

where amide groups align face-to-face with their group moments antiparallel (2). Analytes A1 and A5 have amide groups that could interact either way with CSP1. Generally we do not find dipole stacking but we do observe hydrogen bonding. This conclusion is based upon examination of stereoviews of hundreds of "snapshots" near the global minima on the CSP2-A1 and CSP2-A5 surfaces. A typical stereoview is shown in Figure 4. Again keep in mind that this is only one snapshot of an infinite number of intermolecular arrangements that can take place and it does not adequately represent how the system exists over a long time period (as do our evaluations of  $\bar{E}$  and the fragmentation energies described above) but it clearly shows hydrogen bonding rather than dipole stacking.

### SUMMARY

The differential binding of two chiral analytes on two different Pirkle phases was simulated with atomistic modeling. These results were described and compared with earlier work. The results from this work taken together with our earlier results allow us to state the following: (1) The computational protocol we employ seems to be valid; we always predict the correct retention order and we can qualitatively determine separability factors. (2) The intermolecular potential energy surfaces describing the binding of analytes to these CSP's are very flat. Binding to the front and back sides of CSP1 and CSP3 is possible but a slight preference for binding to the sterically less encumbered face is evident. Generally speaking both *R* and *S* analyte bind to the same region. There is no evidence suggesting one analyte binds selectively to one portion of the CSP while the mirror image binds elsewhere. (3) The fragments on CSP1 most responsible for holding the diastereomeric complexes together usually are the DNB moiety and the amide spacer linkage while the naphthylamine is most responsible in CSP3. These fragments do the majority of the work holding the complexes together regardless of the chirality of the substrate. Again we conclude that one fragment does not selective bind one enantiomer while another fragment binds its mirror image. (4) The fragment bearing the stereogenic center is not the dominant fragment in chiral recognition. This is consonant with Mislow's views (14) that the entire molecule is chiral, not just its asymmetric center.

(5) We conclude that simplification of the myriad of pairwise-additive interactions between molecules into a more manageable caricature or cartoon generally support the chiral recognition models developed by Pirkle.

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## TECHNICAL NOTES

### Maleic Acid/Ammonium Hydroxide Buffer System for Preconcentration of Trace Metals from Seawater

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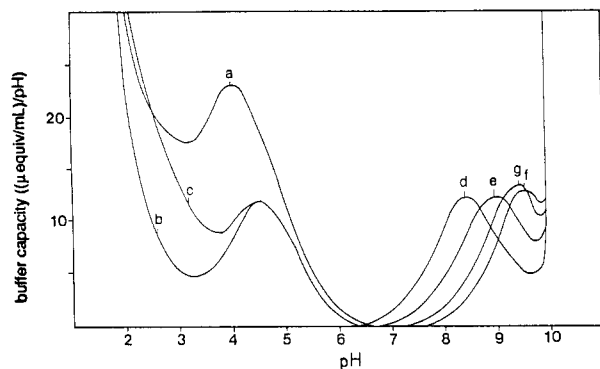
#### INTRODUCTION

Preconcentration is often required in the determination of trace metals in seawater. Among the many proposed preconcentration schemes, the complexation/solvent extraction

(1-3) and the chelating ion-exchange methods (3, 4) are probably the most widely used. The efficiencies of these methods are critically pH-dependent and the optimal pH may vary from method to method and from metal to metal. Thus, the analytical protocols frequently require the addition of buffers to adjust the pH of the sample to the desired value. Furthermore, during the developmental stage of a new method, before the optimal pH has been identified, empirical tests often have to be carried out over a wide pH range at

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**Figure 1.** Buffering capacity curves of various buffer ligands in seawater at a concentration of 0.02 M. The position of each peak on the pH scale reflects the  $pK'$  value: a, citrate; b, acetate; c, phthalate; d, Tris; e, borate; f, ammonium; g, glycine.

regular intervals. In such a situation, different buffers or a combination of various buffer systems would have to be used.

An ideal buffer should provide an adequate buffering capacity over a wide range of pH, have minimal interaction with the metal of interest, not react with other constituents of seawater in a manner that will interfere in the subsequent analysis, and be made up of chemicals that can be easily purified from contaminating trace metals. Presently, the most commonly used buffer ligands are acetate (1, 2, 5), citrate (1, 5, 6), glycine (7), borate (8), and ammonium ions (1, 3, 5, 6). The buffering capacities of these ligands at 0.02 M in seawater are shown in Figure 1. Among these, acetate and ammonium ions are frequently used in combination (2–4, 9, 10). However, it can be seen that a conspicuous gap exists between pH 6 and 7 where none of them can provide an adequate buffering capacity. There are limitations in choosing other buffers. For example, citrate forms strong complexes with Cr and Fe at a certain pH range (7, 11) and phosphate precipitates with Ca and Mg at pH above 6 (1).

In this paper, we will describe a buffer system, the ammonium maleate system, which is made of a mixture of hydrochloric acid, maleic acid, and ammonium hydroxide, and its buffering capacity covers a wide pH between 2 and 9. The working buffers are simple to prepare, easy to use, and can be rendered free from trace metal contaminations readily. The buffer system has been applied successfully to the preconcentration of trace metals in seawater by both the APDC + DDTC/MIBK solvent extraction and the Chelex-100 chelating ion-exchange technique in our laboratories.

## EXPERIMENTAL SECTION

**Reagents. Ammonium Hydroxide Solutions.** Ammonium gas was distilled off a concentrated ammonium hydroxide solution (28%) and redissolved in double distilled water (DDW). The exact concentration of the purified ammonia solution was determined by titration with a standardized acid. A 2 N working solution was prepared by dilution with DDW.

**Hydrochloric and Nitric Acids.** Concentrated HCl and HNO<sub>3</sub> were purified by sub-boiling distillation (12), then stored in Teflon bottles. The exact concentrations were determined by titrations with a standardized base. Working solutions (2 N) were prepared by dilution with DDW.

**Maleic Acid/Ammonium Hydroxide Stock Buffer Solution.** A 58.01-g portion of maleic acid was dissolved in ca. 600 mL of water. The pH of this solution was adjusted to ca. 6.5 with concentrated ammonium hydroxide. The solution was diluted to ca. 1 L and then passed through a Chelex-100 column (20 cm × 2 cm i.d.) packed with 10 g of 100–200 mesh resin in the ammonium form at a flow rate of not more than 2 mL/min. The effluent was collected directly into a Teflon bottle.

**Working Ammonium Maleate Buffers.** The working buffer solutions for prescribed pH values were prepared by diluting 400 mL of the stock buffer solution to 500 mL after various amounts

**Table I. Preparation of the Maleic Acid/Ammonium Hydroxide Working Buffers**

pre-scribed pH	vol (mL) of acid or base added to 400 mL of stock buffer then diluted to 500 mL				pH of working buffer <sup>a</sup>	resultant pH when added to seawater at 5% (v/v) ratio <sup>b</sup> (n = 4)
	conc HCl	2 N HCl	conc NH <sub>4</sub> OH	2 N NH <sub>4</sub> OH		
2.0	27.0				1.12	2.03 ± 0.01
2.5	21.0				1.87	2.49 ± 0.02
3.0	18.8				2.23	2.97 ± 0.03
3.5	17.6				2.51	3.47 ± 0.03
4.0	15.8				2.76	4.01 ± 0.01
4.5		90.0			4.17	4.50 ± 0.00
5.0		64.0			4.98	5.01 ± 0.01
5.5		30.0			5.50	5.51 ± 0.00
6.0			9.0		6.00	6.01 ± 0.01
6.5					6.47	6.50 ± 0.01
7.0				7.0	6.95	6.99 ± 0.03
7.5				15.0	7.59	7.53 ± 0.03
8.0				48.0	8.26	8.02 ± 0.02
8.5			20.0		8.80	8.52 ± 0.01
9.0			40.0		9.28	9.00 ± 0.01

<sup>a</sup> Small pH adjustment of the mixed working buffer is necessary because the HCl and ammonia might vary from batch to batch.

<sup>b</sup> Resultant pH values (25 mL of working buffer added to 500 mL of seawater) measured at 25 °C.

of hydrochloric acid or ammonium hydroxide were added as shown in Table I. The working buffer solutions are thus all ca. 0.4 F with respect to maleate.

**Trace Metal Standards.** The Merck Titrisol standards of Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn were diluted to desired concentrations.

**Seawater.** Coastal seawater was collected in a 25-L polyethylene carboy 10 km east of Keelung Harbor, Taiwan. The sample was filtered through a Whatman GF/C filter, preserved with 1 mL of chloroform, and then stored.

**Instrumentation.** A Hitachi Model Z-8000 atomic absorption spectrometer with Zeeman background correction was used throughout this work for the determination of metals.

**Determination of Buffering Capacity.** The quantitative measure of the buffering strength of a solution at a given pH is usually termed as "buffering capacity", which is defined as the number of millimoles of strong acid/base required per milliliter to cause a unit change in pH. The buffering capacity of a maleate ion added seawater was measured by the following procedure:

Twenty-five milliliters of stock buffer solution was added to 500 mL of seawater to yield a concentration of maleate of ca. 0.02 F. The mixture was titrated with 1 N HCl, NaOH, or NH<sub>4</sub>OH. The pH of the solution after each addition of the titrant was noted. The buffering capacity at a given pH was estimated from the tangent of the titration curve at that pH.

## RESULTS AND DISCUSSION

**Capacity of the Maleic Acid/Ammonium Hydroxide System.** Maleic acid was chosen for detailed study for the following reasons: its  $pK_1$  and  $pK_2$  values are 1.92 and 6.23 (13), so that it may act as a buffer at pH around 6; it does not form strong complexes with most trace metals of interest (13); it has a high chemical stability, a relatively low toxicity, and a reasonably high solubility so that solutions and concentrations below 0.5 M may be prepared readily. The buffering capacity of a mixture of maleic acid, ammonium hydroxide, and seawater (20 mL of stock ammonium maleate buffer added to 500 mL of seawater) at various pH values is shown in Figure 2. Below pH 2.5 and above 7.5, the solution was strongly buffered by water (hydrogen ions) and ammonium ions, respectively. A maximum peak was found at 5.5 which should be equal to  $pK_2'$  of maleic acid in seawater. The shift of  $pK_2$  (6.23) at infinite dilution to  $pK_2'$  (5.5) in seawater reflects the effect on the high ionic strength of seawater of the dissociation constants of acids. Minimum buffering ca-

**Table II. Removal of Heavy Metal Impurities from Ammonium Maleate Stock Buffer (0.5 F with respect to maleate) by Passing through Chelex-100 Columns**

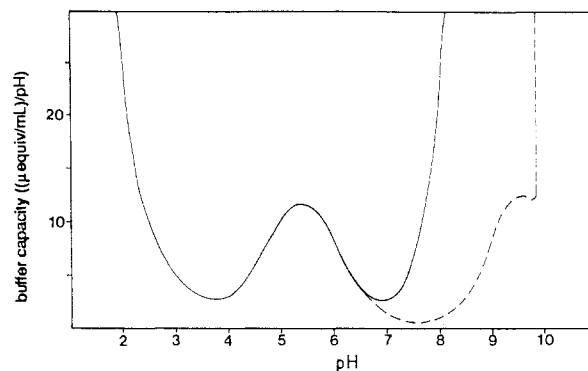
resin wt, g	flow rate, mL/min	pH	removal, <sup>a</sup> %								
			Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn	
0.5	5	3	2	8	95	90	0	54	41	18	
		4	22	73	97	78	5	92	55	88	
		5	53	97	99	76	14	95	64	98	
		6	86	>99	>99	79	42	95	80	>99	
		7	>99	>99	>99	60	85	97	93	>99	
		8	>99	>99	98	5	99	98	95	>99	
		10.0	2	6.5	>99	>99	>99	99	>99	>99	>99

<sup>a</sup>Initial spiking concentration: 10 µg/L for each metal.

capacities were observed at pH 3.5 and 7. Even at these minima, the value exceeded 3 (µequiv/mL)/pH and should be adequate for most analytical purposes. Thus, the maleic acid–ammonium hydroxide and/or hydrochloric acid buffering system alone can cover the entire pH range from pH 2 to 9 without the need of adding other ligands.

A series of working buffers was prepared from these three chemicals as described in Table I. When they were added to seawater at a ratio of 5% (v/v), the resulting pH of the seawater varied progressively from pH 2 to 9 at 0.5 pH intervals with a precision of better than 0.03 pH unit. Thus, the need for monitoring the resulting pH of the sample with a pH meter upon the addition of acid, base, or buffer in order to reach a desired pH may be eliminated. By simplification of the steps needed to bring a sample of seawater to a given pH, it makes the adjustment of a large number of samples to a variety of pH values in the search for an optimal pH during method development much easier. The procedure may be simple enough to allow for the preconcentration of trace metals from seawater to be performed onboard ship.

**Purification of Buffer.** Trace metal impurities in the stock buffer solution can be removed by passing it through a Chelex-100 column. A test was made to find out the optimal pH and removal efficiencies. Each of the eight trace metals, namely, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn, was added to the stock buffer at a concentration of 10 µg/mL. The pH of the buffer solution was then adjusted to pH 3–8 at 1 pH unit intervals, and then passed through a Chelex-100 column containing 0.5 g of resin at a flow rate of ca. 5 mL/min. The trace metals retained by the column were recovered by elution with 2 N nitric acid and determined by atomic absorption spectrometry. The results are given in Table II. The removal efficiencies for most metals showed an increasing trend with increasing pH. Unlike other metals, Fe is poorly removed at higher pH. To compensate for this, an intermediate pH of 6.5 was chosen and the removal efficiencies of the column were further improved by using a larger dimension (containing 10 g resin) and operating at a slower flow rate of 2 mL/min.



**Figure 2.** Buffering capacity curves of a seawater sample (500 mL) added with 25 mL of ammonium maleate stock buffer (ca. 0.02 F with respect to maleate): (solid line) HCl/ammonium hydroxide as the titrant; (dash line) HCl/NaOH as the titrant.

Under these conditions, more than 99% of all metals spiked were removed.

**Application to a Seawater Sample.** Filtered coastal seawater and trace metal added seawater (spiked with Cd, Co, Fe, Mn, Ni, Pb, and Zn) were analyzed by the Chelex-100/graphite furnace atomic absorption spectroscopy (GF-AAS) technique (14) using the nominated buffer and operating at pH 6.5.

A 500-mL aliquot of the sample, with an added 25 mL of the ammonium maleate buffer, was allowed to pass through three consecutively connected Chelex-100 columns (each contained 2 g of 100–200 mesh resin in ammonium form) at a flow rate of 5 mL/min. Each column was rinsed separately with four 5-mL portions of 1 M purified ammonium acetate (pH 5.5) to remove alkali and alkaline-earth elements (3, 4), followed with five 1-mL portions of DDW, and then eluted with four 2-mL portions of 2 N nitric acid. The final eluate (made up to 10.0 mL) from the first column was measured for the heavy metal contents in that seawater; the eluate from the second column was measured to check the residues; and the third column was accounted for the analytical blank. Portions (2.0 mL) of this blank was precisely withdrawn, to which were spiked 0.2 and 0.5 µg of each metals of interest in order to construct calibration curves. The recoveries of the spiked trace metals were calculated from the concentrations of trace metals in the sample and the spiked sample. For comparison, a separate sample was analyzed by the same procedure but using the conventional ammonium acetate buffer at pH 5.5. Both results are given in Table III.

In the experiment where maleate buffer was used, the recoveries of the spiked elements (except for Fe) were all in the range of 95–104%, which should be considered quantitative within experimental uncertainties. The recovery for Fe was comparatively low at 85%. In the experiment where acetate buffer was used at pH 5.5, the recoveries for Cd, Co, Cu, Ni,

**Table III. Analysis of Trace Metals in a Coastal Seawater Sample by Chelex-100 Preconcentration/GF-AAS Using the Ammonium Maleate Buffer at pH 6.5<sup>a</sup>**

element	analytical blank, µg/L	concn found, µg/L		recovery, %	% recovery using acetate buffer (pH 5.5)
		sample	sample + 1 µg/L		
Cd	0.005	0.06 ± 0.01	1.10 ± 0.03	104	99
Co	<0.01	n.d. ± 0.02	0.96 ± 0.07	96	98
Cu	0.02	0.77 ± 0.05	1.75 ± 0.11	98	98
Fe	<0.1	2.10 ± 0.20	3.05 ± 0.24	85	78
Mn	0.01	1.45 ± 0.05	2.42 ± 0.09	97	64
Ni	<0.1	0.23 ± 0.11	1.18 ± 0.08	95	98
Pb	<0.01	n.d. ± 0.01	0.98 ± 0.03	98	102
Zn	0.12	5.70 ± 0.06	6.66 ± 0.11	96	99

<sup>a</sup>All values are the mean of triplicated measurements. Concentrations were calculated from the calibration curves constructed by adding standards to the analytical blank, i.e. the eluate from the third column.

Pb, and Zn were also found satisfactory, ranging between 98% and 102%, but recoveries for Fe and Mn were only 78% and 64%, respectively. These results suggested that the use of the proposed ammonium maleate buffer system has more advantages than that of ammonium acetate for the Chelex-100 operation, by obviously improving the recoveries of Fe and Mn and also providing higher buffering capacity at pH 6.5.

These two buffers were also applied to the APDC + DDTC/MIBK solvent extraction method at pH 4.5 for comparison, but the results did not show significant difference.

### CONCLUSION

The proposed hydrochloric acid/maleic acid/ammonium hydroxide buffer system can be used to bring the pH of seawater samples to predetermined values of pH between 2 and 9 with a high degree of precision. It covers the pH of 6-7 where none of the buffering system presently in use can work effectively. These advantages benefit the search of an optimal pH in developing a new method and make the routine pre-concentration procedure much quicker and easier so that it can be performed onboard ship readily. Since the need of using a pH meter to measure the pH of a seawater sample while adjusting its pH to the desired value is virtually eliminated, the risk of contamination can be largely reduced.

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**Registry No.** NH<sub>4</sub>OH, 1336-21-6; HCl, 7647-01-0; Cd, 7440-43-9; Co, 7440-48-4; Cu, 7440-50-8; Fe, 7439-89-6; Mn, 7439-96-5; Ni, 7440-02-0; Pb, 7439-92-1; Zn, 7440-66-6; maleic acid, 110-16-7; water, 7732-18-5; Chelex-100, 11139-85-8.

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## Design for Field-Flow Fractionation Channels and Split-Flow Lateral-Transport Thin Separation Cells

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Although the mechanisms underlying separation diverge (1), the fundamental technical requirements associated with field-flow fractionation (FFF) channels and split-flow lateral-transport thin (SPLITT) cells are similar in many respects. Both techniques realize the goal of separation through the application of transverse driving forces to various particle populations carried by flow down a thin, unobstructed, ribbonlike channel of regular geometry. In FFF, the more well-established of the two methods, this criterion has usually been met by sandwiching a spacer, i.e., a thin (usually 0.05-0.5 mm) film of Mylar, Teflon, or stainless steel sheeting from which the channel volume has been cut out and removed, between two walls which are constructed such that they allow transmission of the driving force (2) (see ref 3 for a notable exception). This configuration has changed little from that used to carry out some of the first successful thermal FFF experiments more than 20 years ago (4). Split-outlet FFF (5) and SPLITT cell systems (6) have been based upon similar, although somewhat more complicated, configurations. The construction of these systems has been approached through the use of a three-layer channel system, with the layers subject to cut-outs of different geometries and the entire sandwich construction clamped between two appropriate walls.

In order to ensure that the channel area be well-defined, i.e., that a seal be maintained precisely at the channel boundaries and that the channel itself has a high degree of

geometrical integrity, these designs required secure, even clamping of the spacer with rows of bolts. The ideal spacer needed to be both rigid, to maintain the best geometry, and compressible, to maintain the best seal. A compromise serving neither purpose completely was found in Mylar or Teflon spacers, which sealed well when bolted with sufficient force (although the latter possessed little dimensional stability). Wall materials were thereby restricted to those that could withstand these forces of clamping.

This can have serious ramifications for flow FFF and SPLITT systems, which are preferentially constructed from Plexiglass or glass to allow visual monitoring of the channel geometry or contents. In the former, the problem is compounded by the necessity of a porous frit being embedded into the lower channel wall. These frits can be quite brittle and subject to easy breakage. Moreover, clamping of traditional spacers acts to compress the membrane at the channel edges, causing it to bulge slightly into the channel and resulting in uncertain system performance through distortion of the channel geometry. In SPLITT systems, the splitting edges of the necessarily thin splitting layer are insufficiently stable to assure that the passageways above and below remain equally open to flow.

This note describes an uncomplicated new approach to open parallel plate type channel construction which overcomes these obstacles by eliminating the need for a traditional spacer. It