Metal Complexation of Thiacrown Ether Macrocycles by Electrospray Ionization Mass Spectrometry

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In the present study, electrospray ionization mass spectrometry is used to evaluate the metal-binding selectivities of an array of novel caged macrocycles for mercury(II), lead(II), cadmium(II), and zinc(II) ions. In homogeneous methanol/chloroform solutions as well as extractions of metals from aqueous solution by macrocycles in chloroform, it is found that the type of heteroatom (S, O, N), cavity size, and presence of other substituents influence the metal selectivities. Several of the macrocycles in this study bind mercury ion very selectively and efficiently in the presence of many other metal ions and have an avidity toward mercury that was tunable by the size and combination of heteroatoms in the macrocycle ring and the number of cage groups attached. The extraction mechanism was further investigated by determining the variation in extraction selectivity as a function of the counterions of the mercury salts.

Heavy metals, one of the most hazardous classes of pollutants in water sources due to their nonbiodegradability, have caused widespread water endangerment, contamination of fish, and serious health problems.[1] Numerous remediation methods are currently under development,[2−5] including for example, (i) the use of electrolytic recovery of water to reduce metals to their elemental forms, (ii) the use of novel high-capacity ion-exchange resins or zeolites, and (iii) the extraction of metals by microorganisms or plants, such as fungal adsorption, bioleaching with bacteria, or phytoremediation for soil sediment. Novel macrocycles are currently being developed and evaluated for use as selective, recyclable ligands for extraction of heavy metals from contaminated water.[6−16] Fast, efficient feedback about metal selectivities and avidities will aid the design and development process.

Electrospray ionization mass spectrometry (ESI-MS) shows promise for rapid screening of binding selectivities in host−guest chemistry,[17−37] offering versatility in a variety of solvent systems

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and requiring minimal sample consumption. In addition, ESI-MS allows for the analysis of low-micromolar quantities of the macrocycles directly complexed with the metal ions of interest. ESI-MS provides complementary information not available with (i) the purely elemental capabilities of ICPMS and ICP-AES, which are commonly used for heavy metal detection, (ii) NMR analysis, which often requires higher concentrations of analyte and cannot be used for analyzing several complexes simultaneously, and (iii) electrochemical analysis, which is limited to a smaller number of compatible solvents and higher ionic strengths than ESI-MS.

The use of macrocycles for metal binding has been recognized for some time.[38-40] Recently, there has been an increasing interest in using sulfur-containing compounds for complexation of heavy metals for potential applications in the biology of heavy metal complexation with sulfur-containing biomolecules[41-45] and the removal of heavy metals such as silver,[14,46] copper,[13] and mercury[10-15,47,48] from wastewaters. Both acyclic sulfur-containing ligands[47,48] and cyclic thiacrown ethers[10-15] have been used in such applications.

Thiacrown ethers have been attached through covalent linkages to various linker groups which allow them to be incorporated into polymers. In these previous studies, macrocycles with sulfur heteroatoms were incorporated into a polymer by a lariat covalent linkage via an amine nitrogen or ether oxygen on the macrocycle.[10-14] Thiacrown ether macrocycle-based agents have recently been patented for use in extracting heavy metals for wastewater remediation.[49,50] Heavy metal extraction agents could be further improved by using structures that minimize the solubility in an aqueous environment and yet are able to efficiently extract the desired metal ion from the wastewater. For use in acidic, alkaline, or flowing systems, a rugged linkage to a stationary phase is desirable for the extracting agent. Some thiacrown ether structures have been shown to be resistant to the corrosive effects of acidic conditions.[11,12,51,52] The addition of an aliphatic cage structure to thiacrown ethers, such as with those in the present work, has resulted in several macrocycles that are insoluble in protic solvents such as water and methanol.

In the present study, ESI-MS is used to evaluate the metal-binding selectivities of three commercially available thiacrown ethers (**1**–**3** in Figure 1) and an array of novel thiacrown ethers (**4**–**16** in Figure 1) for mercury(II), lead(II), cadmium(II), and zinc(II) ions. The depicted attachment of one or more cage groups to macrocycles of different ring sizes and various combinations of heteroatoms, including sulfur, oxygen, and nitrogen, allows for versatile tuning of the macrocycle's metal-binding cavity and extraction properties. The cage group also provides a remote site for covalent lariat group attachment, which minimizes the alteration of binding properties yet allows incorporation into a polymer for creating resins. It is, in fact, observed in this study that the type of heteroatom (S, O, N), cavity size, and presence of other substituents influence the metal selectivities.

The dual site of carbon–carbon attachment to the thiacrown ether ring and the completely hydrocarbon nature of the cage provide for a rugged, hydrolysis-resistant linkage for potential incorporation into polymers. For this reason, several of these water-insoluble macrocycles that exhibited superior affinity for the heavy metal ions and a high selectivity for mercury in our initial binding assays have been tested for their ability to selectively extract mercury(II) in the presence of other metal ions including lead(II), cadmium(II), zinc(II), copper(II), alkali, and alkaline earth ions from an aqueous environment into an organic environment. These extraction experiments will serve as an initial evaluation of the capabilities of the thiacrown ethers studied herein for the removal of mercury ion from an aqueous environment. Variations in the size, combinations of heteroatoms, and number of attached cage groups resulted in large differences in the complexation measured by ESI-MS which can be semiquantitatively correlated with the macrocycle's avidity for mercury(II) ion.

EXPERIMENTAL METHODS

All mass spectrometry experiments were performed on a ThermoQuest LCQ Duo quadrupole ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) operated in the ESI mode with a needle voltage of 5.0 kV, a heated capillary temperature of 150 °C, and sheath flow nitrogen gas set at 25 arbitrary units. The Harvard syringe pump system (Harvard Apparatus Inc., Holliston, MA) was operated at a flow rate of 10 μL/min for all experiments except the low metal ion concentration experiments where a flow rate of 60 μL/min was used. Lens voltages were set for all experiments by using a tune file created by autotuning the LCQ on the $[\mathbf{1} + \text{Cd} + \text{ClO}_4]^+$ signal with a solution containing 2.5×10^{-5} M macrocycle and each guest metal ion in 50% methanol/ 50% chloroform. Occasional retuning for other complexes showed little variation in spectral quality or intensity of any of the peaks compared to the aforementioned tuning method. The vacuum chamber was operated at a nominal pressure of 1×10^{-5} Torr

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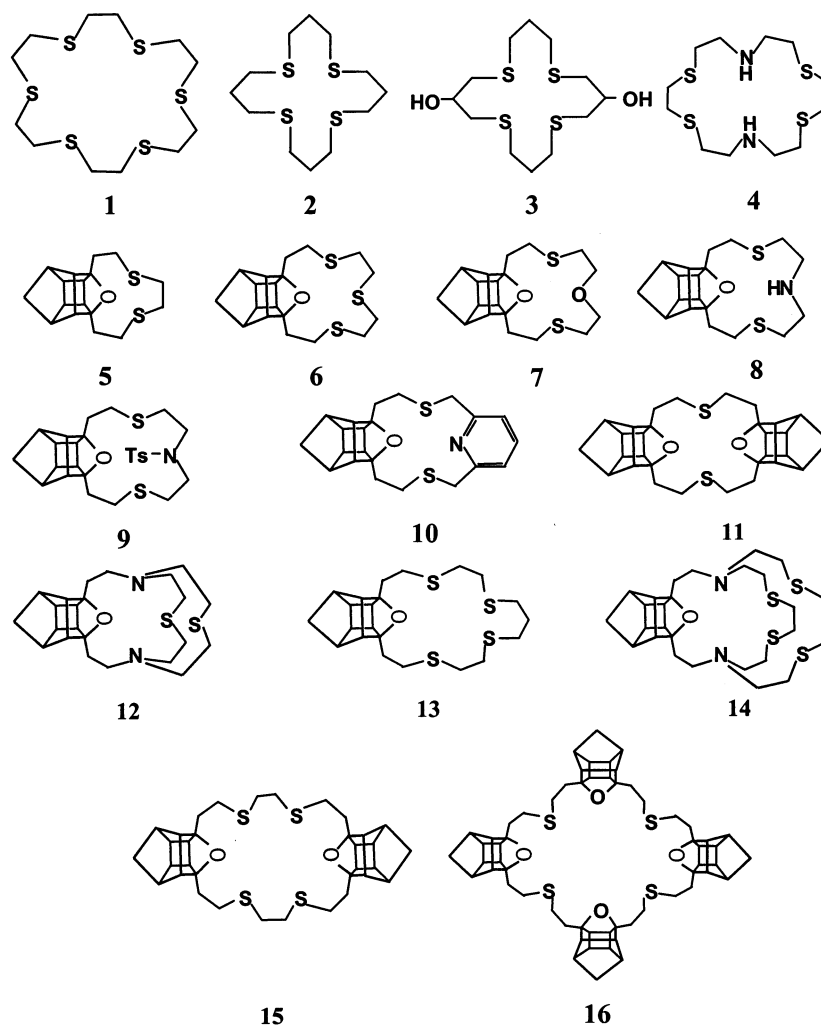
Figure 1. Thiacrown ether macrocycle structures.

with He. Spectra used for this report are an average of 600 scans. All metals salts used for these experiments were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. HPLC-grade OmniSolv methanol and chloroform were purchased from EM Science (Gibbstown, NJ). The water used for these experiments was high purity (18 MΩ) that had been purified on-site.

For the screening of metal cation binding to thiacrown ethers, solutions containing a single macrocycle with multiple metal ions in 50% methanol/50% chloroform were analyzed. Concentrations of macrocycle and each metal perchlorate were 2.5×10^{-5} M. Metal salts added to the solution were perchlorate salts of cadmium(II), lead(II), mercury(II), and zinc(II). For evaluation of spray efficiencies, the correlations between signal intensities of the complexes and their concentrations were estimated using perchlorate salts of cadmium(II), lead(II), and zinc(II) at a 5-fold excess and of mercury(II) at 5- and 2-fold excesses and equivalent concentrations, relative to thiacrown ethers **7** and **13**. Each of the metal perchlorates were mixed individually with each of **7** and **13** (separately) in 50/50 chloroform/methanol solvent systems. Because the binding constants for similar macrocycles with heavy metals in methanol are greater than 10^3 and are likely an order of magnitude greater in 50/50 methanol/chloroform, over 80% of the thiacrown is assumed to be bound. In fact, ions due to

complexes other than (macrocycle + Hg + ClO$_4$)$^+$ were minor or unobserved with the excesses of metal perchlorate versus macrocycle used for spray efficiency comparisons.

For initial extraction experiments, the aqueous phase contained 0.25 M cadmium(II) chloride, mercury(II) chloride, lead chloride, and zinc(II) chloride and the organic phase contained 2.0×10^{-4} M macrocycle in chloroform. For this type of experiment, 1.5 mL of the aqueous phase solution and 1.5 mL of the organic-phase solution were combined in a vial, vortexed for 4 min, and allowed to settle for 5 min. A 1.0-mL portion of the organic phase was then aspirated into a separate vial with an autopipet for ESI-MS analysis. To estimate the spray efficiencies of the different (macrocycle + Hg + Cl)$^+$ complexes observed in the spectra for the extraction experiments, homogeneous solutions of mercury(II) chloride at various concentration ratios relative to each of several thiacrown ethers in solvent systems of 50/50, 80/20, and 90/10 chloroform/methanol were investigated. Since neither mercury chloride nor mercury perchlorate is soluble above very low micromolar concentrations in 99+% pure chloroform, the chloroform/methanol mixtures were used. Signal intensities for (macrocycle + Hg + Cl)$^+$ complexes were determined similar to those for the perchlorates with thiacrown ethers in 50/50 chloroform/methanol solvent systems.
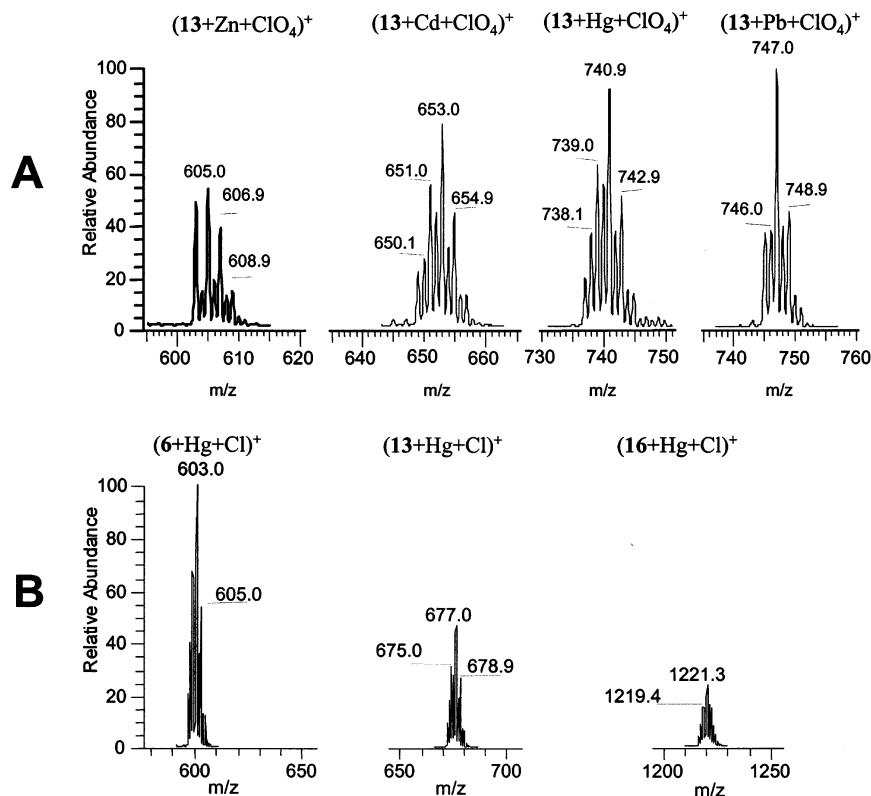
Figure 2. Relative ESI spray efficiencies (based on signal intensities) for (A) (macrocycle + M + ClO$_4$)$^+$ complexes in 50/50 chloroform/methanol solutions containing 2.5 × 10^{-5} M macrocycle and 1.25 × 10^{-4} M HgCl$_2$ and (B) (macrocycle + Hg + Cl)$^+$ complexes in 80/20 chloroform/methanol solutions containing 2.0 × 10^{-5} M macrocycle and 2.25 × 10^{-5} M HgCl$_2$.

For all other extraction experiments, the same method was used except for variations in the metal salts and macrocycles and their concentrations. For the competitive extraction assay for various macrocycles toward mercury(II) chloride, 1 × 10^{-4} M mercury(II) chloride in the aqueous phase and 2 × 10^{-4} M each of **6**, **7**, **13**, **15**, and **16** were used, and the mass spectra signals of each complex in the chloroform were compared. For experiments involving the effects of competing alkali and alkaline earth metal chlorides, **6** or **16** was used for all experiments at a concentration of 1.0 × 10^{-4} M, the concentration of mercury(II) chloride was 0.010 M and 1.0 × 10^{-4} M for the 100:100:1 and 100:1:1 competing ion/metal ion/macrocycle concentration ratio, respectively, and the competing metal chloride was 0.010 M except in the set of experiments where sodium chloride concentration was varied. For the effects of copper salts on mercury ion extraction, **7** and **16** were used at 1.0 × 10^{-4} M, the mercury(II) chloride concentration was 1.0 × 10^{-3} M, and the copper(II) chloride concentration was varied. For the counterion selectivity experiments, two solutions were used. For the first solution, 7 × 10^{-5} M concentration of each mercury salt was mixed with 5 × 10^{-5} M **16**. For the second solution, 2.5 × 10^{-3} M mercury(II) perchlorate and mercury(II) acetate were mixed with 1 × 10^{-4} M **16**. Experiments conducted for the purpose of detecting the extraction of low metal ion concentrations used both a 1:1 **15**/mercury(II) chloride molar ratio with mercury ion concentration varying from 1.0 × 10^{-4} to 1.0 × 10^{-5} M and a mixture with a fixed 2 × 10^{-4} M concentration of **16** in chloroform and an aqueous mercury(II) chloride concentration from 1 × 10^{-4} to 3 × 10^{-6} M. Experiments performed to determine the effects of alkali and alkaline earth metal ions on mercury ion complexation

with the thiacrown ether macrocycles in 50/50 methanol/chloroform solution used equimolar 2.5 × 10^{-5} M concentrations of one or more alkali or alkaline earth metal perchlorates, mercury perchlorate, and **6** or **16**.

RESULTS AND DISCUSSION

To assess the metal selectivities of the macrocycles shown in Figure 1, a variety of ESI-MS experiments were undertaken. First, the approximate binding selectivities of the macrocycles were screened based on ESI-MS analysis of methanol/chloroform solutions containing each macrocycle and an array of metals. Then, an extensive series of extraction experiments was undertaken in which the 10 most promising macrocycles were dissolved in chloroform and used to extract metals from an aqueous solution. For these experiments, the chloroform phase was analyzed by ESI-MS. Counterion effects and competitive binding from nonheavy metals, including alkali, alkaline earth, and copper metal salts, were examined. In addition to these comparisons of binding selectivities involving different metals and ligands, the spray efficiencies for the various complexes were measured.

To estimate the spray efficiencies of complexes containing different ligands or different metals, the signal intensities of [macrocycle + metal(II) + anion]$^+$ complexes in solution were estimated by adding an excess of metal salt to solutions containing one of the thiacrown ether macrocycles. For each 50/50 methanol/chloroform solution containing one metal perchlorate and one thiacrown ether, the signal intensities of the complexes were tallied based on summing the isotopes of the expected complexes. An example is shown in Figure 2, in which the spray efficiencies for complexes containing **13** and different metals (Figure 2A) and
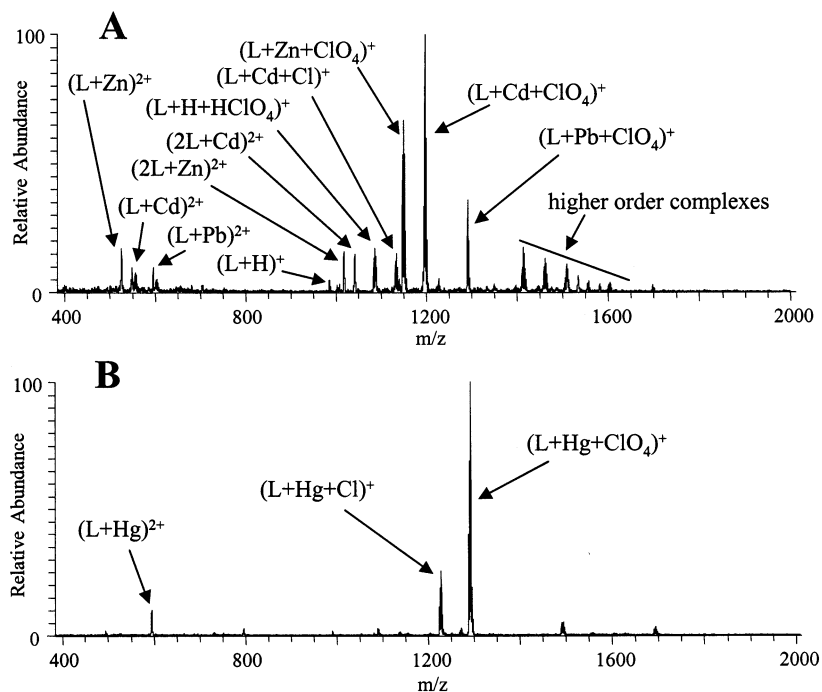
Figure 3. ESI-MS of **16** with metals in 50/50 methanol/chloroform solution (L = **16**). (A) **16** with Cd(ClO$_4$)$_2$, Pb(ClO$_4$)$_2$, and Zn(ClO$_4$)$_2$ (1:1:1:1). (B) **16** with Cd(ClO$_4$)$_2$, Hg(ClO$_4$)$_2$, and Zn(ClO$_4$)$_2$ (1:1:1:1).

for complexes containing Hg and different macrocycles (Figure 2B) are compared. For example, the relative intensities for the complexes involving **13** were (normalized to 100%) 49% for zinc, 73% for Cd, 100% for Pb, and 87% for Hg. The analogous values for the complexes involving **7** were 56% for zinc, 58% for Cd, 100% for Pb, and 40% for Hg. Thus, the [macrocycle + metal(II) ion + ClO$_4$]$^+$ signal intensities for each solution containing a different metal but the same macrocycle typically varied by up to a factor of 2. This means that when solutions containing the same ligand with different metals are compared, the observed intensities for the complexes are influenced by the relative spray efficiencies, in this case resulting in a scaling factor of up to 2.

Similar measurements of ESI spray efficiencies were undertaken for the extraction experiments. To simulate the extraction experiments that involved analysis of only the chloroform phase after extraction of an aqueous solution with a macrocycle-containing chloroform solution, a solvent system of 80/20 chloroform/methanol was chosen in order to approximate the chloroform phase while maintaining the solubility of mercury chloride concentrations in the tens of micromolar level. Again, one metal salt, mercury chloride, was added in excess to a solution of one thiacrown ether, and the intensities of all the isotope peaks for a given complex were tallied in the ESI mass spectra. HgCl$_2$/macrocycle concentration ratios of 9:1 for **6**, **7**, **13**, **15**, and **16**, and a series of HgCl$_2$/macrocycle concentration ratios from 4.5:1 to 1.13:1 for **6**, **13**, and **16** were used to survey the spray efficiencies. For example, the relative intensities for the complexes involving Hg were (normalized to 100%) 100% for **6**, 46% **13**, and 26% for **16** (data shown in Figure 2B). Based on these experiments, the variation in spray efficiencies among the complexes differed by up to a factor of 4. This means that when solutions containing the same metal with different macrocycles are compared, the observed intensities for the complexes are

influenced by the relative spray efficiencies, in this case resulting in a scaling factor of up to 4.

Macrocycle/Heavy Metal Complexation in 50/50 Methanol/Chloroform Solutions. The thiacrown ether macrocycles were found to be insoluble in pure methanol or water at micromolar or higher concentrations, thus making them good candidates for water-insoluble wastewater remediation agents. Micromolar levels of both the macrocycle and salts of cadmium, lead, mercury, and zinc (which are insoluble in chloroform) can be easily dissolved in a homogeneous 50/50 methanol/chloroform solvent solution. This approach can be used to semiquantitatively determine the relative affinities of these metals for the various macrocycles without the added complexities of extraction and proton donation from water. Solutions containing each macrocycle and equimolar concentrations of cadmium, lead, zinc, and with or without mercury were analyzed.

As presented in Figure 3, the ESI-mass spectra for the solutions containing a macrocycle and the metal perchlorates in 50/50 methanol/chloroform typically consist of dominant signals for singly charged tertiary complexes including a macrocycle, a metal ion, and single counterion, (macrocycle + metal + ClO$_4$)$^+$, as well as complexes of a macrocycle and a doubly charged metal ion, (macrocycle + metal)$^{2+}$, which are occasionally dominant. Lower intensity signals due to higher order complexes containing two macrocycles are often seen as well. The results for the spectra of each of the solutions containing one macrocycle with three metal salts (cadmium, lead, and zinc) or four metal salts (cadmium, lead, zinc, and mercury) are compared in Table 1. These selectivity comparisons are semiquantitative, which is reasonable given the similarities of spray efficiencies for the thiacrown complexes with different heavy metals. With this understanding, the quality of the spectra was determined based on (i) the signal-to-noise ratio of the dominant (macrocycle + metal + ClO$_4$)$^+$ complex using

Table 1. Relative Selectivities of Sulfur-Containing Macrocycles for Metal Ions in 50/50 Methanol/Chlororoform

macrocycle	Cd(ClO$_4$)$_2$, Pb(ClO$_4$)$_2$, and Zn(ClO$_4$)$_2$ added to solution of macrocycle			Cd(ClO$_4$)$_2$, Hg(ClO$_4$)$_2$, Zn(ClO$_4$)$_2$, and Pb(ClO$_4$)$_2$[a] added to solution of macrocycle		
	spectrum quality	dominant complex intensity[b]	complexes observed[c]	spectrum quality	dominant complex intensity[b]	complexes observed[c]
1	fair	5.0×10^7	Cd, Pb, Zn > Cu ≫ Na,[e] H[e]	poor[f]		none
2	fair	1.0×10^8	Zn > Cd, Pb > Cu ≫ Na,[e] H[e]	good	6.4×10^7	Hg > Cd, Zn, Cu ≫ Na,[e] H[e]
3	fair	5.3×10^7	Cd, Pb, Zn ≫ Cu,[e] Na,[e] H[e]	fair	3.9×10^7	Hg ≫ Zn, Cd ≫ Na,[e] H[e]
4	good	3.7×10^7	Cd ≫ Pb, Zn, H ≫ Cu,[e] Na[e]	good	3.0×10^7	Hg ≫ H ≫ Cd,[e] Zn,[e] Cu,[e] Na[e]
5	good	4.6×10^7	Na ≫ Cd, Pb, Zn, Cu ≫ H[e]	fair	6.0×10^7	Hg ≫ Cd, Zn, Cu ≫ Na,[e] H[e]
6	very good	3.8×10^7	Zn > Cd > Pb > Cu > H ≫ Na[e]	good	6.1×10^7	Hg > Cd, Zn > Cu > H ≫ Na[e]
7	good	1.3×10^8	Cd, Pb, Zn ≫ Na > Cu, H	very good	3.9×10^8	Hg ≫ all others[e]
8	excellent	5.7×10^7	H ≫ all others	very good	1.9×10^7	H > Hg ≫ Cd,[e] Zn,[e] Cu,[e] Na[e]
9	very good	2.4×10^8	Cd, Pb, Zn ≫ Na, H > Cu	good	3.3×10^6	Hg > Cd, Zn > Cu, H ≫ Na[e]
10	very good	1.2×10^8	H>Cd, Pb, Cu ≫ Zn,[e] Na[e]	good	4.5×10^8	Hg ≫ Cd,[e] Zn,[e] Cu,[e] Na,[e] H[e]
11	good	2.0×10^8	Cd, Zn > Pb, H > Cu, Na	very good	5.9×10^8	Hg ≫ Cd, Zn,H ≫ Cu,[e] Na[e]
12	excellent	1.2×10^9	H ≫ all others[e]	excellent	1.1×10^9	H ≫ all others[e]
13	excellent	1.9×10^8	Pb > Cd > Zn > Cu, H ≫ Na[e]	good	1.4×10^8	Hg > Cd, Cu > Zn,[e] Na,[e] H[e]
14	excellent	1.2×10^9	H > Cd ≫ Cu > Pb > Zn,[e] Na[e]	very good	2.5×10^8	Hg ≫ Cu ≫ Cd,[e] Zn,[e] Na,[e] H[e]
15	very good	9.3×10^7	Pb, Cd, Zn > Cu ≫ Na,[e] H[e]	very good	6.8×10^7	Hg ≫ Cd, Cu ≫ Zn,[e] Na[e], H[e]
16	good	9.0×10^7	Cd, Pb, Zn > H > Cu ≫ Na[e]	good	1.4×10^9	Hg > Zn > Cd, Cu ≫ Na.[e] H[e]

[a] Selectivities of macrocycles with regard to lead ion are not given in this column of the table due to being totally obscured by signal from the complexes of macrocycles with mercury ion. [b] A 10-ms ionization time; 10 μL/min flow rate. [c] Observation of Cu complexes is due to copper sources in the mass spectrometer, it was not added to the solution. Observation of sodium complex is due to residual sodium impurities in chemicals, solvents, glassware, instrumentation, etc.; sodium was not added intentionally to the solutions. Observation of H complexes is not due to protonation from added acid but due to proton sources such as the neutral methanol solvent, water in the atmosphere, and as an impurity in the methanol. [d] The "greater than" symbol (>) indicates a signal intensity difference of greater than 2-fold; the "much greater than" symbol (≫) indicates a signal difference of greater than 5-fold. [e] Undetectable. [f] It is believed that the binding of mercury ion to the macrocycle catalyzes a decomposition reaction; most or all peaks observed are assigned as macrocycle fragments and their complexes with other ions.

ionization times that optimize intensity without detrimental space charging effects and (ii) whether the noise or interference peaks present were large enough in intensity or m/z value to interfere with the detection and identification of species present. The spectrum quality is assessed in Table 1 to indicate the level of signal to noise of the complexes (i.e., excellent, S/N > 100; very good, S/N > 50 and few background ions; good, S/N > 50 with other background ions of significant intensity; fair, S/N up to 25; poor, low S/N and many background ions.)

Signal intensities in Table 1 were determined by summing all of the intensities of the isotope peaks for the (macrocycle + metal ion + ClO$_4$)$^+$ ions for each metal. Based on the relative spray efficiencies determined for the complexes of the different metal ions, the "greater than" symbol (>) indicates a signal intensity difference of greater than 2-fold and the "much greater than" symbol (≫) indicates a signal difference of greater than 5-fold relative to the other ions in the series.

The relative signal intensities for adventitious species, such as cationic sodium, copper, and hydrogen ions, are included in Table 1 even though their concentrations are unknown, to show the degree to which these interferences compete with the intentionally added metal ions. The signal intensities of the singly charged metal complexes in the ESI-mass spectra were used to estimate the relative binding selectivities and avidities of the macrocycles for cadmium(II), lead(II), mercury(II), and zinc ions. The results in Table 1 were used for the purpose of acquiring a preliminary understanding of which thiacrown ethers possessed high avidity or selectivity for particular heavy metal ions.

When the selectivities of the various macrocycles toward zinc, cadmium, and lead ions are compared, it is observed that several of these macrocycles show similar avidities for all three metal ions (1, 3, 7, 9, 15, 16), as in Figure 3A or only small differences in

selectivity (2, 6, 11, 13). Only 4 shows a strong preference for one of the three metal ions, showing a "much greater" selectivity toward cadmium.

Mercury(II) complexes are usually the dominant species or only species observed for the solutions containing this mercury salt, even in the presence of cadmium, lead, and zinc, as exemplified in Figure 3B, a result which suggests that most of the thiacrown ethers have substantial mercury ion selectivities. One macrocycle, 5, with only two sulfur atoms and a small cavity size, appears to prefer to bind to adventitious sodium ion impurity over the much greater quantity of heavy metal ions in solution. This result suggests that the cavity of 5 is too small to permit efficient binding of any of the heavy metals.

Macrocycles 8, 10, 12, and 14 greatly prefer to bind to a proton over the metal ions present, except mercury, with 8 and 12 binding only a proton to the exclusion of metal ions present in the homogeneous solutions, including mercury. Presumably, these results reflect the high basicity of these macrocycles due to the two nitrogen atoms and, for the highly proton-specific 8 and 12, the small cavity size.

These initial findings in homogeneous methanol/chloroform solutions were used to select candidates for extraction of metal ions from an aqueous phase into a chloroform organic phase in order to further evaluate the ability of these macrocycles as agents for extracting heavy metals from an aqueous environment. The ones giving the most intense mercury complexes include 2–4, 6, 7, 9, 11, 13, 15, and 16. By using these 10 selected compounds, the extraction process serves as a model for wastewater extraction and also can be used to determine the selectivities of macrocycles toward heavy metal cations.

Extraction Analysis. *Selective Mercury Ion Extraction from Aqueous Solution.* Due to the exclusive mercury selectivity of most
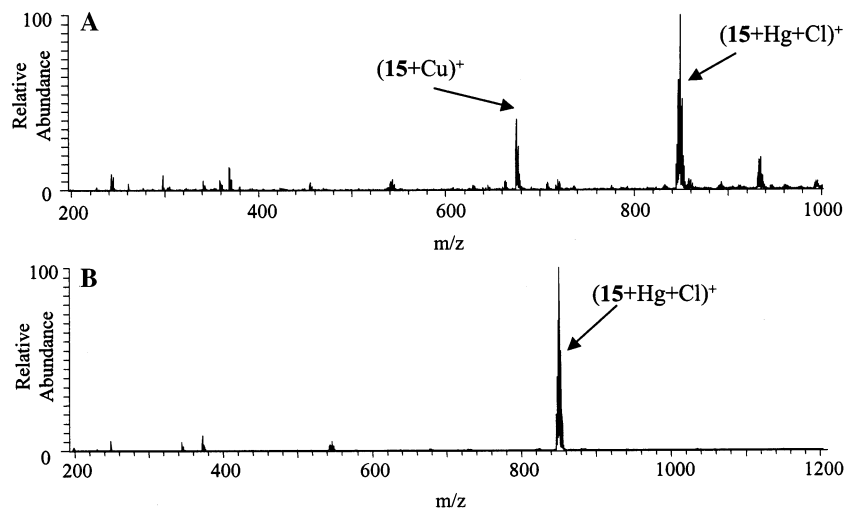
Figure 4. ESI-MS of macrocycle-containing chloroform phase after extraction of aqueous phase. (A) **15** with Cd(ClO$_4$)$_2$, Pb(ClO$_4$)$_2$, Hg(ClO$_4$)$_2$, and Zn(ClO$_4$)$_2$ (1:125:125:125:125). (B) **16** with CdCl$_2$, PbCl$_2$, HgCl$_2$, and ZnCl$_2$ (1:125:125:125:125).

Table 2. Relative Efficiencies of Mercury(II) Extraction by Sulfur-Containing Macrocycles[a]

rank	macrocycle	identified ions	ionization time (ms)	(macrocycle + Hg + Cl)$^+$ intensity $\times 1 \times 10^7$	Ion current/s of ionization time for (macrocycle + Hg + Cl)$^+$ $\times 1 \times 10^8$
best	**15**	(L + Hg + Cl)$^+$	0.5	9.0	1800
good	**16**	(L + Hg + Cl)$^+$	5	18	360
	13	(L + Hg + Cl)$^+$ (L + Cu)$^+$	6	20	330
	7	(L + Hg + Cl)$^+$ (L + Cu)$^+$	10	14	140
	6	(L + Hg + Cl)$^+$	10	12	120
	2	(L + Hg + Cl)$^+$ (L + Cu)$^+$	10	5.0	50
poor	**9**	(L + Hg + Cl)$^+$ (L + Cu)$^+$	300	7.0	2.3
	3	(L + Hg + Cl)$^+$ (L + Cu)$^+$	150	3.0	2.0
	11	(L + Hg + Cl)$^+$ (L + Cu)$^+$	300	3.0	1.0
worst	**4**	noise			

[a] [Macrocycle]$_{(o)}$/[metal chlorides]$_{(aq)}$ 1:125:125:125:125 macrocycle/CdCl$_2$/PbCl$_2$/ZnCl$_2$/HgCl$_2$; flow rate, 10 μL/min.

of the thiacrown ether macrocycles, the 10 macrocycles that displayed the best combination of clean spectra and high signal-to-noise ratio for the corresponding mercury complexes were used to study mercury(II) ion extraction from aqueous solutions. Extractions that employed mercury(II) perchlorate generally were unsuccessful. The observed spectra were similar to that shown for **15** in Figure 4A, with the macrocycle extracting mercury with adventitious chloride ion rather than with either abundant perchlorate ion or no counterion. The appearance of the copper complex for **15** in Figure 4A is related to the presence of adventitious copper and is discussed later.

Extraction of mercury(II) chloride was much more successful, as shown by the spectrum for **15** in Figure 4B and for all the macrocycles tested in Table 2. The data in Table 2 afford a semiquantitative summary of the extraction efficiencies of the macrocycles based on the signal intensities (all isotopes summed) per unit of ionization time (last column in Table 2) of the mercury complexes obtained by using identical extraction conditions and the relative spray efficiencies estimated for the different (macrocycle + Hg + Cl)$^+$ complexes. Over the range of different ionization times used, the signal intensity obtained with the ion trap scales directly with time. This linear relationship of ionization time and signal intensity was mapped for times ranging from 2 to 1000 ms for solutions containing 2.0 $\times$ 10^{-5} M concentrations of

one macrocycle and one metal salt, and correlation coefficients of 1.0 were routinely obtained. The reasons for the more effective extraction of the chloride-containing complexes relative to the perchlorate-containing complexes are discussed in detail below in the discussion of Counterion Influence on Mercury Extraction.

The extraction avidities of the macrocycles for mercury(II) ion appear to be a balance among the number of heteroatoms, the number of sulfur atoms, the size of the metal ion-binding cavity, and the degree of flexibility of the cavity ring. Of the macrocycles tested, **15** gave the best extraction efficiency. Its superior performance is likely due to a combination of several factors. According to X-ray crystallography studies, the central cavity ring of the macrocycle must be a chain of greater than 14 atoms for the mercury(II) ion to sit in the plane of the ring.[53] **13**, **15**, and **16** are the only macrocycles in Table 2 that have central cavity rings with chains of more than 14 atoms. Since mercury(II) ion is known to form coordination numbers from 2 to 8,[54] it is likely that, of these three compounds, **15** provides the best combination of interactions (four S, two O, and one Cl) and cavity fit. For **16**, the two additional oxygens may add more sites for interaction

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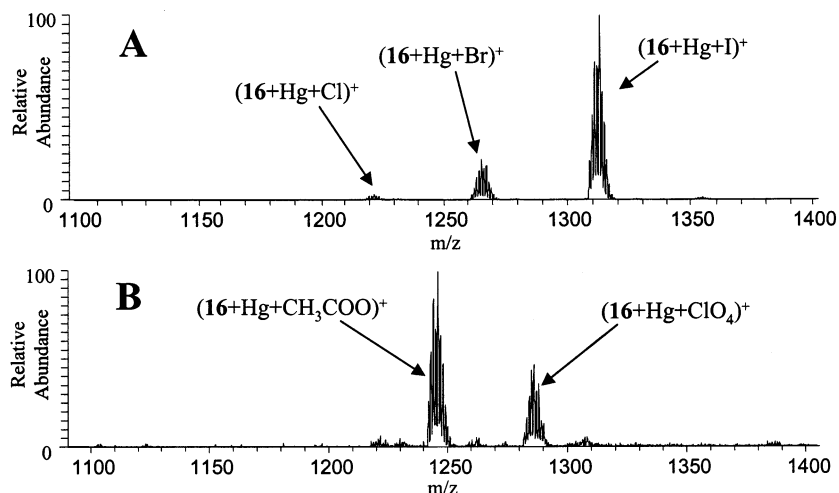
Figure 5. Competitive extraction from H_2O to $CHCl_3$ of mercury(II) ion with various counterions. (A) **16** with $Hg(ClO_4)_2$, $HgCl_2$, $HgBr_2$, HgI_2, and $Hg(Ac)_2$ (1:1.4:1.4:1.4:1.4). (B) **16** with $Hg(ClO_4)_2$ and $Hg(Ac)_2$ (1:25:25).

with the mercury ion, but the significantly larger cavity size and added distance between the sulfurs probably interfere with the ability of sulfurs to attain a geometry as favorable as that which can be achieved for **15**. In addition, **16** is more rigid than **15**, due to the presence of the two additional cages in **16**. **15** has a greater avidity for mercury ion than **16** does because the cavity in **16** is too large and too inflexible (due to the four cages) to permit its reduction to the more effective size of **15**.

The avidity of **13** toward mercury(II) ion is less than **15**, but not because **13** is too large or inflexible, but because it is too flexible and requires a greater loss of entropy to optimally coordinate to the mercury ion than **15**. Macrocycles **6** and **7** are very similar, and the difference in heteroatoms does not appear to make a substantial difference in their mercury ion avidities. Both **6** and **7** show a lower affinity for mercury ion than **13**, **15**, and **16** because their metal ion-binding cavity is too small (14-atom cavity ring).

A competitive extraction assay with 1×10^{-4} M mercury(II) chloride in the aqueous phase and 2×10^{-4} M each of **6**, **7**, **13**, **15**, and **16** was used to further compare the mercury avidities of these five macrocycles, which showed the greatest mercury affinities based on the previous experimental results. The mass spectral signals of each mercury complex obtained upon ESI-MS analysis of the chloroform phase were compared (data not shown). In this assay, **15** emerges as the macrocycle with the greatest mercury affinity, followed by **13** and **16**. This experiment showed a signal ratio for mercury complexes of **15** and **13** of 3:1, respectively. The competitive assay likewise indicated that **13** extracts mercury(II) chloride more competitively than **16** (signal ratio for complexes of **13** and **16** is 30:1, respectively). The signal-to-noise values for the signals of complexes of **6** and **7** in the competitive assay were too low to reasonably estimate signal ratios.

The importance of rigidity and entropy effects in the mercury ion affinities toward the macrocycles is demonstrated by the two commercial macrocycles, **2** and **3**, which, due to the trimethylene groups between heteroatoms and the lack of any cage groups, have lower mercury ion avidities than **6**, **7**, **13**, **15**, and **16**. For **9**, the presence of the tosyl group results in steric hindrance to metal ion binding, which reduces its affinity below that of other

14-atom cavity ring macrocycles such as **6** and **7**. Too much rigidity in a macrocycle coupled with a small cavity size, such as **11**, which has a 16-atom cavity ring and two cage groups, results in a cavity that is unable to effectively coordinate the mercury ion, unlike the less rigid **6**, **7**, and **9**. The poor performance of **4** is due to the proton affinity of the two secondary amine groups in the ring, which allows protons to compete effectively with mercury ion for **4**, a lack of rigidity due to the absence of cage groups, and the relative lack of hydrophobic structure, which renders **4** water-soluble.

Extraction of Mercury at Micromolar Concentrations. To determine the extent to which the extraction of mercury can be monitored by mass spectrometry, the best performing macrocycles in this study, **15** and **16**, were used to determine the lower limit at which extraction of mercury from water into chloroform can be detected. In this part of the study, the two phases were vortexed for 4 min prior to ESI-MS analysis of the macrocycle-containing chloroform phase. For the (macrocycle + Hg + Cl)$^+$ ion with decreasing equimolar concentrations of mercury(II) chloride and **15** in the aqueous and organic phases, respectively, a lower limit of extraction (SNR = 3) of 1.1×10^{-5} M (2 ppm Hg) was determined, although efforts to optimize the limit of extraction were limited by the availability of the macrocycle. In an analogous experiment with **16**, a fixed macrocycle concentration of 2×10^{-4} M was used for each extraction due to greater sample quantities. For this experiment with **16**, a lower limit of extraction (SNR = 3) of 5×10^{-6} M (1 ppm mercury) was determined. The slopes of the log–log plots for these experiments with **15** and **16** are 2 and 1, respectively, which indicates that the extraction process is second order (the concentration of complex is proportional to the product of the mercury chloride and macrocycle concentrations) and is pseudo first order when the macrocycle concentration is held constant. This result indicates that a significant improvement in the detection of the limit of extraction could be obtained by using a fixed concentration of **15** for extractions, as was done with **16**.

Counterion Influence on Mercury Extraction. To further understand the factors that influence the efficiency of mercury extraction, the effect of the counterion was examined. Figure 5A shows the ions observed from competitive complexation of **16** and

Table 3. Solubility, Association Constant, and Thermochemical Radius Data for Various Mercury(II) Salts and Their Counterions

property	$Hg(ClO_4)_2$	$Hg(CH_3COO)_2$	$HgCl_2$	$HgBr_2$	HgI_2
solubility (g of solute/100 g of solution)	>25[a]	20,[b,e] 21,[c,f] 25[d,g]	6.81,[c,e] 7.30[c,e]	0.61[c,e]	0.0048,[c,e] 0.0044,[c,e] 0.0059,[c,e] 0.01[d,e]
$\log K_{rxn}$[h]	<2[a,i]	5.89[j]	6.74[i]	9.00[i]	12.87[i]

	Hg^{2+}	ClO_4^-	CH_3COO^-	Cl^-	Br^-	I^-
thermochemical radius (pm)	112[k]	225[l]	194[l]	168[l]	190[l]	211[l]

[a] Predicted from solubility tests within our laboratory-based and tabulated results for mercury(II) acetate and other perchlorate complexes. [b] From ref 55. [c] From ref 56. [d] From ref 57. [e] 25 °C. [f] −1.5 °C. [g] 10 °C. [h] $\log K_{rxn}$ value is for the reaction, $Hg^{2+}_{(aq)} + X^-_{(aq)} \leftrightarrow HgX^+_{(aq)}$, where X^- is the counterion. [i] 0.5 M, 25 °C, from ref 58. [j] 0.5 M, 25 °C, from ref 59. [k] From ref 60. [l] From refs 61, provided uncertainty for radii of anions is ±19 pm.

mercury(II) ion with chloride, bromide, iodide, acetate, and perchlorate counterions after extraction of an aqueous phase containing the five mercury salts with chloroform containing **16**. Since neither the acetate- nor the perchlorate-containing complexes could be observed in Figure 5A, a second analogous experiment using a larger excess of only acetate and perchlorate salts was performed, with the results shown in Figure 5B. The observed order of counterion selectivity is iodide > bromide > chloride > acetate > perchlorate, most likely due to effects including counterion size and metal/counterion binding energy. The log K values for the reaction

$$Hg^{2+}_{(aq)} + X^-_{(aq)} \leftrightarrow HgX^+_{(aq)}$$
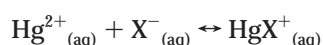
(where X^- is the counterion), the solubilities of the five mercury salts tested, and the thermochemical radii of the five corresponding counterions are presented in Table 3.

With regard to counterion size, the polyatomic ions with branching carbon−oxygen and chloride−oxygen molecular bonds may hinder optimum interaction of mercury with the macrocycle binding cavity, accounting for the selectivity order: halides ≫ acetate > perchlorate. On the other hand, the larger the counterion, the lower its charge density, thereby rendering it more easily transferred from water to chloroform, which accounts for the selectivity order of the halides: iodide > bromide > chloride. However, the selectivity order can also be explained effectively in terms of the metal/counterion binding energy, which affects the distribution of metal ions and metal−macrocycle complexes with two, one, or no counterions attached, which may also influence the ability of the macrocycle to extract the metal ion into chloroform. To maintain charge balance, for every mercury(II) ion that is extracted into the chloroform phase by the thiacrown ether, two chlorides must enter the chloroform phase as well (or, though not observed, some other negative species must be transferred to the chloroform phase or a positive species transported to the chloroform phase). Since the energy required to solvate a naked chloride anion by the chloroform would be prohibitive under the circumstances of the extraction, the chloride ions must transfer into the chloroform in very close proximity to the positive mercury ions. Therefore, it stands to reason that the counterions in mercury salts that have the largest mercury−anion binding energy and lowest solubilities in water will be associated with the mercury ions the most in the aqueous phase and

therefore will facilitate the most mercury extraction. For the series of counterions, chloride, bromide, and iodide, the electronegativities decrease from chloride to iodide, resulting in differences of Pauling electronegativities of 1.16, 0.96, and 0.66, respectively, between mercury and the counterion. In addition, the "effective electronegativity" for the interacting oxygen in acetate ion would be expected to be greater than for chloride and the electronegativity of the interacting oxygen in perchlorate would be expected to be greater than for acetate. However, when mercury is bound to the sulfur atoms in the thiacrown ether cavities, where the mercury−sulfur electronegativity difference is ∼0.58, these essentially covalent interactions will greatly decrease the covalent character of the mercury−anion bonding, making it more electrostatic in nature. Thus, in the extraction process and after the mercury−thiacrown complex is in the chloroform phase, the counterions will likely maintain a weaker, more electrostatic interaction with the (macrocycle + Hg)$^{2+}$ complex as a whole, since the mercury will be well nested within the surrounding heteroatoms of the thaicrown ether cavity, especially for the larger thiacrown ethers **13**, **15**, and **16**. However, it will be much more likely for one chloride ion to maintain an inner- or outer-sphere interaction with the metal center of the thiacrown−mercury complex than for both chloride ions to participate, so that there will probably be one counterion with an inner- or outer-sphere interaction with the complexed mercury and another maintaining a much weaker electrostatic interaction with the (macrocycle + Hg + X)$^+$ complex as a whole.

The order of the solubilities and log K's for the aqueous association constants support this conclusion, and although no solubility or association constant data for mercury(II) perchlorate were found, it was determined in our laboratory that mercury(II) perchlorate is at least twice as soluble in water as mercury(II) acetate, strongly suggesting that the mercury(II) perchlorate has a significantly larger solubility and smaller association constant than mercury(II) acetate.

Interference of Group I, Group II, and Transition Metal Salts on Mercury Extraction. Competitive extraction experiments that involve group I and II metal chlorides at equimolar and 100-fold excess concentrations relative to mercury(II) chloride were undertaken with **6** and **16** in order to evaluate mercury extraction selectivity in the presence of other common metals. For macrocycles **6** and **16**, only mercury was extracted for all of the experiments (data not shown). To determine whether the prefer-

ence for mercury extraction stems from the larger binding constants for mercury or greater water−chloroform transfer efficiency, solutions in 50/50 methanol/chloroform containing equimolar concentratons of **16**, several group I and II metal perchlorates, and mercury(II) perchlorate were analyzed. Once again, the complexation of mercury(II) ion occurs to the exclusion of group I and group II metal ions (data not shown), demonstrating that binding to mercury(II) ion is greatly favored over binding to group I and II ions regardless of the solvent environment.

Although group I and II metals may not complex with the Table 2 macrocycles in the presence of mercury(II) chloride, it was possible that they could indirectly reduce the extraction efficiencies of the macrocycles or suppress the ESI signals when they are present in large excess relative to mercury. To determine whether any observable interference with mercury extraction or detection occurs, the intensities of the $(16 + Hg + Cl)^+$ and $(6 + Hg + Cl)^+$ ions were monitored in separate experiments after extractions of aqueous phase with NaCl added at concentrations from 10- to 100-fold in excess of mercury(II) chloride. For the range $0.001 M \leq [NaCl] \leq 0.1 M$, which includes the range of NaCl concentrations typically found in wastewater, no loss in signal is observed (data not shown). This result demonstrates that typically encountered salt concentrations in wastewater should not significantly inhibit mercury extraction and ESI-MS detection. Similar experiments involving addition of other heavy metals instead of alkali metals showed that all of the macrocycles in Table 2 also show no detectable extraction avidity toward cadmium, lead, and zinc ions when these metal ions are added at the same concentration as mercury in the aqueous phase. These ions are present in much lower concentrations than group I and II metal ions in wastewater, but they have properties that are more similar to those of mercury ion. The lack of avidity shown by the macrocycles toward cadmium, lead, and zinc ions further demonstrates their high level of extraction selectivity toward mercury ions.

The presence of copper complexes with some of the macrocycles in the ESI-mass spectra, due to the presence of copper impurity in the mercury(II) chloride salt or from surfaces of the ESI interface components in contact with the analyte solution, suggests that copper might interfere competitively in mercury extraction. An experiment was undertaken in order to determine the degree to which copper present in the extraction mixture interferes with mercury extraction, detection, or both. The intensities of (i) $(7 + Hg + Cl)^+$ ion, with **7** being one of the smallest macrocycles in Table 2 (14-atom ring), and (ii) $(16 + Hg + Cl)^+$ complex, with **16** being the largest macrocycle in Table 2 (32-atom ring), were monitored after extraction experiments that involved $HgCl_2/CuCl_2$ concentration ratios between 10:1 and 1:10 in the aqueous phase and **16** or **7** in chloroform. The peak for the macrocycle−copper complex corresponds to complexation of a singly charged copper ion in which the ESI process reduces the copper from its +2 to +1 oxidation state, a phenomenon observed by other researchers in ESI experiments and thought to occur by electron capture.[62,63]

Figure 6. ESI-MS of macrocycle-containing chloroform phase after extraction from H_2O to $CHCl_3$. Selectivity for $CuCl_2$ versus $HgCl_2$. (A) Macrocycle **16**. (B) Macrocycle **7**.

The results of these experiments are shown graphically in Figure 6. No appreciable change in the relative intensity of the copper complexes of either **7** or **16** with respect to the analogous (macrocycle + Hg + Cl)$^+$ complexes is seen until the $CuCl_2:HgCl_2$ concentration ratio exceeds 10:1. This result demonstrates that both of these macrocycles have a greater selectivity for mercury than copper. This result also indicates that when copper complexes are observed in the spectra for solutions where no copper was added, it is not due to trace copper in solution but more likely is due to contact with copper elements in the ESI interface during analysis.

CONCLUSIONS

Sulfur-containing crown ether macrocycles have been shown to display potential as agents for selectively extracting and detecting aqueous mercury ion over a large concentration range. Many of the macrocycles in this study bind to mercury very selectively and efficiently in the presence of many other metal ions and have an avidity toward mercury that can be fine-tuned by varying the size and combination of heteroatoms in the macrocycle ring and the number of cage groups attached. Macrocycle cavities with several sulfurs and additional oxygens arranged to bind to mercury with a square-planar geometry appear to be most ideal. Mercury ion extraction is greatly enhanced as the hydrophilicity and the dissociation energy of the metal−anion ion pair in the aqueous medium is reduced. Lower charge density

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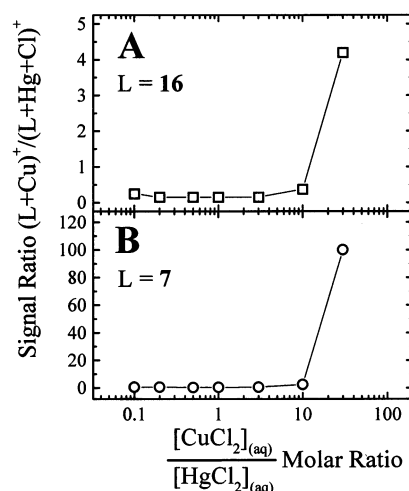
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and more compact, monatomic counterion structure can also reinforce extraction enhancement.