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Optimization of Method for Speciation of Mercury(II), Monomethylmercury Cation, Dimethylmercury and Diethylmercury by Hydride Generation

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This paper describes optimization details of an analytical method development of a hydride-generation procedure for speciation of mercury(II), monomethylmercury cation, dimethylmercury, and diethylmercury using a heated quartz furnace with an atomic absorption spectrophotometer (AAS) as a detector. Typically a new analytical method is developed in steps such as the following: (1) determining analytes individually to confirm retention times and the lack of decomposition during the procedure; (2) comparing peak areas of all analytes as a function of quartz furnace temperature to optimize atomization for AAS detection; (3) conducting factorial experiments to determine which hydride-generation reaction conditions are important in maximizing peak areas and which conditions interact with each other; (4) using the simplex optimization method to give final optimization of reaction conditions. These steps result in conditions that maximize optimized peak areas for analytes while minimizing experimental error.

Keywords: Hydride generation, monomethylmercury, dimethylmercury, statistics

INTRODUCTION

Statistical methods are very helpful in optimizing variable values most efficiently to maximize a signal during development of an analytical method. However, many researchers ignore statistical techniques and do experiments in which only one variable is changed at a time. Such experiments are very inefficient compared with statistically designed ones and, in addition, may miss interactions among variables. Furthermore, researchers who use statistically designed experiments do not usually describe the methods and the reasoning behind them. This paper emphasizes use of factorial experiments and the simplex optimization method during development of an analytical method for speciating inorganic mercury and organomercury compounds by a hydride-generation method.[1]

A factorial experimental design allows testing of several variables at two or more levels.[2] We typically set the variables at two levels (high and low). Consider as an example a study by Ring and Weber[3] of the methylation of tin(II) (Sn(II)) by methyl iodide (CH_3I) in sodium chloride (NaCl) solutions that simulate seawater. In one set of factorial experiments we tested the effects of the variables salinity, methyl iodide concentration, and pH on reaction yield. Testing of this design with two variable settings requires 2^3+4 experiments. The 2^3 experiments represent all combinations of the three variables at low and high levels. The other four experiments (center points) were run at levels intermediate between high and low levels in order to calculate errors. (Performing duplicates of 2^3 experiments also allows error calculations, but requires more experiments.) Calculations based on this experimental design told us that methyl iodide concentration and pH, but not salinity, are significant. The results of this first factorial experiment allowed us to keep salinity constant in future experiments. The only significant two-variable interaction was methyl iodide concentration–pH. An *interaction* is a combination of variables that significantly affects the dependent variable. Researchers miss important interactions between or among variables when they change variables one-at-a-time during method development.

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Simplex optimization, like factorial experiments, is done by changing all variables simultaneously.[2] Consider as an example optimization of variables for determination of inorganic lead and methyllead compounds using ethylation derivatization, a heated quartz furnace, and an atomic absorption spectrophotometer (AAS) detector.[4] While developing the technique, we tested the variables of pH, sodium tetraethylborate concentration, purge time, flow rate of helium carrier gas, and flow rate of hydrogen for the flame in the furnace. In general, to begin the simplex, one chooses $n+1$ sets of experimental conditions for the n variables. For example, the five variables in this example required 5 + 1 initial experiments in which each variable was given a value. After measuring, for example, signal areas, the variable settings along with areas are entered into a simplex program. The output from the program is the next set of variable settings to test. One continues to test sets of variables until integrator areas are approximately constant. This method deletes the least desirable response (area) and replaces it with its mirror image across the remaining points.

EXPERIMENTAL

Inorganic mercury (Hg(II)), monomethylmercury cation (MeHg), dimethylmercury (Me_2Hg), and diethylmercury (Et_2Hg) were determined by hydride- generation volatilization, trapping with liquid nitrogen and separation on a chromatographic column, and detection by AAS in a heated quartz furnace. Full experimental details are in a paper by Puk and Weber,[1] but a summary follows. Water (10 ml) in a hydride-generating flask was adjusted to pH 2 with HCl, and standards (or sample extracts) were added. Et_2Hg was used as an internal standard for samples. The reaction flask was capped, helium was bubbled through the stirred solution to remove oxygen, 0.8 ml of 6% $NaBH_4$ was injected through a septum, and the solution was purged for 6 min. The reaction flask was then bypassed by use of a four-way valve. Simultaneously liquid nitrogen was removed from the column, the Variac for the column was turned on, and the integrator was started. Retention times were: Hg^0, 1.6 min; MeHgH, 2.6 min; Me_2Hg, 3.0 min; Et_2Hg, 4.0 min.

RESULTS AND DISCUSSION

Confirmation of analytes

Volatilization of Hg(II) and MeHg by reactions with $NaBH_4$ reduces Hg(II) to Hg^0 (Eqn [1]) and forms MeHgH from MeHg (Eqn [2]).

$$Hg(II)^{2+} + 2NaBH_4 + 6H_2O \rightarrow Hg^0 + 7H_2 + 2H_3BO_3 + 2Na^+ \quad [1]$$

$$MeHg^+ + NaBH_4 + 3H_2O \rightarrow MeHgH + 3H_2 + H_3BO_3 + Na^+ \quad [2]$$

Filippelli *et al.*[5] and Craig *et al.*[6] identified MeHgH by GC–MS and Craig *et al.*[7] documented it in C_6D_6 by NMR. We formed MeHgH in the headspace of a closed vial after reaction of an aqueous solution of MeHgCl with $NaBH_4$ and identified it by GC–MS[1] in agreement with other groups.[5–7] We observed a headspace MeHgH half-life of 1.5 h, which is easily long enough for determination of MeHg by the method in this research. In our procedure MeHgH is in the reaction flask for only about 10 s and then it is trapped on a column at −196 °C, where it is stable.

When we determine 1 ng of $HgCl_2$, MeHgCl, Me_2Hg, and Et_2Hg standards simultaneously, reproducibility of retention times is less than 5% RSD for all analytes. We confirmed the identity of Hg^0, MeHgH, Me_2Hg, and Et_2Hg derivatized analytes and proved lack of decomposition products by studying each separately in several experiments. We proved that Hg(II) is reduced to Hg^0 because Hg(II) has the same retention time as Hg^0 vapor over liquid mercury. Although our experimental conditions prevent significant volatilization of MeHgCl, we confirmed its retention time from MeHgCl vapor over solid MeHgCl. MeHgCl did not yield any underivatized MeHgCl or rearrangement product Me_2Hg when we used the optimized reaction conditions. We substantiated the retention times of Me_2Hg and Et_2Hg by sampling vapor over solutions in methanol, and by separately placing a methanolic solution of each into a dry hydride-generation flask followed by purging onto the trap. Neither Me_2Hg nor Et_2Hg formed the potential decomposition monoalkylmercury products $MeHg^+$ or $EtHg^+$. MeHgH retention times were substantiated after forming this species in the head space of a closed

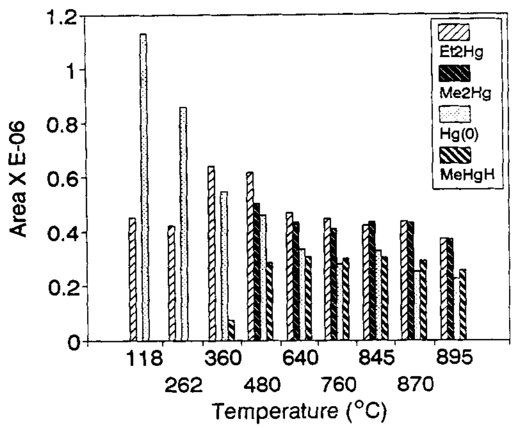

Figure 1 Peak areas for 1 ng of derivatized analytes Hg^0, MeHgH, Me_2Hg, and Et_2Hg at temperatures between 118 °C and 895 °C in the quartz furnace. Areas of Hg^0 were corrected for background.

vial by the method of Filippelli *et al.*;[5] no decomposition or rearrangement occurred.

Optimization of the quartz furnace

Increased atomization efficiency in the quartz furnace contributes to increased method sensitivity. A study of temperature vs volts applied on both sides of the furnace showed that, at any voltage studied, the temperature was equal on both sides of the furnace.

Measurement of peak areas of each analyte as a function of furnace temperature resulted in optimum atomization efficiency. Peak area vs temperature graphs for Hg(II), MeHg, Me_2Hg, and Et_2Hg analytes (Fig. 1) show Et_2Hg, the least stable of the analytes, begins to atomize at less than 118 °C, while MeHgH and Me_2Hg begin to atomize between 360 °C and 480 °C. At temperatures between 600 °C and 825 °C peak areas for MeHgH, Me_2Hg, and Et_2Hg are near their maxima. Because our major interests are environmental MeHg and Me_2Hg, we accept the decreased Hg(II) sensitivity at higher temperatures.

Factorial experiments

Results from several factorial experiments led to important variable and initial conditions for simplex optimization experiments. The following two examples of factorial experiments are representative of those we did. The errors in these early experiments are considerably larger than those in later experiments.

A duplicate 2^3 factorial design tested as variables the effects of concentrations of sodium bromide (NaBr; a possible sensitivity enhancer), acetic acid (HOAc), and $NaBH_4$ on formation of MeHgH (Table 1). The 16 experiments included duplicates of all combinations of high and low levels of the three variables. The only significant variable at the 95% confidence level in the range of variables used was the concentration of $NaBH_4$. Because of this result we discarded the idea of improving sensitivity by addition of halide salts to the hydride-generation flask. The probable reason for the insignificance of HOAc is its excess in the experiments. Because MeHgH areas with high $NaBH_4$ concentration (Table 1, $H = +$) are clearly higher than those at lower concentrations (Table 1, $H = -$), we increased $NaBH_4$ concentration more than 10-fold in the second factorial experiment.

Table 1 Factorial experiment to test the effects of concentrations of sodium bromide, acetic acid, and sodium borohydride on the peak areas of MeHgH from 5 ng MeHgCl

Variable	Level + (High)	 − (Low)
NaBr (*B*)	0.01 M	0.001 M
HOAc (*A*)	0.50 M	0.005 M
$NaBH_4$ (*H*)	4.00 mg	0.040 mg

Expt no.	Variable levels *B*	 *A*	 *H*	Counts × 10^{-6} Expt A	 Expt B
1	+	+	+	0.88	1.73
2	−	+	+	0.57	1.80
3	+	−	+	1.20	1.98
4	−	−	+	0.97	0.37
5	+	+	−	0.09	0.03
6	−	+	−	0.18	0.02
7	+	−	−	0.37	0.42
8	−	−	−	0.63	0.31

Effect	F-value[a]
B	0.61
A	0.42
H	**13.24**
BA	0.85
BH	1.05
AH	0.68
BAH	0.97

[a] Critical *F*-value = 5.32; *F*-value in boldface is significant at the 95% confidence level.

Table 2 Factorial experiment to test the effects of concentrations of acetic acid and sodium borohydride on the peak areas of MeHgH from 5 ng MeHgCl

Variable	Level + (High)	 − (Low)
HOAc (*A*)	0.25 M	0.025 M
$NaBH_4$ (*H*)	47.5 mg	4.75 mg

Expt no.	Variable levels *A*	 *H*	Counts × 10^{-6} Expt A	 Expt B
1	+	+	2.29	2.21
2	−	+	0.99	0.76
3	+	−	1.67	1.20
4	−	−	1.37	1.44

Effect	*F*-values[a]
A	**27.70**
H	1.14
AH	**25.38**

[a] Critical *F*-value = 7.71; *F*-values in boldface are significant at the 95% confidence level.

A second factorial experiment, a duplicate 2^2 one, tested variable concentrations of HOAc and $NaBH_4$ (Table 2). In this set of experiments HOAc concentration and the HOAc–$NaBH_4$ interaction are significant at the 95% confidence level. Particularly noteworthy is the importance of the interaction between HOAc and $NaBH_4$ concentrations; this interaction would be missed in experiments changing only one variable at a time. Although we later replaced HOAc with HCl as an acid,[1] the results of these two factorial experiments led to the inclusion of acid and $NaBH_4$ concentrations in the simplex optimization experiments. Other experiments suggested the third variable of purge time.

Simplex optimization of reaction conditions

We maximized the sensitivity of the technique by studying the derivatization reaction variables HCl concentration, $NaBH_4$ concentration, and purge time by using the simplex optimization method,[2] while other experimental conditions were kept constant. Integrated area was chosen as the response. An entire simplex optimization typically requires only 15–20 experiments and optimum conditions may be reached in fewer experiments.[2]

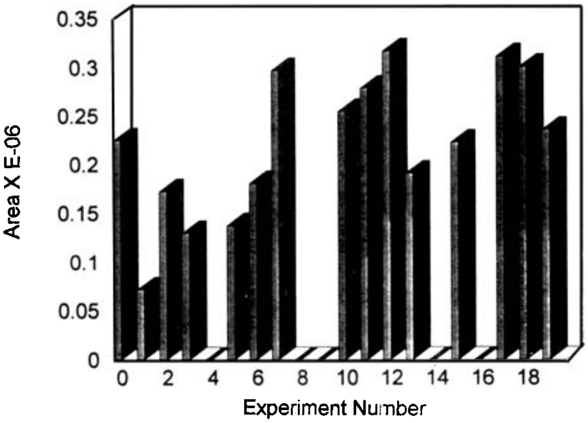

Figure 2 Results of simplex optimization method used to maximize area of MeHgH. For clarity areas of Hg^0, Me_2Hg, and Et_2Hg analytes are not shown. Blank spaces represent experiments in which no MeHgH occurs.

We measured peak areas for 1 ng of mercury as $HgCl_2$, MeHgCl, Me_2Hg, and Et_2Hg by simplex optimization of HCl and $NaBH_4$ concentrations and purge time. Since both Me_2Hg or Et_2Hg are purged unchanged from the reaction solution, their peak areas are relatively insensitive to the reaction conditions. Because the emphasis of this research is to determine MeHg and Me_2Hg in the aquatic environment, we chose conditions that favor maximum areas for MeHgH (Fig. 2, Expts 12, 17, and 18). These conditions are a reaction medium of 0.01 M HCl (pH 2) and addition of 0.8 ml of 6% $NaBH_4$ (48 mg, 0.13 M). The approximately 10-fold excess of $NaBH_4$ is necessary because much of it reacts with H^+ to form hydrogen (H_2). Formation of MeHgH is the rate-limiting step that requires the 6-min purge time.

The reaction of $NaBH_4$ with H^+ at pH 2 results in the rapid formation of considerable amounts of H_2, which probably contributes significantly to effective purging of Hg^0, MeHgH, Me_2Hg, and Et_2Hg from the reaction solution. To determine completeness of the reaction and mass transfer after the 6-min purge, we added a second injection of 0.8 ml of $NaBH_4$ to the original solution previously containing Hg(II) and MeHg at pH 10 (the pH at reaction completion) or after reacidification to pH 2. The absence of appreciable Hg^0 or MeHgH peaks indicates nearly complete (>95%) removal of Hg(II) and MeHg from the reaction flask and of Hg^0 and MeHgH from the transfer lines during the optimized procedure.

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