

distinguished. Conversely, a PDMS or FAB mass spectrum of a even a crude fraction containing peptides of nearly equal hydrophobicity would be likely to produce ion signals from all of the components, which would be distinguished by their masses. For example, Tsorbopoulos et al. (16) were able to record most of the tryptic peptides from recombinant human growth hormone by PDMS analysis of HPLC fractions containing peptides with similar retention times.

Thus, for PDMS as well as FAB, an understanding of the important role of hydrophobicity is essential to the development of effective protocols for determining peptide and protein structure.

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TECHNICAL NOTES

Automated Preconcentration of Trace Metals from Seawater and Freshwater

Alexander van Geen*¹ and Edward Boyle

Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

INTRODUCTION

King and Fritz (1) recently pointed out that separation and concentration of trace metals from sample matrices by solvent extraction (2) is a labor-intensive procedure that is not easily automated. In response, these workers developed an alternative method based on the adsorption of complexed metals onto a resin column. Sodium bis(2-hydroxyethyl) dithiocarbamate (NaHEDC) was shown to form water-soluble hydrophobic complexes with several metals (Cd, Co, Cu, Ni, Pb, and Zn). These complexes adsorbed quantitatively on XAD-4 resin when seawater or distilled water, spiked at concentrations on the order of 10^{-6} M, was passed through a column.

A reliable method capable of processing a large number of samples was required for our work on the distribution of trace metals in coastal waters (3). For this reason we automated a version of the King and Fritz method, which was modified in key aspects to allow for analyses at typical natural dissolved metal concentrations ($<10^{-8}$ M). With this procedure, ten 30-mL samples can be preconcentrated with only 1 h of operator attention; the complete procedure is completed in less than 4 h. The nonautomated manipulations include preparations for a set of extractions and sample loading. The sample throughput rate (samples preconcentrated per hour) is not higher than could be achieved by an experienced worker using solvent extraction, but the analyst's productivity is much higher with the present method since other work can be done while preconcentration is underway. The device is constructed for the most part from commercially available components and could be adapted easily to other column separation

techniques requiring trace-metal-clean conditions.

EXPERIMENTAL SECTION

Apparatus. The main components of the preconcentration device are illustrated in Figure 1. A system of 10 columns and 10 sample reservoirs, including both air and reagent manifolds with their respective control valves, fits in a laminar flow-bench 2 ft \times 2 ft \times 2 ft in size (Environmental Air Control, Inc.). Unless laboratory dust levels are excessive, it is not necessary to work in a completely enclosed clean room in order to obtain acceptably low contamination levels using this preconcentration technique, particularly since most of the apparatus is sealed.

Each of the ten extraction units is built around a custom-designed three-way L-shaped valve block fitted with the Teflon cylinder from a Nalgene stopcock (no. 6470). The work reported here was done with custom blocks made of polypropylene; later experience indicates that fabrication is easier when these blocks are made from ultrahigh molecular weight polyethylene. Maximum operating pressure is 20 psi. Valve blocks include four reagent outlets compatible with standard chromatography fittings (1/4-28) used for 1/8 in. o.d. Teflon tubing. The following connections are made to each valve (Figure 2a):

(1) A polypropylene-syringe sample reservoir (Becton Dickinson & Co.; choice of volume, 10-60 mL) using a Tefzel Luer-lock + 1/4-28 adapter. Each syringe-barrel-reservoir is capped by a section of another syringe mounted upside down. A 1/8 in. indentation is milled into the bottom barrel reservoir; the truncated upper section is fitted into this groove, and epoxy glue is applied on the outside of the joint to maintain an air seal against interior pressure. To avoid contamination, the analyte should not reach the level of this connection. Each reservoir cap is attached to a common air manifold via a small three-way stopcock and a Luer connection.

(2) A small resin column (0.3 mL) made of a section of 1/8 in. o.d. (0.06 in. i.d.) Teflon tubing. The resin is supported by a double

¹Current address: Department of Civil Engineering, Stanford University, Stanford CA 94305.

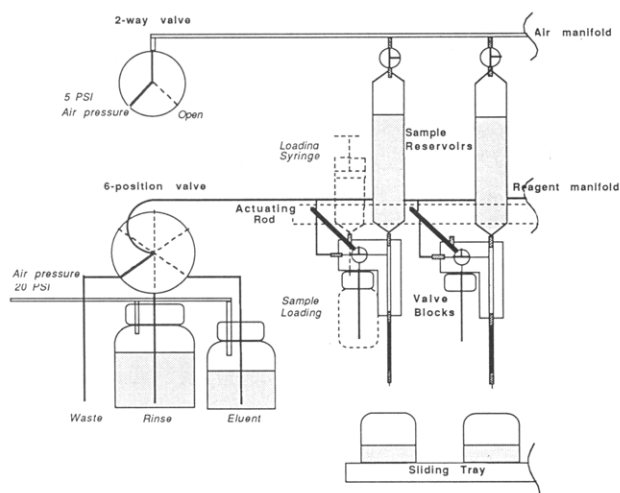


Figure 1. Components of automated preconcentration device. Only 2 out of 10 custom valve blocks and reservoirs linked to the common air and reagent manifolds are shown. Actuating-rod, sliding tray, two-way and six-way valves are controlled by eight solenoid air valves. Also not shown are hand-held computer and interface.

Table I. Correspondence between HP 41CX-Controlled Interface Channels and Control of Device Configurations

channel no. on interface	function of associated solenoid air valves
1	pressure on columns through common three-way Rheodyne valve (5 psi)
2	pressure on reagent bottles, directly from solenoid (20 psi)
3	actuating rod on run (up) through two syringe pistons (20 psi)
4	actuating rod on load (down) through two opposing syringes (20 psi)
5	tray to second position through two small syringes (5 psi)
6	tray to third position through two additional syringes (5 psi)
7	six-way valve ^a switch, directly from solenoid (20 psi)
8	six-way valve reset, directly from solenoid (20 psi)

^a Positions of six-way valve: 1, rinse agent; 2, waste; 3, not used; 4, eluent; 5, rinse; 6, waste.

layer of 8- μ m Nuclepore polycarbonate membrane filter inserted between a standard flanged tubing-to-tubing 1/4-28 connection.

(3) A sample loading tube (Teflon 1/4-28) with bottle cap matching the sample bottle, attached air-tight using a nylon screw and silicone rubber gasket. An additional diagonal conduit in the valve block with 1/4-28 connections directs air from an external syringe which provides pressure, forcing the sample into the reservoir. (4) A branch of the manifold linking each valve unit to the reagent input valve. When the reagent manifold is opened to the waste position, air pressure (applied over the reservoirs) blows out reagent in excess of a fixed volume (1 mL \pm 0.1) contained within the custom valve block. In principle, each custom valve block (which directs flow along three different paths) could be replaced by a set of two three-port Teflon valves (one of which could be electrically actuated), a Teflon T connector, and a different support structure for parts connected to the valves. The solution described here is simpler and less expensive, hence overcoming our reluctance to use noncommercial custom parts.

The ten valve units are mounted onto a Plexiglas frame which also supports a valve actuating rod and a three-position sliding tray (Figure 3). Both actuating rod and sliding tray are controlled by pairs of air-actuated pistons (polypropylene syringes). Pressure at the air manifold (Fig. 1) is controlled by a three-way pneumatically controlled slider valve (Rheodyne Model 5302). A six-position, chemically inert, valve (Rheodyne Model 5012) selects

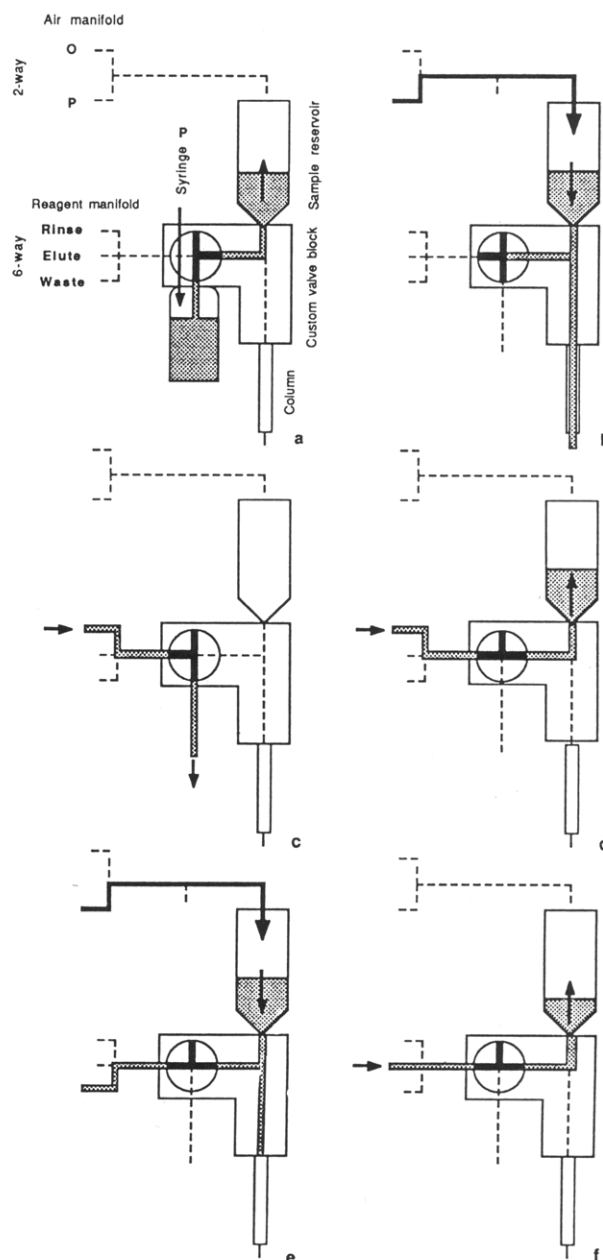


Figure 2. Flow paths for six configurations of the device: (a) sample loading; (b) column loading, rinsing, eluting depending on reservoir content; (c) reagent manifold rinsing; (d) rinse solution loading; (e) purging of excess reagent to waste (rinse solution or eluent); (f) eluent solution loading.

rinse solution, eluent, or waste. The outflow from this valve is connected to the reagent manifold. Each action of the device is controlled by a programmable HP 41CX calculator-timer connected to an Oceanic Scientific 1000A interface and a simple relay circuit. This circuit controls eight solenoid air valves which activate all the components of the device. The function of each solenoid air valve is listed Table I. Any programmable timed device that can activate the relays could be substituted for the HP 41CX and Ocean Scientific 1000A.

Reagents. Benson Co. (Reno, NE) kindly supplied the hydrophobic resin in 20–30 μ m size range (Neutral Porous, BPR-80). Columns were slurry packed with 0.3 mL of resin in ethanol. Two to three complete extraction-elution cycles are usually sufficient to clean new columns. (With the Amberlite XAD-4 resin used by King and Fritz, we could not decrease blanks to acceptable levels, even after extensive cleaning. This may be due to impurities in the resin or to contamination during grinding which is necessary to reduce the size of the large resin beads manufactured by Amberlite.)

NaHEDC was synthesized following the procedure of King and

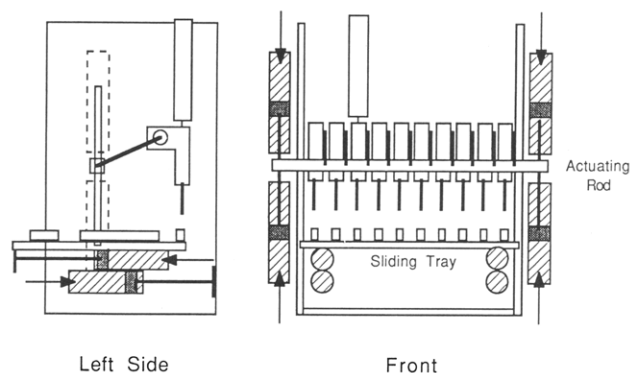


Figure 3. Function of air-actuated pistons (syringes) attached to Plexiglas frame. Side view from inside the frame. Pistons are hatched diagonally. Side view shows pair of pistons attached to frame at one end and sliding tray on the other. Direction of air input is indicated by arrows. At initial tray position both pistons are compressed and the first set of ten collection cups (at left on figure) are under the resin columns. After pressure is applied briefly to one syringe on each side of tray, rinse solution can be collected in the next set of (larger) cups. Finally, with pressure applied briefly to all four tray pistons simultaneously, Teflon eluent cups are brought under the columns. This fully extended position is depicted. Also shown in side view are the vertical slot which directs the actuating rod, and one of ten stopcock control handles (diagonal). Front view shows position of paired pistons which lower and raise the actuating rod depending on where pressure is applied. This motion rotates the stopcocks by 90° via the control handles. Only one of ten sample reservoirs on top of valve blocks is shown.

Fritz. Ligand crystals have been preserved for 2 years in a freezer without noticeable degradation. A 6% by weight ligand solution is prepared, buffered at pH = 8.3, which should be kept in a refrigerator and used within 6 days. Beyond this period, recoveries decrease rapidly due to degradation of the ligand. In our work, NaHEDC solution is purified by two passages through a resin column similar to that used for samples; blanks could be reduced by further passes through this preparative column.

Water is purified with a Corning Mega-Pure glass still after passage through a deionizer, a mixed-bed high-purity exchange column, and activated charcoal. The 0.1 N $\text{Na}_2\text{B}_4\text{O}_7$ buffer solution is cleaned by passage through a resin column after addition of 1 mL of NaHEDC/1000 mL. Ammonia is added to the buffer (to 0.5 N) in order to neutralize acidified samples. Ammonia is sub-boiling distilled by gently swirling reagent grade ammonia next to an open bottle of distilled water overnight in a closed container. One liter of water rinse solution is buffered to pH = 9.0 by addition of 1 mL of purified 0.1 N $\text{Na}_2\text{B}_4\text{O}_7$. The elution solution is composed of glass-distilled ethanol with 3-times-Vycor-distilled NHO_3 added to 1.0N (elution is not complete if only 1N nitric acid is used). Phosphoric acid is cleaned by passing a 0.25 N solution through a small Dowex AG 50W-X8 cation-exchange column. These purifications were applied to reagent grade solutions.

Procedure. Upon collection, 250-mL seawater samples are acidified with 1 mL of 6 N HCl (3 times Vycor-distilled). In order to obtain high recoveries of Cd, Cu, Ni, and Zn simultaneously, 30-mL samples are first neutralized to pH = 8.4 with 1 mL of $\text{Na}_2\text{B}_4\text{O}_7/\text{NH}_4\text{OH}$ buffer. Then, 0.1 mL of ligand solution (sufficient to complex a solution 250 μM in divalent cations) is added to each neutralized sample. Samples are routinely spiked with Co before NaHEDC is added to reach an effective concentration of 10^{-9} M (which is more than an order of magnitude higher than natural seawater concentrations). Co recovery monitoring (by graphite furnace atomic absorption spectroscopy, GFAAS) provides a simple check on ligand degradation, column performance, or errors in reagent additions. After reagent addition to each sample, extraction should proceed without delay. The typical sequence of steps outlined in Figure 2 is as follows:

In order to fill each reservoir manually with sample solution, the custom valve-blocks are disengaged from the actuating rod so that each can be operated individually. A 60-mL Nalgene bottle (containing 30 mL of buffered sample and ligand) is tightly

Table II. Program Controlling Eight Solenoid Air Valves Which Determine the Configurations of the Device Described in Figure 2

Main Timer Program	
1	initialize clock
2	pressure on reservoirs
3	delay for 2 h, configuration Figure 2b
4	pressure off
5	tray to 2nd position
6	rinse subroutine
7	six-way valve switched to position 5
8	rinse subroutine
9	six-way valve switched to position 1
10	rinse subroutine
11	six-way valve switched to position 4
12	elute subroutine
13	end
Rinse Subroutine	
1	pressure on reagent bottles, configuration Figure 2c
2	actuating rod to load position
3	delay for 2 min, configuration Figure 2d
4	pressure off
5	six-way valve to next position (6 or 2, waste)
6	pressure on reservoirs
7	delay for 1 min, configuration Figure 2e
8	actuating rod to run position
9	delay for 15 min, configuration Figure 2b
10	return
Elute Subroutine	
1	pressure on reagent bottles, configuration Figure 2c
2	actuating rod to load position
3	delay for 30 s, configuration Figure 2f
4	pressure off
5	six-way valve to position 6 (waste)
6	tray to third position
7	pressure on reservoirs
8	delay for 1 min, configuration Figure 2e
9	actuating rod to run position
10	delay for 45 min, configuration 2b
11	return

fastened onto the block-mounted cap temporarily. The sample solution is then forced in from the bottle manually with an external syringe connected through the diagonal conduit of the valve block (Figure 2a). When the loading bottle is disconnected (i.e., in the automated mode), this flow path permits rinsing of the reagent manifold and loading tubes prior to rinse or elution steps (Figure 2c). Ten samples can be loaded in approximately one-half hour.

After all of the reservoirs have been filled with buffered sample, the device can be switched to the automated cycle, which lasts 3 h. During the first step, metal-ligand complexes are retained on the column. In order to sustain a flow rate of 15 mL/h, 5 psi air pressure is applied over each column reservoir via the air manifold (Figure 2b). Samples passing through each column are collected in cups at the first position of the sliding tray (for subsequent weighing to determine the exact sample size). Passage of air through the columns toward the end of this step does not adversely affect reproducibility.

While the ten samples are forced through the resin columns for 2 h, the reservoirs are isolated from one another by selecting the appropriate position for the special three-way valve block. Following this step, the ten valves are connected to a common reagent manifold (by vertical motion of the actuating rod; Figure 1). The collection tray moves to the second (waste) position for the three rinse cycles. Table II contains a detailed description of the program steps involved in loading the rinse solution from the six-position pneumatic reagent flow valve. The sequence of steps includes manifold rinsing (Figure 2c) with the actuating rod in the "run" position, loading of the rinse solution into each reservoir ("load", Figure 2d), purging of excess rinse solution to waste (Figure 2e), and, finally, return of the actuating rod to the "run" position for column rinsing (configuration similar to Figure 2b, with only 1 mL on top of each column). Rinsing is necessary to remove residual salts, which interfere with the GFAAS analysis of the concentrate.

Table III. Metal Concentrations in Sargasso Sea Surface Water Determined by Extraction at pH = 8.4, from 30-mL Sample Volume to 0.5-mL Final Volume^a

element	SW (<i>n</i> = 13)	SW + spike (<i>n</i> = 13)	% av recovery	blank range
Cd	0.001 ± 0.001	0.076 ± 0.005	98	<0.001
Cu	0.83 ± 0.1	3.08 ± 0.2	93	0.05–0.15
Ni	1.95 ± 0.2	5.08 ± 0.3	94	0.05–0.10
Zn	0.1 ± 0.1	3.38 ± 0.3	98	0.2–0.4

^a Concentrations in nM. One liter of Sargasso Sea surface water was spiked with 0.8 mL of a solution containing Cd, Cu, Ni, and Zn at 95.5, 3041, 4162, and 4038 nM concentration, respectively, to determine recovery.

Next, the concentrated metals are eluted from the columns with 1 mL of 1 N HNO₃ in ethanol (Figure 2f). The eluent is retained in Teflon beakers placed under the columns in the final position of the sliding tray. This step concludes the automated cycle. The eluent solution is subsequently evaporated to dryness by placing the Teflon beakers in a hot water bath in a covered glass container purged by a stream of filtered air, using heat lamps to prevent condensation on the glass. These dried beakers can be stored indefinitely (in a clean air-tight container) before analysis.

After the final elution step, oxidation of the resin by nitric acid is minimized by rinsing the columns with water. This step is particularly important if the system is not going to be used within a day. This rinsing prevents formation of cation-exchange sites on the resin which retain major seawater cations until the final elution step. When this precaution is taken, Na, Mg, K, and Ca levels in the final 0.5 mL of concentrate are typically 0.2% of seawater concentrations. Since concentrates from seawater samples run without ligand contain similar salt concentrations, mechanical trapping of seawater in the first prototype of this system may be the most important contribution to the residual salt; we have refined the plumbing to reduce dead volume in subsequent versions.

On the day of analysis, concentrates are taken up to 0.5 mL of 0.3 N HNO₃ containing 0.025 N H₃PO₄ as a matrix modifier. Trace metal concentrates were analyzed with a Perkin-Elmer Zeeman 5000 graphite-furnace atomic absorption photometer equipped with an HGA-500 furnace controller/AS-40 autosampler. Standard lamp and furnace conditions were used. For Zn and Cd analyses, a L'vov platform is used for best reproducibility. Pyrocoated graphite tubes were used for Cu, Co, and Ni determinations. Standard curves were established by spiking concentrates from distilled water processed as a sample. The sensitivity in this matrix relative to a pure HNO₃/H₃PO₄ solution is depressed significantly (10–20%) for Cd and Zn, but it is unchanged for Cu or Ni. The presence of phosphoric acid improves reproducibility of Cd and Zn analyses and is not detrimental to Cu, Co, and Ni results.

RESULTS AND DISCUSSION

Acidified North Atlantic surface water from the Sargasso Sea was spiked with Cd, Cu, Ni, and Zn at levels comparable to the midrange of concentrations encountered in oceanic waters. Replicate analyses of both spiked and unspiked samples are listed in Table III. Reproducibility on spiked samples between days ranged from 6 to 9%. Within a day, reproducibility of replicate analyses on different columns improves to 3–4%. Recoveries range between 93 and 98%; when the recovery is significantly less than 100%, these are incorporated into the calculation of metal concentrations in the original samples. Trace metal concentrations found in Sargasso Sea surface water are comparable to earlier determinations by different methods in this laboratory as well as by other workers (4, 5): Cd, ≤1 pM; Cu, 0.83 nM; Ni, 1.95 nM; Zn, <0.1 nM.

Although our modifications of the King and Fritz preconcentration method enable analyses of metal concentrations lower by 3 orders of magnitude, the working pH range has been narrowed as well. Spiked seawater samples buffered to

Table IV. Metal Concentrations in Guadiana River Water Determined by Extraction at pH = 8.4, from 30-mL Sample Volume to 3-mL Final Volume^a

element	RW (<i>n</i> = 3)	RW + spike (<i>n</i> = 2)	% av recovery
Cd	0.019 ± 0.001	0.259 ± 0.001	101
Cu	11.6 ± 0.6	19.5 ± 0.6	101
Ni	12.2 ± 1	22.8 ± 0.8	103
Zn	2.4 ± 0.2	10.5 ± 0.1	85

^a Concentrations in nM. 150 μL of the same spike as in Table III solution was added to a 60 mL river sample to determine recovery.

pH = 5.0 with 250 μL of 0.3 M ammonium acetate and 5 N ammonia, instead of Na₂B₄D₇, show 100% recoveries for Cu and Ni, but Cd and Zn are not retained on the column under these conditions. Depending on the elements of interest, compromises may have to be made between recovery efficiencies for each element. Alternatively, extractions could be repeated under optimum conditions for each element.

Blanks were determined by passing Sargasso Sea surface through the procedure once and then repeating the procedure on the eluted seawater after another addition of all reagents (except ammonia, whose negligible contribution was checked separately). Results listed in Table III indicate blanks are small relative to open ocean surface concentrations for Cu and Ni. The Cd blank of 1 pM (at the detection limit for a 60:1 concentration ratio) is small relative to subsurface concentrations which increase by up to 3 orders of magnitude. Even though the Zn blank is well above the detection limit and central gyre surface water concentrations, this blank is small relative to Zn concentrations encountered in coastal waters and subsurface open ocean waters. Contributions from the NaHEDC ligand accounts for more than 80% of the total blanks for Cu, Ni, and Zn. Further purification of the ligand could reduce the blank if necessary. We did not do so because the concentration levels in our Mediterranean and coastal water samples did not require such low blanks.

In order to determine the applicability of this method to freshwater systems, Guadiana River water (Spain) has also been analyzed. After filtration through a 0.4-μm Nuclepore polycarbonate membrane, freshwater samples were processed in the same way as seawater samples. As indicated in Table IV, recoveries cannot be distinguished from 100% for Cd, Cu, and Ni. Even though recovery for Zn is lower (85%), reproducibility remains high for this element. We have no explanation for the 10–15% lower Zn recoveries we observed for distilled water.

CONCLUSION

This device automates the preconcentration of trace metals from natural fresh- and seawaters by HEDC–metal complex retention on a hydrophobic column. The method has proved reliable for more than 300 individual preconcentrations. Previous work on dithiocarbamates (6) indicates that other elements should be amenable to this procedure as well. In particular, high recoveries of Ag, Fe, U, V, and Bi were obtained by King and Fritz. Our preliminary studies indicate that Pb analysis is also possible. Due to its flexibility in programming, eluent choice, and sample size, this device should allow automation of other column separation techniques requiring trace-metal-clean conditions. Chelex and Fractogel immobilized hydroxyquinoline resins, for instance, have been used successfully to preconcentrate trace elements such as Cd and Zn (7), Mn (8), Al (9), and Ga (10) from seawater. Such methods could easily be automated with this system. Minimal sample handling (and consequently lower risks of contamination) involved in the automated column extraction approach should allow a greater number of labo-

ratories to determine trace metal concentrations reliably in natural waters.

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Registry No. HEDC, 1528-72-9; Cu, 7440-50-8; Ni, 7440-02-0; Zn, 7440-66-6; Cd, 7440-43-9; water, 7732-18-5.

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Characterization of Arrays of Microelectrodes for Fast Voltammetry

William E. Strohhben, Diane K. Smith, and Dennis H. Evans*

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

INTRODUCTION

Microelectrodes have been crucial to the recent rapid development of high-speed electrochemical techniques. For disk electrodes, both the iR error and RC time constant are proportional to the disk radius, so both can be made negligibly small by using sufficiently small electrodes. Microdisk electrodes on the order of 10 μm in diameter have allowed cyclic voltammetric measurements at scan rates up to 1 MV/s (1-6) and submicrosecond chronoamperometric measurements (7).

In these fast experiments, radial diffusion contributes little to the flux of reactant at the electrode. Thus the cell current, which is then proportional to the disk area, plummets rapidly as smaller and smaller disks are used. Consequently, it is necessary to use a high-gain current-to-voltage converter, often with two or more stages of amplification, and careful attention must be paid to noise and bandwidth considerations (1-9).

A direct way of increasing the current to be measured is to use more than one microelectrode, i.e., arrays of N widely separated and noninteracting disks that will provide N times the current from a single disk. Such arrays should facilitate the step of current-to-voltage conversion, allowing less sophisticated, perhaps even commercial, instrumentation to be used. However, it has not been previously demonstrated that the advantages of small iR and RC will be retained with arrays of microdisks. Here we report the construction of robust arrays of up to 30 5- and 12.5- μm -radius microdisks and the characterization of these "polytrodes" for fast voltammetric experiments.

There have been many reports of microelectrode arrays, but in most cases the individual microelectrodes were not widely separated from one another and the arrays were not intended for fast experiments. Caudill et al. (10) prepared arrays of 100 widely spaced microdisks and demonstrated that the steady-state diffusion fields did not overlap. Other arrangements of well-dispersed microelectrodes have been reported (11, 12). Resistance effects (13-15), dispersion in the capacitance (16), and noise levels (17, 18) with microelectrode arrays have also been discussed. Arrays of carbon paste electrodes in porous polycarbonate membranes have been used at moderate scan rates where radial diffusion was of minor consequence (19, 20) and arrays of 10 gold-band electrodes (10- μm width) have been used at scan rates approaching 10^4 V/s (21). Another interesting robust microelectrode array

comprises epoxy-filled grooves that have been cut in a glassy carbon surface (22).

EXPERIMENTAL SECTION

Construction of Microelectrode Arrays. The method that was used was a scale-up of the procedure for preparing individual microdisks. The holder for the array was a length of 6-mm diameter soft glass tubing, the end of which was compressed to reduce the inside diameter to about 2 mm for a length of 1 cm. Next, a glass capillary was pulled from 6-mm-diameter soft glass tubing. The inside diameter of the capillary ranged from 50 to 75 μm . The capillary was cut in lengths of 1 cm, cleaned with acetone, and dried at 120 $^{\circ}\text{C}$. Similarly platinum wire of 10- μm diameter (Goodfellow Metals, Cambridge, England) or 25- μm diameter (Johnson Matthey, Inc.) was cut into 2-cm lengths, cleaned in acetone, and dried at 120 $^{\circ}\text{C}$.

A wire was then carefully inserted into each capillary until the platinum reached the end. The portion of each capillary with the wire flush with the end was sealed over about a 1-mm length with a small flame. The desired number of these capillaries were placed in the constricted end of the soft glass housing, a vacuum was pulled on the end of the housing opposite to the bundle of capillaries, and the wires, capillaries, and housing were slowly fused with a small flame.

The assembly was placed in an annealing oven to remove strain, then the tip of the assembly was cut to expose the platinum microdisks and polished with 400-grit alumina (Buehler). A smooth finish was achieved by polishing with successively finer grades of diamond polishing compound (Buehler, 15, 6, 1, 0.25 μm) and completed by polishing with 0.05- μm alumina suspension (Buehler). Connection to an electrical lead inside the tube was achieved by using a low-melting alloy, Wood's metal.

Micrographs of arrays of 17 12.5- μm -radius disks and 29 5- μm -radius disks are shown in Figure 1. The disks are widely separated in both arrays. For the 17-electrode array, the average of the 17 center-to-center nearest-neighbor distances was 180 μm and the smallest nearest-neighbor distance was 160 μm or 13 disk radii. In scanning electron microscopy (SEM) at higher magnifications the polished disk surfaces appear to be flush with the glass surface with no discernible cracks or fissures at the metal-glass seal. The disks are not perfectly circular as shown by the SEM micrograph of a single 12.5- μm -radius disk (Figure 1). However, the distortions are small, and the electrodes can be expected to give responses close to those predicted for perfectly circular disks.

Many arrays have been constructed containing from 1 to 30 disks. The electrodes are very robust. Typical treatments of the arrays include polishing with 0.05- μm alumina between experi-