PRE-CONCENTRATION EFFICIENCY OF CHELEX-100 RESIN FOR HEAVY METALS IN SEAWATER
Part 2. Distribution of Heavy Metals on a Chelex-100 Column and Optimization of the Column Efficiency by a Plate Simulation Method

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SUMMARY

Seawater was spiked with heavy metals and passed through a Chelex-100 column string consisting of ten minicolumns. The recoveries of metals from each minicolumn are used to study their distribution on a column. A simplified model was constructed to simulate the chelating efficiency of columns of various sizes, at various pH and flow rates. It is shown that a column containing 2 g of resin in the magnesium form with a flow rate of 4 ml min^{-1} is suitable for pre-concentration of Cd, Cu, Co, Mn, Ni, Pb, and Zn in seawater after adjustment to pH 6.5.

The chelating ion-exchange technique has been widely applied to pre-concentrate very low levels of trace metals from seawater during the past 15 years [1, 2]. However, some recent evidence has indicated that this technique may not be as efficient as was previously thought [3, 4]. Most recent investigators have adopted the procedure suggested by Kingston et al. [5] for Chelex-100 column operation, but have achieved various degrees of success. To clarify this, Sturgeon et al. [3] repeated the process in standard spiking experiments and showed that the recoveries of spiked heavy metals from seawater were as low as 44% for manganese, 57% for iron and 77% for copper. Because of uncertainties in their experiments, they admitted that the recoveries should be termed "apparent" recoveries rather than "true" recoveries. Even though the efficiency of the method was doubtful, it was still feasible to determine the concentration of heavy metals in natural seawater based on standard-addition calibration. A comparative study made by Boniforti et al. [4] showed that the results obtained with Chelex-100 agreed fairly well with those by other methods such as APDC/MIBK extraction or co-precipitation. However, if analytical losses occur, many investigators believed that they are caused by the organic matter present in seawater which forms complexes with trace metals and so causes incomplete absorption of metals on the resin [3, 6, 7].

In Part 1 of this series [8], it was shown that the efficiency of the Chelex-

100 is strongly related to the resin affinity for the element of interest, the operational pH and the salt matrix. The operating pH of 4–5 suggested by Leyden and Underwood [9] and the pH of 5.0–5.5 suggested by Kingston et al. [5], proved to be suitable for freshwater analysis, but is obviously not the optimum pH for seawater analysis. This was demonstrated by the low recovery of manganese in seawater at pH 5.4 by Sturgeon et al. [3], and the higher recovery at pH 7.6 [10]. However, if the column capacity were large enough or the flow rate were slow, one could still obtain satisfactory recovery, as reported by Kingston et al. [5] who obtained nearly 100% recovery for manganese. Thus, the column dimensions and flow rate are also important criteria for column performance. However, in many previous publications, the choice of column size and flow rate were quite arbitrary. The lack of general criteria on those parameters has made optimization of the Chelex-100 technique a matter of trial and error.

In the work described below, the distribution of heavy metals on a Chelex-100 column after seawater had passed through was studied. The column was divided into small sections and the spiked trace metals were recovered from each individual section. In so doing, a simplified model was derived to describe the effect of column size and flow rate on the recovery of heavy metals. The efficiency of any given column can therefore be predicted.

EXPERIMENTAL

Materials and instrumentation

Chelex-100 columns. Chelex-100 resin (sodium form, 100–200 mesh; Bio-Rad Laboratories) was weighed in 0.4 ± 0.02-g portions, which were sealed in 50-ml beakers until required. Each portion was packed into a tygon minicolumn as described in Part 1 [8]. It was convenient to use a column rack which held twenty minicolumns in a row, so that to pack and wash twenty minicolumns simultaneously required no more than 1 h. Ten minicolumns were connected together to give a column string containing a total resin weight of 4 g. When connections were made, a layer of water was maintained above the resin to prevent air entering the resin bed (see Fig. 1). The column string was converted to the ammonium form by washing in sequence with 20 ml of 2 M nitric acid, 60 ml of distilled water, 20 ml of 1 M ammonia water, and 100 ml of distilled water.

Purified coastal seawater. Freshly collected coastal seawater was stored in a 25-l polyethylene tank. To this was added 50 mg of iron as iron(III) chloride, and the pH was maintained at its original 8.0. The mixture was stirred and allowed to stand for 2 or 3 days until the brownish precipitate settled on the bottom. The supernatant liquid was led to a column containing 10 g of Chelex-100 (sodium form) and the effluent was filtered through a 0.45-μm membrane filter. The co-precipitation process with hydrated iron(III) oxide absorbs par-
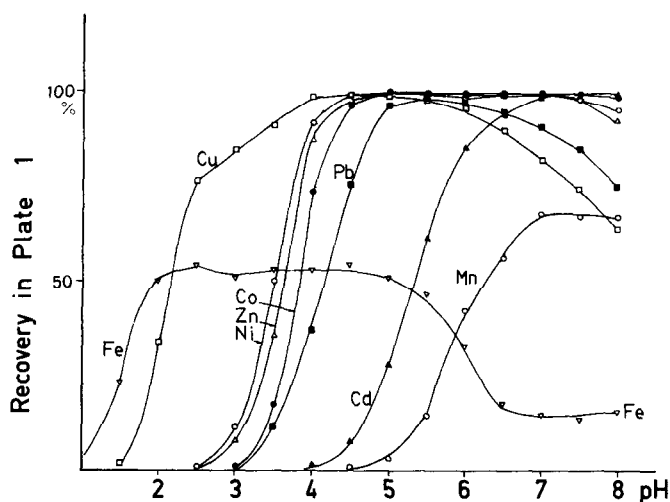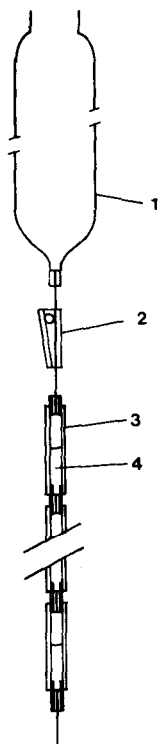
Fig. 1. The sectional Chelex-100 column string: (1) 500-ml sample reservoir; (2) plastic clamp; (3) column made of tygon tubing; (4) Chelex-100 resin, 100-200 mesh.

Fig. 2. The recovery of trace metals in the first section (0.4 g of resin) of a Chelex-100 column at various pH values in presence of ammonium acetate. Sample (500 ml of seawater) was spiked with 10 μg of each element and loaded at a flow rate of 4 ml min^{-1}.

ticles and most heavy metals such as chromium, copper or zinc, whereas the later Chelex-100 treatment further removes cadmium, remaining heavy metals, and part of the colloidal iron.

Atomic absorption spectrometry (a.a.s.). A Hitachi Model Z-8000 (polarized Zeeman effect) atomic absorption spectrometer was used to determine heavy metals. Both the flame and graphite-furnace atomizers are equipped with strong magnetic fields to give a double-beam correction system based on the Zeeman effect. The operational conditions for the graphite furnace were as recommended by the manufacturer.

General procedure

An aliquot (500 ml) of the purified seawater was spiked with 10 μg of each heavy metal (including Cd, Cu, Cr(III), Fe, Mn, Ni, Pb, and Zn) to give a concentration level of 20 μg l^{-1}. To the solution was added 20 ml of 1 M am-

monium acetate, and the pH was adjusted to 5.4 (or 8.0) with ammonia. The sample was loaded from a pyrex reservoir onto the Chelex-100 column string (consisting of ten minicolumns in the ammonium form), at a flow rate of 4 ml min^{-1} controlled by a Pharmacia peristaltic pump. After the sample had passed through, the column string was washed with 40 ml of distilled water and then disconnected into its ten sections. To recover the heavy metals from each section, elution with four 2-ml portions of 2 M nitric acid was applied; the volume of eluate was made up to exactly 10 ml in a test tube. The metals were determined directly by flame a.a.s.

Recommended procedure for natural seawater

An aliquot (500 ml) of the seawater was mixed with 20 ml of 1 M ammonium acetate (pH 6.1) and the pH was adjusted to 6.5 with ammonia. The mixture was transferred to a pyrex reservoir and loaded onto a Chelex-100 column containing 2 g of the resin in the magnesium form at a flow rate of not more than 4 ml min^{-1} controlled by a plastic clamp (about 1 drop per second). After all the sample had passed through, the column was disconnected from the reservoir and a pyrex funnel was plugged into the top. The column was washed with 20 ml of distilled water, five 4-ml portions of 1 M ammonium acetate (pH 5.5) to remove major cations, and 40 ml of distilled water. Heavy metals were then recovered with four 2-ml portions of 2 M nitric acid. The eluate was collected in a calibrated test tube and diluted to exactly 10 ml with distilled water. The final solution was stirred vigorously and was then ready for graphite-furnace a.a.s. determination of Cd, Co, Cu, Fe, Mn, Ni, and Pb. Zinc was determined by flame a.a.s. because of its high sensitivity. The analysis was repeated five times, as were analyses of blanks and spiked samples (see Results and Discussion).

RESULTS AND DISCUSSION

Distribution of metals on the column sections

The recoveries of trace metals from the Chelex-100 column string at pH 5.4 and 8.0 are shown in Table 1. It is interesting to note that, at these two pH values, the general distribution patterns are distinctly different for several elements. At pH 5.4, the first section of the column collects more than 97% of Co, Cu, Ni, Pb, and Zn, but only 51% of Cd, 40% of Fe, and 14% of Mn. At pH 8.0, only Co, Ni, and Zn retain similar patterns as at pH 5.4; the recoveries of Cu, Pb, and Fe in the first plate drop to 68%, 67%, and 21%, respectively, while the recoveries of cadmium and manganese increase to 93% and 64%. The significance of these results is that they explain why Sturgeon et al. [3] obtained low recovery of manganese working at pH 5.4, and why Bruland et al. [11] found that the Chelex-100 method gave low results for copper at pH 8.0. The reason for these analytical losses was not the inadequacy of the Chelex-100

TABLE 1

Distribution of spiked heavy metals on the sectional Chelex-100 column at pH 5.4 or pH 8.0 (both in presence of ammonium acetate)[a]

Section	Recovery (% of the spiked amount)								
	Cd	Co	Cu	Cr^{3+}	Fe	Mn	Ni	Pb	Zn
Distribution at pH 5.4									
1	51	97	98	9	40	14	99	97	105
2	27	2	2	<	13	11	<	<	4
3	13	<	<	<	6	9	<	<	<
4	6	<	<	<	5	8	<	<	<
5	2	<	<	<	4	5	<	<	<
6	1	<	<	<	4	5	<	<	<
7	1	<	<	<	3	5	<	<	<
8	<	<	<	<	3	4	<	<	<
9	<	<	<	<	3	3	<	<	<
10	<	<	<	<	2	3	<	<	<
Total	101	99	100	9	83	67	99	97	109
Distribution at pH 8.0									
1	93	96	68	4	21	64	96	67	95
2	6	4	9	2	12	22	<	11	12
3	1	<	5	<	8	7	<	5	2
4	<	<	2	<	5	4	<	<	<
5	<	<	<	<	4	1	<	<	<
6	<	<	<	<	3	<	<	<	<
7	<	<	<	<	3	<	<	<	<
8	<	<	<	<	3	<	<	<	<
9	<	<	<	<	2	<	<	<	<
10	<	<	<	<	3	<	<	<	<
Total	100	100	84	6	64	98	96	83	109

[a]10 μg of each metal was spiked to 500 ml of seawater; the loading flow rate was controlled at 4.0 ml min^{-1}. Each section of the column contained 0.4 g of 100–200-mesh resin. "<" denotes less than the detection limit of Zeeman-effect flame a.a.s.

method, but the selection of a non-optimal pH so that the apparent capacity of the column was insufficient for those elements. It should be borne in mind that the use of pH 5.4 is not wrong; nearly 100% recovery can still be obtained by using a big column or a slow flow rate. It can be assumed that if Sturgeon et al. [3] had used more resin, they would have obtained higher recovery of manganese; by contrast, if they had used less resin, they might have found that the recovery for Cd was also incomplete.

Empirical equation to estimate column efficiency

Because the distribution of metal on the sectional column has a gradually decreasing trend with increasing section number, an empirical equation can be derived to fit the experimental results in Table 1. If it is assumed that the metal-removing efficiency of each individual section (plate) is identical, the recovery of each metal from the first plate (R_1, %) can be used to estimate the recoveries from the second or other plates:

$R_2 = R_1 (1-R_1); R_3 = R_1 (1-R_1)^2; R_n = R_1 (1-R_1)^{n-1}$

The total percentage recovery of a column consisting of n plates will be R (total) $= R_1 + R_2 + .. + R_n$

Based on these equations, and from the experimental data for R_1 (%), computer simulation can be used to predict the efficiency of a Chelex-100 column of given dimensions and to assess the optimal pH for better performance. To test this, additional spiking experiments were made at various pH values (1–8), and the results of the recoveries in the first plate (R_1 %) were plotted against pH as shown in Fig. 2.

The computer-simulated distributions matched amazingly well with the actual measured distributions for Cd, Co, Mn, Ni, and Zn at all pH values tested. For example, the calculated recoveries of manganese at various pH values are shown in Table 2, which clearly illustrates the pH effect on the column efficiency. For a Chelex-100 column containing 2 g of resin, operated at pH 5.5 and at a flow rate of 4 ml min^{-1}, the total recovery of manganese is predicted to be 55.6%. Doubling the column size to 4 g of resin increases the recovery to 80.3%: a large 11.6-g resin column would be required to obtain an efficiency of 99%. At the same flow rate but at pH 6.5, the same 2-g resin column would give 98.3% recovery of manganese. The recovery can be further improved to 99.7% if the solution is adjusted to pH 7.0. The agreement of calculated and experimental results also applied to iron at pH ≯ 5, and to copper and lead at pH ≯ 7.

TABLE 2

Simulated distribution of manganese on a Chelex-100 column operated at a flow rate of 4 ml min^{-1}

Plate number[a]	Recovery (%) at different pH				
	5.0	5.5	6.0	6.5	7.0
1	4.0	15.0	42.0	56.0	68.0
2	3.8	12.7	24.4	24.6	21.8
3	3.7	10.8	14.1	10.8	7.0
4	3.5	9.2	8.2	4.8	2.2
5	3.4	7.8	4.8	2.2	0.7
Total	18.4	55.5	93.5	98.4	99.7

[a]Each plate is assumed to contain 0.4 g of resin. Recoveries on plate 1 were taken from Fig. 2; other data were calculated by the proposed equations.

Above these pH thresholds, part of these elements may appear in the effluent. This can be ascribed to the fact that at high pH Fe, Cu, and Pb form hydroxide complexes which cannot enter the bore structure of the resin and are therefore lost to the sorption. For these reasons, the choice of pH for multi-element collection was limited to 7.0, or preferably 6.5. The effort of improving recovery of iron was abandoned.

Flow-rate

The efficiency of a Chelex-100 column is also affected by the flow rate. It should be noted that the sample usually passes through a column at a rate so
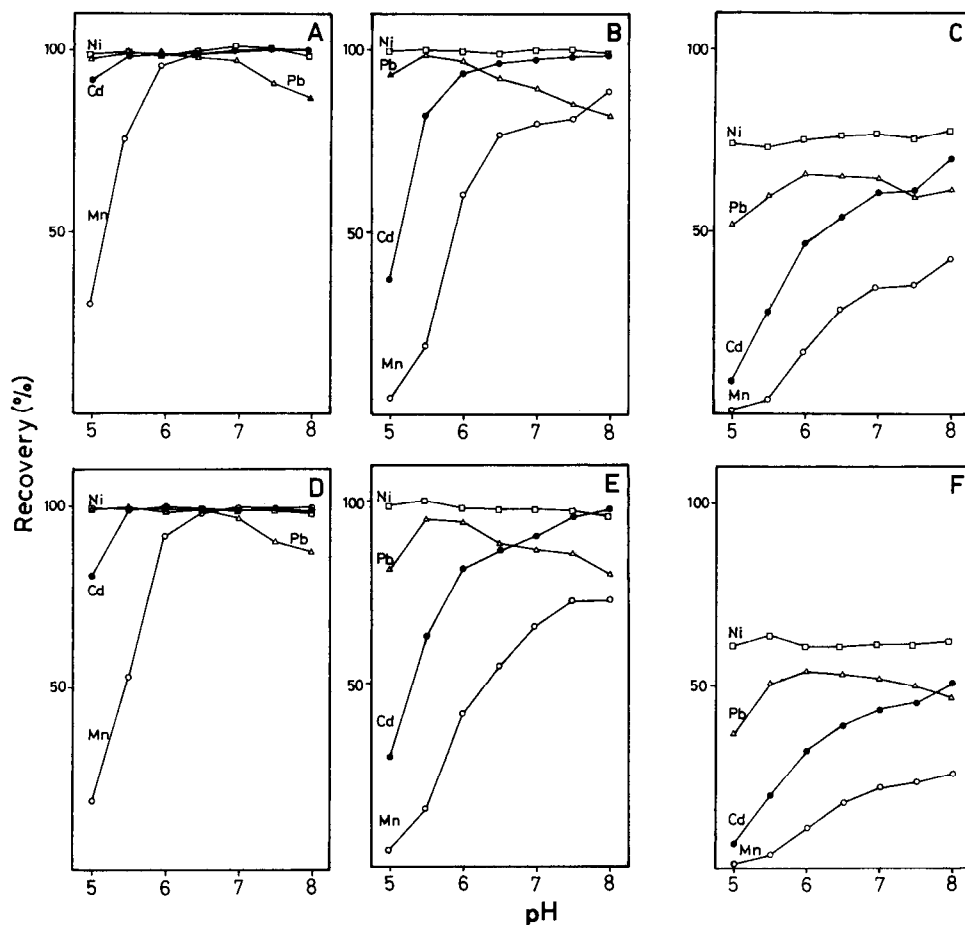

Fig. 3. Recovery of Cd, Mn, Ni, and Pb from seawater at various pH values on Chelex-100 columns with different resin weights at various flow rates; 10 μg of each metal was added to 500 ml of seawater. Resin weight: (A, D) 2.0 g; (B, E) 0.4 g; (C, F) 0.1 g. Flow rate: (A–C) 1 ml min^{-1}; (D–F) 4 ml min^{-1}.

quick that complete chelation is unlikely to be achieved. The slower the flow rate, the more chance for heavy metals to be chelated (retained). De Mora and Harrison [12] applied the term "contact time" to describe the combined effect of column size and flow rate. The contact time (t_c) is defined as the average time of water to contact the column packing: t_c = (void volume of column)/ (flow rate).

For a Chelex-100 column having a void bed volume of 0.5 ml, operated at a flow rate of 4 ml min^{-1}, the contact time would be only 7.5 s. If this concept is correct, then the improvement of column efficiency by halving the flow rate should be equivalent to that obtained by doubling the column size. To check this, tests were conducted with different columns (containing 0.1, 0.4 g, and 2.0 g of resin) at various flow rates (1 and 4 ml min^{-1}). To avoid the problems of measuring the void volume, only the resin weight was considered. Theoretically, 0.1 g of resin with a flow rate of 1 ml min^{-1} should give the same results as those obtained with 0.4 g of resin and 4 ml min^{-1}. However, the experimental results shown in Fig. 3 do not support this simple idea. Calculations showed that reducing the flow rate from 4 to 1 ml min^{-1} is approximately equivalent to doubling the column size. Paulson [7] has demonstrated that the most effective way to enhance the column efficiency is to use slow flow rates (e.g., 0.2 ml min^{-1}). However, considering the total loading time for each sample, the use of high flow rates is obviously preferable. It was found here that a column containing 2 g of Chelex-100 resin, with the seawater at pH 6.5, and a flow rate of 4 ml min^{-1} provided the most appropriate combination for pre-concentrating Cd, Co, Cr, Mn, Ni, Pb, and Zn from seawater at > 98% of efficiency.

Buffer and resin form

In preliminary experiments, sodium acetate was used as buffer instead of ammonium acetate. No significant difference was found. Both the magnesium and the ammonium forms of the resin were tested for efficiency, and gave quite similar results. The reason for interest in the magnesium form of the resin was simply that it gives much more stable pH than does the ammonium form. Usually when 20 ml of 1 M ammonium acetate (pH 6.1) is added to 500 ml of unacidified seawater, the pH goes to 6.5 right away. At this pH, the ammonium acetate does not provide enough buffering capacity and the pH may change during passage through an ammonium column. However, use of the magnesium form of the resin usually introduces high blanks, probably from the magnesium chloride used to prepare it. To compensate for this, the reagent was carefully purified by adding a little sodium acetate buffer and passing through a Chelex-100 column at pH 6.5. The solution was stored in a pyrex glass container over Chelex-100 resin beads.

Application to natural seawater

All the previous spiked experiments at the 20 μg l^{-1} level proved that the Chelex-100 technique, if used under the nominally optimal conditions, is more

than 98% efficient for pre-concentrating heavy metals from seawater (except for iron and chromium). The technique should work even better at $< 1\ \mu g\ l^{-1}$ levels, but the real efficiency at such concentrations could not be proved because of the limit of determination of graphite-furnace a.a.s.

The recommended Chelex-100 procedure (see Experimental) was applied to a natural seawater, to demonstrate its precision and detection limit. The seawater tested was collected by the staff of the R.V. Ocean Researcher I from the surface of the West Philippine Sea. It was filtered, acidified and stored in a polyethylene tank.

The final solution obtained by the recommended procedure contains a high concentration of ammonium ions, which may interfere with the determination of the metals by graphite-furnace a.a.s. [13] because of variable behaviour during the drying and ashing stages. Accordingly, the heavy metal contents cannot be calculated directly from calibration grahps prepared with standards in either distilled water or in 2 M nitric acid and the standard addition method must be applied. The seawater sample was spiked with $0.1\ \mu g\ l^{-1}$ Cd and $1\ \mu g\ l^{-1}$ of the other metals and treated by the same pre-concentration procedure as the sample . The mean readings and results obtained are shown in Table 3. The sample was probably contaminated before it reached the laboratory, judged from the high contents of zinc and lead, and compared with literature data. However, the low detection limits and the relative standard deviations given

TABLE 3

Analysis of a Philippine Sea seawater sample by the Chelex-100 technique and graphite-furnace a.a.s.

	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn[a]
Raw data[b]								
Analytical								
blank	34	34	39	151	670	90	36	25
Seawater	180	35	420	518	3436	257	780	588
Spiked								
seawater	1445	1349	1732	1984	6265	804	1999	812
Calculated results								
Detection								
limit[c] (ng l^{-1})	1	18	30	85	50	100	15	24
Conc. in								
sample (ng l^{-1})	12	$<$[d]	290	250	978	302	611	2500
S.d. (ng l^{-1})	1	—	20	50	58	48	16	160
R.s.d. (%)	6	—	7	10	6	16	3	6

[a]Zinc was determined by flame a.a.s. [b]Each number (absorbance $\times 10^4$) is the mean of five readings. [c]Detection limits were estimated from 4.65 times the deviation of blank readings. [d]Below detection limit.

by the proposed procedure, with its 50-fold concentration factor, provide enough sensitivity for studying the vertical profiles of Cd, Cu, Fe, Mn, Ni, and Zn in open oceans. It is also feasible to detect Co and Pb in seawater by increasing the concentration factor to 1000-fold.

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