Evaluation and Application of Dialysis Titration Technique for Determination of Complexing Capacity of Freshwaters

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ABSTRACT

The purpose of Part 1 is to evaluate dialysis titration as a method of determining the complexing capacity (metal ion binding ability) of 10 mg/L soil-derived fulvic acid (SFA) solutions. We determined the complexing capacity of SFA for Cu^{2+} or Cd^{2+} at pH 5, 6 and 7 during dialysis experiments. SFA complexing capacity values are greater for Cu^{2+} than Cd^{2+} at the same pH and generally increase for either Cu^{2+} or Cd^{2+} as pH increases. A maximum of 10% of SFA complexing capacity permeates the dialysis membrane. A statistical comparison of dialysis and Cu^{2+} selective electrode results show no difference in the ability of the two techniques to measure complexing capacity.

Part 2 describes the ability of naturally occurring ligands in seven New Hampshire freshwater samples to bind Cu^{2+} and Cd^{2+} by a dialysis titration technique. The resulting values called potential binding levels (PBL) are the residual Cu^{2+}-and Cd^{2+}-binding properties of the water samples. Cu^{2+} PBL were 1.1 to 15.1 μM and Cd^{2+} PBL were 0.0 to 9.7 μM. We correlated alkalinity, pH, dissolved organic carbon, hardness, conductance, and UV absorbance to Cu^{2+} and Cd^{2+} PBL values. At the 90% confidence level Cu^{2+} PBL values had significant correlations with inorganic properties but not with organic properties of the samples, and Cd^{2+} PBL values had no significant trends.

Keywords: Humic Material, Fulvic Acid, Dialysis, Complexing Capacity

PART 1: EVALUATION OF DIALYSIS TITRATION TECHNIQUE FOR DETERMINATION OF SOIL FULVIC ACID COMPLEXING CAPACITY

INTRODUCTION

The complexing capacity of isolated humic matter (fulvic and humic acids) and humic matter dissolved in natural waters is the ability to complex free hydrated metal ions. The analytical difficulty inherent with complexing capacity measurements is to distinguish the free metal ion species from the complexed species, while having minimal effect on chemical equilibria. Past researchers have employed biological and chemical techniques to make these exacting measurements. Biological methods often shift equilibria less than chemical methods, but offer a smaller range of metal ions appropriate for study [1-3]. Chemical methods offer precision, convenience, sensitivity, and a greater number of metal ions that can be studied.

It is convenient to place chemical methods in three categories: miscellaneous, electrochemical, and membrane separation or gel filtration chromatography (GFC). The miscellaneous category includes a copper(II) solubilization method [4,5], a cobalt(III) complexation method [6,7], and chromatographic methods using MnO_2 [8,9] or Chelex-100 resin [10-12].

Electrochemical methods such as metal ion selective electrode (ISE) potentiometric titrations are commonly employed to determine complexing capacities [13-19]. Many research groups also use voltametric titrations to measure metal ion complexing capacities

[20-30], although this application of voltammetry is controversial because of potential interferences with the working electrode [31-37].

A group of analytical methods to determine metal ion binding capacities by separation use GFC [38-40], ultrafiltration [41], or dialysis [42-46] to segregate free metal ions from metal complexes during a titration. Determination of concentrations of both metal species after separation by various analytical techniques is routine for many metal ions. An advantage offered by dialysis is the ability to make _in situ_ measurements of natural metal ion abundances [46]. This molecular size separation technique is attractive compared to the other complexing capacity determination methods because it is versatile, and it avoids possible sample alterations caused by the addition of complexing agents or electrolytes. An important advantage of dialysis titrations is that chemical equilibria are undisturbed.

The purpose of this study is to evaluate a dialysis titration method for the determination of humic material complexing capacities. We used soil-derived fulvic acid (SFA) as a model of naturally-occurring dissolved organic matter and atomic absorption spectrometry (AAS) for metal ion determinations. We evaluated the effectiveness of dialysis segregation of free and complexed metal ion, dialysis titration reproducibility, and dialysis membrane permeability to metal ions and uncomplexed ligand.

EXPERIMENTAL SECTION

Materials and Reagents

All solutions were prepared with water that was doubly deionized and distilled from $KMnO_4$ solution. Analytical grade reagents were used to prepare all solutions with two exceptions. The metal ion standard solutions were all prepared from Fisher 1000 ppm Atomic Absorption solutions, and all soil-derived fulvic acid solutions were prepared from SFA isolated and characterized by Weber and Wilson [47].

Glass, polycarbonate, polypropylene and polyethylene apparatus and vessels were cleaned by soaking in 10% HNO_3 followed by rinses with distilled water. All metal ion solutions were prepared in polypropylene volumetric flasks using disposable polycarbonate-tipped pipets. Dialyses and sample storage were carried out in polypropylene bottles.

The 1000 molecular weight cut-off (m w c o) Spectra/Por 6 dialysis bags (Spectrum Industries, Los Angeles, CA) were cleaned prior to use with a modification of a procedure of Guy [48]. The bags were rinsed and soaked in warm water to remove the preservatives. They were soaked in 0.1% Na_2S solution at about 60°C for 15 min, rinsed with warm distilled water, soaked in 3% H_2SO_4 at about 60°C for 5 min., rinsed in warm distilled water again, and stored in distilled water until needed.

Complexing Capacity Measurements by Dialysis

The dialysis titration procedure involved immersing a dialysis bag with 80 mL 0.001 M KNO_3 in 2L of sample solution. The internal dialysis solution (diffusate solution) pH was adjusted with 0.1 M KOH or 0.1 M HNO_3 to match the external dialysis solution (retentate solution). Dialysis was performed at room temperature, with constant stirring for an equilibration period of 48 h between titrant additions. After this equilibration period 5 mL aliquots of dialysis retentate and diffusate solutions were taken and acidified with 50 µL 16 M HNO_3. Metal ion concentration measurements were performed by flame AAS (Techtron AA5 Spectrometer) or flameless AAS (Instrumentation Laboratories Model 351) using a calibration curve of standard solutions. All complexing capacity titration data was obtained and evaluated in the same way. Sequential additions of metal ion are made to the solution of the complexing agent, then free (uncomplexed) metal ion concentration and total (uncomplexed and bound) metal ion concentration are measured after an appropriate equilibration period. Total metal ion concentrations are measured rather than calculated to distinguish the amount of metal ion added from the amount actually available for coordination, should any metal ion be lost from solution by wall effects, precipitation, etc. In the dialysis technique, the internal dialysis bag (diffusate) solution metal ions are considered free, and the external (retentate) solution metal ions are both free and coordinated (total). Since the retentate to diffusate solution volume ratio is 2000/80, the retentate volume approximates the total volume. This procedure was used to measure the complexing capacity of 6.25 μM EDTA, 0.001 M KNO_3, and 15.5 μM SFA.

Each titration curve obtained has two linear branches. The lower branch of early data points has essentially zero slope, and the upper branch of data after the titration endpoint is markedly sloped. The complexing capacity is the x-axis intercept of the upper branch, which is determined by linear regression and expressed in μM total ion.

Paired t-Test Experiments: Dialysis and ISE

A paired t-test of dialysis titration and Cu^{2+} ISE titration complexing capacities was conducted to determine whether the two titration techniques provide the same results. Five 15.5 μM SFA solutions were prepared; two at pH 6, two at pH 7, and one at pH 5, and the complexing capacity of each was determined by both methods.

Calibration titration curves (0.100 M KNO_3) were taken three times daily to measure the ISE response to Cu^{2+} as in previously described Cu^{2+} ISE experiments [49]. The calibration curves were run at exactly the same pH as the SFA titrations since constant pH is critical to the electrode response at pH > 4 [50]. All SFA titrations were carried out in 0.01 M KNO_3 to maintain a constant ionic strength throughout the experiment and the titrations were performed at 25.0°C with light excluded from the titration vessel. The electrode required frequent polishing during the titration of high pH solutions in order to retain a Nernstian response. Electrode response times were slower at pH 7 than at pH 5, but 5 min to 1 hr equilibration periods were used between additions at all pH values.

Dialysis Membrane Permeability Study

The 1000 m w c o dialysis membrane permeability to Cu^{2+}, Cd^{2+}, and SFA was determined by sampling another series of metal ion-SFA dialysis diffusate and retentate solutions as a function of time. In these permeability studies, 2 L of either 10 mg/L SFA or 0.001 M KNO_3 were dialyzed against 80 mL 0.001 M KNO_3. The Cu^{2+} and Cd^{2+} concentrations were 31.5 μM and 17.8 μM, respectively. The pH of all internal and external dialysis solutions was adjusted to the appropriate level with minute volumes of 0.01 M KOH.

In the permeation experiments, the membrane diffusion rate of the metal ion and organic matter was monitored by taking aliquots of internal and external dialysis solutions periodically. SFA diffusion was monitored by taking 10 mL solution aliquots, raising their pH to 7.6, then measuring their 260 nm absorbance, i.e. "color" (Cary 14, Applied Physics Corp., Monrovia, CA), and 435 nm fluorescence emission after excitation at 350 nm (Perkin Elmer 204 Fluorometer, Norwalk, CT). The results were compared to a pH 7.6 SFA calibration curve. To determine metal ion diffusivity, 5 mL aliquots of dialysis retentate and diffusate solution were taken, acidified with 50 μL 16 M HNO_3, and analyzed for metal ion concentration by flame AAS.

Dialysis of 62 μM (40 ppm) SFA against 0.001 M KNO_3 at pH 5 for 36 days with no metal ions added was also performed. At the end of this dialysis period the absorbance, fluorescence, and Cu^{2+}

binding capacity in the diffusate solution were measured. Cu^{2+} binding capacities of the dialysis retentate and diffusate solutions were determined in a fluorometric titration at pH 5 where, after each addition of Cu^{2+} and pH adjustment, the 435 nm fluorescence emission intensity of the dialysis solution was measured. Distilled water was the reference solution. Plots of fluorescence intensity vs. total Cu^{2+} added were used to calculate the intersection of the upper and lower titration curve branches. The total Cu^{2+} added value of this intersection is the binding capacity of the diffusate solution.

RESULTS AND DISCUSSION

Dialysis Membrane Permeability to Cu^{2+}, Cd^{2+}, and SFA

The validity of complexing capacity determinations by dialysis titration rests on two requirements. First, the dialysis membrane should effectively exclude complexed metal ion and uncomplexed ligand from the diffusate solution since its metal ion concentration is assumed to be free metal ion. Complexed metal in the internal solution causes an underestimation of the complexing capacity of the ligand. Similarly, free ligand that passes the membrane, not being available for complexation in the retentate solution, also results in an underestimation of the complexing capacity. Second, uncomplexed metal ion diffusion through the dialysis membrane must be unhindered so that the uncomplexed metal ion levels are the same in the internal and external solutions. This requirement is necessary because the internal dialysis solution metal ion concentration is taken as the equilibrium "free" metal ion concentration in dialysis complexing capacity determinations. We tested both of these requirements in three sets of dialysis experiments.

M^{2+}- KNO_3 vs. KNO_3 Dialyses. The M^{2+}-KNO3 vs. KNO3 dialysis permeation experiments (solutions A-F in Table 1-1) are experimental blank dialyses. That is, these diffusate and retentate solution values of absorbance, fluorescence and metal ion concentrations are background levels for comparison with SFA dialysis permeation measurements. These measurements also determine metal ion diffusion that is independent of the influence of SFA. We found that metal ion equilibrium between the diffusate and retentate solutions occurs in less than one day, and that diffusion appears to be pH independent from pH 5-7. The absorbance of all the diffusate and retentate solutions gradually increases over the period of a dialysis titration. Moreover, the color increases seem pH dependent (Table 1-1). Fluorescence values of the M^{2+}-KNO_3 vs. KNO_3 control dialysis solutions do not change with time on the 1-27 day experimental time scale, and are independent of pH. The material responsible for the gradually increasing UV absorbance in the dialysis control solutions has very weak fluorescence properties since the KNO_3 blank dialysis solutions all have very small 0.1 to 0.4 (mg/L as SFA) fluorescence emission intensity (Table 1-1). The cellulose membranes are probably the source of the UV absorbing and fluorescing materials seen in these blank experiments.

M^{2+}-SFA vs. KNO_3 Dialyses. The second set of permeation experiments were M^{2+}-SFA vs. KNO_3 dialyses. We determined the

Table 1-1. Dialyses Used to Determine Membrane Permeability to SFA[a], Cu^{2+}, and Cd^{2+}

Dialysis Solution[b]		pH	M^{2+}	UV-Absorbance[c]		Fluorescence[c]	
				Retentate	Diffusate	Retentate	Diffusate
0.001 M KNO_3							
	A	5	Cu	3.3	3.4	0.6	0.4
	B	6	Cu	1.7	3.5	0.2	0.1
	C	7	Cu	2.0	1.8	0.2	0.1
	D	5	Cd	0.4	0.8	0.1	0.1
	E	6	Cd	0.5	0.6	0.1	0.1
	F	7	Cd	0.5	0.8	0.1	0.2
15.5 μM SFA							
	G	5	Cu	10.0	3.0	3.0	2.0
	H	6	Cu	10.0	3.0	3.0	3.0
	I	7	Cu	10.0	0.5	3.0	2.0
	J	5	Cd	9.5	2.0	8.0	5.5
	K	6	Cd	10.0	1.5	9.5	5.0
	L	7	Cd	8.0	1.8	10.0	5.5
62.0 μM SFA		5	--	35	3.5	40	9

[a] SFA is soil-derived fulvic acid.

[b] All solutions were dialyzed against 0.001 M KNO_3.

[c] Mg/L as SFA.

permeability of Spectra/Por 6 1000 m w c o dialysis membranes to SFA diffusion from a series of dialyses of 15.5 μM (10 mg/L) SFA-M^{2+} vs. 0.001 M KNO_3 (solutions G-L in Table 1-1) by monitoring dialysis retentate and diffusate solution color and fluorescence as a function of time, just as in the above blank experiments. Point for point subtraction of blank dialysis color and fluorescence intensities from the SFA measurements isolated the contribution of SFA from that of the dialysis membrane material which gradually appears in solution.

There is a limited pH dependence of metal ion diffusion in the M^{2+}-SFA vs. KNO_3 dialyses. Because of the greater metal ion binding ability of SFA at higher pH, there are smaller Cd^{2+} and Cu^{2+} diffusate concentrations at pH 7 than at pH 5. At pH 7 the free Cd^{2+} levels are approximately twice the comparable free Cu^{2+} concentrations which shows the relative extent of Cd^{2+} and Cu^{2+} complexation by SFA in the equilibrium diffusate concentrations of the two metal ions. Metal ion diffusate solution concentrations reach equilibrium across the dialysis membrane rapidly, just as they did in the M^{2+}-KNO_3 vs. KNO_3 blank dialyses.

Diffusate solution absorbance in the M^{2+}-SFA vs. KNO_3 experiments increases up to ca. 15 days of dialysis before achieving a constant value in all SFA dialyses. The final values increase as pH increases in the Cu^{2+} dialyses (Table 1-1). Diffusate color rises to ca. 30% of the retentate solution color at pH 5, ca. 30% at pH 6, and less than 5% at pH 7. This pH dependence is much less distinct for the Cd^{2+} dialyses as Cd^{2+} dialysis diffusate color rises to only ca. 20% of the retentate solution absorbance at all pH values.

In M^{2+}-SFA vs. KNO_3 experiments, the diffusate solution fluorescence intensities increased gradually over the dialysis period. The average dialysis fluorescence diffusion rate was 0.05 mg/L/day for Cu^{2+} and 0.21 mg/L/day for Cd^{2+}. The fluorescence data show an important metal ion dependence. The fluorescence of the diffusate Cu^{2+} solutions is 40% of the analogous Cd^{2+} values. Saar [51], in studies of Cu^{2+} complexes of SFA, showed that the 435 nm fluorescence of SFA, but not its UV absorbance, is quenched in direct proportion to its complexation of Cu^{2+}. The Cu^{2+} dialysis fluorescence data then are a measure of uncomplexed SFA. Therefore, a large fraction of the diffusate molecules have little complexing capacity for Cu^{2+}. SFA complexation by Cd^{2+} has no effect on SFA fluorescence [51], and therefore, no free ligand information can be obtained from the Cd^{2+} dialysis solution fluorescence.

In the above two experiments, we determined the extent that absorbance and fluorescence of 15.5 $\mu\underline{M}$ SFA permeated the membrane in the presence of 31.5 $\mu\underline{M}$ Cu^{2+} or 17.8 $\mu\underline{M}$ Cd^{2+}. The fluorescence of the Cu^{2+} diffusate solution is not indicative of internal SFA complexing capacity. Nor is there evidence that the unquenched fluorescence or absorbance of the Cd^{2+} experiments is proportional to complexing capacity.

Diffusate Complexing Capacity Experiment. The third experiment, a permeation study of 62.0 $\mu\underline{M}$ (40 mg/L) SFA at pH 5, directly measures the diffusate complexing capacity. After 30 days of dialysis of SFA vs. 0.001 $\underline{M}$ KNO_3 at pH 5, the diffusate-to-retentate fluorescence ratio was 22% and the absorbance ratio was 10%.

Thus, 22% of the fluorescence and 10% of the absorbance are inside the membrane. This indicates that the material diffusing through the dialysis membrane had greater fluorescence than UV absorbing tendency. We also measured the complexing capacity of the dialysis diffusate and retentate solutions by monitoring SFA fluorescence quenching during a Cu^{2+} titration [51]. Two trials yielded 8 and 10% of Cu^{2+} complexing capacity inside the dialysis bag.

In related work with our SFA sample, Templeton and Chasteen [52] found that initial fractionation by GFC produced two fractions: 91% by weight of large molecules and 9% of small molecules. Their electron paramagnetic resonance (EPR) study of VO^{2+} complexing properties of each fraction revealed that the conditional stability constants of the small molecules are only 2% that of the large molecules. It is likely that this 9% weakly-binding organic fraction predominates in our diffusate solutions.

GFC fractionation [52] and dialysis studies (this work) both suggest that about 10% of metal ion binding capacity of SFA will permeate the 1000 m w c o dialysis membrane. This lost binding capacity limits the usefulness of the dialysis technique and results in underestimation of the metal ion complexing capacity determined in this study. However, the 10% complexing capacity in the diffusate is a high estimate for several reasons. (1) As mentioned above, the smaller molecules are weaker ligands [52]. (2) Permeation of SFA absorbance and fluorescence is greater at pH 5 than pH 6 or 7. (3) The average size of our SFA molecules increases at higher pH values [52]. (4) As demonstrated by our dialysis experiments, SFA complexes permeate the membrane less than uncomplexed SFA molecules.

Dialysis/AAS Complexing Capacity Measurements

Complexing Capacity of 6.25 μM EDTA. Having established the metal ion and SFA diffusion properties with 1000 m w c o membranes, the next step in evaluating complexing capacity measurements by dialysis/AAS was to perform a series of titrations on EDTA and SFA solutions. In a preliminary experiment we measured the complexing capacity of the strong ligand EDTA for Cu^{2+} and Cd^{2+} at pH 6. The result for 6.25 μM EDTA is a 6.4 μM complexing capacity for Cu^{2+} and Cd^{2+} (Table 1-2). The dialysis titration technique provided an experimental EDTA complexing capacity that deviated only 2.4% from theoretical Cu^{2+} and Cd^{2+} complexing capacities.

Complexing Capacity of 15.5 μM SFA. Next, we did a series of Cu^{2+} and Cd^{2+} titrations of SFA at pH 5, 6, and 7. SFA, a complicated mixture of compounds, does not form metal ion complexes with well-defined stoichiometries. Rather, SFA complexation is a function of the metal ion, pH, ligand concentration, degree of complexation, and ionic strength [49, 51, 53]. Complexing capacities from our dialysis experiments should show previously observed trends. Titration of 15.5 μM (10 mg/L) SFA by Cu^{2+} and Cd^{2+} yield Cu^{2+} complexing capacities (μM) of 16.2 for pH 5, 24.1 for pH 6 and 28.7 for pH 7; and Cd^{2+} complexing capacities (μM) of 8.0 for pH 5, 15.7 for pH 6, and 19.3 for pH 7 (Table 1-2). These results are typical because complexing capacities of SFA are greater for Cu^{2+} than Cd^{2+} at the same pH, and increase for both metal ions as pH increases [49, 51, 53].

Table 1-2. Dialysis/AAS Complexing Capacity Results[a]

Titrated Solution		Titrant	Complexing Capacity (µM)	Titration Curve Slope
EDTA (6.25 µM)				
	pH 6	Cu	6.4	1.2
		Cd	6.4	1.2
SFA (15.5 µM)				
	pH 5	Cu	19.2[b]	1.0
			16.4	1.0
			13.1	1.1
	Ave(sd)[a]		16.2 (±3.1)	1.08 (±0.04)
	pH 6	Cu	21.8[b]	0.6
			32.2[b]	0.7
			18.4	0.5
	Ave(sd)[a]		24.1 (±7.2)	0.57 (±0.08)
	pH 7	Cu	31.4[b]	0.2
			15.1[b]	0.2
			39.5	0.3
	Ave(sd)[a]		28.7 (±12.4)	0.22 (±0.08)
	pH 5	Cd	8.0	1.1
	pH 6	Cd	15.7	1.1
	pH 7	Cd	19.3	1.1
KNO_3 (0.001 M)				
	pH 5	Cu	0.5	0.9
	pH 6	Cu	-0.5	1.0
		Cd	-1.9	1.0
	pH 7	Cu	1.0	1.0[c]
		Cd	0.0	1.0

---

[a] SFA is soil-derived fulvic acid, EDTA is ethylenediaminetetraacetic acid, sd is standard derivation.

[b] Data used in the paired t-test method comparison.

[c] Curve levels out beyond 25 µM total Cu^{2+}.

The slopes of the upper curves (Table 1-2) are surprisingly varied. Well past the metal ion complexing capacity, we expect titration slopes of unity, because the metal ion complexing ability of SFA is exhausted and all metal ion additions should cause an equal increase in free metal ion. This expected behavior occurs in the EDTA complexing capacity titrations and Cd^{2+} titrations of SFA (Table 1-2). In contrast, the mean Cu^{2+} titration slopes of 0.57 at pH 6 and 0.22 at pH 7 vary appreciably from unity. The diminished slopes indicate the presence of soluble Cu^{2+} species that do not permeate the membrane at pH 6 and 7 and show that Cu^{2+} diffusion is diminished negligibly at pH 5, by 60% at pH 6, and by 90% at pH 7. Under our experimental conditions, the best candidate for the non-permeating copper(II) species is $Cu_2(OH)_2^{2+}$ [54]. McCrady and Chapman also attribute non-unity slopes from Cu^{2+} titrations of natural waters to inorganic complexation [15].

In M^{2+}-KNO_3 vs. KNO_3 control experiments in the absence of SFA, the titration curve slopes are unity with the exception of Cu^{2+} at pH 7 and the intercepts always near zero (Table 1-2). The Cu^{2+} titration curve at pH 7, however, is greatly distorted by inorganic copper(II) species formation. The slope flattens and there is a great increase in data scatter for free Cu^{2+} beyond about 25 $\mu \underline{M}$. In this titration a green solid precipitated from an unstirred dialysis cell solution, but it soon turned black. Since we did not exclude CO_2 from our experiments, we suspected that the green precipitate was probably $Cu_2(OH)_2CO_3$ (malachite) which decomposed to CuO (tenorite). The conditions of the experiment are near the borderline of malachite-tenorite stability [54,55].

Also, the pH 7 Cu^{2+}-SFA titration was limited to about 25 μM free Cu^{2+} as predicted for the maximum Cu^{2+} solubility in the presence of CO_2 at pH 7 [55]. An X-ray powder pattern determination confirmed that the green precipitate is malachite.

The reproducibility of the experiments shown in Table 1-2 is dependent on several effects. (1) Titration reproducibility problems are mostly caused by pH drift in the 48 h between titrant additions. This problem is greatest at pH 7 where carbonate buffering acts to lower the solution pH to about 5.5. (2) Titration reproducibility would be affected if SFA degraded to different extents in replicate experiments due to oxidation or polymerization. This effect would also be greatest at pH 7. (3) Varying amounts of microorganism growth on the dialysis membranes might inhibit metal ion diffusion or might enlarge the pores. (4) Inhomogeneity in the dialysis membrane porosities would cause different SFA retentions to occur in the dialysis cells, thereby altering the measured complexing capacities and titration slopes.

Comparison of Dialysis and ISE Titration Methods

Prior fulvic acid complexing capacity determinations and the permeation properties of the dialysis membranes discussed earlier suggest that fulvic acid complexing capacity results are method dependent [11,16,48]. In this study we statistically compared dialysis/AAS and ISE results of five SFA titrations to determine if these methods yield the same complexing capacity value. This comparison, which is appropriate since Cu^{2+} complexing capacity is most often determind by ISE potentiometry, was achieved by conducting a paired t-test where the free Cu^{2+} in each SFA solution is determined by both methods. In the paired t-test, Cu^{2+} complexing capacities of five 15.5 μM SFA solutions (pH 5, 6, and 7, Table 1-2) were measured by dialysis/AAS and ISE potentiometry. A statistically significant difference between the paired measurements means that dialysis/AAS and ISE potentiometric titrations of identical solutions do not yield the same result. In fact, the calculated t (1.17) is smaller than the critical t for 4 degrees of freedom at the 95% confidence level (2.78). The t-test conclusion, therefore, is that we cannot reject the hypothesis of no difference between dialysis/AA and ISE potentiometry results.

CONCLUSIONS

In this study we demonstrated that dialysis titration/AAS is an effective way to measure the complexation of SFA by strongly bonding (Cu^{2+}) and weakly bonding (Cd^{2+}) metal ions. At the 95% confidence level, no significant difference could be found between dialysis titration and ISE titration complexing capacities of five SFA solutions. Of all the methods available for the measurement of metal ion complexing capacity to fulvic acid samples, dialysis titration is potentially the most versatile. The method is not limited to paramagnetic metal ions like EPR or fluorescence studies, by relatively low sensitivity like ISE measurements or to a small selection of metal ions like voltammetric determinations. The wide range of metal ions and their concentrations and the capability of low ionic strength (e.g. 0.001 M KNO_3) experiments suggest that the dialysis titration technique is appropriate for the measurement of complexing capacities of unmodified natural water samples. In Part 2, we will demonstrate that dialysis titration is an effective means of determining complexing capacities of freshwater samples.

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PART 2: DETERMINATION OF COPPER(II) AND CADMIUM(II) COMPLEXING ABILITIES OF SOME NEW HAMPSHIRE FRESHWATERS

INTRODUCTION

Since some free (aquo) heavy metal ions are toxic to aquatic life and to man, the chemistry of metal ions in natural waters, particularly drinking water supplies, is an active field of research. The concentration of free metal ions is regulated by processes such as coordination with naturally occurring organic ligands, adsorption to particulates on sediment, and precipitation of metal complexes. A measure of free metal ion regulation of a sample as its "complexing capacity" or "complexing ability". "Complexing capacity" is a measure of the free metal ion binding ability of a water sample just as alkalinity is a measure of its proton binding ability.

The term "complexing capacity" has no conventional definition. Some researchers determine sorption to suspended material in the measurement of free metal ion sequestering mechanisms, recognizing that sorption to clays, colloidal material and sediment is the usual fate of many dissolved metal ions in drinking water sources (1). Most researchers, however, first remove suspended material by microfiltration (ca. 0.4 μm porosity) to isolate dissolved species and greatly simplify the experimental system. In either case, metal ion binding capacity is measured by some kind of titration of the water sample with the metal ion of interest. The titration endpoint is detected by the sudden increase in uncomplexed metal ion when the binding ability of the water sample is exhausted. This unused portion of the binding capacity is the fraction of interest, and it is in fact what is commonly called the "complexing capacity."

Since natural water samples contain bound metal ions, some of the complexing capability of the sample is expended before addition of metal ion. In this paper we carefully distinguish expended metal ion binding capacity from the potential amount available for added metal ions. Total binding capacity, the amount of metal ion binding material present in solution, refers to a specific ion (or group of ions) and is expressed as mg/L or micromolarity of bound species. In our definition total binding capacity has two components. The portion of the capacity already used by ions found in the same is called the natural binding level (NBL). Experimentally, the difference between total and free (unbound) metal ion concentrations is the NBL of the sample. The remaining portion still available for complexation is called the potential binding level (PBL). During a titration of a sample with metal ions the point at which no more metal ion is bound marks its PBL. Total binding capacity is the sum of the PBL and NBL.

Recent work on the measurement of the PBL of natural water samples includes biological (2), ion selective electrode (3,4), chromatographic (5,6), and voltammetric (7) studies. Truitt (8) critically compared these approaches, and noted that each is limited to relatively few metal ions and they all may compromise sample integrity during the analysis. Dialysis titrations, however, are more versatile and affect sample equilibria the least. In

Part 1 we (9) evaluated the dialysis titration technique by measuring Cu^{2+} and Cd^{2+} complexing capacities of soil-derived fulvic acid samples. In Part 2 we used the dialysis titration method to measure PBL values of seven southeastern New Hampshire freshwater samples, and correlated the results with several other properties of the samples.

EXPERIMENTAL SECTION

Materials and Reagents

All solutions were prepared with doubly deionized water that was distilled from $KMnO_4$ solution. Analytical grade reagents were used to prepare all solutions with three exceptions. Ultrex nitric acid (Ventron Corp., Beverly, Mass.) was used to acidify samples for atomic absorption spectrometry (AAS) determination. Metal ion standard solutions were prepared from Fisher 1000 ppm Atomic Absorption solutions and soil-derived fulvic acid (SFA) reference solutions were prepared from SFA isolated and characterized by Weber and Wilson (10). This material is used as a model humic compound and a spectrometric reference compound (8).

Glass, polycarbonate, polypropylene and polyethylene apparatus and vessels were cleaned by soaking in 10% HNO_3 followed by rinses with distilled water. Metal ion solutions were prepared in polypropylene volumetric flasks using disposal polycarbonate tipped pipets. Dialyses experiments and sample storage used polypropylene bottles.

The 1000 molecular weight cut-off (m w c o) Spectra/Por 6 dialysis bags (Spectrum Industries, Los Angeles, CA) were cleaned with a procedure recommended by Guy (11) as modified by us (Part 1). Natural water filtration was accomplished with the polycarbonate filter assembly described by us (12) but a 142 mm Teflon and plexiglass filter support (Nuclepore Corp., Pleasanton, CA) was substituted for the 47 mm polycarbonate support.

Natural Water Sampling

Water samples were taken from seven locations in southeastern New Hampshire including the Oyster River, the Exeter River, the Lamprey River, the Durham Reservoir, and the Portsmouth Reservoir, as representatives of local drinking water supplies. Barrington Swamp and Drew Pond samples were taken for comparison with the drinking water supply samples.

The water samples were all obtained by submerging acid-washed polypropylene bottles 15 cm below the surface before releasing the bottle cap. The bottles were rinsed with sample before an aliquot was collected. Approximately 10L of sample was taken at each site. The samples were taken back to the laboratory and immediately filtered through 0.45 μM polycarbonate membranes using the filter apparatus previously described (12). The samples were packed in ice prior to filtration and were finally stored in polypropylene bottles at 4°C.

Characterization of the Water Samples

Several properties of each natural water sample were determined. Except for the spectrometric and PBL measurements, all characterization procedures were taken from Standard Methods for the Examination of Water and Wastewater (13).

A. Natural water sample pH measurements were made with an Orion 701A electrometer (Orion Research Inc., Cambridge, Mass.) and a Corning model 476050 combination pH electrode (Corning Science Products, Medfield, Mass.).

B. Alkalinity determinations were made by titrating each sample with 0.0288 M HCl to pH 3.7 in triplicate, with the same apparatus used to measure pH. Alkalinity was obtained from the titration endpoint.

C. Dissolved organic carbon (DOC) was determined with a Sybron/Barnstead PHOTOchem Organic Carbon analyzer (Barnstead Company, Boston, Mass.), using 0.21254 g/L KHP (100 mg/L C) as a reference standard. At least triply replicated measurements were made.

D. Water sample hardness was measured by triplicate titrations with 0.01003 M EDTA to an Eriochrome Black endpoint.

E. All metal ion levels were measured by AAS. Iron measurements were made on a Techtron AA5 spectrometer (Varian Corp., Melbourne, Australia) with an air-acetylene flame. Lead, copper and cadmium measurements were made with an IL351 spectrometer and IL555 atomization programmer using pyrolytic graphite furnaces (Intrumentation Laboratory Inc., Wilmington, Mass.).

F. Color measurements were made with a Cary 14 double beam spectrophotometer (Applied Physics Corp., Monrovia, CA). Using distilled water as a reference solution, color was determined by scanning the 300-260 nm spectrum of each sample at the natural pH and at pH 7.6. The reported color is their 260 nm absorbance in a 1 cm path length cell at pH 7.6 compared to a calibration curve produced with a dilution series of pH 7.6 SFA standard solutions.

G. Fluorescence measurements were made with a Perkin-Elmer 204 spectrofluorometer and a Perkin-Elmer 150 Xenon lamp power supply (Perkin-Elmer Corp., Norwalk, Conn.). Fluorescence emissions were determined against distilled H_2O using 1 cm quartz sample cells. Samples were excited at 350 nm and emission was measured at 435 nm. Fluorescence measurements of all samples and standard SFA solutions were made at pH 7.6, using the same calibration procedure as in the color measurements.

H. Conductance was measured at 25.0°C with a YSI 31 conductance bridge (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) and a Pt black conductance cell having a cell constant of 0.260 cm^{-1}. KCl standard solutions were used to determine the cell constant.

I. Cu^{2+} and Cd^{2+} potential binding level (PBL) measurements were made by the dialysis/AAS technique described in detail in Part 1. The technique used for PBL measurements involved titrating samples while they were dialyzing against a small amount of electrolyte solution. The dialyzable (free) metal ion levels were measured by AAS after every addition of titrant. Cu^{2+} and Cd^{2+} PBL results were obtained after linear regression from x-axis intercepts of plots of free vs. total metal ion levels for each water sample.

RESULTS AND DISCUSSION

Water Sample Characteristics

The seven southeastern New Hampshire freshwater samples are a range of freshwater types, including pond water, swamp water, and drinking water sources like rivers and reservoirs. We took samples from the Durham Reservoir, Portsmouth Reservoir, Lamprey River, Exeter River, Oyster River, Drew Pond and Barrington Swamp. All the river samples were taken just upstream from water treatment plants.

We measured several chemical properties of the freshwater samples using established methods (13) to provide a context for the PBL values of each natural water sample, and for a water property correlation study. Each filtered natural water sample was examined for pH, alkalinity, hardness, conductance, dissolved organic carbon content (DOC), color, dissolved iron, copper and cadmium levels, and Cu^{2+} and Cd^{2+} PBL values (Table 2-1). All samples were taken at the end of a month-long rainy period and some were dark brown colored. Soil run-off and groundwater upwelling mechanisms are responsible for increased organic matter and suspended matter in river reservoir water after heavy rain periods (14,15).

Potential Binding Level (PBL) Values

Table 2-1 indicates Cu^{2+} and Cd^{2+} titration data for the freshwater samples. We can make comparisons of Cu^{2+} and Cd^{2+} PBL values of all the water samples except for the Oyster River and Exeter River samples. Cu^{2+} PBL values (2 to 12 $\mu\underline{M}$) are larger

Table 2-1. Natural Water Sample Characteristics

Water[a] Characteristic	Portsmouth Reservoir	Lamprey River	Oyster River	Exeter River	Durham Reservoir	Drew Pond	Barrington Swamp
pH	6.3	6.6	7.3	7.4	7.4	6.4	5.7
DOC (mg/L as KHP)	6.8	11.8	11.4	12.5	7.7	12.0	11.8
Hardness (mg/L as $CaCO_3$)	25	22	30	31	23	7.8	5.3
Color (mg/L as SFA)	12.8	19.6	16.3	11.0	19.0	16.5	17.0
Alkalinity (mg/L as $CaCO_3$)	7.4	20	41	43.4	30.7	4.2	1.7
Conductance (μmhos/cm)	55.2	81.3	115	140	118	65.0	54.8
[Fe] μM	5.30	6.39	3.99	6.75	2.26	6.00	7.23
[Cu] μM	0.044	0.031	0.047	0.015	0.047	0.016	0.011
[Cd] μM	0.020	0.007	0.010	0.036	0.003	0.002	0.00
Cu PBL μM	8.6	10.7	1.1	2.1[b]	5.0	11.9	15.1
Cd PBL μM	0	0.4	n.d.	4.3	3.1	9.7	2.0
Cu Slope	1.0	0.92	0.61	0.67	0.56	0.89	1.15
Cd Slope	0.95	0.97	n.d.	1.03	1.00	1.04	0.98

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[a] KHP is potassium hydrogen phthalate, SFA is soil-derived fulvic acid, PBL is potential binding level, and slope refers to the slope of the dialysis titration curve past the endpoint.

[b] Less reliable value; see the text.

than the corresponding Cd^{2+} values (0 to 10 μM). Only in the doubtful example of the Exeter River (see below) is the Cu^{2+} PBL lower than the Cd^{2+} value. Past ion selective electrode work by this group with Cu^{2+} (16) and with Cd^{2+} (17), and dialysis studies with Cu^{2+} and Cd^{2+} (Part 1) on isolated fulvic acid samples show that the complexing ability of Cu^{2+} is greater than that of Cd^{2+}.

We can make no comparison between the Cu^{2+} and Cd^{2+} PBL values in the Oyster River sample because the Cd^{2+} titration failed when microorganisms attacked the dialysis membrane. Cu^{2+} apparently inhibited this microbial activity since there was no evidence of these organisms in the Cu^{2+} titration solution or in the titration data. Only the Oyster River sample exhibited this type of analytical interference, but obviously any water sample with biota is susceptible to adverse effects using the dialysis/AAS measurement method. The difficult is aggravated over long titration periods because biological growth conditions are optimized. First, the medium is provided with nutrients in the form of the cellulose membrane. Then an incubation period is provided while the titration progresses. In the failed Oyster River sample titration, both the observed spots of growth and the titration data reflected a sudden inhibition of Cd^{2+} diffusion after two weeks of the titration.

The Exeter River Cu^{2+} titration was short-lived due to the solubility limit of some copper(II) species. Soon after the titration began, a green precipitate appeared and uncomplexed Cu^{2+} levels did not increase above 30-50 μM with subsequent Cu^{2+} additions. The Cu^{2+} PBL value of the Exeter River reported in Table 2-1 is calculated from the few data prior to the interference and, as a result, is of dubious validity. The Exeter River has the highest pH (7.4), alkalinity, and conductance of our seven samples. These properties would cooperate to lower Cu^{2+} solubility through the formation of oxide ion, hydroxide ion, carbonate ion or mixed inorganic copper(II) precipitates. A solubility diagram (18) suggests that the green precipitate is malachite ($Cu_2(OH)_2CO_3$), and an x-ray powder pattern identified it.

Table 2-1 also has the PBL titration curve slopes. The slopes are part of the list of natural water sample properties. The x-axis intercept of the free metal ion vs. total metal ion curve is the PBL value due to complexing organic matter. In the absence of a solid any deviation of the slope from unity beyond the endpoint is due to a soluble species that cannot pass the dialysis membrane. Table 2-1 shows that all Cd^{2+} slopes are near unity, but the Oyster River, Exeter River, and Durham Reservoir Cu^{2+} slopes are significantly lower than one. $Cu_2(OH)_2^{2+}$ is the most likely membrane impermeable species on the basis of distribution diagrams (19).

Correlation of Natural Water Sample Characteristics

Correlation coefficients were calculated for all of the properties (Table 2-2) of the Portsmouth Reservoir, Lamprey River, Exeter River, Durham Reservoir, Drew Pond and Barrington Swamp water samples. (The Oyster River sample properties were not included since we did not determine its Cd^{2+} PBL value.) These coefficients reveal statistically significant relationships among the sample properties. Underlined coefficients are correlations

Table 2-2. Correlations of the Natural Water Characteristics[a]

Characteristic[b]	DOC	Hard.	Color	Alk.	Cond.	[Fe]	Copper PBL	Cadmium PBL	Copper Slope	Cadmium Slope
pH	-.119	.755	-.144	.925	.933	-.529	-.930	.156	-.967	.640
DOC		-.316	0.004	.073	.122	.745	.226	.501	.094	.504
Hardness			-.435	.800	.685	-.272	-.880	-.407	-.569	.173
Color				-.250	-.221	-.338	.462	.013	-.009	-.250
Alkalinity					.980	-.259	-.910	-.001	-.844	.670
Conductance						-.296	-.887	.164	-.897	.788
[Fe]							.414	.045	.617	-.014
Copper PBL								.015	.832	-.555
Cadmium PBL									-.317	.669
Copper Slope										-.702

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[a] Underlined correlation coefficients have 90% confidence level significance (d.f = 4, R = .729).

[b] PBL is potential binding level, and slopes refer to dialysis titration data.

assigned significance with a 0.1 probability of error (there are 4 degrees of of freedom, R = .729). Correlations in Table 2-2 lead to two general conclusions. First, the strong correlations are found exclusively among the inorganic aspects of the water samples. These include the positive trends of alkalinity with conductance, hardness with alkalinity, and the pH correlations with hardness, alkalinity and conductance. In general pH trends seem to influence the other water qualities with the notable exceptions of color, DOC and the Cd^{2+} titration parameters. The correlations of the inorganic water properties are not surprising considering their mutual dependence by definition. Second, the organic matter content of the water samples is remarkably inconsistent in its trends with other water properties, as shown in the almost universal poor correlations of DOC and color with other properties. The positive correlation of DOC with iron content is indicative of iron complexation and solubilization by dissolved humic matter. An interesting observation is that color and DOC have essentially no correlation with each other. Indeed, color shows no trends at all. While DOC and color were supposed to be indicators of humic content and binding capacity, their mutual independence confirms the conclusions of our fulvic acid permeation study in Part 1 that color is not a good indicator of humic matter binding capacity. The correlation results could also reflect the small range of colors between the water samples (11.0 to 19.6 mg/L as SFA). That is, the statistics were unable to detect any trends in this small range of values.

The correlations originally of interest are those involving the dialysis titration parmaters, slopes, and PBL values (x-axis intercepts). The Cd^{2+} titration results show only one trend: when the conductance increased, the slope increased. There was little spread among the Cd^{2+} titration slopes, and it is difficult to discern trends. In addition, Cd^{2+} PBL values were generally small (with the exception of Drew Pond), further obscuring trends. In contrast, the Cu^{2+} titration results varied more than the Cd^{2+} results, and we identified some trends. Cu^{2+} PBL values showed negative trends with pH, alkalinity, conductance and hardness, and Cu^{2+} slopes correlated negatively with pH, alkalinity and conductance and positively with Cu^{2+} PCL values.

We collected some of our samples during a prolonged rainy period when extensive soil run-off contributed to the water sample constituency. Under these conditions high concentrations of hardness cations such as Ca^{2+} and Mg^{2+} and Na^+ and K^+ are present. Accompanying anions HCO_3^- and OH^- would contribute to high alkalinity values. The monovalent ions Na^+ and K^+ are unlikely to compete with Cd^{2+} or Cu^{2+} for organic matter, but Ca^{2+} and/or Mg^{2+} might effectively compete when in large excess. The strong negative correlation of Cu^{2+} PBL values with water properties measuring $[Ca^{2+}]$, $[Mg^{2+}]$, $[HCO_3^-]$, and $[OH^-]$ might be indicative of competition of Ca^{2+} and/or Mg^{2+} with Cu^{2+} for the organic ligands. Because Cd^{2+} complexes of isolated aquatic fulvic acid are much weaker than those of Cu^{2+} (9,16,17), Cd^{2+} binding would be more affected by excess Ca^{2+} and Mg^{2+}. We see no negative correlations at the 90% confidence level between Cd^{2+} PBL values and inorganic water properties due to the small magnitude and range of Cd^{2+} PBL values (Table 2-1).

CONCLUSIONS

The correlation study results indicate that inorganic constituents, like alkalinity and pH, influence the Cu^{2+} and Cd^{2+} PBL values more than the organic parameters, DOC and color. Two recently published investigations, in which the authors correlated Cu^{2+}-selective electrode titration binding capacities to other natural water sample properties, have similar conclusions about the relative importance of the organic matter in metal ion binding ability. McCrady and Chapman (4) studied natural river water, well water, and artifically reconstituted water. They concluded that complexing agents other than simple inorganic species (OH^-, CO_3^{-2}) define the titration curve at low Cu^{2+} levels, but pH and alkalinity dominate the upper titration curve slope. Their river samples, taken in the northwestern U.S., had higher pH values (7.0 to 8.5), and higher alkalinities (24 to 219 mg/L as $CaCO_3$) than our samples. The samples of Giesy et al. (3), who sampled southern Maine rivers and lakes, had higher pH values (4.6 to 6.3), and lower alkalinities (1 to 30 mg/L as $CaCO_3$) than our samples. They concluded that Cu^{2+} complexation was largely associated with organic matter while Cd^{2+} binding was chiefly inorganic species controlled. The differences between the three studies in apparently due to the differences in the composition of the water samples. In soft, non-alkaline, acidic, colored, northeastern water systems dissolved organic matter has an appreciable metal binding influence (3). Nearby water systems, sampled during an extended rainy period, exhibit different metal binding properties due to the soil run-off loading of the water, principally with inorganic compounds (this study). The metal ion binding chemistry of the low organic concentration, high pH, and alkaline northwestern waters, however, is principally dominated by inorganic species (4).

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