the change in spin state of Fe(III) upon bis-N-methylimidazole complex formation. In the case of Fe(III), the unsymmetrical electron configuration of the low-spin Fe(III) product apparently makes the metal sensitive to both the symmetry and the nature of the substituents, probably due to extensive mixing of metal and porphyrin π-symmetry orbitals (the d_{xz}, d_{yz}, e-symmetry metal orbitals are unsymmetrically filled). The results imply that the nature and pattern of porphyrin substituents in naturally-occurring heme proteins (*i.e.*, cytochromes b, c, a, hemoglobin, etc.) are carefully chosen to maximize the stability of metal-ligand bonds, in addition to controlling other physical properties.

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M4

'Non-Coordinating' Buffers for Studies Involving Metal Ions

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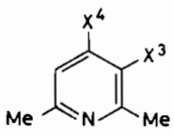
Buffer systems are of outstanding importance for solution chemistry. When it comes to kinetic and thermodynamic studies on metal ions in buffered aqueous solution the extent of complex formation between the buffer applied and the metal ions studied should be negligibly small. In addition, there should be no or at least only a minor catalytic effect of the buffer on systems which are subject to general acid catalysis.

2,6-Lutidine (=2,6-dimethylpyridine) and 2,4,6-collidine (=2,4,6-trimethylpyridine) have often been applied as buffer compounds for the pH range 6.5–8.0 because of their restricted coordination properties due to steric hindrance through the two methyl groups neighbouring the donor nitrogen. A series of lutidines L carrying substituents in the 3- and/or 4-position has been synthesized and characterized with respect to yield upon synthesis, solubility in water, and UV absorption.



L ≙ 3-X^3-4-X^4-2,6-dimethylpyridine

pK_a values of the free bases L and complex formation constants for the aquo ions Ag^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, and Ce^{3+} as determined by potentiometric titration in aqueous solution are presented. A sequence of 2,6-lutidine type buffers is suggested covering the pH range 3–8 in small steps. The formation constants for the 1:1 complexes of divalent and trivalent aquo metal cations are small (mean value: $K = 1.7\ M^{-1}$) and nearly independent of both the nature of the metal and the pK_a of the substituted 2,6-lutidines studied. These results are interpreted as being indicative of weak complex formation sterically restricted to 'outer sphere' interaction.

It is shown that the acids LH^+ do not act as catalysts for the dissociation of a nickel(II) triglycine complex which is known to be subject to general acid catalysis.

M5

Stability and Structure of Complexes of Transition Metal Ions with Nucleotides and Related Compounds

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Potentiometric, spectrophotometric and kinetic techniques have been used to determine the stabilities of complexes of transition metal ions with phosphoric acid and ribose-phosphate, purine nucleosides and nucleotides [1–4]. At ionic strength 0.1 *M*, dinegative phosphate groups bind to Ni^{2+} and Co^{2+} with stability constants close to 100 M^{-1} [1, 5]. The neutral purine nucleosides form only weak complexes; the binding constants depend markedly on the base involved, *e.g.* K = 14 for Ni^{2+}–inosine, and K = 2 M^{-1} for Ni^{2+}–adenosine [3]. The data are consistent with the assumption that the N7 atom of the imidazole ring is the predominant binding site. Similar differences are observed also for the complex stabilities of the nucleotides: K(Ni–IMP) = 920 M^{-1}, and K(Ni–AMP) = 300 [3]. The experimental overall stabilities of the nucleotide complexes can be rationalized only by assuming a chelate structure, with the metal ion being bound to the phosphate group *and* to the base. Space-filling models indicate that in the nucleotide complexes only the N7 atom can act as the binding site of the base. The kinetic data, too, can be interpreted only by a stepwise chelate formation process. Moreover, the kinetic data enable also the evaluation of the stepwise equilibria. In the case of NiAMP, about 2/3 of the complexes are present in the chelate form, 1/3 in the monodentate form.

Complex formation of Ni^{2+} with the dinucleoside-monophosphate ApA^- is weaker ($K = 2.6\ M^{-1}$) than with $AMPH^-$ (K = 11) [4], despite the availability of an additional adenosine group in ApA^-. This observation is attributed to the conformational properties of ApA^- in solution. The ligand prefers a conformation in which the two adenines are in a stacked position.

In this conformation the N7 atoms are far away from the phosphate group, and the metal ion can bind either to the phosphate or to the base, but not simultaneously to both [4].

Strong interactions were observed between Ni^{2+} ions and the polynucleotide poly(A) [4]. Quantitative data were obtained by using murexide as an indicator for the concentration of free metal ions. The difference from the total metal concentration gives the amount of Ni^{2+} bound to poly(A). This technique is applicable for free Ni^{2+} concentrations 10^{-5}–10^{-3} *M*. At the upper limit, 1 Ni^{2+} is bound per 4.4 mononucleotide units (ionic strength 0.1 *M*). A Scatchard plot yields a straight line, *i.e.* the process can formally be described as the binding of Ni^{2+} to one class of independent binding sites at poly(A). Slope and intercept of the plot yield a value of 0.26 for the number of binding sites per monomer (*i.e.* 4 monomers form one binding site), and a stability constant $K = 8.2 \times 10^3\ M^{-1}$ for the binding of Ni^{2+} to these binding sites. In kinetic studies of this system two reaction effects were detected. The kinetic data can be rationalized in terms of a mechanism which involves a second-order (outer-sphere) association process followed by 3 first-order steps (inner-sphere binding, probably to two phosphates and one base [6]). The high overall stability constant is mainly due to strong outer-sphere association (high charge density at poly(A)).

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M6

Factors Influencing the Affinities between Metal Ions and Donor Atoms in Ligands of Biological Interest

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The biochemistry of a metal in a certain oxidation state is very much determined by its affinity for various ligands. Not only the absolute strengths of the metal–ligand interactions are important, however, but also the relative affinities between ligands of various properties and types.

Two classes of metal ion acceptors have been discerned, characterized by different ligand affinity sequences. These classes termed (a), or hard, and (b), of soft, show the sequences [1–4]:

(a)	(b)
$F \gg Cl > Br > I$	$F \ll Cl < Br < I$
$O \gg S > Se > Te$	$O \ll S < Se \simeq Te$
$N \gg P > As > Sb > Bi$	$N \ll P > As > Sb > Bi$

Ligands preferring hard, or soft, acceptors are also termed hard, or soft, respectively.

The soft acceptors are situated in a roughly triangular area of the periodic system, with the apex of the triangle at copper and the base extending approximately from osmium to bismuth. For a certain element, however, the character depends very much upon the oxidation state. Also, the border-line is not sharp. Thus, copper(I) is typically soft while copper(II) is a border-line case. Though the transition is gradual, the difference in properties between the same oxidation state of neighbour metals across the border-line is considerable. Thus, while mercury(II) is typically soft, and cadmium(II) mildly so, zinc(II) generally behaves as a fairly hard acceptor.

The classification of the metal acceptors was done from stability measurements in aqueous solutions. The stabilities measured evidently depend upon the dielectric and solvating properties of the medium used, however. A high dielectric constant will decrease the strength of all ionic interactions. A further decrease of the stabilities of the complexes will result if both the metal ion and the ligand are strongly solvated; the solvation processes generally compete strongly with the complex formation [5, 6].

If the solvation of the ligands does not present any special features, the change of solvation from one medium to another will be much the same for all ligands in the group. The stabilities of the complexes might thus change considerably, but with much the same factor for all ligands; the relative order will stay the same. If, on the other hand, the ligands of a group are differently preferred by different solvents, changes in the affinity sequence do occur [5].

In the case of the halide ions, the ability to form hydrogen bonds decreases sharply along the series, being very strong for F^- and hardly perceptible for I^-. In the same sequence, the preference for protic solvents where such bonds can be formed sharply decreases. Several examples are known where this factor brings about switches in the affinity sequence.

As mentioned, zinc(II) generally behaves as a hard acceptor, with the affinity sequence $F^- \gg Cl^- > Br^- > I$, as illustrated by the stability constants K_1 measured for the reactions $Zn^{2+} + X^- \rightleftharpoons ZnX^+$ in aqueous solution, Table I. Built into the enzyme carbonic anhydrase, zinc(II) displays the opposite affinity sequence, however, Table I. The reason is certainly that in the enzyme, the coordination site of zinc(II) is situated in a hydrophobic crevice. In order to enter, the ligands have to undergo an extensive