Long-Term Monitoring of Bioavailable Copper in the Aquatic Environment Using a Resin-Filled Dialysis Membrane

S. Tao, T. Liang

Department of Urban and Environmental Sciences, Peking University, Beijing 100871, People's Republic of China

It has been demonstrated that only a relatively small portion of the total amount of metals in an aquatic system is biologically available. To evaluate the ecotoxicity of the metals in a water body, the measurement of such a fraction is often critical (Wachnik 1988; Jonnalagadda and Rao 1993). A number of methods, including anodic stripping voltammetry have been developed for collecting information on metal speciation. None of them, however, can measure the biologically available fraction for routine monitoring purposes. This is due to both extremely low concentrations of such a fraction and shift of the equilibrium among species during the analysis (Tao 1987). Furthermore, most efforts on metal speciation have focused on the determination of the available metal concentration in a single grab sample. The level of individual species may, however, fluctuate randomly.

The information on the overall bioavailability of the metals over a relatively long period of time is necessary for ecotoxicity assessment. This, however, cannot be provided by measured results from a limited number of samples collected individually. Sodergren (1987) used a dialysis membrane filled with organic solvent to simulate the absorption of organic pollutants by an organism. This technique was applied to predict the levels of bioavailable lipophilic pollutants in organisms and to study bioaccumulation mechanisms in aquatic environments (Sodergren 1990). A similar approach using dialysis with receiving resins was reported by Morrison (1987). A cellulose dialysis membrane, with a molecular weight cut-off of 1000 dalton, filled with Chelex-100 chelating resin was employed to simulate the slow membrane-limited metal transport process across a cell membrane. The method was tested for urban stormwater and other polluted waters. The membrane uptake rate, receiving resin uptake rate, as well as the relationship between metal uptake and rainfall duration were investigated. It was found that the metal uptake rates measured at three stormwater outfalls reflected the sporadic and variable nature of bioavailable metals in stormwater discharge (Morrison 1987, 1989). The dialysis-resin technique has also been employed to determine the free-ion fraction of cadmium in water samples (Berggren 1990). The purpose of the study was to measure the instant concentration of a single sample. Information on overall bioavailability of the metals cannot be provided by this approach.

Correspondence to: S. Tao
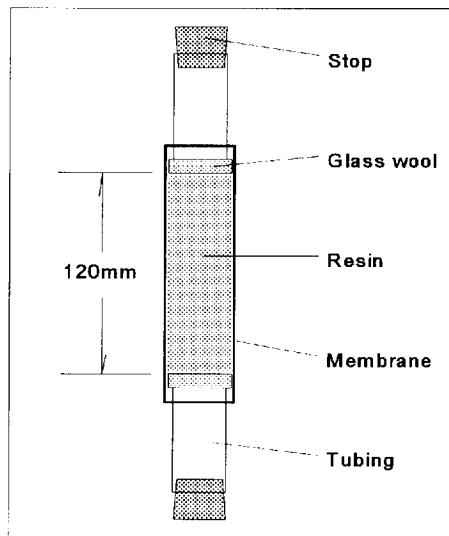
Figure 1. Schematic diagram of a resin-filled dialysis cell

A better understanding of the mechanism of metal uptake, the factors affecting the uptake, and the correlation between membrane and organism uptake is necessary before the technique can be put in full use for ecotoxicity evaluation. The objective of this study was to investigate the feasibility of using the dialysis membrane filled with receiving resin for long-term *in-situ* monitoring of metal availability. As the first step of such an investigation, the results of the study on a number of factors affecting the metal uptake are presented in this paper. These data provide a sound basis for an ongoing comparative study between metal uptake by fish and by the membrane.

MATERIALS AND METHODS

Six types of cation exchange resin (IR-120, IRC-718, TJ-732, Chelex-100, CG-120, TJ-724) have been tested for their affinity to copper. Cellulose dialysis membrane (Spectra/Pro) with pore sizes of 100, 500, and 1000 MWCO were tested. Dialysis membranes of 8-mm diameter were filled with the resin to 25 mm in height. The resin was previously rinsed with 7% HCl (overnight) and deionized water. Both ends of the cells were connected to polyethylene tubing with tiny rubber stoppers (Figure 1). The cells were rinsed with 2 ml of 3% nitric acid (v/v) and 20 ml deionized water before use.

The cells were fixed on specially designed racks in 1000 ml beakers. For static simulation, the solution with copper and other reagents added to the beakers were replaced daily. The concentrations of copper remained unchanged, relatively. The cells were eluted with 2 ml of 3% nitric acid (v/v) after the experiment.

Resin types and membrane pore size were compared for their copper uptake ability. Effects of copper concentration in water, temperature, existence of EDAT and fulvic acid were studied experimentally.

Copper contents were determined using a PAR polarograph, model 303, equipped with a hanging mercury drop electrode, in a differential pulse anodic stripping mode. All instrumental settings were the same as in a previous study (Tao 1987).

The fulvic acid was collected from Yichun River using XAD-8 resin column (Tao *et al.* 1990). The NaOH eluted fulvic acid was deionized by passing it through a cation exchange resin column and dialyzing it in a dialysis membrane (100 MWCO) for 4 days with occasional changes of water. Natural water sample was collected from Jinmi Canal, Beijing and was passed through a 0.45μ filter. All reagents were analytical grade or better and all glassware was soaked in 5% nitric acid (v/v) for 24 hours. Double distilled deionized water was used throughout.

RESULTS AND DISCUSSION

Six types of resin were tested in a preliminary experiment that lasted for 40 days. The concentrations of Cu, Ca, Mg, K, and Na were 0.030, 20, 5, 5, and 5 mg/L, respectively. Table 1 shows the total amounts of copper absorbed by the cells filled with various types of resins. The significant difference among the resins was obvious. Of all resins tested, Chelex-100 was the best one as far as its affinity to copper is concerned. Chelex-100 is a chelate resin and it can adsorb trace metals including copper specifically. Its affinity to copper is almost four-fold higher than that to calcium. Chelex-100 resin was, therefore, selected to fill the dialysis membrane cell for the remainder of the experiments.

Table 1. Copper absorbed by the membrane filled with various types of resins (Cu, Ca, Mg, K, and Na: 0.030, 20, 5, 5, and 5 mg/L, 40 days).

Resin	IR-120	IRC-718	TJ-732	Chelex-100	CG-120	TJ-724
Cu (μg/cell)	9.9	4.7	2.1	14.5	1.9	2.3

The dialysis membrane with pore sizes of 100, 500, and 1000 MWCO was tested for the absorption of copper in solutions with concentrations of 0, 100, 200, or 300 mg/L, respectively. The amounts of copper absorbed by the cells within 120 hours were measured. It was shown that the amount of copper absorbed by a cell was positively proportional to the copper concentration in the solution, indicating a fist-order kinetics of the metal transfer into the cell. The plot of the copper absorbed and the pore size of the membrane shows a curvilinear relation that can be described by a power fiction with non-integral exponent.

Theoretically, when the concentration of copper in the solution or the pore size of the membrane is zero, the amount of copper absorbed by the cell should be zero as well. In other words, both functions for describing relations between the amount of
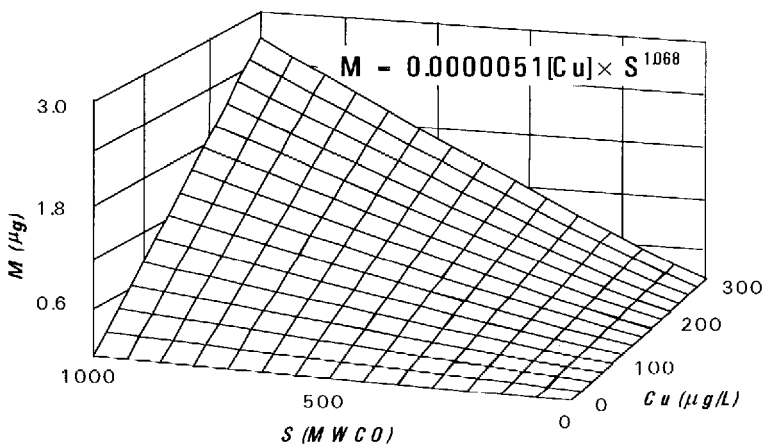
Figure 2. Effect of Cu concentration and pore size on the absorption of Cu by the fesin-filled dialysis cell (the surface was derived by least-square fitting).

metal absorbed (M) and metal concentration in the solution ([CU]) or pore size (S) should be forced to origin:

$$M = a_1 [Cu], \quad M = a_2 S^n.$$

where a_1 and a_2 are constants and n is a non-integral number. When the two equations combined, M can be calculated using the following equation in which n is a pure experiential constant while a is copper absorbed by the cell under the conditions of unit concentration and pore size.

$$M = a [Cu] S^n.$$

The two parameters of a (0.0000051) and n (1.068) were derived by least-square non-linear fitting based on the measured data. The curvilinear surface fitted is shown in Figure 2.

A 160-hr absorption experiment was carried out at temperatures of 0, 10, 22.8, and 30°C (S=1000 MWCO). The initial copper concentration was 60 µg/L. The measured results are shown in Figure 3 as circles and they indicate a nearly linear relationship between copper absorbed and temperature within the tested range.

The increase in the amount of copper absorbed at elevated temperatures was due mainly to the increase in diffusion rate across the stagnant film between the cell wall and the bulk solution. According to Wilke-Chang's equation, the diffusion rate is proportional positively to the temperature and negatively to the viscosity of the solution (Thibodeaux 1979). A curvilinear equation with both temperature (T) and viscosity (η) in it, therefore, can fit the measured data better than a linear one and both of them are shown in Figure 3.
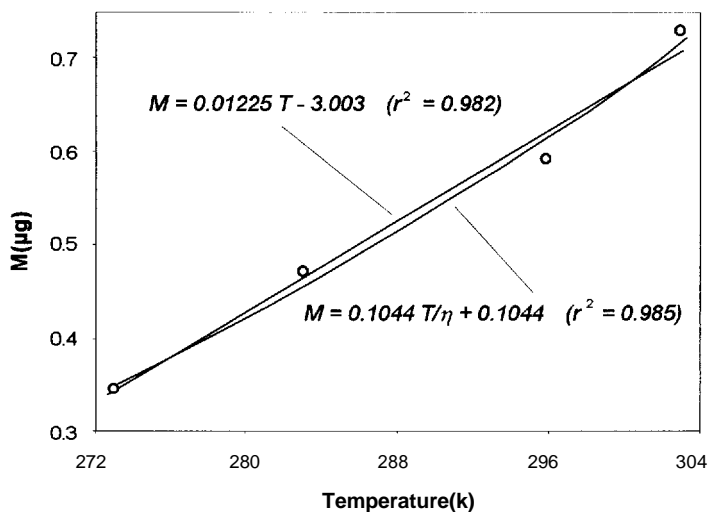
Figure 3. Effect of temperature on the absorption of Cu by the dialysis cell.
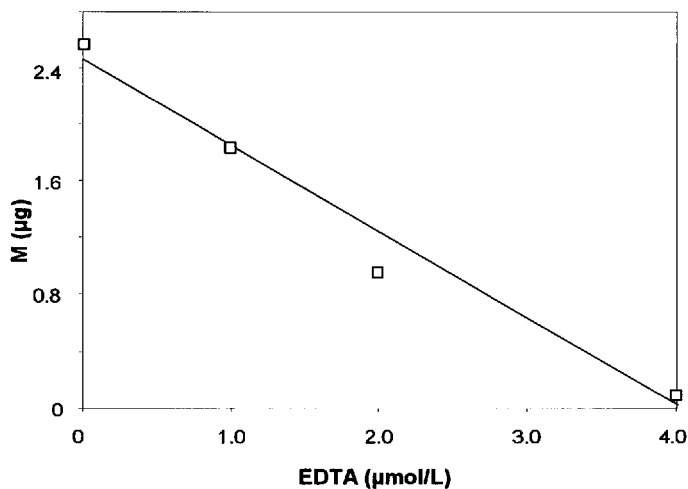
Figure 4. Effect of EDTA on the absorption of Cu by the dialysis cell.

Natural and synthetic organic ligands play an important role in influencing the speciation of copper and affecting the interaction between the organism and the metal (Hansen *et al.* 1990). It is assumed that only free copper ions can pass through the wall of the dialysis membrane while the copper complex with various kinds of organic ligands will not be absorbed by the dialysis cells. EDTA and aquatic fulvic acid were tested for their effects on the absorption of copper by the membrane. The effect of EDTA is shown in Figure 4.
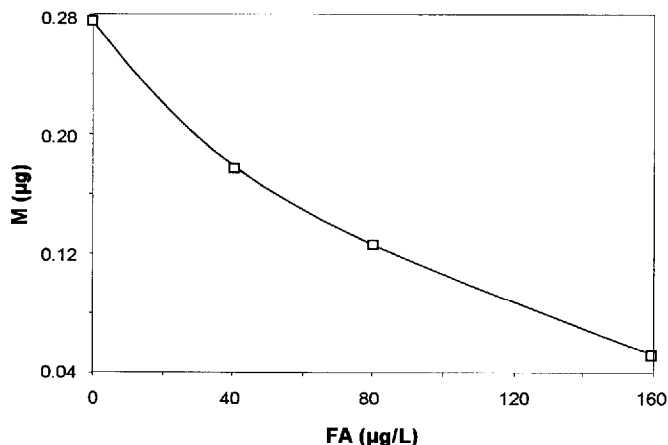
Figure 5. Effect of fulvic acid on the absorption of Cu by the dialysis cell.

The initial concentration of copper in the system was 4 μmol/L, with 0, 1, 2, and 4 μmol/L EDTA added. Since EDTA has a very strong affinity to copper at a completing ratio of 1:1, the free copper ions that remained in the solution were almost equal to the difference between the total concentration of copper and EDTA. With equal molar concentrations of copper and EDTA, the concentration of free copper in the solution was almost zero, as well as the amount of copper absorbed by the cell. With a decrease in the EDTA concentration, the amount of copper accumulated in the cell increased proportionally, indicating that the Cu-EDTA complex was not able to be absorbed by the cell.

A similar experiment using fulvic acid collected from Yichun River water was carried out. The relation between copper absorbed by the cell and the fulvic acid added is plotted in Figure 5. The quantitative relationship between concentrations of metal and ligands is not readily calculated, owing to the heterogeneity of the humic material and complicated stoichiometry of copper-humate binding. Still, the amount of copper absorbed by the cell was negatively proportional to the dosage of fulvic acid as demonstrated in the Figure 5. Like Cu-EDAT, the Cu-fulvic acid complex was not available for absorption by the dialysis membrane cell as well.

The effects of naturally occurring ligands of all kinds were demonstrated by comparison experiments using distilled water and uncontaminated river water (filtrated through a 0.45 μ filter). After the addition of copper into the two systems, the absorption of copper by dialysis membranes was measured during a 40-hr experiment. The result shown in Figure 6 indicated that around 50% of the copper (100 μg/L) added into the natural river water was complexed by naturally occurring ligands and was not available to dialysis cell absorption. If the total concentration of
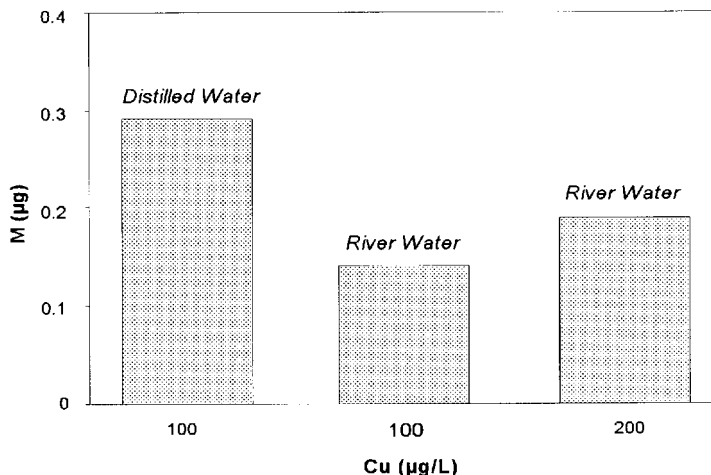
Figure 6. Effect of natural occurring ligands in river water on the absorption of Cu by the dialysis cell.

copper was doubled to 200 μg/L, the absorbed quantity of copper was not proportional to the amount added, indicating again the non-linear stoichiometry of complexation.

In many cases, the free ions are the only metal fraction available biologically to aquatic organisms. The results previously described indicate the feasibility of applying a resin-filled dialysis membrane to monitor this fraction in natural water if the stoichiometrical relationship between the membrane absorption and organism uptake is established.

It is concluded that free metal ions in an aquatic system can be absorbed specifically by a dialysis membrane filled with cation exchange resin. Chelex 100 is the best one among those tested regarding their affinity to copper. The absorption rate of copper by the dialysis membrane is positively correlated to copper concentration in the water, water temperature and an exponential fiction with a non-integrated exponent of membrane pore-size. Only free copper could be absorbed by the dialysis cells, while the complex of copper with EDTA, fulvic acid or other ligands from natural water samples would not transfer across the wall of the dialysis membrane.

REFERENCES

Berggren D (1990) Speciation of cadmiun(II) using donnan dialysis and differential pulse anodic stripping voltammetry in a flow-injection system. Int J Environ Anal Chem 41:133-148

Hansen AM, Leckie LO, Mandelli EF, Altmann RS (1990) Study of copper association with dissolved organic matter in surface waters of three Mexican coastal lagoons. Environ Sci Tech 24:683-688

Jonnalagadda SB, Rao PVVP (1993) Toxicity, bioavailability and metal speciation. Compar Biochem Physiol C-Compar Pharma 106:585-595

Morrison GMP (1987) Bioavailable metal uptake rate determination in polluted waters by dialysis with receiving resins. Environ Tech Lett 8:393-402

Morrison GMP (1989) Bioavailable metal uptake rate in urban stormwater determined by dialysis with receiving resins, Hydrobiologia 176/177:491-495

Sodergren A (1987) Solvent-filled dialysis membranes simulate uptake of pollutants by aquatic organisms. Environ Sci Tech 21:855-859

Sodergren A (1990) Monitoring of persistent, lipophilic pollutants in water and sediment by solvent-filled dialysis membranes. Ecotoxi Environ Saf 19:143-149

Tao S (1987) Zero-deposition time extrapolation DPASV for determination of the complexation capacity. Environ Tech Lett 8:433-440

Tao S, Chen JS, Deng BS (1990) Contents of dissolved humic substances in the rivers of eastern China. J Chinese Geog 1:54-63

Thibodeaux LJ (1979) Chemodynamics, Environmental Movement of Chemicals in Air, Water, and Soil. John Wiley & Sons Inc, NY

Wachnik A (1988) The physiological role of copper and the problems of copper nutritional deficiency. Nahrung 32:755-765