Competitive Complexation of Trace Metals with Dissolved Humic Acid

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In this study we investigated the effects of competing trace metals and Ca2+ on Cd(II), Pb(II), and Cu(II) complexation by humic acid extracted from groundwater in Orange County, California. Two types of titration experiments were conducted, those using a single metal and those in which the humic acid had been pre-equilibrated with a competing metal (either a trace metal or Ca2+). The labile metal concentration in the titration was determined by differential pulse polarography (DPP). Results show the different effects of competing trace metal ions and the effect of Ca2+. Both trace metals and Ca2+ do not compete effectively with Cd(II) complexation. While no effects of Cu(II) on Pb(II) complexation were observed, the presence of Cd(II) appeared to slightly enhance the binding between Pb(II) and humic acid. The addition of Pb(II) decreased the amount of Cu(II) complexation, but Cd(II) caused a slight increase at the lower concentrations. Calcium, however, decreased the amount of complexation for all three metals. Results indicate that the metals are not necessarily competing for the same sites. Conformational changes that occur when trace metals bind to the different sites may cause this competing or enchanced effect. Since Ca(II) is introduced at two orders of magnitude higher in concentration than the trace metals, it can outcompete the trace metal for sites where electrostatic interactions dominate. The results indicate that in groundwater situations, where more than one metal is present, the effect of other metals must be considered in predicting metal speciation. — Environ Health Perspect 103(Suppl 1):29-32 (1995)

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Introduction

Relative toxicities of dissolved trace metals to plants and animals depend on the free metal concentrations. The presence of ligands in natural water systems controls free metal speciation. One such category of ligands is humic substances, which arise from the chemical and biological degradation of plant and animal residues. Metal complexation by humic substances can play an important role in regulating the toxicity, bioavailability, and transport properties of trace metals in aquatic systems.

The current study investigated the complexation of Cd(II), Pb(II), and Cu(II) by humic acid extracted from Orange County groundwater as a function of pH, competing trace metal, and competing Ca2+ concentrations. Cd(II), Pb(II), and Cu(II) were chosen based on toxicity considerations and their individual characteristics. In accordance with hard and soft acid concepts (1), Cd(II) is a soft acid, while Pb(II) and Cu(II) are on the borderline between soft and hard acids. That is, in a multiligand complexation system, Cd(II) shows its attachment preference for P and S ligand atoms, but Pb(II) and Cu(II) show their relative attachment preferences for O and N ligand atoms. Ca2+ was also added to our system; it is considered a hard acid, forming mainly outer-sphere complexes (1).

Humic acid was used as a model ligand in our study. A typical humic acid molecule contains aromatic rings and aliphatic chains that host numerous carboxylic, phenolic, hydroxyl, and other functional groups. The O-containing groups are the most numerous; there is a small percentage of N and S binding sites as well.

Because of the large number of possible binding sites on humic acid, the model used to interpret experimental data is often based on some simplifying assumptions (2). In this study, we use the discrete ligand model of metal–humic acid binding, assuming 1:1 metal to binding site stoichiometry:

\[ [MeL] = \sum_{i=1}^{n} (K_i \times [Me][L_i]) \]

where \([MeL]\) stands for bound-metal concentration, \([Me]\) stands for free metal concentration, \(L_i\) is the \(i^{th}\) ligand concentration, and \(K_i\) is the conditional binding constant corresponding to \(i^{th}\) ligand.

Typically, when fitting titration data, one conditional binding constant is needed for each order of magnitude of bound metal concentration (3,4). The binding constant determined this way is a fitting parameter; it depends on experimental conditions.

Materials and Methods

Titration were carried out by adding a metal solution into a buffered solution containing humic acid. A Metrohm Polarographic Analyzer E506 626 (Metrohm Ltd.) was used to determine the concentration of labile metal species (assumed to be free metal ion and metal hydroxide) in the titration. Labile metal concentration was measured at a dropping mercury electrode, together with a reference electrode (Ag/AgCl) and a counter electrode (Pt). The DP 10 mode was selected, with scan rate set at 5 mV/sec, mercury drop rate set at 0.5 drop/sec, gap scale set at 1, and mercury reservoir set 40 cm high. The water-jacketed analytical cell was thermostated at Isotemp Refrigerated Circulator Model 9100 (Fisher Scientific) at 25°C. To remove dissolved O2, N2 gas was used to purge the analytical cell for 5 min before measurements.

Titration were carried out at pH 6, 7, and 8. MES monohydrate (4-morpholineethanesulfonic acid, \(pK_a = 6.15\)) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, \(pK_a = 7.35\)) were used to buffer the pH of electrolyte solution at pH 6 and pH 7, respectively. Due to the interference caused by interaction between HEPES and trace...
metal–humate complexes, PIPES (1,4-piperazinebis(ethanesulfonic acid), pK_a = 6.8), also purchased from Aldrich Chemical Co., was used to buffer the electrolyte solution at pH 7 for part of the experiments. TRIS (Tris(hydroxymethyl)aminomethane, pK_a = 8.3), purchased from Fisher Scientific, was used to buffer the electrolyte solution at pH 8. All these buffers were chosen because they have a very low or zero trace-metal binding capacity (5). Sodium nitrate (NaNO_3) was used to fix the ionic strength of electrolyte solution at 0.05 M. Humic acid stock solution was extracted from Orange County groundwater according to the procedure described by Thurman and Malcolm (6), 5 ppm DOC was used in all experiments. The elemental composition of the humic acid is C: 54.77%, H: 5.52%, O: 33.57%, N: 2.12%, S: 1.83%. Two types of titrations were conducted, those using a single metal and those in which the humic acid had been pre-equilibrated with a competing metal (either a trace metal or Ca^{2+}). Metal concentrations ranged from 4 to 94 μM. Competing trace metal concentration used was 10 μM and Ca^{2+} concentration used was 5 mM. The time interval between each metal aliquot delivery was at least 2 min since it was shown in our study that 2 min gives a sufficient time for metal–humic acid mixtures to reach equilibrium. Each measurement was repeated three times. The data were analyzed using a single ligand model:

\[ Me + L \leftrightarrow MeL \]  \hspace{1cm} \text{(2)}

where Me represents trace metals and L is the representative ligand. Charges are omitted for simplicity. The conditional formation constant for this complexation at a constant pH is:

\[ K = \frac{[MeL]}{[Me][L]} \]  \hspace{1cm} \text{(3)}

[Me] and [MeL] are, respectively, the free, and bound concentrations of trace metal, and [L] is the free concentration of ligand. [Me] concentration was calculated by subtracting metal-hydroxide concentration from the labile metal concentration, and [MeL] and [L] values were then determined using the mass balance equations:

\[ L_r = [L] + [MeL] \]  \hspace{1cm} \text{(4)}

\[ Me_r = [Me]_L + [MeL] \]  \hspace{1cm} \text{(5)}

where [Me]_r is the labile metal concentration. Substitute Equation 4 into Equation 3 and rearrange:

\[ [MeL] = \frac{KL_r[Me]}{1 + K[Me]} \]  \hspace{1cm} \text{(6)}

A nonlinear regression method was chosen to determine K and L, parameters in the functions of [MeL] = f([Me]). In the fitting processes of the titration data, free metal concentration instead of the bound metal concentration was used as the independent variable, while Me_r was the dependent variable, according to Cabaniss and Shuman (3). All the nonlinear regressions were done using SYSTAT program (7).

**Results**

Three experimental variables were studied for each metal: pH, the effect of a second trace metal, and the effect of adding Ca^{2+}. Three pH values were studied (6, 7, and 8), with the pH effects on trace metal complexation by humic acid shown in Figure 1. For Cd(II) complexation, since log K_{CdOH} = 4.0, the magnitude of displacement between the calibration curve and the titration curves are the measures of Cd(II) binding capacity of humic acid in the pH range of 6 to 8. Cd(II) does not bind strongly to the humic acid at pH 6 and 7. There is a slight enhancement at pH 8. For Pb(II) complexation, with log K_{PbOH} = 6.0, the displacement distance between calibration curve and titration curve indicates both humic acid and OH^- complexation occur. At pH 8, equilibrium calculations indicate that PbOH^- represents 50% of the total metal concentration when no humic acid is present, while at pH 6, it is negligible. These plots suggest that Pb(II) can bind strongly to humic acid and Pb(II) complexation pattern is similar to Pb(II). The only difference is that OH^- complexes significant amounts of Cu(II) at pH 7. The results of these trace metal suggest that the relative magnitude of the binding constants should be Cu(II) ~ Pb(II) > Cd(II).

The values of conditional binding constants (K) and total ligand site concentrations (L_r) determined from single ligand model (Equation 6) for Cd(II), Pb(II), and Cu(II) at pH 6, 7, and 8 are listed in Table 1. Modeling results indicate that conditional binding constants for Cd(II) are about 10^4 for all pH values. Similarly, site concentrations are approximately the same for all pH values. The conditional binding constants for Pb(II) and Cu(II) are one

**Figure 1.** pH effects on (A) Cd(III), (B) Pb(II), and (C) Cu(II) complexation by humic acid.

**Table 1.** Conditional binding constants and total ligand concentrations for Cd(II), Pb(II), and Cu(II) complexation by humic acid.

| pH | Cd(II) | Pb(II) | Cu(II) |
|----|--------|--------|--------|
| 6  | 0.06 ± 0.01 | 0.16 ± 0.01 | 0.30 ± 0.07 |
| 7  | 0.09 ± 0.02 | 0.13 ± 0.01 | 0.14 ± 0.03 |
| 8  | 0.11 ± 0.03 | 0.09 ± 0.01 | 0.04 ± 0.00 |
|    | L_r μM |        |        |        |
| 6  | 3.43 ± 0.22 | 11.25 ± 0.35 | 7.96 ± 0.38 |
| 7  | 2.30 ± 0.58 | 23.73 ± 0.88 | 18.75 ± 2.74 |
| 8  | 5.49 ± 0.69 | 50.57 ± 6.83 | 81.95 ± 3.75 |
order of magnitude higher, $10^5$. They are more sensitive to pH than those for Cd(II), and decrease with pH. Conversely, the binding site concentrations increase with increasing pH. Thus, the two variables are inversely correlated.

Competing effects of trace metals and Ca$^{2+}$ at pH 6 are shown in Figure 2. For Cd(II), the titration curves obtained in the presence of Pb(II), Cu(II), and Ca$^{2+}$ are almost the same as the titration curve with no additional metals. It suggests that both trace metals and Ca$^{2+}$ have very little competing effect. This could imply that competing metals bind to different sites than Cd$^{2+}$. From the titration curve and the competitive titration curves of Pb(II), we can see that while Cu(II) has no effect on the Pb(II) complexation, the presence of Cd(II) appears to slightly enhance the binding between Pb(II) and humic acid. Of the competing metals investigated, only the presence of 5 mM Ca$^{2+}$ decreases the amount of Pb(II) complexed by humic acid. The competing effects of other metals with Cu(II) show different trends. While Ca$^{2+}$ competes effectively with Cu(II) as well as Pb(II), Pb(II) displaces Cu(II) from humic acid at the high Cu(II) loading level. On the other hand, the Cd(II) also appears to enhance the bonding between Cu(II) and humic acid at the low Cu(II) loading range.

The effects of pH on systems containing both Ca$^{2+}$ and trace metals are illustrated in Figure 3. The plots are obtained by subtracting the titration curves with Ca$^{2+}$ from those obtained without Ca$^{2+}$. At pH values of 6 and 7, Ca$^{2+}$ has little effect on Cd(II) binding. A relatively large amount of Cd(II) is displaced by Ca$^{2+}$ at pH 8; the magnitude of displacement increases with Cd(II) loading. For Pb(II), we observe fairly large displacements at both pH 6 and 7; similar to Cd(II), the magnitude of displacement increases with metal loading. At pH 8, however, there is less competition. The competing effects of Ca$^{2+}$ on Cu(II) binding are similar to Pb(II), but due to the difference in hydrolysis constants, the competing effects of Ca$^{2+}$ on Cu(II) are shown to decrease at both pH 7 and 8.

**Discussion**

As shown in the results, the binding strengths of Cd(II), and Pb(II), and Cu(II) with humic acid are in the order Cu(II) > Pb(II) > Cd(II), and the conditional binding constant for Cd(II) is about one order of magnitude smaller than those of Pb(II) and Cu(II). The same trends have been described in many similar investigations studying trace metals with fulvic acid (8,9). This is because both Pb(II) and Cu(II) are on the borderline between hard and soft acids, and they have similar strong preferences for ligand atoms such as O and N. For example, they have similar binding constants for a variety of carboxylic acids (10). Cd(II), however, prefers soft ligands such as S. As our humic substances have only 1.8% S versus 36% O plus N, it is not surprising that we observe less Cd(II) binding.

According to Cabaniss and Shuman (3), changing pH affects metal–ligand binding due to a) competition between H$^+$ and Me for binding sites; b) competition between humic acid and inorganic ligands (OH$^-$) for the metals; and c) potential formation of mixed ligand species such as hydrolyzed metal–ligand complexes. Electrostatic and conformational effects at different pH values may also be important. Examining the change in binding strength for all the metals between pH 7 and 8 suggests that a) phenol-containing sites become important with increasing pH, or b) the concomitant change in conformation increases the electrostatic interaction between humic acid and metals. The relative decrease in Cu(II) binding strength at pH 8 is due to the OH$^-$ competing with humic acid for Cu(II).

Previous studies (11) have shown that competition between Cu(II) and Cd(II) is
minor, and no report has indicated that Cd(II) can enhance the binding of Pb(II) and Cu(II) with humic acid. We suspect that the small enhancement observed in this work may be due to an experimental artifact such as the humic acid coating the Hg electrode surface. However, it is also possible that the initial binding of Cd(II) to humic acid causes a conformational change and therefore enhances the binding (2). While it is understandable that Pb(II) and Cu(II) have no competing effect on Cd(II) binding and Pb(II) competes with Cu(II) for binding sites, the observation of no effect of Cu(II) on Pb(II) binding is not what we expected. The possible explanation is that Cu(II) at low concentration binds to some Cu(II)-specific sites.

In previous studies, Ca\(^{2+}\) was reported to have no effect on Cu(II)-humate binding (12). In this work, we observed that Ca\(^{2+}\) does have a measurable effect on all the trace metals studied. The effect, however, was pH dependent. Since Ca\(^{2+}\) mainly forms outer-sphere complexes with ligands, the effect of adding Ca\(^{2+}\) might be due to an overall change in electrostatic forces. The effect of adding Ca\(^{2+}\) is, perhaps, analogous to the ionic strength effect on Cu(II)-fulvic acid binding reported by Cabaniss and Shuman (3). Ca\(^{2+}\) has the smaller effect on Cd(II) binding at pH 6 than at pH 8; this might be due to partial neutralization of the negative charge on humic acid by protons at pH 6. The observation that Ca\(^{2+}\) has a smaller effect on Pb(II) and Cu(II) binding at pH 8 relative to pH 6 may be explained by the conformational changes or increased binding of PbOH and CuOH relative to pH 7. This explanation is also supported by the observation that at pH 7, Ca\(^{2+}\) has a larger effect on Pb(II) binding than on Cu(II) binding.

Contrary to the report by Cabaniss and Shuman (3) that ionic strength effects are smaller at high metal loading than at low metal loading, in the current study we observed that the Ca\(^{2+}\) effect increases with trace metal loading. This may indicate that the competing effect is cumulative and proportional to the bound metal concentration. In this discussion, we have referred to the binding strengths based on the relative displacement of the titration curves from the blank curve. We have determined \(K_L\), for each curve and found that they are inversely correlated. However, the \(K_L\) values for Cu(II) are well within the range of reported values (13). Since \(K_L\) are inversely correlated fitting parameters, they cannot be evaluated separately because a specific value of \(K\) represents an overall binding capacity at a specific value of \(L\). Their products might have physical meaning, since they indicate the binding strength of trace metals under the experimental conditions (Table 2) and they follow the displacement trends of the titration curves.

Results obtained in this study indicate that trace metals do not necessarily compete for the same sites on the humic acid, since they have different affinities for different binding sites. Conformational changes that can occur when trace metals bind to the different sites may cause either a competing or an enhanced effect. Ca\(^{2+}\), however, is two orders of magnitude higher in concentration than the trace metals and outcompetes the trace metal for sites where electrostatic interaction dominate. The results also indicate that in groundwater situations where more than one metal is present, the effect of other metals must be considered in predicting metal speciation.

Although these experiments were conducted in the laboratory, the results have some implications about the movement of trace metals in groundwater: a) at pH values of natural waters (6–10), the presence of Ca\(^{2+}\) may prevent the trace metal from binding to humic acid and therefore reduce the importance of metal–humate complexes in natural water systems; and b) when more than one metal is present, the effect of other metals must be considered in predicting metal speciation.

### Table 2. Binding strength of Cd(II), Pb(II), and Cu(II) to humic acid at different pH values.

| pH  | Cd(II)   | Pb(II)   | Cu(II)   |
|-----|----------|----------|----------|
| 6   | 0.20 ± 0.02 | 1.63 ± 0.08 | 2.41 ± 0.44 |
| 7   | 0.19 ± 0.00 | 2.98 ± 0.06 | 2.62 ± 0.19 |
| 8   | 0.61 ± 0.12 | 4.14 ± 0.14 | 2.86 ± 0.03 |

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