



## Project Summary

# Control of Cadmium Carbonate Precipitation Interferences During the Dialysis of Cadmium in High Bicarbonate Alkalinity Aquatic-Life Bioassay Waters

John E. Poldoski

The precipitation of cadmium carbonate during the dialysis of cadmium in a high bicarbonate alkalinity natural water, was linked to a significant source of error when determining dialyzate cadmium concentrations. The relative standard deviation was reduced by approximately four-fold when this precipitation was controlled by adding a particular preparation of humic acid to the dialysis bag filling solution. Linear regression correlation coefficients for sample-by-sample comparisons between resultant dialysis values and corresponding free cadmium values, obtained by cadmium ion selective electrode, were 0.90 or greater for concentrations in the range of 2  $\mu\text{g}/\text{L}$  to 132  $\mu\text{g}/\text{L}$   $\text{Cd}^{2+}$ .

*This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

### Introduction

Research papers (1-4) have appeared recently concerning the use of dialysis for investigating the metal ion binding

properties of natural waters and the dissolved state of metal ions. In particular, Truitt and Weber (4) have reported using equilibrium dialysis for studying binding from macromolecular natural organics found in several bodies of water of the northeastern U.S., but they noted some interferences, presumably associated with metal carbonate precipitation within the dialysis bag. In the work presented here, some similar problems in the dialysis of cadmium occurred which were linked to cadmium carbonate precipitation in a high bicarbonate alkalinity water that was used for conducting aquatic-life bioassays in this laboratory. In other related chemical kinetics studies of cadmium precipitation in this water, it was discovered that the presence of Aldrich humic acid (HA) drastically reduced this rate of precipitation. Thus, it followed that this observation could be put to constructive use. If an appropriate preparation of humic acid (quenching reagent) was incorporated as part of the filling solution for the dialysis bag, it should be largely incapable of permeating the membrane and into the retentate solution. Therefore, it would be available within the dialysis bag to control cadmium carbonate precipitation. The purpose of this summary is to report on this novel approach to dialysis in natural waters and to illustrate some of its characteristics.



## Experimental Section

### Apparatus

Experiments were conducted using two different molecular weight cut-off (MWCO) dialysis bags (Spectrum Medical Industries, SMI), specifically the 1,000 MWCO (#132634) and 12,000-14,000 MWCO (#132700) sizes clamped with SMI closures.

The flow-through mini-diluters were operated as part of an aquatic-life related study in this laboratory and details of this work, including functioning of the mini-diluters are described elsewhere (5,6).

Atomic absorption spectrometric (AAS) measurements of total cadmium and other elements were made with a Perkin Elmer model 5000 atomic absorption spectrophotometer equipped with deuterium arc background correction, a model HGA-500 graphite furnace, a model AS-40 autosampler, a model AS-50 auto-sampler, and a model 56 strip chart recorder.

Free cadmium ion and pH measurements were made with an Orion model 801 pH meter equipped with a 500 mL FEP teflon cell, an Orion model 9448A cadmium ion selective electrode (ISE), an Orion double junction reference electrode (#900200), an Orion model 605 electrode switch, and a Perkin Elmer model 165 strip chart recorder.

Calculations were performed with a Texas Instruments model T159 programmable calculator equipped with the statistics solid-state software module.

### Chemicals and Reagents

Unless indicated otherwise, all chemicals were of reagent grade quality or better and deionized distilled water (Millipore Super Q) was used for preparing solutions. The high calcium hardness-high bicarbonate alkalinity natural water (hardwater), used as the dilutant water for aquatic-life bioassays, was prepared by dissolving  $\text{CaCO}_3$  from limestone (5,7) in Lake Superior water (softwater) followed by filtration. It was subsequently aerated with 0.45  $\mu$  filtered air until a constant pH of approximately 8.2 was maintained. Non-complexing dialysis retentate solutions (labelled as A or B) were prepared to contain  $4 \times 10^{-3}$  M borate buffer (pH 8) and either  $4 \times 10^{-4}$  M (A) or  $2 \times 10^{-3}$  M (B)  $\text{Ca}(\text{NO}_3)_2$ .

### Quenching Reagent Preparation

The quenching reagent was prepared by adding 1,000 mg of humic acid (Aldrich, H1675-2) to one liter of hardwater and shaking the mixture for 1 hr.

Portions of this solution were then pressure-filtered at 3 atm through a pre-cleaned Millipore 0.45  $\mu$ , 47 mm diameter membrane filter. The 0-10 mL fraction was discarded and the 10-50 mL fraction was collected and saved. This filtration process was repeated with a new filter until the entire 1 L volume was processed. The collected filtrate was diluted ten-fold with deionized distilled water and stored in the absence of light at 4°C until use.

## Results And Discussion

### Reproducibility at High and Low Alkalinity

Table 1 presents replication data for 5 h dialyses of cadmium and other cations in the mini-diluter bioassay system, in addition to cadmium dialysis from retentate solution B. For these cases, deionized distilled water was used as the filling solution to compare the effect of various retentate solutions on reproducibility. Data are generally presented as the mean and relative standard deviation of ratios of total metal concentration in the dialyzate to total metal concentration in the unfiltered solution. In addition, for just hardwater, the average ratio (and its relative standard deviation) of total

cadmium concentration in the dialyzate to total cadmium concentration in the corresponding 0.45  $\mu$  filtrate is also given. Further, the means of the ratios of each filtered concentration to each corresponding total metal concentration are given for comparison. As expected for the softwaters, with little or no precipitation occurring, reproducible ratios close to 1 were obtained. These comparisons show that the filtrate and dialyzate ratios are very similar, suggesting a general equilibrium of cadmium and other dissolved components between dialyzate and retentate solutions. For hardwater, ratios significantly less than 1 were due to a contribution from particulate forms in the bioassay waters. In a typical hardwater flow-through bioassay system,  $\text{Cd}^{2+}$  concentrations of up to 1 mg/L were added to the high bicarbonate bearing bioassay waters. This resulted in the solubility product of cadmium carbonate being greatly exceeded with resultant precipitation continually occurring in the water column and sticking onto the surfaces of the tanks. Obviously, this process could also be occurring inside the dialysis bags, possibly giving rise to a significant source of imprecision and positive bias. These reasons are likely to explain the unusually high average R

Table 1. Reproducibility of 5 h Dialyses in Various Carbonate Alkalinity Waters Using Deionized Distilled Water as the Dialysis Bag Filling Solution

Retentate Water Type	Metal	$R^a$	Percent Relative Standard Deviation	Number	
Retentate Solution B, No carbonate alkalinity	Cd	1.02	6.1	12	
Hardwater, 212 mg/L carbonate, alkalinity bioassay water	Cd	0.366	45.4	10	
		$1.19^b$ (0.312) <sup>c</sup>	34.0	10	
Softwater, 48 mg/L carbonate, alkalinity bioassay water	Cd	0.878 (0.948)	4.4	14	
		Ca	0.912 (0.968)	1.0	4
		Mg	0.757 (0.816)	1.1	4
		Na	0.968 (0.948)	1.0	4

<sup>a</sup> Mean ratio of (total [metal] in dialyzate) / (total [Metal] in retentate).

<sup>b</sup> Indicates mean ratio of (total [Cd] in dialyzate) / (total [Cd] in 0.45  $\mu$  filtrate).

<sup>c</sup> Parentheses indicate mean ratio of (total [metal] in 0.45  $\mu$  filtrate) / (total [metal] in unfiltered solution).

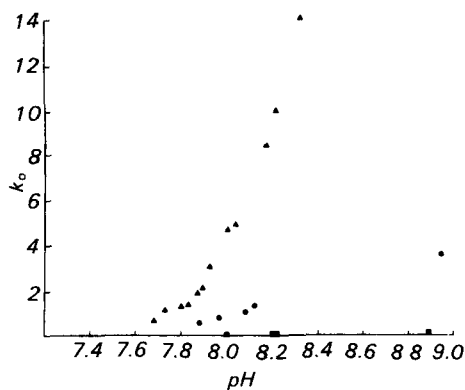
value of 1.19 given for hardwater and the corresponding high relative standard deviation (34%-45%), as presented in Table 1. Subsequent details will further describe this problem and how it might be avoided.

### Quenching Effect of Humic Acid

Chemical kinetics studies (8) of  $\text{CO}_3^{2-}$  added to hardwater, with and without added clay, indicated that the initial reaction rates exhibited pseudo first-order behavior with respect to loss of  $\text{Cd}^{2+}$  from solution and that they were greatly affected by a variety of other parameters, but most importantly, humic acid presence (Figure 1). In the absence of humic acid, observed pseudo first-order rate constants (arbitrary units) ranged from moderate to relatively high values, particularly at high pH and high clay concentration. However, under similar conditions with 50 mg/L humic acid present, observed rate constants were at near zero values over a range of pH conditions. In softwater, with or without clay or humic acid present, loss of  $\text{Cd}^{2+}$  from solution as a function of time was not observed. These specific observations demonstrated the problem and the likely benefit of employing humic acid in the dialyzate.

### Effect of Variables on the Conditional Concentration Factor (F)

The effect of using the quenching reagent preparation as the dialysis bag filling solution was investigated with



**Figure 1.** Water quality variables affecting the pseudo first-order rate constant (arbitrary units) for loss of free cadmium from solution.  
 ▲ - hardwater + clay (90 NTU turbidity) + 10 mg/L  $\text{Cd}^{2+}$ , ● - hardwater + 10 mg/L  $\text{Cd}^{2+}$ , ■ - hardwater + clay (90 NTU turbidity) + humic acid (50 mg/L) + 10 mg/L  $\text{Cd}^{2+}$ .

regard to equilibration time, reproducibility, and possible accuracy of dialysis. Since this solution would undoubtedly bind cadmium ions, there would obviously be a natural tendency for cadmium to concentrate in the dialyzate. Therefore, it was necessary to determine this concentration factor and some factors affecting it. This information could permit calculation of the concentration that would have normally dialyzed in the absence of the concentration effect of the quenching reagent.

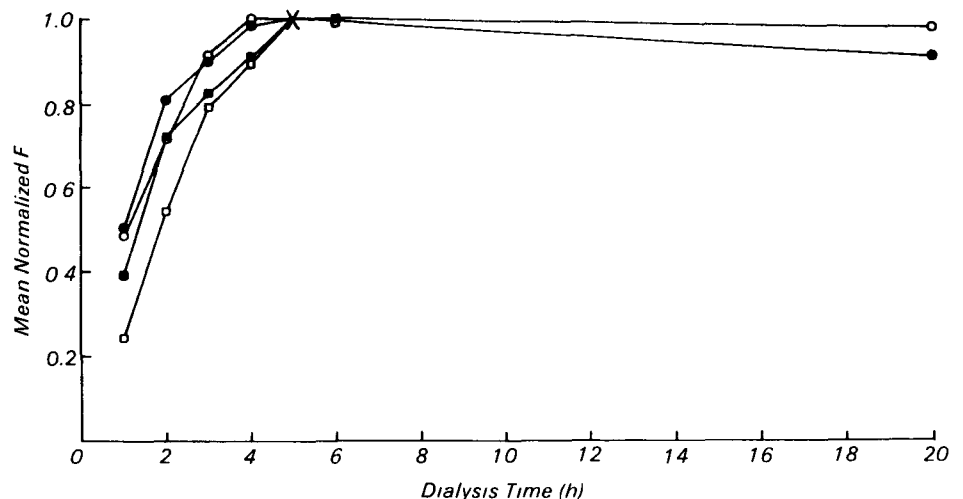
It is appropriate to mention at this time that the experimental system studied (flow-through and fixed volume) had two noteworthy characteristics: 1) the effective retentate volume was infinitely large relative to the dialyzate volume, and 2) the humic acid in the quenching reagent contributed insignificantly to the overall ionic content of the dialyzate. Therefore, the phenomena commonly referred to as Donnan membrane equilibria become insignificant in this case and should not be an additional factor complicating the interpretation of data. Definition of F may be attempted by first considering retentate media containing only dissolved cadmium species capable of equilibrating with the dialyzate, with F given by the following expression:

$$F = \frac{\text{total [Cd] in dialyzate}}{\text{total [Cd] in retentate}} = \frac{[\text{Cd}]_d}{[\text{Cd}]_r}$$

Retentate solutions A or B fulfill these requirements and, therefore, the concentration of the species that are both inside and outside the membrane should be equal at equilibrium. For bioassay media, the concentration of other metals and ligands were considered negligibly low in concentration, except for cadmium, calcium, and bicarbonate. Calcium and bicarbonate concentrations were kept at a constant level, consequently concentrations of cadmium, pH, and dialysis time were studied as main parameters primarily affecting the value of F.

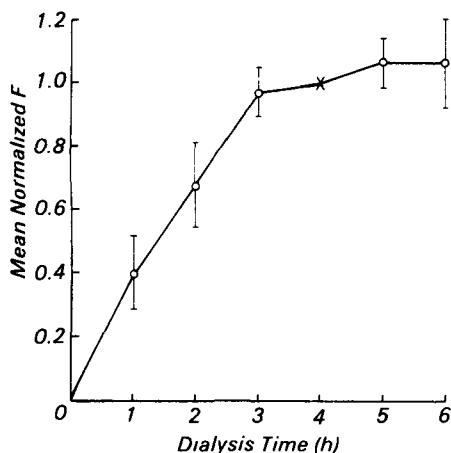
Figure 2 shows the effect of dialysis time (1-20 h) on values of F for retentate solutions A and B, all normalized to each corresponding mean 5 h F value representing a set of conditions. Conditions varied by using either 1,000 MWCO or 12,000 MWCO membranes and total cadmium concentrations in the range of <math>10\text{-}200\mu\text{g/L}</math>. As a result, very similar dialysis rates and equilibrium values were obtained regardless of the particular membrane. It can be seen that the value of F stabilized after 4 h and changed about 10% or less in the 4-20 h range. Reproducibility was in the range of 10% or less for the plateau region.

As anticipated, experiments that were conducted in high bicarbonate alkalinity bioassay water produced similar results with respect to dialysis rate and reproducibility (Figure 3). Under actual



**Figure 2.** Change in mean 5 h normalized conditional concentration factor as a function of dialysis time for retentate solutions A and B  
 Retentate solution A: ○ - 1,000 MWCO, ● - 12,000-14,000 MWCO, Retentate solution B: □ - 1,000 MWCO, ■ - 12,000-14,000 MWCO. X - data to which other data are normalized.

bioassay conditions, a plateau was reached in 4-5 h. In addition, the improvement in reproducibility, that can be seen by comparing data in Figure 3 (error bars indicate standard deviation) to corresponding data in Table 1, strongly indicates that the high error associated with dialysis in this type of water can be significantly reduced by using an appropriate quenching reagent in the dialyzate.



**Figure 3.** Change in mean 4 h normalized conditional concentration factor as a function of dialysis time for high bicarbonate alkalinity flow-through bioassay waters. X - data to which other data are normalized. The number of data points averaged for the 1, 2, 3, 5, and 6 hour periods were 7, 7, 2, 4, and 3, respectively.

## Literature Cited

- 1) Benes, P. Semicontinuous Monitoring of Truly Dissolved Forms of Trace Elements in Streams Using Dialysis In Situ I. Principle and Conditions. *Water Res.* 1980, 14, 511-513.
- 2) Rainville, D.P. and Weber, J.H. Complexing Capacity of Soil Fulvic Acid for  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  Measured by Dialysis Titration: A Model Based on Soil Fulvic Acid Aggregation. *Can. J. Chem.* 1982, 60, 1-5.
- 3) Truitt, R.E. and Weber, J.H. Determination of Complexing Capacity of Fulvic Acid for Copper (II) and Cadmium (II) by Dialysis Titration. *Anal. Chem.* 1981, 53, 337-342.
- 4) Truitt, R.E. and Weber, J.H. Copper(II)- and Cadmium(II)-Binding Abilities of Some New Hampshire Freshwaters Determined by Dialysis Titration. *Environ. Sci. Technol.* 1987, 15, 1204-1208.
- 5) Benoit, D.A. U.S. Environmental Protection Agency, Duluth, MN, Personal Communication, 1981-1983.
- 6) Benoit, D.A., Mattson, V.M., and Olson, D.M. A Continuous-Flow Mini-Diluter System for Toxicity Testing. *Water Res.* 1982, 16, 457-464.
- 7) Lemke, A.E. A Water Hardener for Experimental Use. *J. Am. Water Works Assoc.* 1969, 61, 415-416.
- 8) Poldoski, J.E. U.S. Environmental Protection Agency, Duluth, MN. Unpublished work, 1981-1983.

The EPA author, **John E. Poldoski** (also the EPA Project Officer, see below), is with Environmental Research Laboratory, Duluth, MN 55804.

The complete report, entitled "Control of Cadmium Carbonate Precipitation Interferences During the Dialysis of Cadmium in High Bicarbonate Alkalinity Aquatic-Life Bioassay Waters," (Order No. PB 86-145 620/AS; Cost: \$9.95, subject to change) will be available only from:

National Technical Information Service  
5285 Port Royal Road  
Springfield, VA 22161  
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:  
Environmental Research Laboratory  
U.S. Environmental Protection Agency  
Duluth, MN 55804.

United States  
Environmental Protection  
Agency

Center for Environmental Research  
Information  
Cincinnati OH 45268

Official Business  
Penalty for Private Use \$300

EPA/600/S3-86/001

0000329 PS  
U S ENVIR PROTECTION AGENCY  
REGION 5 LIBRARY  
230 S DEARBORN STREET  
CHICAGO IL 60604