Technical Note

A Low Contamination Chelex-100 Technique for Shipboard Pre-concentration of Heavy Metals in Seawater

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ABSTRACT

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An easy to handle, on-board procedure is proposed which uses a Chelex-100 ion-exchange column to pre-concentrate Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn from seawater with minimal risk of contamination. Immediately after collection, the seawater sample is poured into a polyethylene terephthalate (PET) bottle containing ammonium maleate buffer at pH 6.5. The bottle is then connected to a pre-packed Chelex-100 column set, and hung upside-down to allow the sample to flow through the column in dust-free conditions. Finally, the whole batch of columns is brought back to the land-based laboratory for further elution and analysis. A concentration factor of 250 is achieved.

INTRODUCTION

The Chelex-100 chelating ion-exchange technique has a long history of application in trace metal analysis for seawater (Riley and Taylor, 1972; Abdullah et al., 1976; Bruland et al., 1985). Most investigators considered this technique the easiest and cleanest method to pre-concentrate trace metals when compared with other methods such as solvent extraction and co-precipitation and it is as efficient as those methods (Bruland et al., 1985; Sturgeon et al., 1980; Boniforti et al., 1984). Also, as the Chelex-100 column method does not require specific reagents and fragile glassware, it has been recommended for on-board treatment of seawater samples immediately after collection (Boniforti et al., 1984).

Surprisingly, a review of past literature revealed only a few cases of on-board application (Riley and Taylor, 1972; Bruland et al., 1979). This is mainly be-

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cause the conventional Chelex-100 procedure cannot be carried out consistently under unpredictable weather conditions. The lack of clean environment also prohibits the on-board operation. As a result, many investigators stored samples after acidification, then brought them back to a land-based laboratory for further treatment (Hirota et al., 1985).

It should be noted that once seawater is acidified without filtration, the final result could be ambiguous because the measured concentrations cannot be classified as dissolved, total, or acid leachable. If the sample is filtered upon collection, the risk of contamination increases. Storage and transportation of large amounts of sample may give rise to other problems. Furthermore, to adjust the acidified samples to the desired pH before pre-concentration is tedious and also subject to contamination. Direct on-board pre-concentration, without filtering, storing and transporting samples may provide a more straightforward resolution, even though the results can only be classified as 'labile' or 'extractable' fraction and its definition depends solely upon the method chosen.

This paper describes a routine procedure for on-board operation of the Chelex-100 pre-concentration, with minimal maneuver. The principle is based generally on the method reported previously (Pai, 1988; Pai et al., 1988) but uses ammonium maleate as a buffer (Pai et al., 1990) instead of conventional ammonium acetate, and uses a more practical hanging bottle technique to avoid operational contamination. To demonstrate the applicability and precision of the method, a spiking experiment was carried out on board the R/V "Ocean Researcher I" at a station in the northern South China Sea, 100 km from Kaoshuing Harbour, Taiwan. The analysis by atomic absorption spectrometry (AAS) was completed within 3 days after returning from the cruise.

MATERIALS

Distilled water (DDW): the water used to prepare reagents was double distilled in a borosilicate vessel, and stored in a polypropylene (PP) tank.

Ammonium hydroxide: ammonia vapor was bubbled through distilled water until saturation was reached. The ammonia concentration was checked by acid titration.

Nitric acid: Merck GR grade concentrated nitric acid was sub-boiled in a Pyrex glass container and stored in a Teflon bottle (Mattinson, 1972). The concentration of the sub-boiled nitric acid was checked by titration against a standard base.

Ammonium maleate buffer: Merck GR grade maleic acid (58.03 g) was dissolved in ~700 ml of distilled water in a 1-l Pyrex beaker and filtered with Whatman GF/C filter to remove particles. The pH was gradually adjusted to ~6.5 using purified ammonium hydroxide. The final volume was made up to 1 l with DDW.

Ammonium acetate buffer: it was prepared by diluting 57 ml of glacial acetic acid in a 1-l beaker, and the pH was adjusted to 6–6.5 with purified ammonium

Fig. 1. (a) The pre-packed Chelex-100 column set which was sealed in a PE bag, folded into three sections and placed in a carrying box to keep the column vertical. (b) The modified PET bottle containing 30 ml of ammonium buffer. (c) The column on the working rack for packing and elution. 1 - silicon rubber stopper, No. 7, with holes; 2 - nipple; 3 - silicon rubber tubing, 15×0.2 cm i.d.; 4 - roller clamp; 5 - adaptor, made of Tygon tubing; 6 - Chelex-100 column, 12×0.794 cm i.d., packed with 2 g of resin, 100–200 mesh, ammonium form; 7 - silicon rubber tubing, 4×0.2 cm i.d.; 8 - ventilation tube, 40×0.2 cm i.d.; 9 - PE bag; 10 - strengthened with a tape; 11 - hanging string; 12 - silicon rubber stopper, No. 7; 13 - Pyrex glass funnel; 14 - wooden rack, holding 20 columns in a row.

hydroxide. The final volume was made up to 1 l. After purification by a Chelex-100 column, the pH of this buffer was adjusted back to 5.5 using sub-boiled nitric acid.

Purification of buffers: both maleate and acetate buffers were purified by passage through a Chelex-100 column containing 10 g of 100–200 mesh resin in ammonium form at a flow rate not more than 2 ml min^{-1} . The first 20 ml of the effluent was discarded and the remaining effluent was stored in Teflon bottles.

Metal standards: a mixed metal standard solution was prepared by combining and diluting Merck Titrisol Standards to a concentration equivalent to $1 \text{ ml} = 10 \text{ } \mu\text{g}$ each of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn. A $50\text{-}\mu\text{l}$ micropipet was used for on-board spiking.

Instrument: a Hitachi Z-8000 Polarized Zeeman Effect atomic absorption spectrometer was used for the detection of trace metals. Two types of graphite furnace were used: a cup-type furnace was used to detect high-sensitivity elements such as Cd, Mn and Zn, and a pyro-coated tube-type furnace was used for other elements.

Chelex-100 column set: Chelex-100 resin (2 g, 100–200 mesh), manufactured by Bio-Rad Laboratories (Richmond, CA, U.S.A.), was weighed in a glass beaker, acidified with 1 ml of acid (this makes it easy to pack), and packed into a column made of Tygon tubing ($12 \times 0.794 \text{ cm i.d.}$). The column was washed in a stepwise manner with three 1-ml portions of 2 N nitric acid, five 1-ml portions of DDW, three 1-ml portions of 1 N ammonium hydroxide, and at least five 4-ml portions of DDW. The packed column was connected to adaptors with silicon rubber tubings at both ends, fitted with roller clamps and a stopper plug, and sealed into a polyethylene (PE) bag (Fig. 1).

Sample reservoir: a 1280-ml polyethylene terephthalate (PET) bottle (Coke bottle) was used as the sample reservoir. The bottom part of the bottle, strengthened by tape, was attached to a string (Fig. 1). The bottle was washed in sequence with detergent, mild acid and Dextran, and finally rinsed with DDW. To each bottle 30 ml of ammonium maleate buffer (pH 6.5) was added. The bottle was plugged with a silicon rubber stopper, and placed in a plastic bag.

PROCEDURE

Pre-boarding

Individually sealed Chelex-100 column sets and PET bottles sets were all kept in a vertical position in a carrying box. Go-Flo sampling bottles (2.5 l Type 1080 General Oceanics Inc., Miami, FL) were acid cleaned in the laboratory and wrapped with PE film.

On-board

Twelve Go-Flo bottles were installed onto a rosette sampler (General Oceanics Inc., Miami, FL, U.S.A.) which was attached to an SBE-9 conductivity-temperature-depth (CTD) unit (Seabird Electronics Inc., Bellevue, WA). Seawater samples were collected at required depths.

When the rosette was retrieved, the valve of the Go-Flo sampler was opened and at least 100 ml of seawater was allowed to flush through the outlet. The sample was then poured directly into the PET bottle, filling it to the neck. The bottle was immediately plugged with a Chelex-100 column set (still wrapped in a PE bag, with only the stopper and the outflow tubing pulled out). The ventilation tubing was tied to the bottle with a rubber band and the whole combination was hung upside-down on a hook (Fig. 2). The rolling clamps were then opened and the seawater outflow was adjusted to $\sim 4\text{--}5\text{ ml min}^{-1}$. Each sample took 4–6 h to finish. The column sets were disconnected from the bottles (kept hanging until the ship returned), folded to keep the column in a vertical position, and then packed into the carrying box.

Fig. 2. The layout of the hanging bottle technique for passing the sample through the Chelex-100 column, which was protected by a PE bag throughout the expedition.

In the laboratory

When the Chelex-100 column sets were brought back to the land-based laboratory, they were disconnected from tubings and the adaptor, fixed on a wooden rack with room for 20 columns, and washed sequentially with the following solutions: five 1-ml portions of DDW, four 5-ml portions of 1 M ammonium acetate at pH 5.5 and five 1-ml portions of DDW. Stepwise elution was carried out with 1-ml portions of 2 N nitric acid. The effluent of the first two portions was discarded (to reduce the void bed volume), and the effluent of the further five 1-ml portions of 2 N nitric acid was collected into a previously calibrated Pyrex glass test tube which was kept underneath the column until the final yield reached exactly 5.0 ml. The heavy metal contents were detected by graphite furnace atomic absorption spectrometry (GFAAS).

On-board spiking test

Deep ocean water was collected by 12 Go-Flo samplers at 1000-m depth in one cast, and water from each sampling bottle was poured into separate PET bottles. To four of the bottles 0.02 ml of the mixed standard solution had already been added by micropipet, so the spiked concentration was equivalent to 0.2 μg of each metal in 1250 ml of seawater (or 0.16 $\mu\text{g l}^{-1}$); another four bottles were spiked with 0.1 ml of the mixed standard to give a spiked concentration of 2 μg of each metal in 1250 ml (or 0.8 $\mu\text{g l}^{-1}$). The remaining bottles were unspiked. All samples were immediately treated by Chelex-100 columns following the procedures described above.

Preparation of analytical blank

The Chelex-100 column effluent of the non-spiked seawater sample was collected and was considered as the theoretical blank seawater. It was poured directly into another PET bottle containing 30 ml of ammonium maleate buffer, and allowed to pass through a new Chelex-100 column in the same manner as for other samples. The final eluate from this column was detected by GFAAS and the signals produced for each element were accounted for as the analytical blank. Four replicates were made.

RESULTS AND DISCUSSION

Contamination

The possible sources of reagent contamination were from distilled water, nitric acid, maleic acid, acetic acid, ammonia and the resin itself. All these reagents had been carefully purified and thus gave very low reagent blanks. The risks of operational contamination came from the sampler, the PET bottle, the column set and the test tube. As the preparation of the columns, the elution, and the final determination were all carried out in a dust-controlled laboratory, and during the deck operation all bottles and columns were pro-

TABLE 1

Determination of heavy metals in seawater from 1000-m depth at a station in the South China Sea

Element	Analytical blank ($\mu\text{g l}^{-1}$)		Concentrations found ($\mu\text{g l}^{-1}$) ^a			
	Mean	sd ($n=4$)	Detected ^b	sd ($n=4$)	Corrected ^c	rsd (%)
Cd	0.006	0.002	0.078	0.004	0.072	6
Co	0.011	0.005	0.015	0.007	0.004 (nd)	175
Cu	0.013	0.004	0.131	0.012	0.118	11
Fe	0.06	0.02	0.21	0.04	0.14	28
Mn	0.030	0.006	0.111	0.010	0.081	12
Ni	0.076	0.011	0.529	0.014	0.453	3
Pb	0.027	0.010	0.040	0.015	0.013 (nd)	116
Zn	0.115	0.014	0.521	0.021	0.406	5

^aAll measurements were replicated four times.

^bConcentrations were estimated directly from the standard addition curves.

^cConcentrations were corrected by deducting the analytical blanks. (nd): considered as 'not detectable', i.e. less than 4.65 times the standard deviation of the analytical blank.

tected by PE bags from aerosols and seawater splashing, the chance of operational contamination was also very small. Additional tests showed that the PET bottle did not absorb or release detectable heavy metals over 48 h when filled with seawater at pH 6.5.

Although the contaminations from these possible sources were not identified individually, the total analytical blanks are shown in Table 1. The possible contamination from the Go-Flo sampler, however, was not included.

Stepwise washing and elution

Several washings were necessary before the final recovery of heavy metals from the column. The washing with distilled water was to get rid of seawater or reagent residues from the resin bed. The rinsing of ammonium acetate was to remove major ions from the resin (Kingston et al., 1978). After these washings, the final elution with nitric acid recovered heavy metals in the absence of interfering major ions. As the 100–200 mesh resin bed held liquid under hydrostatic pressure without letting the column dry out, a stepwise washing or elution technique was applied.

In practice, the eluent liquid was added in small portions to the top of the resin bed using a dispenser. The liquid was allowed to drain before the second portion was added, thus mixing could be kept to the minimum. Figure 3 shows the elution profiles of Na, K, Mg, Ca, NH_4^+ , NO_3^- and Mn from a Chelex-100 column (1280 ml of sample had passed through previously). Other elements,

Fig. 3. Elution profiles of Na, K, Mg, Ca, Na⁺, NO₃⁻ and Mn from a Chelex-100 column by distilled water, ammonium acetate and nitric acid; after 1280 ml of spiked and buffered seawater had passed through. Vertical scale represents concentrations of 10 000 ppm Na, 1000 ppm K, 2000 ppm Mg, 1000 ppm Ca, 1 M NH₄⁺, 2 M NO₃⁻ and 2 ppm Mn.

such as Cd, Cu and Pb, were also monitored in the same effluent portions, but as their eluting positions were identical to that of Mn, for simplicity they are not plotted. The elutions were made by measuring the concentrations of the analytes in the effluent after each 1-ml portion of elution. It can be seen that elution with small portions gave reasonably good results. For instance, the residue seawater could be washed out by three 1-ml portions of distilled water, but would give a longer tailing if 2-ml or 5-ml portions were used.

The removal of Na, K, Mg and Ca from the Chelex-100 column could be nearly completed by stepwise elution with 20 1-ml portions of 1 M ammonium acetate at pH 5.5. However, this would be too tedious in practice. For simpler operation, elution with four 5-ml portions of 1 M ammonium acetate is recommended; this removed >99% of Na and K and >95% of Mg and Ca from the column.

The volume of nitric acid required for complete recovery of heavy metals from a Chelex-100 column has been discussed in several earlier publications. Pakalns et al. (1978) suggested that a minimum of 25 ml of diluted acid was necessary to elute the Chelex-100 column for complete recovery of metals. Sturgeon et al. (1980) discussed the difficulties of quantitative recovery of heavy metals by up to even 100 ml of acid, and finally abandoned the column operation. However, results of this work show that it is possible to obtain complete recovery using small amounts of acid in a stepwise manner. It has been observed that if a large volume of nitric acid is loaded on the column, vertical convection occurs in the top layer, which leads to long tailing of the eluate. The elution in 1-ml steps proved to give very little mixing effect; complete recovery could be achieved with 10 1-ml portions of acid using a 100–200 mesh resin in an 0.8-cm bore column. The elution profiles shown in Fig. 3 illustrate that nearly 99% of the trace metals were collected in the fractions from 43 to 47 of the acid elutions with 1-ml portions. If the first two fractions of the eluate were discarded, the final eluate volume could be reduced to 5 ml. Additional tests using 2- and 5-ml portions of acid showed that the minimum elution volume required would be 7 and 20 ml respectively.

The matrix in the final yield (5 ml) consisted of ~1.4–1.6 M of nitrate, ~0.2 M of ammonium ions, not more than 1 mM of Ca and 0.5 mM of Mg. The effect of such matrix may interfere strongly with the absorption signals of the atomic absorption spectrometer (Danielsson et al., 1982). To compensate for matrix interference in the quantitative determination, the standard addition technique was applied to a solution of the same matrix composition, i.e. the analytical blank. Noise or background effects were corrected by the Zeeman effect optical system of the spectrometer.

Analysis of the non-spiked and spiked seawater

Trace metals in seawater from 1000-m depth in the South China Sea (located at 22°00'N, 119°25'E) were pre-concentrated on board by the Chelex-

100 column using the proposed hanging bottle technique, and were detected by GFAAS. The results for the reagent blank and for the non-spiked samples are shown in Table 1. The results for the spiked samples are presented as standard addition curves as shown in Fig. 4. The concentrations of heavy metals that are estimated directly from the standard addition curves are considered as 'detected', and the concentrations that are deducted from the analytical blanks are considered as 'corrected'. It should be emphasized that the analytical and the reagent blanks are different in definition, and are difficult to distinguish from each other in practice. For example, in Table 1 the analytical blank for Cd is $0.006 \mu\text{g l}^{-1}$, which means that the GFAAS signal produced by the blank sample is equivalent to a Cd concentration of $0.75 \mu\text{g l}^{-1}$ (after 125-fold pre-concentration). The blank signal includes the possible contamination from reagents and containers during the operation, as well as the background noise of the instrument.

Five elements, i.e. Cd, Cu, Ni, Mn and Zn, were measured with good preci-

Fig. 4. Standard addition curves of trace metals for South China Sea water from 1000-m depth. Amounts of 0.2 and $1 \mu\text{g}$ of each metal were added to 1.25-l aliquots of sample, to give spiked concentrations of 0.16 and $0.8 \mu\text{g l}^{-1}$ respectively. Absorbance readings were not deducted from the analytical blanks. Vertical bars represent the standard deviation of four measurements.

sion (rsd 3–11%), and the concentration levels measured were all higher than the analytical blanks; this indicates that this technique is suitable for on-board measurement of these five elements. The precision for iron was only fair (rsd 28%) at the $0.14 \mu\text{g l}^{-1}$ level, but the result could still be considered as 'detectable'. For Co and Pb, even after 250-fold pre-concentration, GFAAS apparently was not sufficiently sensitive for the determination of these elements at their natural concentrations below $0.013 \mu\text{g l}^{-1}$. Nonetheless, this technique may still be useful for measuring Co, Fe and Pb in nearshore waters at higher concentrations.

SUMMARY

The proposed hanging bottle technique provides a fast, clean and easy to handle means to perform Chelex-100 pre-concentration on board a research vessel. As the seawater sample is directly poured into the reservoir bottle, and the pH is precisely controlled by the ammonium maleate buffer, the need for volumetric measuring and transfers and for a pH-meter are all eliminated. The only actions which need to be taken on board are to collect the sample and pour it into the bottle, connect the column set, hang it upside-down, and let it flow. Thus problems usually associated with rough weather no longer exist. The whole pre-concentration scheme is designed to be contamination-protected at low cost, and may be recommended for routine fieldwork.

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