Binding of MeHg to Dissolved Organic Matter

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Abstract

The fate and transport of methylmercury ($MeHg^+$) in the environment is tied closely to the cycling of organic matter. Dissolved organic matter (DOM), in particular, transports $MeHg^+$ away from methylation zones and the binding constant between DOM and $MeHg^+$ is an important parameter used to predict the fate and transport of $MeHg^+$ in the environment. The Competitive Ligand Exchange – Solid Phase Extraction (CLE-SPE) method was utilized in this work to estimate the binding constant of $MeHg^+$ to dissolved organic matter (DOM). DOM isolates from 4 sites in the St Louis River watershed were utilized and compared to each other as well as well-characterized model DOM isolates. The binding constants determined in this study were similar between sites, but larger than others reported in the literature. However, the binding coefficients for $MeHg^+$ found in this study are lower than those reported for other mercury (Hg(II)) species. Similar binding coefficients between sites effectively rules out that $MeHg^+$ differs between sites due to differences in DOM between the sites.

Introduction

Methylmercury is a neurotoxin and the prevalent form of mercury in fish consumed by humans [1]. In aquatic environments $MeHg^+$ binds with ligands (L) to form MeHgL species. If equilibrium is achieved, the concentration of ligands and their binding constants with $MeHg^+$ will determine which MeHgL species exist in various aquatic environments. Mercury ions are classified as soft Lewis acids and bind strongly to soft Lewis bases such as sulfides and reduced thiols. Thiols are sometimes referred to as mercaptans based on the Latin *mercurium captans* or capturing mercury. Reduced thiols are abundant in organic matter leading to equilibrium models predicting $MeHg^+$ ions being bonded to reduced thiol ligands contained in dissolved organic matter (DOM) in most freshwater lakes, wetlands and rivers [2].

$MeHg^+$ interaction with DOM impacts its fate and transport in aquatic systems. As the predominant ligand for $MeHg^+$, DOM is responsible for transporting $MeHg^+$ away from methylation zones such as lake sediments, wetlands, and other anoxic environments. The uptake of $MeHg^+$ by algae and other organisms near the bottom of aquatic food chains is influenced by DOM. $MeHg^+$ bonding to DOM can also affect the photodegradation rates of $MeHg^+$ in aquatic systems [3]. Thus, when attempting to model fate and transport of $MeHg^+$ in aquatic systems, the binding coefficient between $MeHg^+$ and DOM is of utmost importance.

Few studies have been conducted to quantify partitioning between $MeHg^+$ and DOM resulting in low confidence in predicting $MeHg^+$ speciation with equilibrium modeling. More attention has been paid to Hg^{2+} binding to reduced sulfur groups in DOM and inorganic sulfides as these processes directly impact methylation. Furthermore, Hg^{2+} interactions with reduced thiols in DOM compete with the formation of HgS^o and further aggregation of HgS^o nano- and micro-particles [4] which are less available for methylation

[5]. These studies on Hg^{2+}have used CLE-SPE [6], equilibrium dialysis ligand exchange [7], competitive complexation with bromide [8], reducible Hg titrations [9], and others. A study by Gasper et al. [10] suggested that CLE-SPE was the most appropriate method of three methods reviewed. On the other hand, $MeHg^{+}$ binding with reduced sulfur groups on DOM has received less attention and no published study has used CLE-SPE. Hintelmann et al. [11, 12] used a membrane equilibrium dialysis technique to measure a binding constant between $MeHg^{+}$ and humic substances and calculated log K values ranging from 12.15 to 14.48. Khwaja et al. used a similar technique as Hintelmann et al. and found log K to range from 15.5 to 16.0 These values are contrasted with log K for Hg(II) and reduced thiol groups on DOM of 28 to 33 [13].

In this study, binding of $MeHg^{+}$ to Suwanee River Fulvic Acid, Williams Lake DOM, and DOM isolated from St Louis River watershed systems with elevated sulfate levels due to mining activities was investigated.

Methods

Overview of the CLE-SPE method

The binding of $MeHg^{+}$ to DOM can be described by the equation:

$$MeHg^{+} + DOM \Leftrightarrow MeHgDOM \quad (1)$$

Where $MeHg^{+}$ is truly dissolved and MeHgDOM is $MeHg^{+}$ complexed with DOM. Since $MeHg^{+}$ will preferentially bind to reduced sulfur groups (RS^{-}) on DOM (ref), we can rewrite (1) based on

$$RSH \overset{K_a}{\Leftrightarrow} RS^{-} + H^{+} \quad (2)$$

$$RS^{-} + MeHg^{+} \overset{K_{MeHgSR}}{\Longleftrightarrow} MeHg(RS) \quad (3)$$

Which leads to an over conditional stability constant (K') of $MeHg^{+}$ bound to DOM of:

$$K' = \frac{[MeHg(RS)]}{[RS^{-}][MeHg^{+}]} \quad (4)$$

Very few binding coefficients exist in the scientific literature since it is not possible to separate MeHg bound to DOM (MeHgRS) and truly dissolved $MeHg^{+}$. Strictly model approaches and competitive exchange approaches have been used in the past to determine MeHg binding constants [14]. In this study we used a competitive ligand exchange – solid phase extraction method (CLE-SPE), an approach used to estimate binding constants for Hg(II) [6, 10].

Similarly to the equilibrium with DOM (eq. 1), we can describe the binding of $MeHg^{+}$ to a variety reduced-sulfur containing ligands (L^{-}) with a set of equations.

$$MeHg^{+} + L^{-} \Leftrightarrow MeHgL_{RS} \quad (5)$$

$$K = \frac{[MeHgL]}{[L^-][MeHg^+]} \qquad (6)$$

The value of K has previously been measured for several polar, sulfur-containing organic molecules [15]. Equation 6 can be solved for [$MeHg^+$], which can then be substituted into equation 4, yielding:

$$K' = \frac{K[L^-][MeHg(RS)]}{[RS^-][MeHgL]} \qquad (7)$$

Equation 7 is the key equation to determine the binding constant between $MeHg^+$ and DOM using the CLE-SPE method.

In practice, a two-step procedure is necessary to determine K' using equation 7. In the first step, DOM is dissolved in a $MeHg^+$ solution and pumped through a glass column packed with silica particles which have nonpolar octadecyl (C18) chains attached to their surface. A certain portion of the $MeHg^+$ bound to the DOM will pass through the column at a given pH, and this is operationally defined as the hydrophilic fraction. Conversely, $MeHg^+$ retained on the C18 is defined as the hydrophobic fraction.

In the second step, the process is repeated with the DOM/$MeHg^+$ solution and the addition of a competing ligand with a known binding constant with $MeHg^+$. If the ligand is hydrophilic in nature, then it will compete for $MeHg^+$ with the hydrophobic portion of the DOM. Similarly, a hydrophobic ligand will compete with the hydrophilic fraction of the DOM. In these experiments, l-cysteine and mercaptoacetic acid were utilized as hydrophilic ligands and dodecanethiol was the hydrophobic ligand.

In equation 7, K, [L^-], and [MeHg(RS)] can be measured directly. For example, in determining K' for hydrophilic DOM, the hydrophobic ligand dodecanethiol is used as the competing ligand. In this case, K for dodecanethiol is known, [L-] is known, and [MeHg(RS)] is measured directly as the $MeHg^+$ attached to the hydrophilic DOM which passed through the column in the second step. Ultimately, exact values of [RS^-] and [MeHgL] in equation 7 cannot be measured, but a minimum and maximum value for each is assigned, resulting in a range of values for K' over a few orders of magnitude. The minimum and maximum values for [RS^-] were estimated based on the total reduced sulfur present in each DOM isolate. In the example using dodecanethiol as the competing ligand, [RS^-] is the reduced sulfur concentration present on the hydrophilic DOM. It was assumed that 30% of total S was reduced S^2. The minimum value for [RS^-] was assumed to be the [$MeHg^+$] in the hydrophilic fraction with no completing ligand present (in this example). This minimum value assumes all of the hydrophilic DOM RS^- sites are filled with $MeHg^+$. The maximum [RS^-] value assumes all of the reduced sulfur sites are unprotonated RS^- sites. For example, for a typical total S content of 1.0% by weight, the maximum [RS^-] is 9.36×10^{-7}M. Both the minimum and maximum values are extremes as is discussed later. [MeHgL] maximum assumes all $MeHg^+$ is attached to the competing ligand while [MeHgL] minimum assumes all the $MeHg^+$ is attached to the DOM. In the example above using dodecanethiol as the competing ligand, the maximum value assumes all of the $MeHg^+$ retained on the column (hydrophobic fraction) is attached to the dodecanethiol, while the minimum value assumes that only the additional $MeHg^+$ retained on the C18 in step 2 relative to step 1 is attached to the dodecanethiol.

Table 3 contains all of the parameters used to determine binding constants for each isolate. Figures 1-4 contain the actual experimental results used to calculate the binding constants for the recently collected St Louis River watershed isolates.

Experimental Methods

DOM collection and isolation. Commercially available Suwannee River Fulvic Acid I (SRFA; International Humic Substances Society), DOM isolated from Williams Lake, MN, or DOM isolated from four sites in the St. Louis River watershed were used in the CLE-SPE experiments. For the DOM isolates collected in the St Louis River watershed, water was collected from four sites in June of 2012 by pumping 60 to 150 L of water through 0.2 μm capsule filters (Table 1). The water was shipped to the US Geological Lab in Boulder, CO where the hydrophobic organic acid fraction (HPOA) and transphilic acid (TPIA) fractions of the DOM were isolated following the procedures given by Aiken et al. [16]. The HPOA fraction is operationally defined as that fraction of the DOM that sorbs to Amberlite XAD-8 resin at pH 2 and can be eluted with 0.1 N NaOH, and, is generally comprised of 90-95% fulvic acid with the remainder being humic acid. The HPOA fraction was retained on the XAD-8 resin and then back-eluted with 0.1 M NaOH. The eluate was desalted, proton saturated, lyophilized, and stored for later use. The TPIA fraction passes through the XAD-8 and an additional XAD-4 resin which is used to isolate a hydrophilic fraction (not utilized in this work). CLE-SPE experiments were performed on both HPOA and TPIA fractions.

CLE-SPE Method. All experiments were conducted at a $MeHg^+$ concentration of 100 $ng.L^{-1}$ made from a one $mg.L^{-1}$ MeHgCl standard (Brooks Rand Laboratories Inc.) and at a DOM concentration of 10 $mg.L^{-1}$. MeHg-DOM solutions were equilibrate at 4°C overnight. After equilibration, trace-metal grade acetate buffer (pH = 4.8) and phosphate/citrate buffer (pH = 5.5) was added to each solution. When competing ligands were used they were added at this time. All ligand stock solutions were 1 mM in Milli Q water and sonicated to ensure homogeneity. After addition of buffer and ligands, the solution was equilibrated for two hours before solid-phase extraction chromatography. Solid-phase extraction was carried out in a 1 cm diameter Spectrum Chromatography glass column packed with 0.5 grams of C_{18} resin (Supelco ENVI-18) and set with methanol. The resin was cleaned for 20 minutes prior to any extraction with sequential rinses of ultrapure water and 5 mM HCl at a flow rate of 4 $mL.min^{-1}$ using a peristaltic pump. Detailed CLE-SPE experimental methods are contained in Appendix A.

The MeHg-DOM solution was pumped through the column, and six 40 mL fractions were collected. After the solution was pumped through, air was pushed through the resin for 15 minutes followed two 40 mL 0.24 M HCl fractions to elute $MeHg^+$ attached to the C18. Samples were immediately preserved with 200 μL of 12 M HCl and refrigerated until analysis.

$MeHg^+$ Analysis. An isotope dilution technique was used in analysis of the samples and batch samples were also analyzed to compare with actual concentrations of each specific stock solution. Briefly, one mL of sample and 50 pg of $Me^{201}Hg^+$ were diluted with Milli-Q water in a glass 50-mL autosampler vial. $MeHg^+$ concentrations were measured using standard ethylation/isotope dilution techniques [17, 18]. The pH was adjusted to ~4.8 after neutralization of added acid with potassium hydroxide by addition of a

sodium acetate buffer. $MeHg^+$ species were then ethylated using sodium tetraethyl borate to create volatile methylethylmercury species. Following ethylation, samples were analyzed on an Agilent 7700 ICP-MS connected to a Brooks Rand MERX system using an isotope dilution method [19].

Results and Discussion

The results that we present in Table 2 are still preliminary in nature and need to be further validated using multiple ligands in both the hydrophobic and hydrophilic fractions. The binding constants for a single fraction will not change relative to each other. The absolute magnitude of the binding constant depends on the literature binding constants utilized for the competing ligands and the assumptions that are made. Equation (7) relies on a known binding constant between $MeHg^+$ and the ligand of interest in equation 5. In this research we used l-cysteine as the hydrophilic ligand and dodecanethiol as the hydrophobic ligand. L-cysteine forms a protonated complex with $MeHg^+$ at the experimental pH used [15]:

$$MeHg^+ + H^+ + L^{2-} \Leftrightarrow MeHgL_{RS}H \qquad \log \beta = 26.05 \qquad (8)$$

Results from this work are highly dependent on the log β value of 26.05 in equation 8. As a check, we adid one experiment using mercaptoacetic acid which has a log K value of 20.69 with $MeHg^+$. A log K value for dodecanthiol and MeHg does not exist in the literature so CLE-SPE in the absence of DOM was used to determine a log K for dodecanethiol. Log K for dodecanethiol was determined to range from 17.76 to 20.39 using L-cysteine as the competing ligand and the geometric mean (20.01) was used to calculate the log K for DOM in the hydrophilic fraction.

The highest log K was found in the hydrophobic portion of the HPOA isolates with significantly lower values determined for the hydrophilic portion of the HPOA and the TPIA. Log K values did not change significantly within hydrophobic or hydrophilic fractions suggesting that similar binding regimes are present across the isolates. For example, if all of the $MeHg^+$ is bound to reduced thiol groups, similar log K values might be expected across isolates. This is observed even as SUVA ranges from 1.8 to 4.8 in the HPOA fractions. These initial results suggest lower binding constants between $MeHg^+$ and the hydrophilic fractions of the HPOA and TPIA. If the binding constants in the hydrophilic fractions are lower it could be due to a different binding regime, but it is unclear what that regime is without analysis with x-ray absorption, which has not been completed at this time. We are hesitant to conclude this as the ranges do overlap and the binding constants on the hydrophilic fractions are dependent on the binding constant of dodecanethiol.

Compared to other studies using different methods, our log K values ranging from 14-22 overlap, but are generally larger than previously reported. Strictly modeling studies conducted by Hintelmann et al. [12] and Amirbahman et al.[20] reported a range of 13.02-14.8, while Karlsson and Skyllberg[21] used a competitive exchange approach and determined log K= 15.6 at pH 5.1 and Khwaja et al.[14] reported log K's ranging from 15.5 to 16 using a competitive ligand, equilibrium dialysis technique. We are unclear at this point why our values are higher, but the CLE-SPE is a more widely accepted approach than what was previously used. Using our values in equilibrium modeling would lead to RS^- groups on DOM controlling $MeHg^+$ speciation over an even larger range of DOM values. This would be particularly important in

systems with low DOM and high levels of dissolved sulfides as might be present waters with elevated sulfate loading.

Conclusions

It was demonstrated that the CLE-SPE method is suitable for determining binding constants between $MeHg^+$ and dissolved organic matter. The method worked for determining binding constants in the hydrophobic and hydrophilic portions of HPOA isolates and for the hydrophilic portion of TPIA isolates. No significant differences were found across isolates for any given portion of the DOM. Differences were noted between the hydrophobic portion of HPOA and the hydrophilic portions of the HPOA and TPIA isolates. It is not clear if these are real differences or an artifact of the thiol ligand binding constants utilized. The lower range of binding constants determined between $MeHg^+$ and DOM by the CLE-SPE method overlapped with literature values, but were generally higher than reported values.

Table 1. Summary of collected water samples.

Aiken ID	SampleType	Isolate Origin	Sample Date	Volume
IS12-0010MN	isolation	Lake Manganika	6/26/2012	150 L
IS12-0011MN	isolation	West Swan River	6/26/2012	90 L
IS12-0012MN	isolation	St. Louis River, Mile 94	6/27/2012	60 L
IS12-0013MN	isolation	LLC Downstream of Wetland (site 5)	6/26/2012	60 L

Table 1a. Isolate Properties

Isolate	Fraction	%C	%S	SUVA
Manganika	HPOA	52.2	1.1	4.2
	TPIA	45.5	2.1	2.9
Swan River	HPOA	51.4	0.8	N/A
	TPIA	46.7	1.3	N/A
SLR	HPOA	51.4	0.5	4.8
	TPIA	45.3	1.1	3.5
LLC Wetland	HPOA	51.1	0.9	4.7
	TPIA	45.2	1.4	3.7
Williams	HPOA	55.2	0.8	1.8
	TPIA	49.6	1.0	1.2
SRFA		52.44	0.4	3.2

Table 2. Summary table of Log K values assuming binding is occurring to RS^- sites on DOM. All of our experiments were at pH=5.2-5.3, and Log $K_{MeHgDOM}$ would change depending on the actual pK_as of the reduced thiol groups on the DOM.

DOM Fraction	Log K_{MeHgRS} Hydrophilic Fraction	Log K_{MeHgRS} Hydrophobic Fraction[a]
Lake Manganika HPOA	14.43 – 18.33	17.24 – 21.27
Lake Manganika TPIA	14.70 – 18.40	‡
West Swan River HPOA	14.84 – 18.64	17.35-21.14
West Swan River HPOA		15.79 – 19.71 (pH 6 using MAA)
West Swan River TPIA	15.13-18.90	‡
St. Louis River Mile 94 HPOA	14.96 -18.40	17.23 – 21.23
St. Louis River Mile 94 TPIA	15.20 – 18.70	‡
LLC Downstream of Wetland HPOA	14.37 – 17.97	17.64 – 22.10
LLC Downstream of Wetland TPIA	15.06 – 18.67	‡
Williams Lake HPOA	14.73 – 18.27	17.25 - 20.82
Williams Lake TPIA	14.95 – 18.86	‡
Suwanee River Fulvic Acid	15.71 – 19.34	17.25 – 20.70

[a]Note that for the TPIA fractions we were unable to determine a log K for the hydrophobic fraction since most of the DOM passes through the C18 column even without adding a hydrophilic ligand. Thus, only the dodecanethiol ligand could be utilized to determine log K for the hydrophilic fraction of the DOM.

Table 3. Parameters for calculating K_{MeHgRS} for *hydrophobic portion* of the HPOA isolates.

Parameter	Explanation	Max value	Min value
Table 3a. Lake Manganika HPOA			
$K_{MeHg,Cys}[H^+]$	K for $MeHg^+$/Cyst[a]	7.07×10^{20}	--
[L]	[Protonated Cysteine]	7.5×10^{-9} M	--
[MeHg(RS)]	Concentration retained on C18	1.68×10^{-11} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	2.03×10^{-6} M[b]	2.49×10^{-10} M[c]
[MeHgL]	$MeHg^+$ attached to cysteine	4.95×10^{-10} M[e]	1.69×10^{-10} M[f]
Table 3b. West Swan River HPOA			
[L]	[Protonated Cysteine]	7.5×10^{-9} M	--
[MeHg(RS)]	Concentration retained on C18	1.82×10^{-11} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	7.49×10^{-7} M[b]	1.90×10^{-10} M[c]
[MeHgL]	$MeHg^+$ attached to cysteine	5.78×10^{-10} M[e]	3.73×10^{-10} M[f]
Table 3c. St Louis River Mile 94 HPOA			
[L]	[Protonated Cysteine]	2.11×10^{-9} M	--
[MeHg(RS)]	Concentration retained on C18	3.03×10^{-11} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	4.68×10^{-7} M[b]	1.83×10^{-10} M[c]
[MeHgL]	$MeHg^+$ attached to cysteine	5.12×10^{-10} M[e]	1.29×10^{-10} M[f]
Table 3d. Long Lake Creek Site 5 HPOA			
[L]	[Protonated Cysteine]	2.86×10^{-9} M	--
[MeHg(RS)]	Concentration retained on C18	8.28×10^{-11} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	8.42×10^{-7} M[b]	1.62×10^{-10} M[c]
[MeHgL]	$MeHg^+$ attached to cysteine	5.02×10^{-10} M[e]	9.02×10^{-11} M[f]

[a]Cysteine exists in a protonated form at our experimental pH (5.2) thus we multiply the K by $[H^+]$ as explained in Cardiano et al. [15]. $K = 1.12 \times 10^{26}$; $[H^+] = 6.31 \times 10^{-6}$.

[b]Calculated based on 10 mg/L DOM, S content, and 30% of reduced sulfur is assumed to be reduced thiol groups

[c]This is $[MeHg^+]$ retained on C18 column with no ligand present and assumes all RS^- sites are occupied by $MeHg^+$

[e]Assumes all of the $MeHg^+$ is attached to L-cysteine in the eluent.

[f]Assumes all of the $MeHg^+$ is attached to hydrophilic DOM in the eluent. Calculated as [MeHgL]max – $[MeHg^+]$ in the eluent when no ligand is present.

Table 4. Parameters for calculating K_{MeHgRS} for *hydrophilic portion* of the HPOA isolates.

Parameter	Explanation	Max value	Min value
Table 4a. Lake Manganika HPOA			
$K_{MeHg,dodec}$	K for $MeHg^+$/Dodec.[a]	1.02×10^{20}	--
[L]	[deprotonated dodecanethiol][b]	5.02×10^{-12} M	--
[MeHg(RS)]	Concentration of eluent	2.05×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	1.03×10^{-6} M[c]	3.26×10^{-10} M[e]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	3.83×10^{-10} M[f]	1.51×10^{-10} M[g]
Table 4b. West Swan River HPOA			
[L]	[deprotonated dodecanethiol][b]	1.37×10^{-11} M	--
[MeHg(RS)]	Concentration of eluent	1.41×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	7.49×10^{-7} M[c]	1.90×10^{-10} M[e]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	3.80×10^{-10} M[f]	2.03×10^{-10} M[g]
Table 4c. St Louis River Mile 94 HPOA			
[L]	[deprotonated dodecanethiol][b]	5.77×10^{-12} M	--
[MeHg(RS)]	Concentration of eluent	2.14×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	4.68×10^{-7} M[c]	1.83×10^{-10} M[e]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	2.93×10^{-10} M[f]	1.22×10^{-10} M[g]
Table 4d. Long Lake Creek Site 5 HPOA			
[L]	[deprotonated dodecanethiol][b]	2.51×10^{-12} M	--
[MeHg(RS)]	Concentration of eluent	2.41×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	8.42×10^{-7} M[c]	1.62×10^{-10} M[e]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	3.10×10^{-10} M[f]	1.60×10^{-11} M[g]

[a]No literature values exists for MeHg+ and dodecanthiol binding. The binding constant was determined using CLE-SPE with L-cysteine as the competing ligand. Log K for dodecanethiol was determined to range from 17.76 to 20.39. A mean value of 20.01 was used in all calculations.

[b]Used pKa of 10.8 for dodecanthiol

[c]Calculated based on 10 mg/L DOM, S content, and 30% of reduced sulfur is assumed to be reduced thiol groups

[e]Assuming $[RS^-]+[RSH] = 2.3x10^{-7}$ M, pH = 5.2, and pK_a = 10 for RSH groups (as in [2, 14]) on the DOM (alternatively the max value above could be used as the min and max=$2.3x10^{-7}$ M; or different pK_as could be tested as in [14]).

[f]Assumes all of the $MeHg^+$ is attached to dodecanethiol on the C18.

[g]Assumes all of the $MeHg^+$ is attached to hydrophobic DOM on the C18. Calculated as $[MeHgL]_{max}$ – $[MeHg^+]$ on the C18 when no competing ligand is present.

Table 5. Parameters for calculating K_{MeHgRS} for *hydrophilic portion* of the TPIA isolates using dodecanethiol as the competing ligand.

Table 5a. Lake Manganika HPOA			
Parameter	Explanation	Max value	Min value
$K_{MeHg,dodec}$	K for $MeHg^+$/Dodec.[a]	1.02×10^{20}	--
[L]	[deprotonated dodecanethiol][b]	9.60×10^{-12} M	--
[MeHg(RS)]	Concentration of eluent	3.54×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	1.97×10^{-6} M[c]	4.86×10^{-10} M[d]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	3.53×10^{-10} M[f]	2.80×10^{-10} M[g]
Table 5b. West Swan River HPOA			
[L]	[deprotonated dodecanethiol][b]	1.61×10^{-11} M	--
[MeHg(RS)]	Concentration of eluent	2.59×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	1.22×10^{-6} M[c]	3.96×10^{-10} M[d]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	2.59×10^{-10} M[f]	1.27×10^{-10} M[g]
Table 5c. St Louis River Mile 94 HPOA			
[L]	[deprotonated dodecanethiol][b]	1.60×10^{-11} M	--
[MeHg(RS)]	Concentration of eluent	2.90×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	1.03×10^{-6} M[c]	4.91×10^{-10} M[d]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	2.90×10^{-10} M[f]	1.95×10^{-10} M[g]
Table 5d. Long Lake Creek Site 5 HPOA			
[L]	[deprotonated dodecanethiol][b]	1.48×10^{-11} M	--
[MeHg(RS)]	Concentration of eluent	2.50×10^{-10} M	--
$[RS^-]$	Unprotonated reduced thiol groups on DOM	1.31×10^{-6} M[c]	4.53×10^{-10} M[d]
[MeHgL]	$MeHg^+$ attached to dodecanethiol	2.50×10^{-10} M[f]	1.74×10^{-10} M[g]

[a]No literature values exists for MeHg+ and dodecanethiol binding. The binding constant was determined using CLE-SPE with L-cysteine as the competing ligand. Log K for dodecanethiol was determined to range from 17.76 to 20.39. A mean value of 20.01 was used in all calculations.

[b]Used pKa of 10.8 for dodecanethiol

[c]Calculated based on 10 mg/L DOM, S content, and 30% of reduced sulfur is assumed to be reduced thiol groups

[d]This is [$MeHg^+$] passing through the C18 column with no ligand present and assumes all RS^- sites are occupied by $MeHg^+$.

[f]Assumes all of the $MeHg^+$ is attached to dodecanethiol on the C18.

[g]Assumes all of the $MeHg^+$ is attached to hydrophobic DOM on the C18. Calculated as $[MeHgL]_{max}$ – [$MeHg^+$] on the C18 when no competing ligand is present.

Experimental Results: Graphs of CLE-SPE experiments for Minnesota Isolates

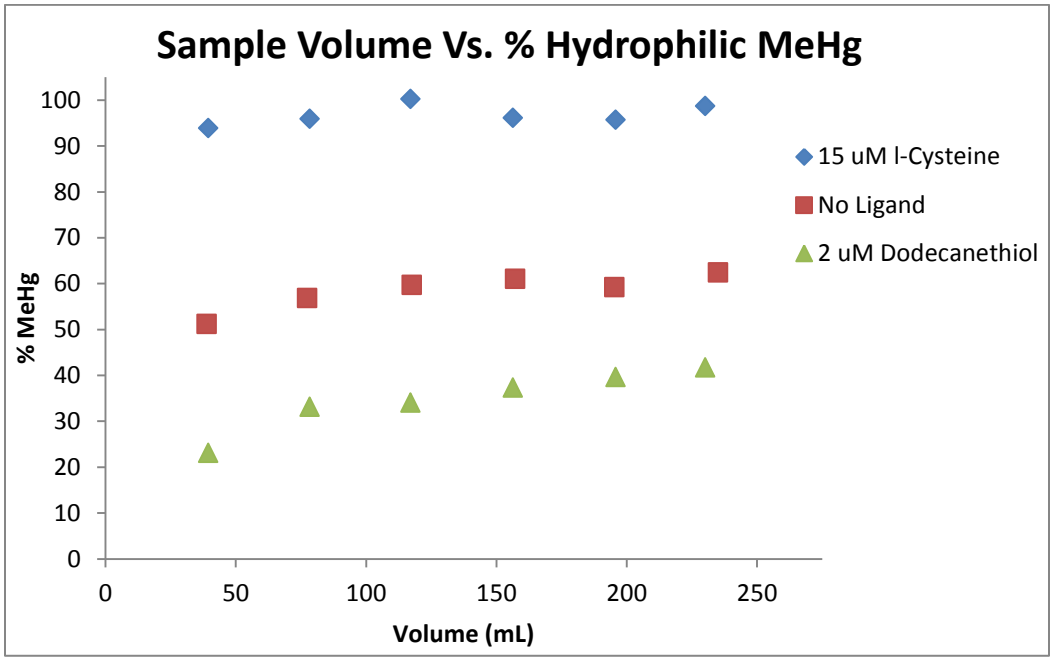

Figure 1a. Lake Manganika HPOA CLE-SPE experiments.

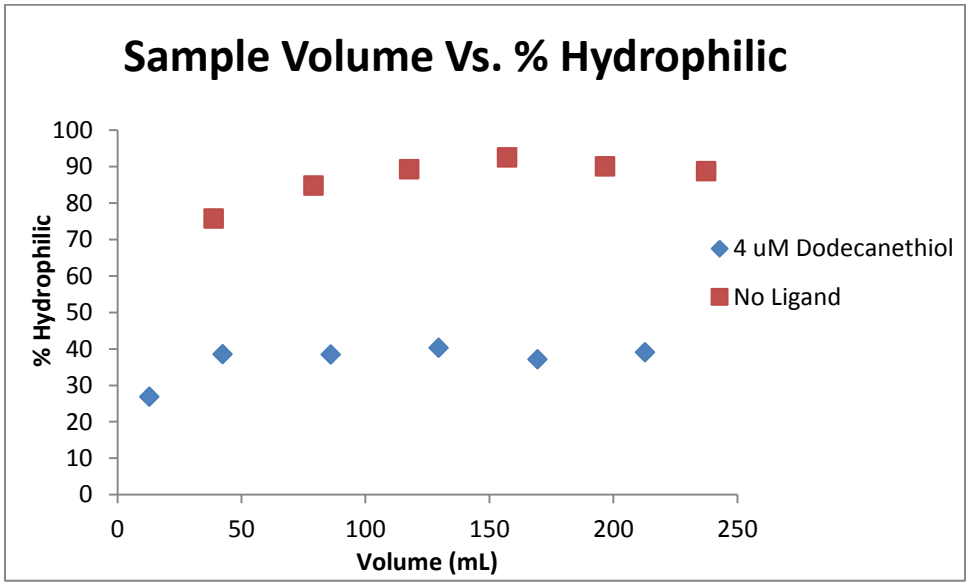

Figure 1b Lake Manganika TPIA

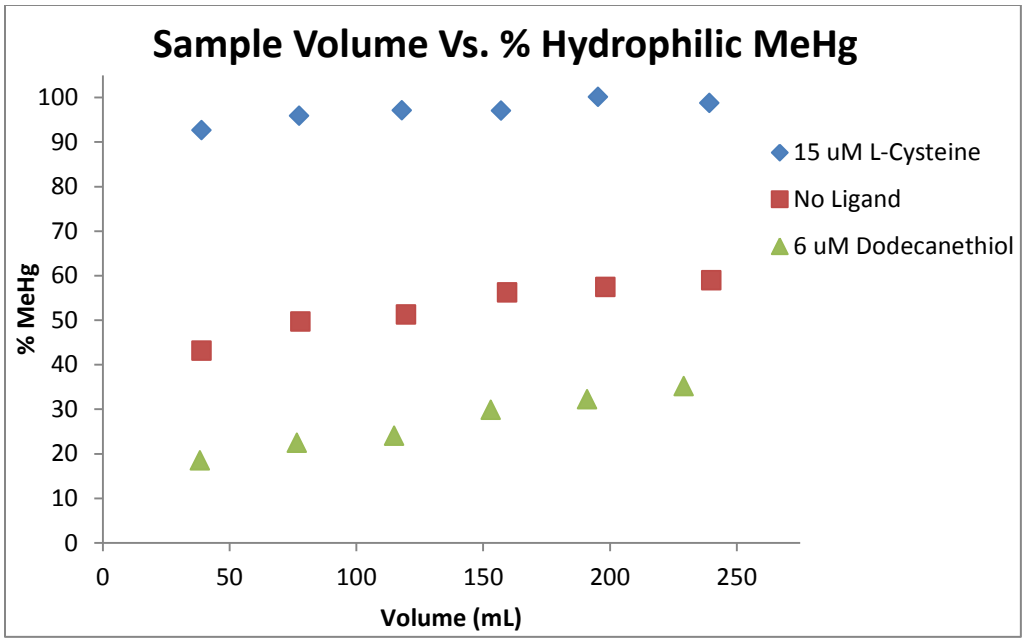

Figure 2a. W Swan River HPOA

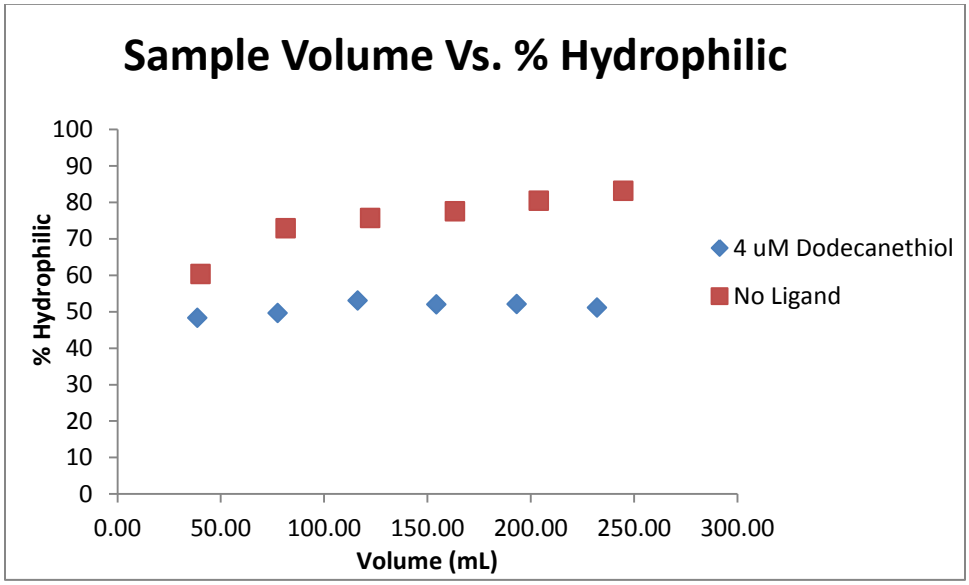

Figure 2b. W Swan River TPIA

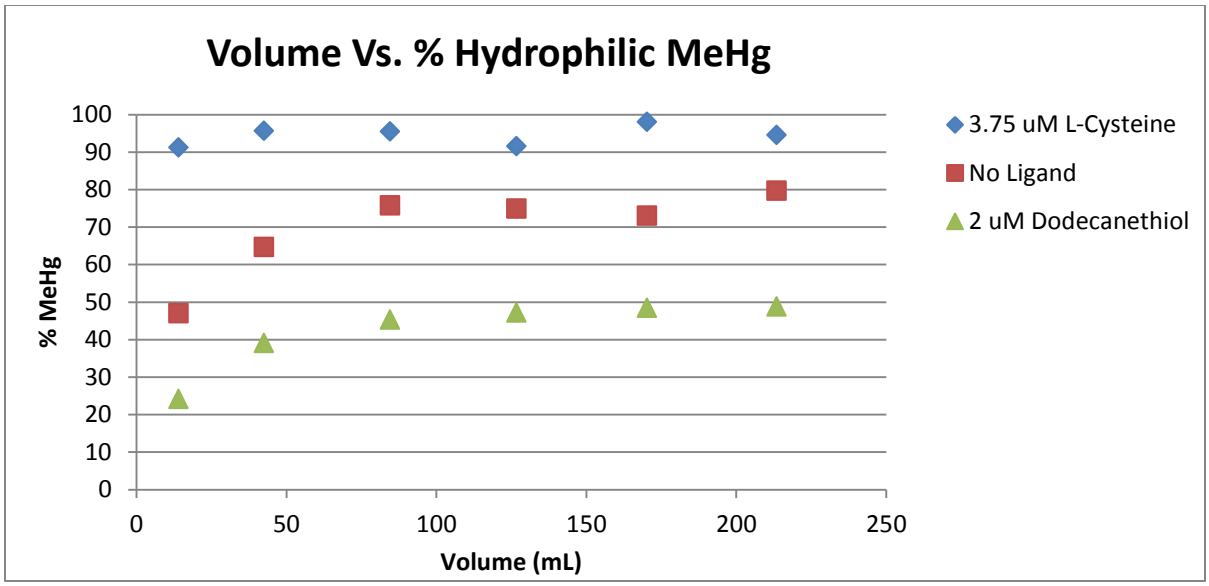

Figure 3a. St Louis River HPOA

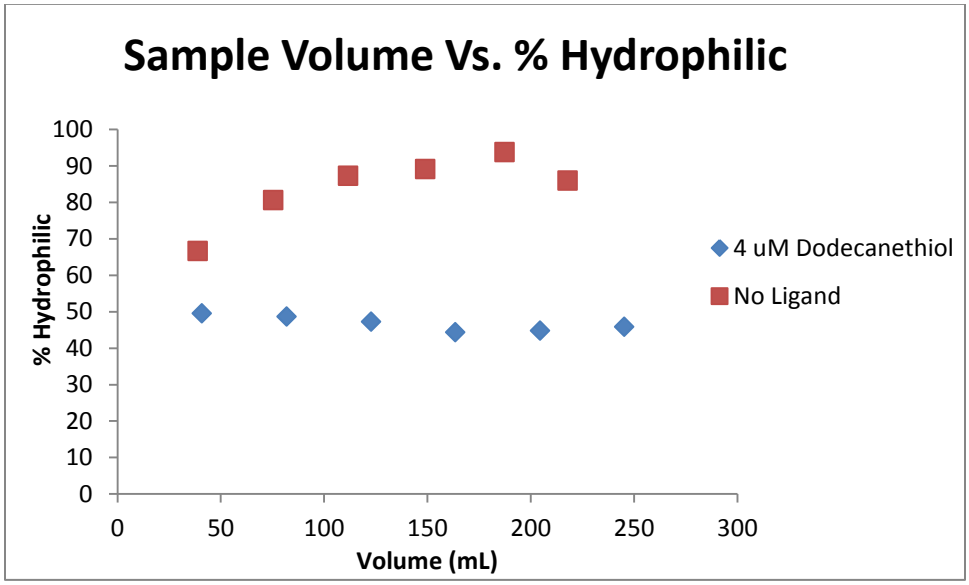

Figure 3b. St Louis River TPIA

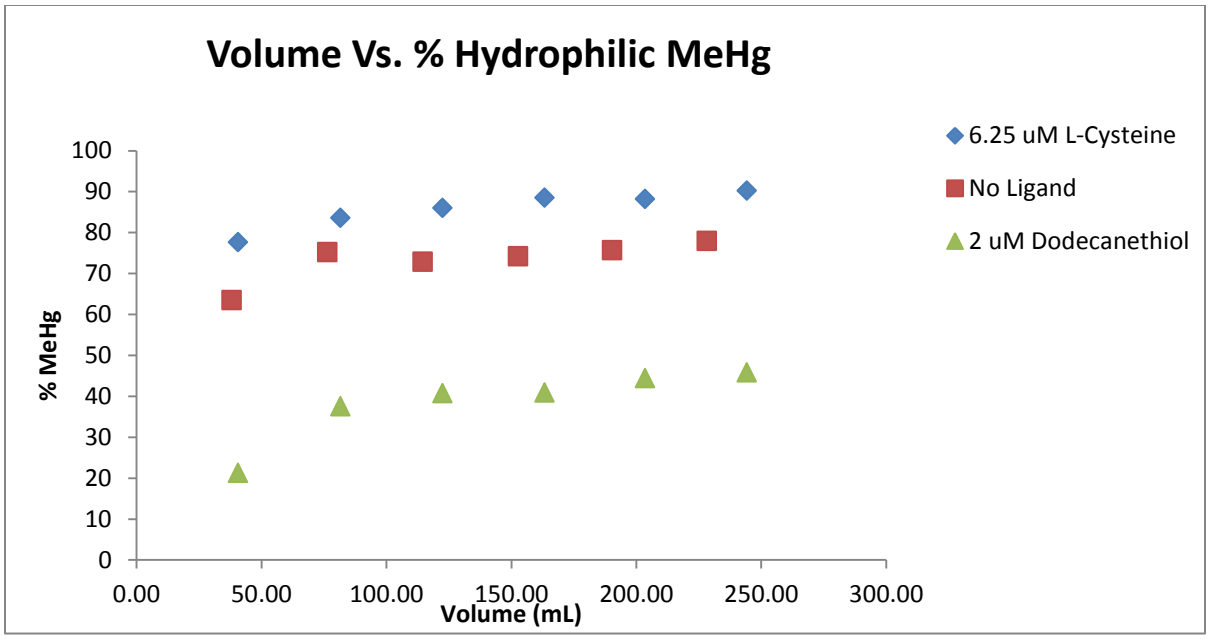

Figure 4a. Long Lake Creek Site 5 HPOA

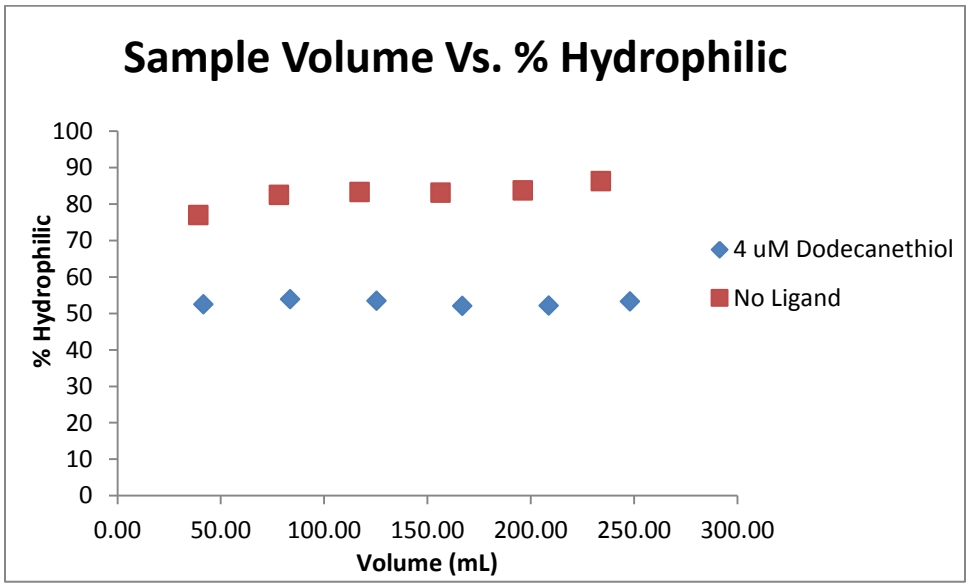

Figure 4b. Long Lake Creek Site 5 TPIA

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Appendix A. Detailed CLE-SPE instructions

General CLE-SPE Procedure for Methylmercury Isolation

Preparing the MeHg Solution

1. Clean a one-Liter Teflon bottle by rinsing three times with MQ water
2. Using the specific Fill Settings on the MQ Dispenser, fill the bottle with one liter of MQ water
3. Add 100.0 µL MeHgCl (MeHg Standard of 1 ppm) to the Teflon bottle – In fridge and double-bagged
4. Mix and measure out 250 mL of solution in a volumetric flask and dump into a 400 mL beaker
5. Repeat step 4 for another 400 mL beaker
6. Mass out 2.5 of DOM for each beaker (10 mg/L concentration)
7. Add to each solution and stir thoroughly
8. Cover with Parafilm and let equilibrate for 22 hours in the biochemistry lab freezer

CLE-SPE Procedure

1. Add approx. 200 µL of 5.0 pH phosphate buffer to each beaker, measure the sample and adjust until pH is 5.2-5.3 and wait two hours before CLE-SPE
2. Set up Engine Pump * Change lines through the pump every two runs *
3. Screw bottom of Teflon resin system (White valve part) to the free black nut
4. Screw nut/bottom to the middle glass port with the two black threaded nuts
5. Measure out 5.0 grams of ENVI-18
6. Pour measured resin into the opening in the glass
7. Put 2 mL Methanol into each system to rinse resin down to the bottom
8. Vigorously tap bubbles out of each resin chamber
9. Connect pump line to the top of free threaded Teflon plunger and screw line tight
10. Turn pump on and run MQ water until the glass chamber area is full and domed
11. Open bottom valve to each CLE-SPE system
12. Push down free threaded Teflon plunger into the glass chamber
13. Leave a reasonable amount of space between the top of the resin and the plunger head
14. Screw last black nut connecting the plunger and glass chamber area
15. Flush systems with MQ water for 5 minutes *make sure to turn off pump when switching reservoirs*
16. Flush systems with 5 mM HCl for 5 minutes *make sure to turn off pump when switching reservoirs*
17. Repeat steps 15-16 for each system
18. Optional – Adjust pump rate to about 4 mL/min
19. Label 16 centrifuge tubes 1-1 through 1-6 then 1-e1 and 1-e2, then 2-1 through 2-6 then 2-e1 and 2-e2
20. Turn of pump and load the previously prepared samples into the lines *leave Parafilm on the tops of samples by inserting plastic line through the beaker mouth and into the sample*

21. Turn on pump *do not collect the 1st portion of samples* – let a couple mL through after insertion into the sample for the 5 mM HCl to be entirely flushed out of the system
22. Collect centrifuge tube samples to 40 mL for first 6 tubes – let run dry from samples at 35-36 mL of last tube
23. Let sit 10 minutes with the pump on and bottom nozzle open so no pressure builds up
24. Rinse column with about 4 mL Methanol combined for both lines
25. Flush through with 2% HCl, collecting in the e-class centrifuge tubes
26. Mass centrifuge tubes once filled for volume
27. Add 200 µL 12 M HCl to each centrifuge tube using mechanical pipette
28. Add 200 µL antifoaming agent to each sample tube
29. Refrigerate until ready to run through the ICP-MS

Preparation for ICP-MS

1. Take samples out of fridge and measure 3-4 g (mL) of each sample into AutoSampler vials using a zeroed scale
2. Put approximately 30 mL MQ water until liquid is to the top of the regular vial area
3. Put 200 µL Sodium Acetate Buffer into each vial with a repeating pipette
4. Put 50 µL 201 MeHg isotope into each vial
5. Measure pH of each vial using a pH sensor and adjust each vial using 3 M KOH until the pH = 4.5-5.0 -> use 100 µL increments and mix after each addition
6. Rinse pH sensor after each trial
7. Put 75 µL STEB into each vial under hood rapidly so because of possible ethylation, cap and shake each vial, uncap and fill with MQ water until the vial becomes domed
8. Put in AutoSampler rack and prepare blanks for ICP-MS analyzation
9. Prepare relative samples using beginning/end bottles from made batches and fill to 40 mL or until liquid is to the top of the regular vial area
10. Put 200 µL 12 M HCl in each vial using an adjustable pipette *Use special pipette for high Molar Acid (Old pipette in drawer)*
11. Put 200 µL Antifoaming agent using a mechanical repeating pipette

Blank order from beginning of AutoSampler –

Three rinses – Just MQ Water

Three Calibration blanks – MQ water, 200 µL acetate buffer, and 75 µL STEB

Three calibration standards – MQ water, 200 µL acetate buffer, 50 µL MethyMercury standard (B-R) and 50 µL Hg Isotope (star on top) , and 75 µL STEB w/procedural measures

One more Calibration blank

12. After every 10 samples in sample set, one calibration blank like above, then one precision and recovery blank (a calibration standard with both standard and isotope)

13. Run ICP-MS

Data Analysis from ICP-MS

1. Take counts from ICP-MS record and translate them to the MeHg data analysis Microsoft Excel spreadsheet and use them along with volumes of sample used for each to determine concentration and total count of MeHg in each sample and the total
2. Do spreadsheet analysis to figure out all data sets and values and translate into CLE-SPE Jan 2013 file set and analyze percentages and counts for consistency and good data results

Troubleshooting:

1. When flushing the CLE-SPE system with either MQ water, 5 mM HCl, or 2% HCl, always turn pump off when transferring lines to a different liquid. If air gets in the lines, stop the pump as soon as possible. Fill two beakers/caps/reservoirs with the substance previously used and put the end of the Teflon system into that liquid. Turn the pump switch direction the other way and turn the pump on until the air bubbles are fully released from the lines. Then, turn engine off and turn the pump switch back. Put lines into desired liquid and then turn pump on. Make sure resin is not sucked way back up into resin chamber by the reverse action, and continue procedure.
2. Also, if air or liquid gets in the lines when it is not supposed to, disconnect the first section of lines from the pump line that goes through the pump engine itself. Lift far edge above end of line and empty liquid/air out the beginning of the lines. Reconnect and reattempt desired action.
3. If distribution cap in Step 12 of Teflon plunger falls off the front of the plunger when inserting into the glass chamber, stop pump and empty out chamber and obtain the lost cap. Empty the resin and dry chamber. Reattach the cap to the plunger by pulling the plastic ring back over it, securing the cap to the plunger. Re-mass more resin, repeat procedure and proceed as normal.
4. If a large amount of air gets in the lines in the middle of a CLE-SPE run and the lines get disconnected and drained so reverse engine will not suffice (Troubleshoot 1), take a 20-200 µL adjustable repeating pipette and manually insert sample liquid back into the lines until the lines are full again (may need two people). Connect lines again, and then start engine and proceed.