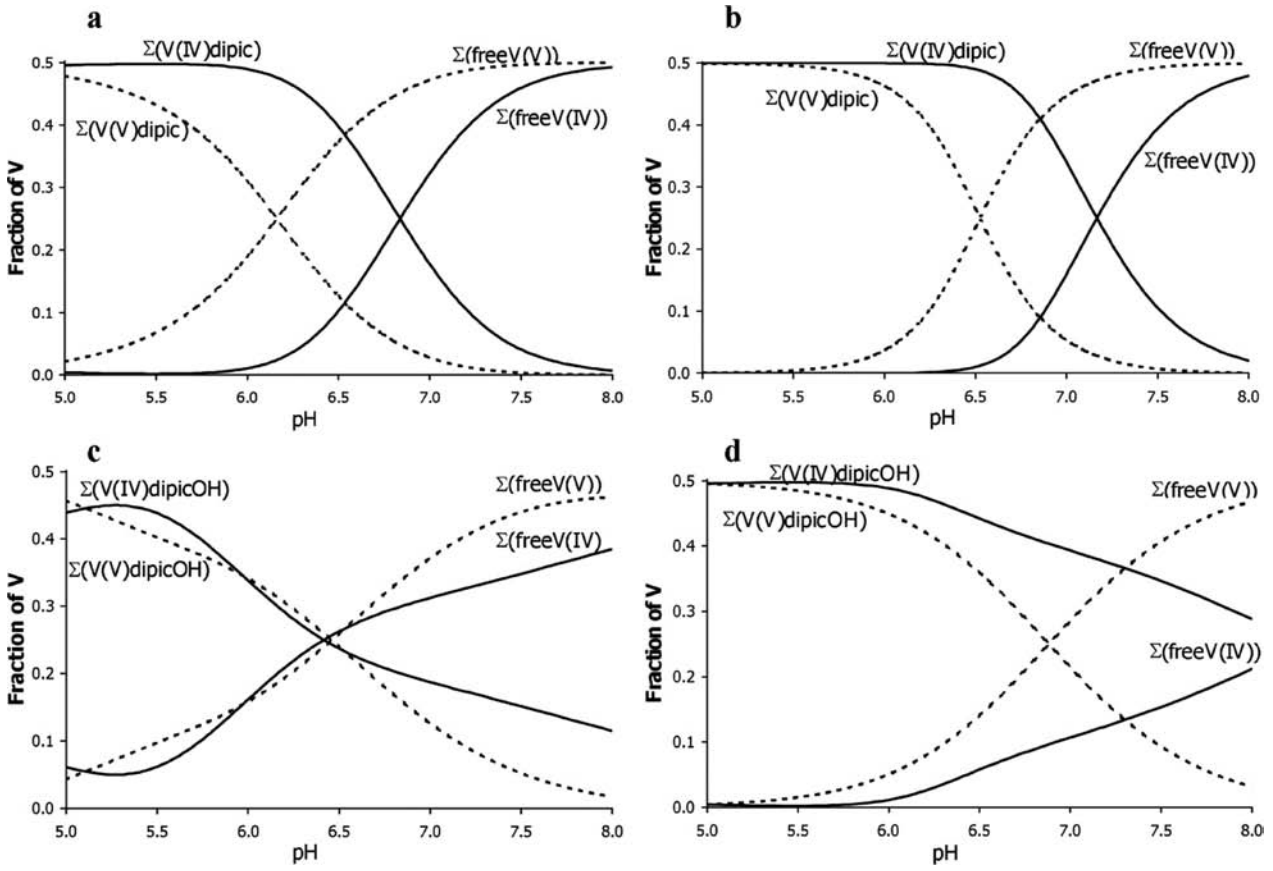

Figure 2. pH dependence of the mole fraction of the ligand bound and free vanadium species (a) in the 2:1:1 dipic–$V^{V}O$–$V^{IV}O$ system, (c) in the 2:1:1 dipic–OH–$V^{V}O$–$V^{IV}O$ system, (b) in the 4:1:1 dipic–$V^{V}O$–$V^{IV}O$ system, and (d) in the 4:1:1 dipic–OH–$V^{V}O$–$V^{IV}O$ system, at a concentration of 2 mM for all components. Adapted with permission from ref 23. Copyright 2003 Elsevier.

$$10VO_3^- + 8H_3O^+ \rightleftharpoons H_4V_{10}O_{28}^{2-} + 10H_2O \quad (6)$$

$$5V_2O_5 + 9H_2O \rightleftharpoons V_{10}O_{28}^{4-} + 6H_3O^+ \quad (7)$$

Redox. The solution redox chemistry with vanadium is complex and very sensitive to the pH, the concentration of the metal, and the presence of oxygen or other ligands.[47,89,90,93,98,99] Solutions of V^{IV} at acidic pH are stable. However, as the pH increases above 4, V^{IV} oxidizes to V^{V} in the presence of oxygen.[47,89,90,93,98,99] As the pH increases above 10, V^{IV} forms the $VO(OH)_3^-$ species, and under these basic conditions, the aqueous solutions containing V^{IV} are stable against oxidation. Solutions of V^{V} are stable over the entire pH range.[47,90,93] If a reducing environment is produced by the presence of reducing ligands, however, V^{V} may reduce to form V^{IV}.

The major naturally existing reductants in biological systems include the tripeptide gluthathione, the amino acid cysteine, and ascorbate. Numerous studies have been reported with V^{III},[100] V^{IV},[47,89,99] and V^{V} [47,89,99] with gluthathione. In the case of V^{IV}, EPR spectroscopy has been very informative and documents the formation of a range of complexes. In the case of V^{V} and gluthathione, however, reports are in conflict with some suggesting that V^{V} is reduced,[101,102] while others suggest no reduction.[103−105] A recent study of V^{V} with 2-mercaptoethanol showed that these conflicting reports are a consequence of specific experimental conditions. The investigation demonstrates that, at low pH and high concentrations, it is more likely that reduction will be observed.[99] However, at higher pH and at lower concentrations, V^{V} is stable in the presence of thiols. Studies have shown that V^{V} complexes with thiol ligands can be characterized both in solution and in the solid state.[99]

The speciation distribution for vanadium is complex even in simple, controlled, classical speciation studies. This speciation is critical for biological responses, nonetheless. For example, the vanadate monomer is a potent inhibitor for enzymes catalyzing phosphorylation reactions,[106] but some phosphatases appear to be inhibited by the higher oligomeric oxovanadates.[94,107] Other enzymes such as 6-phosphogluconate dehydrogenase are inhibited by the tetramer but not the other vanadate oligomers.[108] In animal and cellular studies, knowledge of vanadium-induced inhibition before and during the formation of complexes is less well understood because of the complicated composition of biological fluids. However, the above reactions do provide the underlying processes that will be key when considering analysis of the speciation distribution in future, more complex biological conditions.

Reactions in Biological Fluids. Biological systems contain several fluids of differing composition. In animal studies, the biological fluids predominately consist of serum, saliva, and urine. For cellular studies, the most common biological fluid considered is the cytoplasm. The lmm metabolites in these biological fluids and cellular compartments readily form coordination complexes with V^{IV} and V^{V} systems.[83−85,98] Extensive work on modeling species distribution in blood plasma by Kiss and co-workers.[17,25] The details on each specific drug investigated and the fact that other hmm protein

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complexes form with serum albumin and immunoglobin attest to the diversity that exists (Figure 2). We refer readers to the original work reported by these workers for details for each of these systems.[17,25] These studies show that vanadium is extracted from its complexes in the presence of citrate and other molecules in blood plasma and corresponding fluids.

As described above, vanadium exhibits antidiabetic properties that render the metal attractive for therapeutic use. Because ligands may alter the bioprocessing of any drug, it is of interest to examine how the redox reactions affect binding. In one study, the affinity of ligands for different oxidation states was investigated, and a summary of the results is shown in Figure 2.[23] Solution chemists calculate β values and report them in tables, but the data shown in Figure 2 illustrate how two different but related ligands, dipicolinate (pyridine-2,6-dicarboxylate) and 4-hydroxydipicolinate (4-hydroxypyridine-2,6-dicarboxylate), affect the overall distribution of species. In Figure 2a, the dipic ligand is shown to bind more effectively to V^{IV} than V^{V} because the former complex exists at higher pH. In contrast, the dipic−OH ligand binds equally with each oxidation state. However, when excess ligand is added, the dipic−OH binds more to V^{IV}. This summary shows a direct comparison of the ligand binding to metal ions in two different oxidation states. This is important because potential drugs and nutritional additives will form complexes in the presence of ligands and lose ligands once they reach the bloodstream or any other biological fluids.

Because of the low levels of compounds in animal fluids, often the detection limits for spectroscopic investigations are not sufficiently high for full spectroscopic characterization, and measurements are made of the total vanadium content. The distribution of species is measured when vanadium is added as a simple vanadyl cation salt[86] and as the antidiabetic drug bis(maltolato)oxovanadium(IV).[79] A series of EPR studies were carried out by Sanna et al. to experimentally test these predictions.[83−85] The majority of the vanadium will be bound to hmm and specifically to transferrin. Although other proteins also bind vanadium, their affinities are much lower. The fraction of vanadium bound to lmm is in the range of 10−20% of the total vanadium and represents the mobile fraction of vanadium in the blood. This fraction plays an important role in the mode of action of vanadium.[17,25] In Figure 3, the distribution of vanadium in the drugs is illustrated. A simulation of how 0.100 mM vanadium-based drugs distribute in the blood was used to evaluate how different drugs will react in blood.[17,25] These studies show that vanadium will bind mainly to transferrin and that citrate is the main metabolite that binds to vanadium. In summary, these studies demonstrate that vanadium citrate and vanadium transferrin complexes are the major coordination complexes that form in serum when vanadium salts and complexes are administered.[83−85]

A recent study investigated whether the biodistribution of V^{IV} originated from the oral administration of $VOSO_4$ for 6 weeks to patients with type 2 diabetes.[86] Serum levels were found to correlate with the dose administered. However, no correlation was found for total serum and insulin-like response. Because of the properties of these complexes, it is likely that the drug alters character as it encounters different conditions, including substantial changes in the pH, while traveling through the stomach and digestive tract and then during hepatic circulation/metabolism and finally systemic circulation. During this process, several vanadium pools could form. The possibility that the insulin-enhancing properties of vanadium will be

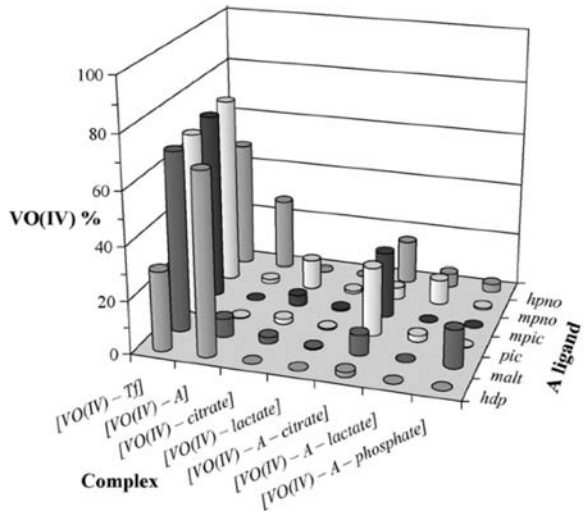

Figure 3. Speciation of various potentially antidiabetic $V^{IV}O$ compounds (0.100 mM) in serum at pH 7.4 (malt = maltolate, pic = picolinate, Mepic = 6-Me-picolinate, HpNo = 2-HO-pyridine *N*-oxide, TpNo = 2-HS-pyridine *N*-oxide, and Hdp = 3-HO-1,2-diMe-4-pyridone). Adapted with permission from ref 17. Copyright 2007 Chemical Society of Japan.

proportional to one of these pools seems reasonable. Indeed, the data show that the effectiveness of the treatments is proportional to the total vanadium pool in the serum, and further investigations of the coordination chemistry may help to identify the vanadium pool that correlates with insulin-like activity.

Vanadium as a Cofactor in Enzymes. Vanadium is a known cofactor in two classes of enzymes, haloperoxidases and nitrogenases[61,63−65,109] and possible also in a third class, vanabins.[71−73] The coordination chemistry of the first class, haloperoxidases, will briefly be summarized here. There are three different haloperoxidases—chloro-, bromo-, and iodoperoxidase—all of which have vanadium in the oxidation state V. Importantly, vanadium remains in this oxidation state throughout the catalytic cycle. The crystal structure of a 67 kDa vanadium-dependent chloroperoxidase was determined for the native enzyme in its resting state at pH 8.0.[110−112] Vanadium is located in a trigonal-bipyramidal binding site with three equatorial oxygen atoms with bond distances of around 1.65 Å. The presumed OH and His-496 groups in apical positions have bond lengths of 1.93 and 1.96 Å, respectively. The vanadate binding site is located at the bottom of an ∼10 Å solvent-accessible channel. The overall negative charge on the vanadate is compensated for by hydrogen bonds to several positively charged amino acid side chains (e.g., Lys-353, Arg-360, and Arg-490).[110] Other important residues, including Lys-353, Arg-360, His-404, and Arg-490, form hydrogen bonds with the nonprotein oxygen atoms of vanadate. Given that the bond distance for the apical OH ligand is in the range for the vanadium(V)−hydroxyl bond,[113] its hydrogen bonding with the His-404 residue suggests that the fifth ligand is a hydroxyl group.

The binding site in vanadium haloperoxidases contains a $[V(OH)_2(O)_2Im]^-$ cofactor contained in a binding pocket of basic residues (lysine and arginine) that facilitate the metal's conversion between the native vanadate and peroxo forms during catalysis.[114] The residues at the catalytic site provide a coordination environment consistent with that of a high-

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oxidation-state binding site. This binding site is very different from that in the nitrogenase and the vanabin, where the coordination environment for vanadium is consistent with a low-oxidation-state environment. In nitrogenase, vanadium is coordinated to an accompanying organic cofactor and lower pK_a residues (namely, cysteine) from the enzyme, which, because of the electronic nature of vanadium, allow for both CO and N_2 reduction.[115]

Effect of Vanadium on DNA. Some vanadium compounds have been investigated and found to interact with DNA.[116,117] Peroxovanadium compounds are able to nick DNA and have been reported to be very toxic[47,74,117−120] upon oral administration. As a result, these derivatives were abandoned despite their early promise as drug candidates in cell culture studies.[47,117,118,121,122] By comparison, vanadate shows no apparent affect. These same compounds were previously reported to exhibit insulin-enhancing effects, although when administered orally in animals, their effectiveness was overshadowed by their toxicity. These observations further underline the importance of studies focusing on compound transport and uptake.

How Vanadium Interacts with and Traverses Membrane Interfaces. Recent studies in our laboratory have focused on the exploration of how vanadium compounds associate with interfaces of membrane model systems and subsequent verification in cell studies. These studies have shown that the coordination compounds bis(maltolato)-oxovanadium(IV),[79] dioxovanadium dipicolate,[80] decavanadate[123,124] and vanadate[125] interact very differently with an interface. For example, bis(maltolato)oxovanadium(IV) was found to associate with the interface of the model system, and one consequence of this association was compound hydrolysis.[79] In contrast, dioxovanadium(V) dipicolinate penetrates the interface[56] and therefore demonstrates that the ligand can impact the interaction with the interface.[79,126,127] More recently, the chemistry of these complexes has been found to drastically change in a hydrophobic environment, which may be important to the mode of action of this class of compounds.[56,128,129] Finally, although decavanadate remains in aqueous environments, it shows the ability of associating and impacting membrane interfaces.[130] The counterion was found to have a profound effect here and even in very small amounts may significantly change the nature of the decavanadate interaction.[130] These results document the variety of responses that these compounds exert and underscore the need for more detailed information on these systems.

Summary. Speciation is important for all metal ions but particularly for metal ions such as vanadium with multiple activities and functions.[47] Vanadium is a good case study, in part, because it is an early transition metal and, as such, is exceedingly sensitive to its environment, including potential ligands, pH, and ionic strength.[56] The compound's environment will impact the properties of the resulting species that form. Some generalizations can cautiously be made as an initial guideline on how to interpret the observations reported. For example, vanadium in oxidation state 4+ is generally acting as a cation, whereas vanadium in oxidation state 5+ tends to act as an anion unless complexed by a ligand.[47] However, all of these forms exchange ligands and form complexes with transferrin and citrate, the two major vanadium-containing components in blood plasma.[17] The application of metal-based drugs or supplements undoubtedly will result in bioprocessing, leading to new species.[86] With the studies on vanadium, we demonstrate the need to consider each compound separately and highlight the fact that different species may exhibit different biological responses, and referring to a metal as a single entity is not conducive to an understanding of how it truly acts.[86]

ILLUSTRATION OF THE IMPORTANCE OF SPECIATION IN BIOLOGICAL ACTIVITY: IRON

Background. Iron is an essential element in biology with multiple roles both in the structure and in the function of multiple proteins and enzymes.[33,131−149] Proteins include, but are not limited to, heme-containing proteins such as hemoglobin, myoglobin, and cytochrome P450. These proteins are responsible for oxygen uptake, oxidation of toxicants for metabolism, and many other functions. Nonheme enzymes also contain iron and exhibit a greater variety of properties and functions. Examples include nonheme iron oxidases, nitrogenases, and other proteins containing iron−sulfur clusters. These clusters play key roles in electron-transfer reactions within proteins and are required for cellular respiration. In addition, iron-containing metalloenzymes are involved in DNA synthesis, oxygen fixation, phosphate ester hydrolysis, and iron storage.

Although iron exists in many oxidation states, it is protected in an enzyme complex so higher oxidation states are generally only observed during catalytic conversions of the enzymes.[144] Two oxidation states, the 2+ (Fe^{II}) and 3+ (Fe^{III}) states, are most commonly encountered in biology. Although it performs essential functions, when in excess, iron can cause the formation of reactive oxygen species (ROS) that can cause tissue and organ damage.[7,46,150,151] Iron overload diseases are frequent disorders and are generally treated by control of the iron intake and with various types of chelation therapies.[46,152,153]

Aqueous Chemistry. The low solubility of both Fe^{2+} and Fe^{3+} causes the aqueous chemistry of these cations to be closely linked to iron chelators present in solution.[31−33,132,137,150−162] Even at low concentrations and in the absence of other ligands, both Fe^{2+} and Fe^{3+} undergo very complex hydrolytic and redox reactions in aqueous or semiaqueous environments.[20,109,158] For example, upon dissolution of simple salts, e.g., $FeCl_2$ and $FeCl_3$, in aqueous solution several species emerge, with the vast majority being biologically relevant Fe^{2+} and Fe^{3+} forms in different protonation and oligomerization states.[20,31,32,133,137,150−162] When the hydrated Fe^{2+} and Fe^{3+} ions form in aqueous solution, one would anticipate the reactions shown in eqs 8 and 9 because these species are acidic and deprotonate to form the corresponding $Fe(H_2O)_5(OH)^+$ and $Fe(H_2O)_5(OH)^{2+}$ species. This is a simple deprotonation reaction that takes place when any iron salts are dissolved, so solutions of these ions, in the absence of buffers, are acidic.

$$Fe(H_2O)_6^{2+} \rightleftharpoons Fe(H_2O)_5(OH)^+ + H^+ \quad (8)$$

$$Fe(H_2O)_6^{3+} \rightleftharpoons Fe(H_2O)_5(OH)^{2+} + H^+ \quad (9)$$

The speciation distributions for both of the common oxidation states are illustrated in Figures 4 and 5 at comparable conditions because a number of reactions are dependent on the iron concentration, oxidation state, and pH. The reader is referred elsewhere for details.[20,33,132] In Figure 4, the y axis shows the percent of a particular species and the x axis shows the pH of 0.001 M Fe^{II}.[20] Although we only show this figure here, these types of figures should be analyzed in conjunction

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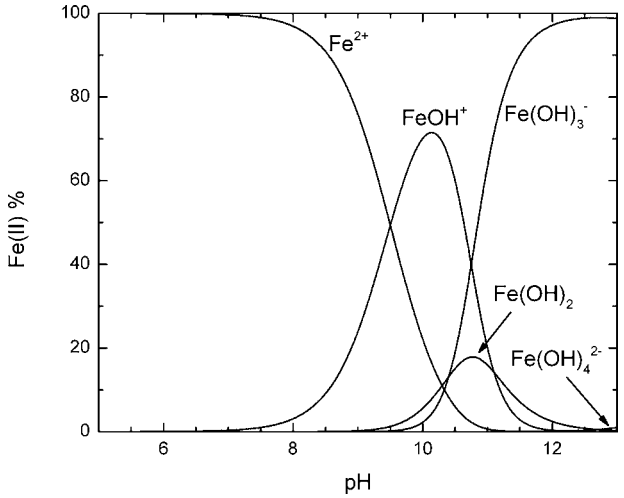

Figure 4. Distribution of hydrolysis products $(Fe^{2+})_x(OH)_y$ at I = 1.0 M at 25 °C in 0.001 M Fe^{II}. The species observed are Fe^{2+} [the free hydrated ion has a formula of $Fe(H_2O)_6^{2+}$], $Fe(OH)^+$, $Fe(OH)_2$, $Fe(OH)_3^-$, and $Fe(OH)_4^{2-}$ based on the formation constants reported in ref 20.

with figures or information regarding the solubility product of the most insoluble related material, which, in the case of Fe^{2+} (Figure 4), is $Fe(OH)_2$, also referred to as green rust when the initially white $Fe(OH)_2$ turns green upon exposure to small amounts of oxygen.[163] Further oxidation leads to a red-brown Fe^{3+} compound, referred to as γ-FeO(OH) or lepidocrocite.[164] The low solubility of $Fe(OH)_2$ at neutral and basic pH emphasizes the fact that at these pH values the metal ion must be complexed to ligands in order to be available in a soluble form unless the concentrations are very low. Figure 4 shows that Fe^{2+} is a major component of solutions at 1 mM and lower concentrations at neutral pH. When the pH is increased above 9, the metal ion begins to hydrolyze and a second species forms, $Fe(H_2O)_5(OH)^+$ [abbreviated as $Fe(OH)^+$ in Figure 4]. Above pH 10, three additional species exist, and although their concentrations are very low, the $Fe(OH)_2$ species further hydrolyzes to form two anionic species that are soluble at high pH: $Fe(OH)_3^-$ [abbreviated from $Fe(H_2O)_3(OH)_3^-$] and $Fe(OH)_4^{2-}$ [abbreviated from $Fe(H_2O)_2(OH)_4^{2-}$].

In Figure 5, we show an example of a speciation diagram at two concentrations of Fe^{3+} (0.1 M and 0.01 mM) prepared from a pure salt.[20,157−159,165] The aqueous Fe^{III} ion undergoes a series of complex oligomerization reactions, and the speciation diagrams represent the stoichiometry of the species that form. Therefore, even though a single iron compound was used, several Fe^{III} species are formed upon dissolution. In Figure 5a, the species in solution at a low concentration of Fe^{III} (10^{-5} M) are identified as follows: $Fe(H_2O)_6^{3+}$ (abbreviated as Fe^{3+}), $Fe(H_2O)_5(OH)^{2+}$ [abbreviated as $Fe(OH)^{2+}$], $Fe(H_2O)_4(OH)_2^+$ [abbreviated as $Fe(OH)_2^+$], $Fe(H_2O)_3(OH)_3$ [abbreviated as $Fe(OH)_3$], and $Fe(H_2O)_2(OH)_4^-$ [abbreviated as $Fe(OH)_4^-$]. This low-concentration plot shows that the species distribution contains mainly mononuclear species but, depending on the pH, several species may form. The solubility of Fe^{III} ions with respect to α-FeO(OH) [also known as goethite, one form of $Fe(OH)_3(s)$] is significantly less than that for $Fe(OH)_2$ and has the lowest solubility in the neutral pH range. This plot illustrates the complexity of the speciation for Fe^{3+} and underlines the fact that ligands are needed to stabilize systems under physiological conditions.

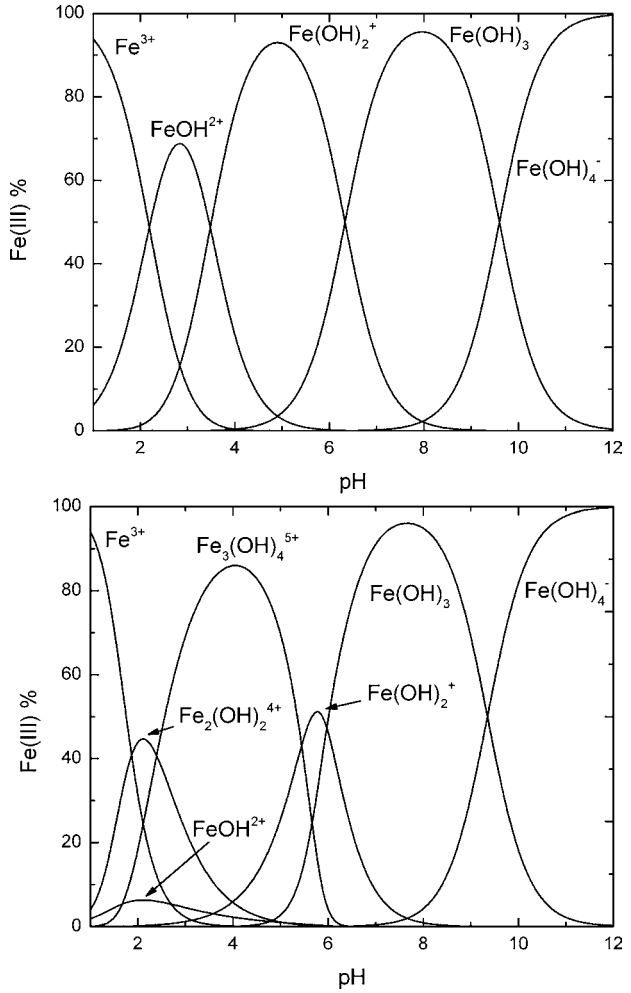

Figure 5. Distribution of hydrolysis products $(Fe^{3+})_x(OH)_y$ at I = 1.0 M at 25 °C in (a) 0.01 mM Fe^{III} {the species observed are Fe^{3+} [which is the abbreviation for $Fe(H_2O)_6^{3+}$], $Fe(OH)^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$} and (b) 0.1 M Fe^{III} [the species observed are Fe^{3+}, $Fe(OH)^{2+}$, $Fe_2(OH)_2^{4+}$, $Fe_3(OH)_4^{5+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$ based on the formation constants reported by ref 20].

In Figure 5b, the species in a solution at the higher concentration of Fe^{III} (0.1 M) is significantly different from the speciation observed at 0.01 mM. The speciation at higher Fe^{III} concentration forms some of the same species as those at lower concentration and also a few oligomeric species containing three or four iron atoms. The species observed are Fe^{3+}, $Fe(OH)^{2+}$, $Fe_2(OH)_2^{4+}$, $Fe_3(OH)_4^{5+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$.[20] However, if this solution is added to a biological system, the metabolites, including proteins, will bind Fe^{3+} and shift the equilibrium to reduce the amounts of free Fe^{3+} and fewer if any di- and trinuclear species form. However, this is a simplification,[33,131,132] because in addition to the hydrolytic chemistry, redox processes are also possible. In biological fluids, the iron ions can interconvert via reaction with metabolites present in a particular biological environment.

Reactions in Biological Fluids. What are the consequences of the speciation reactions? With regard to solution studies, speciation chemists have learned how to manipulate these solutions to maintain the solubility of the ions either by forming complexes containing Fe^{2+} and Fe^{3+} or by using the complexes to deliver Fe^{2+} and Fe^{3+} to a particular system.[33,131,132] Nature has overcome this issue by involving

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very strong chelating ligand systems to stabilize the iron with respect to hydrolysis and redox chemistry. In biological fluids, several metabolites are noninnocent and associate strongly with the iron ions to dramatically affect their speciation. The properties of these systems are conveniently examined using β_{III}/β_{II}, which allows detailed analysis of when one oxidation state of the coordination complex is more stable.[132] Specifically, the range of phosphates, citrates, and many amino acids and other acids in biological fluids are potent ligating agents. Although ligands possessing alkoxy or carboxyl groups are generally weak ligands in aqueous solutions, when present as part of a multiple-functionality ligand constellation, even these groups can strongly bind iron. Citrate is an example of a potent ligand, and multiple studies have been carried out to characterize this system.[165−170]

Computational analysis of the speciation in serum has been done using computer programs and databases developed to calculate the speciation in serum.[165] The *Joint Expert Speciation System* (*JESS*) software package allows for modeling in complex aqueous environments and is frequently used for applications in biological and environmental systems.[165,171−174] Table 1 illustrates how the speciation of the fraction of mobile iron ions is modified by the presence of citrate based on the *JESS* database.[165] The minor iron species calculated to be present in the biological fluids are not listed. However, the carbonate and amino acid complexes consist of about 45% of the iron at low citrate concentrations and 27% at higher citrate concentrations. Although this modeling prediction showed a higher concentration of free metal ion than anticipated, this study was the first in which the Fe^{II}/Fe^{III} redox pair was considered in these analyses. Since the modeling shown in Table 1 was reported, some of the problems with the speciation of the iron(III) citrate system have been addressed. It is important to point out that only 90−94% of the iron species were accounted for in this table, and other iron species must be present at low concentrations.[166−169,175−179]

In order to illustrate the effect of citrate on the Fe^{II} and Fe^{III} speciation directly, we carried out a calculation introducing citrate to the system. For these studies, we used the average value of iron reported in human blood serum, 1.2 mg/L (i.e., 0.02 mmol/L[180]), the physiological concentration of citrate of 0.13 mmol/L,[170] and the reported composition and stability constants for citrate verified by Martell and co-workers.[167−169] In Figures 6 and 7, the changes in speciation compared to the speciation shown in Figures 4 and 5 are a result of citrate and the 100-fold decreased concentration of Fe^{II} and Fe^{III}. As shown for these low and physiologically relevant concentrations, much of the free iron ion in the presence of citrate becomes complexed. However, depending on the concentration, some of the iron ions will remain free in the presence of citrate alone. The addition of other metabolites will further affect the speciation, as shown for the modeling calculation shown in Table 2. These figures underline the fact that speciation will change in biological fluids because of the metabolites that exist under biological conditions. The results shown in Figures 6 and 7 illustrate the importance of considering oxidation states and concentrations of the form of the iron coordination complex.

It is known that hmm entities also complex iron in addition to the lmm modeled above. Particularly, transferrin is a very potent ligand for iron. The reason that iron speciation is regulated so tightly in biological systems is because of the

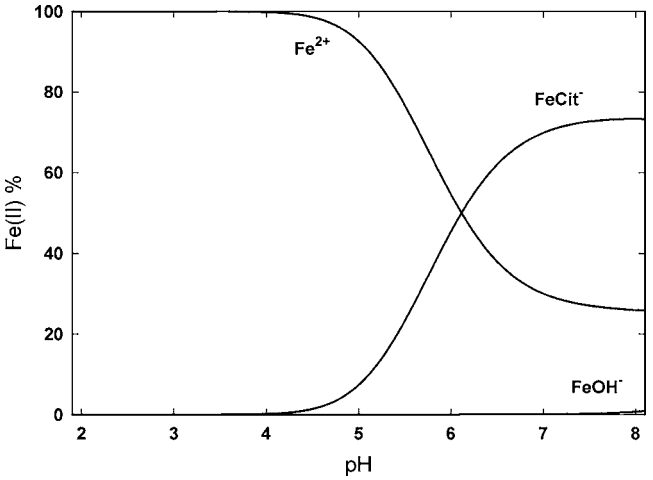

Figure 6. Distribution of hydrolysis products and citrate complexes at 0.02 mM Fe^{II} and 0.13 mM citrate shown as a function of the pH. The calculations were done using the constants reported by refs 20 and 167−169.

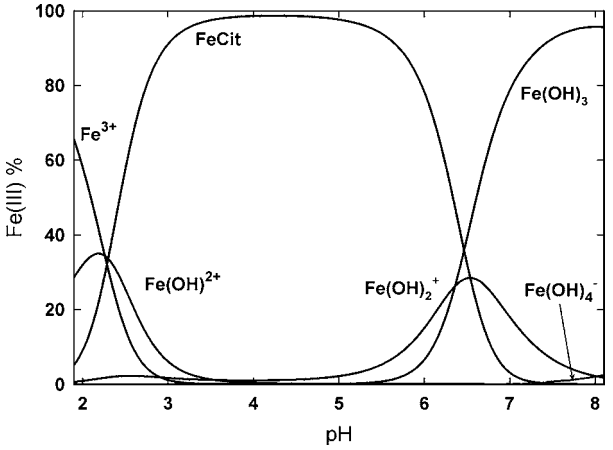

Figure 7. Distribution of hydrolysis products and citrate complexes at 0.02 M Fe^{III} and 0.13 mM citrate. The data are shown as a function of the pH. The calculations were done using the constants reported by refs 20 and 167−169.

Table 2. Iron Speciation in Human Blood Plasma Calculated Using Computer Simulations Based on the *JESS* Database (Performed at 105 mV Redox Potential of Human Blood Plasma) Considering Contributions of Both the Fe^{2+} and Fe^{3+} Parts of the Redox Pair, with $[Fe]_{total}$ = 1 μM[165]

species	% total iron from 0.22 mM citrate	% total iron from 1.0 mM citrate
$(Fe^{2+})(Cit)^-$	19	51
(Fe^{2+})	18	11
$(Fe^{2+})(CO_3)^0$	16	10
$(Fe^{2+})(ThrH)^{2+}$	15	9
$(Fe^{2+})(His)^+$	13	8
$(Fe^{3+})(Cys)_2^-$	9	5

reactions that take place when iron ions are present in excess.[5−7,181,182]

The reaction of Fe^{2+} with hydrogen peroxide is referred to as the Fenton reaction[183] and has been studied for over a century. This reaction has been implicated in a variety of biological pathways.[184] Equation 10 shows the reaction of Fe^{2+} with

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H_2O_2, which traditionally was thought to form iron(III) hydroxides and hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \rightleftharpoons FeOH^{2+} + OH^{\bullet} \quad (10)$$

Recently mechanistic details of this simple reaction have been elucidated.[185] The mechanism is presumably a homolytic cleavage of the O−O bond of hydrogen peroxide. It was demonstrated that this reaction only exclusively occurs in aqueous solution at pH 3 or lower. Aqueous reactions at higher pH values are not this simple.

In aqueous solution at neutral pH values an Fe^{IV} species is produced instead of the hydroxyl radicals. It is the Fe^{IV} species that carries out the observed oxidation reactions. This new species is formed via eqs 11−13, in a series of reactions that are a combination of iron hydrolysis, complexation, and redox reactions. These reactions show heterolytic cleavage of the coordinated hydrogen peroxide in contrast to the homolytic cleavage shown in eq 10.[185,186] It is also interesting to note that the reactivity of the species produced under neutral pH values is significantly greater than the aqueous Fe^{IV} species isolated from the oxidation of Fe^{II} with ozone in acidic pH values.[186] These two Fe^{IV} species must be different.

$$[Fe(OH_2)_6]^{2+} \rightleftharpoons [Fe(OH_2)_5OH]^{+} + H^{+} \quad (11)$$

$$[Fe(OH_2)_5OH]^{+} + H_2O_2 \rightleftharpoons [Fe(OH_2)_4(OH)(H_2O_2)]^{+} + H_2O \quad (12)$$

$$[Fe(OH_2)_4(OH)(H_2O_2)]^{+} \rightleftharpoons [Fe(OH_2)_4(OH)O]^{+} + H_2O \quad (13)$$

The pH regions where these two mechanisms predominate are <3 and >6. Reactions between these two pH regions will probably have a mixture of both pathways. This is a prime example of pH-induced changes in the mechanism, and reactivity, that result from changes in speciation. In the past, many studies have not been done with strict control of the pH, possibly explaining the ambiguity that has plagued Fenton chemistry.

Not only can hydrogen peroxide act as an oxidizing agent, it can, under appropriate conditions, become a reducing agent. The reaction of Fe^{III} with hydrogen peroxide to produce Fe^{II} and superoxide is shown in eq 14. The subsequent reduction of Fe^{III} by superoxide to Fe^{II} and molecular oxygen is shown in eq 15.

$$Fe^{3+} + H_2O_2 \rightleftharpoons Fe^{2+} + O_2^{-} + 2H^{+} \quad (14)$$

$$Fe^{3+} + O_2^{-} \rightleftharpoons Fe^{2+} + O_2 \quad (15)$$

Because of the work in aqueous media,[185,186] traditional schemes regarding ROS, discussed below, may need revision based on changes in Fenton chemistry when the iron is complexed by biological ligands.

The hydroxyl radical and superoxide are well-known strong ROS, which cause many problems in biological systems unless contained. With the recent advance in Fenton chemistry, Fe^{IV} [185,186] may also contribute to the many problems in biological systems attributed to ROS. Indeed, it is the coordination chemistry of the iron ions with the lmm and hmm metabolites that maintains the health of the biological system. Importantly, upon complexation, the properties of the iron ions change because the ability of an iron chelator to prevent and disengage the Fenton reactions is a function of its redox potential.[132] In Figure 8, the redox potentials for a series

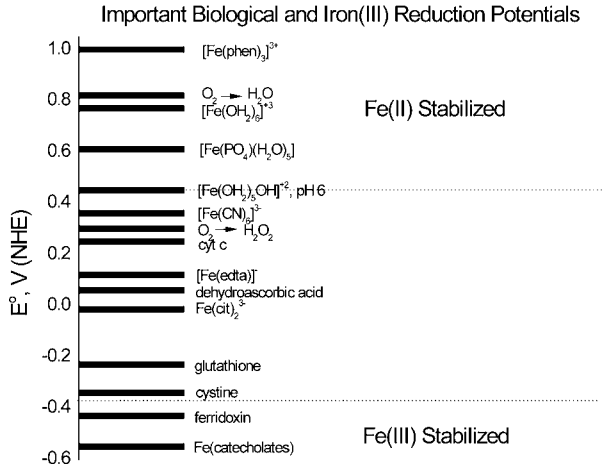

Figure 8. Number of important metabolic ligands and reduction potentials for the resulting Fe^{II} and Fe^{III} complexes. The scale will show what oxidation state is likely to be stable under a particular set of conditions. The dotted lines represent the oxidation and reduction of water.

of ligands that are commonly found in biological fluids are given. The dotted lines represent the oxidation and reduction of water. This figure illustrates the different complexes that forms and what types of ligands that stabilize Fe^{II} and Fe^{III}, respectively. If the ligand does support electron transfer or keep the iron ion tightly bound, the reactions in eqs 10−15 will not occur.

When an iron imbalance occurs, the iron is mobilized from being bound to protein and will be able to undergo redox reactions, leading to ROS formation.[5,31,32,137,161,182] When the levels exceed the antioxidant defense, cells undergo oxidative stress, leading to significant, and often irreversible, cellular damage. Medical chelators, such as desferral, protect the cell by keeping the Fe^{2+} and Fe^{3+} ions coordinated. When the resulting iron complex has a redox potential outside the redox region defined by the reactions of H_2O_2 and NAD/NADH (outside the −425 to +400 mV range), the Fenton reactions are prevented. Siderophores and metal-chelated complexes are effective in keeping the iron bound and away from engaging in redox reactions.[31,132,133,150] Indeed, metal chelation is a successful strategy for the treatment of iron overload diseases.

Iron as a Cofactor in Multiple Enzymes Essential for Life. It is widely accepted that iron plays a central role in a number of critical metalloproteins in biology. Elaborate systems have been developed to properly process Fe^{2+}/Fe^{3+}. They require that specific coordination chemistry be designed around the binding site in each of the proteins.[187,188] Indeed, it is a nontrivial matter for the iron ions to be taken up, transported to the ferritins, and stored until they are needed.[189−191] However, the consequences when these processes are not functioning properly are devastating[7,140,190] and in some cases can cause metabolic changes.[143,192]

Tight Regulation of Iron Uptake. The iron-ion level in the human body is monitored very closely because of its vital role in fundamental molecular pathways and its deleterious effects when misregulated.[134,189−191] Because iron demonstrates no nonpathological source of elimination, iron levels are regulated solely by uptake. Diseases associated with both an

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iron excess and an iron deficiency are very problematic. Because iron demonstrates redox activity and plays a critical role in cellular respiration, both forms of imbalance lead to ROS generation and oxidative stress. These pathological outcomes have been associated with imbalances in the iron metabolism and its production, storage, and detoxification.[189,190,7,140,191] It is important that the proteins processing iron ions[187,188] as well as the enzymes deactivating unwanted ROS are present.[7,140] For example, in the case of peroxodiferritin, structural differences may direct how diiron sites can function either as a substrate (in ferritin biomineralization) or as a cofactor (in O_2 activation).[143] The coordination chemistry at the iron sites is assisting in directing the enzyme activity and metabolism. Furthermore, the generation of ROS in terms of the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), among others, can be directed/inhibited by the enzyme.

Summary. What are the consequences of the speciation reactions? The high reactivity of free iron with its potential toxicity when present in excess makes it essential that the free iron concentrations are maintained at a minimum. In the nonpathological state, most of the iron enzymes are able to bind the iron ion, but the reactivity and toxicity of iron-iron misregulation impact the cells dramatically, leading to severe diseases. Understanding the details associated with the speciation of iron and the potential reactions that the metal ion can undergo is significant to the life scientist. Because studies in this area often make alterations to a system, they also largely involve modification of a coordination site of the iron ion, either directly or indirectly. Indeed, subtle changes in a binding site can have dramatic effects on the enzyme activity. Therefore, consideration of the coordination chemistry of the system is likely to facilitate discoveries that will further the understanding of the role and effect of the metal ion and the ultimate development of treatments of potential disorders.

PERSPECTIVE

Metal complexes in both the discrete chemical and biological context have been established as major players with regard to structure, catalysis, and activity in biology. With increasing interdisciplinary approaches to research, embracing the principles of coordination chemistry has the potential to advance the field of speciation analysis significantly and is beginning to be advocated by solution speciation chemists. Classical solution speciation chemistry determines metal speciation in terms of composition but provides little characterization of these often labile complexes that both influence and are influenced by the surrounding molecular environment. The contributions of coordination and bioinorganic chemists to speciation analysis may affect the application of metals in medicine and environmental studies. In this Forum Article, we described definitions of speciation and two representative metal systems, vanadium and iron, to illustrate why metal speciation is different from the speciation of other classes of compounds. Importantly, upon elucidation of the composition, reactivity, and stereochemistry of metal compounds, combined with an understanding of the coordination chemistry will lead to a better understanding of the labile complexes containing the remaining metals in the periodic table and their role in biology and medicine.

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Notes

The authors declare no competing financial interest.

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