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Selective Determination of Methylmercury by Flow-Injection Fast-Scan Voltammetry

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Abstract
A simple flow-injection system, suitable for solution flow rates at microliters-per-minute, has been combined with fast-scan voltammetry for selective determination of methylmercury. A thin Hg film was formed at a Pt microelectrode prior to the measurement. Detection of methylmercury is carried out by measuring the oxidation of methylmercury radicals that have been generated at the Hg microelectrode. At slow scan rates, the electrogenerated methylmercury radicals undergo a follow-up dimerization reaction to form dimethyldimercury ($E_rC_i^2$ mechanism). At fast scan rates, it was found that methylmercury radicals can be quantitatively reoxidized (reversible electron-transfer). Optimization of the experimental conditions of the system was performed based on studies of the relationship between the scan rate and the dimerization rate of the methylmercury radical. Under optimized conditions, detection level of subnanomole was obtained with a sample consumption of less than 10 μL and the concentration detection limit for methylmercury at 50 V/s was estimated to be about 0.56 μM. To demonstrate the applicability of this method to automatic analyses, repetitive fast-scan cyclic voltammetry was conducted in conjunction with multiple sample injections. Determination of methylmercury in the presence of excess inorganic mercury was also conducted. This new approach to methylmercury determination was successfully applied to the analysis of elevated dogfish muscle samples.

Keywords: fast-scan voltammetry, flow-injection analysis, methylmercury, platinum-based mercury microelectrodes

1. Introduction

The toxicity of mercury, like that of many other heavy metals, varies with its chemical forms. Methylmercury (CH_3Hg^+) is much more toxic than Hg^{2+} due to its higher membrane permeability and greater tissue fixation [1]. Methylmercury is of particular environmental importance because it can be converted from inorganic mercury by microorganisms present in the environment. Various analytical techniques have been developed to determine total mercury at trace levels and/or to speciate methylmercury from inorganic mercury. To speciate methylmercury from inorganic mercury, a separation or extraction step is generally employed to isolate methylmercury for subsequent measurements. For example, Evans and McKee reported the combination of high-performance liquid chromatography with amperometric detection [2, 3] and Saouter and Blattman used gas chromatography/atomic fluorescence spectrometry [4]. In other reports, various extraction procedures were performed prior to the detection by inductively coupled plasma-mass spectrometry [5–8]. Selective analytical techniques for methylmercury detection have also used complexation reactions with ammonium tetramethylenethiocarbamate [9] and mercaptobenzothiazole [10] or binding to tissue [11], sediment [12], or yeast [13].

While most of the established techniques are highly selective and sensitive, the procedures and instrumentation are usually rather complicated and some of the approaches can be time-consuming. Voltammetry is an attractive analytical technique due to its simplicity and good sensitivity [14, 15]. Coupled with a flow-injection device, voltammetry conducted in an electrochemical flow cell can yield high sample throughputs [16, 17]. However, for the analysis of methylmercury, conventional voltammetry has not been the method of choice because the reduction of methylmercury, like that of other organomercurials, is a relatively complicated process [18–20].

We recently demonstrated that fast-scan anodic stripping voltammetry (FS-ASV) can be conducted in a miniaturized electrochemical flow cell that is also part of a microflow system [21]. We have shown that heavy metals (e.g., Cd and Pb) can be accumulated into and analyzed at hemispherical Hg microelectrodes deposited onto Pt microelectrodes [21]. In this article, we report the adaptation of this system to the studies of the electrochemical reduction of methylmercury at Pt-based Hg microelectrodes and the selective determination of methylmercury at low levels.

2. Experimental

2.1. Reagents and Chemicals

Methylmercury(II) chloride standard solution (1000 ppm or 4.60 mM) was purchased from Alpha-AESAR (Ward Hill, MA). *Caution: methylmercury (CH_3Hg^+) chloride is highly toxic and must be handled with extreme care. Since CH_3Hg^+ was found to be stable in 1.0 M nitric acid/0.01 % (v/v) Triton X-100 (Rohm and Haas, Philadelphia, PA) for at least 1–2 mo., a dilute stock solution (e.g., 1 mM CH_3Hg^+) was used for preparing the subsequent sample solutions. The original bottle containing the methylmercury(II) chloride standard was then stored in a glass jar. Gloves must be worn when making solutions. The disposable pipet tips were rinsed with 1 % HNO_3 solution before being discarded.* Nitric acid used for preparing the carrier solution (0.02 M HNO_3 in deionized water) was double distilled from Vycor (GFS Chemicals, Columbus, OH). Water was purified by a Barnstead System (Boston, MA). Certified dogfish muscle sample (DORM-2) was obtained from National Research Council of Canada (Ottawa, Canada).

2.2. Instruments

The microflow system used in the flow-injection fast-scan voltammetric experiments has been described in our previous work [21]. Fast-scan voltammograms were obtained with an EI-400 bipotentiostat using either the CE-6000 software, designed to collect voltammograms for scanning electrochemical microscopy experiments, or the CV6 software (Univer-

sity of Pittsburgh) written for flow-injection analysis with the repetitive cyclic voltammetric detection. For verification of methylmercury concentrations in the various steps of the sample digestion procedure, a SpectroFlame inductively coupled plasma-atomic emission spectrometer (Spectro Analytical Instruments, Fitchburg, MA) was used. A cyclonic spray chamber and a high flow-rate (ca. 1 mL/min) glass nebulizer (Spectro Analytical Instruments) were used to create the aerosol.

2.3. Electrodes and Cells

The design of the flow-onto thin-layer microflow electrochemical cell was described elsewhere [21]. Pt microelectrodes were fabricated by sealing 10-or 25-μm-diameter Pt wires (Goodfellow Corp., Cambridge, England) into glass capillaries. The glass-imbedded Pt microelectrode was then affixed to the center of a round PEEK block. The electrode was polished with diamond paste down to 1 μm (Buehler, Lake Bluff, IL) and sonicated in deionized water.

2.4. Procedure

Fresh methylmercury chloride solutions were made from a 1.0 mM stock solution by dilution with a 1 M HCl/0.01 % Triton X-100 (v/v) solution. 0.020 M HNO_3 carrier solution was degassed with N_2 and transferred into the pump reservoir. The procedure for making a Pt-based Hg thin film microelectrode has been described in our previous work [21]. After the formation of the Hg film microelectrode, a 250-μL loop was replaced with a 54-μL sample loop to avoid generating large amounts of methylmercury waste. After the valve was switched from the load position to the inject position, cyclic voltammetry was continuously run until voltammograms with constant peak currents were observed. A representative voltammogram was then recorded. This step was followed by switching the valve back to the load position to record a background voltammogram from the carrier solution.

Isolation of methylmercury from elevated dogfish muscle was performed in a modified procedure provided by Berman et al. [5]. Specifically, 1.5045 g DORM-2 certified sample was spiked with 20 μL of a 4.60 mM methylmercury chloride standard. 6.00 mL of a 0.10 M copper sulfate solution and 3.00 mL of a 2.50 M hydrobromic acid solution were then added into the sample powder. The resulting slurry was extracted three times with 4.00 mL of toluene. This was then followed by a backextraction using 6.00 mL of a 2.42 mM sodium thiosulfate solution. The methylmercury concentration in the final extract (ca. 5.30 mL) was verified to be 15.5 μM by inductively coupled plasma-atomic emission spectrometry. This value corresponded to a recovery of about 74 % of the total methylmercury in the spiked sample (ca. 21.5 μM).

3. Results and Discussion

3.1. Electrochemical Reduction of Methylmercury Cation

The electrochemical reduction reaction of organomercury compounds was first studied by polarography [18]. The first cyclic voltammetric study on the methylmercury reduction in aqueous media was reported by Heaton and Laitinen [19]. These researchers systematically investigated the voltammetric behavior of methylmercury cation at a dropping mercury electrode in various basic and acidic solutions. In acidic solutions, the mechanism of the electrochemical reduction of methylmercury cation was described as follows:

$$CH_3Hg^+ + e^- \rightleftharpoons CH_3Hg^\bullet \qquad (1)$$

$$2CH_3Hg^\bullet \rightarrow (CH_3Hg)_2 \qquad (2)$$

$$(CH_3Hg)_2 \rightarrow (CH_3)_2Hg + Hg \qquad (3)$$
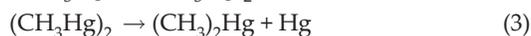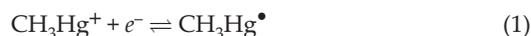
Reaction 1 is a reversible electron-transfer process, which is followed by a fast dimerization reaction, yielding dimethyldimercury (Reaction 2). The formation of dimethylmercury and elemental mercury (Reaction 3) is much slower than the dimerization process. Heaton and Laitinen also suggested that Reactions 2 and 3 might be surface processes based on the appearance of electrocapillary curves of methylmercury cation [19]. They attributed the surface-like anodic wave to the oxidation of the methylmercury radical that had absorbed onto the mercury electrode.

Although the mechanism associated with methylmercury reduction was exquisitely elucidated by Heaton and Latinen [19], the analytical implication of the electrochemical reduction of methylmercury (e.g., detection of methylmercury at low levels) was not explored. In a more recent report, Ireland-Rispert et al. measured methylmercury in the presence of inorganic mercury at a gold film electrode [20]. They accumulated and determined using differential pulse stripping voltammetry the inorganic mercury that was produced in Reaction 3. Because inorganic mercury in the same solution was also preconcentrated, a rather complicated double standard addition procedure had to be employed in order to quantify both methylmercury and inorganic mercury. The treatment based on the double standard addition procedure was not very useful due to the incomplete transformation of methylmercury to inorganic mercury and the influence of gold electrode surface on the stripping of the preconcentrated inorganic mercury. Consequently, a poor %RSD(20–40%) was obtained [20]. Since Reaction 1 is reversible, we decided to rely on outrunning Reaction 2 to quantitatively determine methylmercury. To achieve this, fast-scan voltammetry at Hg film microelectrodes has to be resorted to. We envisioned that the use of Pt-based Hg microelectrodes should obviate the necessity of employing dropping mercury electrodes. Dropping mercury electrodes generally produce higher charging currents and are more difficult to be incorporated into miniaturized, low dead-volume flow electrochemical cells [16, 17].

A typical fast-scan voltammogram of methylmercury obtained at a Pt-based Hg film electrode is shown in Figure 1.
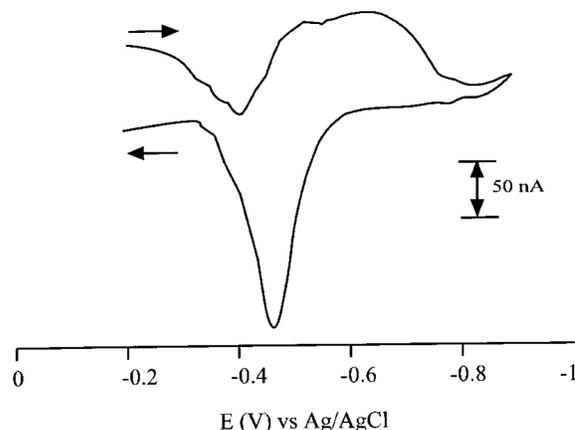
Figure 1. Background-subtracted, fast scan cyclic voltammogram of 0.5mM CH_3Hg^+ in a 1.0M HCl/0.01% Triton X-100 solution at 100 V/s. The initial potential was –0.2V (vs. Ag/AgCl) and the switching potential was –0.9 V. The thin Hg film was deposited onto a 25-μm-diameter Pt microelectrode. Arrows indicate the scan directions.

The shape of the voltammogram and the peak potentials are very similar to that obtained at dropping mercury electrodes [19]. As can be seen in Figure 1, the reduction of methylmercury (the forward process of Reaction 1) produces a broad wave with a peak potential at about –0.63 V. The broad shape of the reduction wave is probably due to the follow-up chemical reactions (Reactions 2 and 3). The oxidation wave with a peak potential at E = –0.48 V, on the other hand, is sharp and well defined. As mentioned above, the oxidation wave has been ascribed to the oxidation reaction of the methylmercury radical (the reverse process of Reaction 1) that is confined to the Hg electrode surface. Upon oxidation, the adsorbed methylmercury radicals are stripped off the electrode. This conclusion is supported by the fact that the anodic wave is absent in the background voltammograms collected from the carrier solution.

We carried out further studies on the relationship between the scan rate and the peak current of the methylmercury radical oxidation. Shown in Figure 2 is a series of fast-scan voltammograms of a 0.5 mM solution of methylmercury obtained at various scan rates. In Figure 2, only the segments in the anodic-going direction are presented to illustrate the scan-rate dependence of the anodic peak currents. For 0.5 mM methylmercury solutions, we noticed that scan rates below 50 V/s were not fast enough to completely outrun the dimerization reaction of methylmercury radicals. When the scan rate was increased to 50 V/s or higher, methylmercury radicals began to be reversibly oxidized. This is evidenced by the proportionality between the scan rate and the anodic peak height. For example, the data in Figure 2 show that i_p at 100 V/s is 1.92 times as much as i_p at 50 V/s, whereas i_p(50 V/s)/ i_p(25 V/s) is only 1.73. This proportionality indicates that methylmercury radical can rapidly adsorb onto the mercury film electrode and becomes reoxidized during the scan reversal. To verify the concentration dependence of the dimerization reaction, we also analyzed the relationship between the peak current and the methylmercury concentration for a given scan rate. This point will be discussed below in connection with the calibration plot construction.

3.2. Analytical Performance

The quantitative aspect of the flow-injection fast-scan voltammetric analysis of methylmercury was examined. A
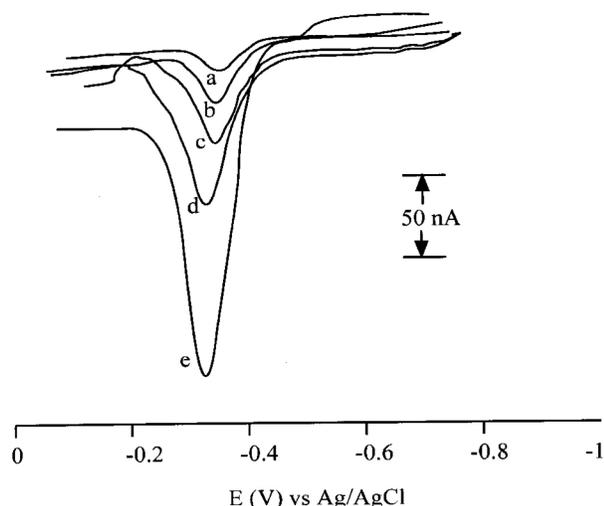
Figure 2. Background-subtracted, fast-scan linear-sweep voltammograms of 0.5 mM CH$_3$Hg$^+$ obtained at different scan rates: a) 5 V/s; b) 10 V/s; c) 25 V/s; d) 50 V/s; and e) 100 V/s. The thin Hg film was deposited onto a 25-μm-diameter Pt microelectrode.
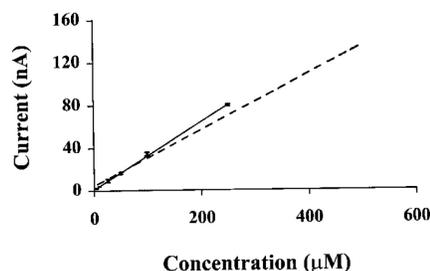
Figure 3. Calibration plot for methylmercury determination at 50 V/s. The concentrations determined are 5, 10, 25, 50, 100, and 250 μM, respectively. Each point was an averaged value of three peak currents deduced from background-subtracted voltammograms. Absolute standard deviations are shown as the error bars. Dotted calibration plot included the 500 μM methylmercury concentration and produced a much larger intercept.

calibration plot for a concentration range at an appropriate scan rate was constructed. Figure 3 shows the calibration plot for methylmercury in the concentration range between 5 μM and 250 μM. The relationship between the averaged peak current i_p in nA and concentration C in μM is given by the regression equation: i_p = 0.32 C +1.09. A very good linear relationship between the peak current and the concentration, reflected by an excellent regression coefficient of 0.9994, was observed. It is interesting to note that the regression including the 500 μM methylmercury solution (dotted line curve in Figure 3) yielded a smaller regression coefficient, 0.9877, and a greater intercept, 4.18. These values suggest that the current-concentration relationship begins to deviate from the expected linearity and 50 V/s was simply not fast enough for 500 μM of methylmercury. This trend is consistent with the aforementioned studies on the scan-rate dependence of the peak current. When the methylmercury concentration is high, the dimerization rate increases and starts to compete with the methylmercury radical oxidation. As a consequence, faster scan rates must be utilized to construct a quantitative calibration plot to include higher concentrations.

The reproducibility of the measurements based on this technique was also investigated. The relative standard deviation measured from five consecutive voltammetric runs was found to be typically in the 3–10% range. Absolute standard deviations are represented by the error bars in Figure 3.

The concentration detection limit of the flow-injection fast-scan analysis was estimated to be 0.56 μM for methylmercury at a scan rate of 50 V/s (S/N = 3). Such a detection limit is within the concentration range of methylmercury present in many polluted organisms [1]. For example, Grieb et al. measured methylmercury content in yellow perch collected from a drainage lake [22] and reported a methylmercury weight ratio around 1–2 μg/g. This 1– 2 μg/g methylmercury weight ratio would correspond to about 0.88–1.78 μM in a 5.30-mL extract for the digestion procedure described in Section 2. The certified methylmercury value (4.64 μg/g) in the DORM-2 dogfish muscle sample, similar to that determined from many other marine species that are capable of accumulating methylmercury, is even higher. Since the oxidation peak current is proportional to the scan rate [23], much lower detection limits should be expected at very fast scan rates (e.g., 1 kV/s or higher). We could not attempt such experiments because of the scan-rate limitation of our instrument (about 500 V/s).

3.3. Repetitive Cyclic Voltammetry

To demonstrate the feasibility of performing automatic analysis using fast-scan voltammetry in our microflow system,
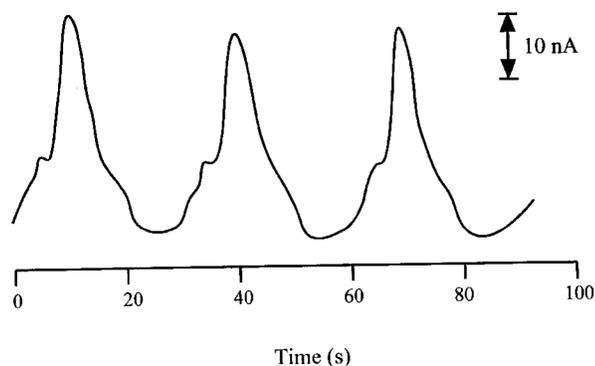
Figure 4. Current-time curve obtained during a repetitive cyclic voltammetric experiment conducted in a programmed flow-injection procedure. Experimental conditions: initial potential = –0.2 V and switching potential = –0.9 V. The scan rate was 50 V/s and the methylmercury concentration was 100 μM. Each injection lasted for 15 s and the valve was switched back to the load position for 10 s for each sample loading. A 10-μm-diameter Pt microelectrode was used for forming the Hg film microelectrode.

Figure 5. Fast linear-scan voltammograms of a) 25 μM CH$_3$Hg^{++} and 250 μM Hg^{2+} (solid line curve), and b) 250 μM Hg^{2+} obtained at Hg film microelectrode deposited onto a 25-μm-diameter Pt substrate (dotted line curve). The scan rate employed was 50 V/s.

we carried out repetitive cyclic voltammetric experiments with multiple injections. Figure 4 shows a representative current-time curve obtained from a programmed procedure during which three injections of a 100-μM methylmercury solution were made. Each injection lasted 15 s and corresponds to a 7.5-μL sample consumption. This means that only 0.75 nmol of methylmercury is needed for each flow-injection analysis. In this experiment, the potential at the Hg microelectrode was continuously scanned between –0.2 V and –0.9 V, while currents were integrated from voltammograms between –0.4 V and –0.65 V in the anodic segment. The CV6 software can plot the current integrated over the oxidation peak as a function of the acquisition time. As can be seen in Figure 4, the repetitive cyclic voltammetric experiment yielded reproducible peaks with a standard deviation of about 4.8 % for the concentration determined. Owing to the fact that a few seconds are needed to flush the methylmercury out of the cell at microbore flow rates, relatively broad peaks were observed. We estimated that, for the internal cell volume of about 7 μL and a flow rate of 30 μL/min, it would take at least 14 s to wash the analyte from the previous injection out of the cell system. Such an estimate appears to be consistent with the time between the peak and the baseline displayed in Figure 4.

The good signal-to-noise ratio and the relatively high currents suggest that fast-scan voltammetry is a sensitive technique. Since the Pt-based Hg film microelectrode is hydrodynamically stable, this methodology provides an attractive avenue for continuous monitoring of methylmercury species at low levels.

3.4. Determination of Methylmercury in the Presence of Inorganic Mercury

The potential utilization of our methodology for mercury speciation was also explored. Curve a in Figure 5 shows the linear fast-scan voltammogram obtained from injecting a sample containing 25 μM CH$_3$Hg$^+$ and 250 μM Hg^{2+}. Again, the anodic peak at –0.44 V arose from the oxidation reaction of methylmercury radical. The voltammogram obtained under the same experimental condition after injecting a 250 μM Hg^{2+} is shown as Curve b. Clearly, the oxidation of inorganic mercury does not occur in the potential range where methylmercury radical oxidizes. In fact, we observed that the deposited Hg film would be stripped at 0.35 V, a value that is more positive than the poten-

tial regime depicted in Figure 5. The large difference between the oxidation potential of methylmercury and that of elemental mercury provides the basis for selective determination of methylmercury in the presence of inorganic mercury.
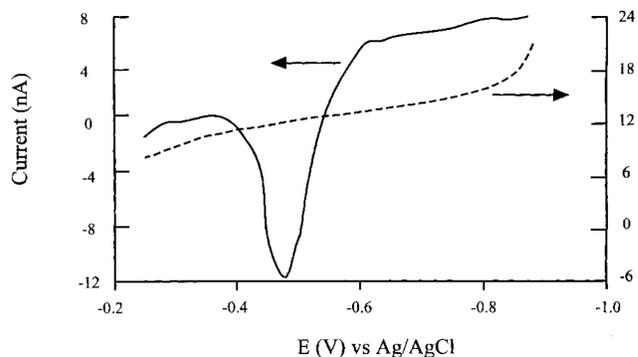
3.5. Real Sample Analysis

Finally, we applied our approach to the analysis of a real world sample. A digested/extracted dogfish muscle sample was analyzed for methylmercury using the above calibration plot. The level of methylmercury in this sample was elevated by spiking 20 μL of a 4.60 mM standard methylmercury chloride solution into 1.5045 g of the powdery sample. We determined the methylmercury concentration in the final extract to be 16.7 μM. This value is in good agreement with that measured from a separate ICP-AES experiment (15.5 μM). Since the extraction procedure has a recovery of about 74 %, the methylmercury concentration would be around 3.0 μM in the final extract for the original DORM-2 sample. This concentration would be close to the detection limit and towards the end of the above calibration plot. Therefore, we elevated the methylmercury content of the untreated dogfish muscle slightly to validate our method with a somewhat higher concentration that can be more accurately measured.

It is worth noting that toluene and thiosulfate present in the extract are problematic to the operation of the ICP sample introduction and plasma. Consequently, a 10-fold dilution had to be made to decrease the concentrations of residual toluene and sodium thiosulfate introduced to the ICP sample introduction system. The flow-injection fast-scan voltammetry, however, is less prone to possible interferences caused by species used for sample extractions, suggesting that this method should be amenable to methylmercury analysis in complex sample media.

4. Conclusions

Fast-scan voltammetry conducted in our microflow system, originally designed for anodic stripping analysis, has been extended to the selective determination of methylmercury. The mechanism and cyclic voltammetric behavior of methylmercury at Pt-based Hg film microelectrodes were found to be very similar to that observed at the dropping mercury electrode. Flow-injection fast-scan voltammetry was demonstrated to be simple, reproducible, and rapid for methylmercury analysis. A concentration detection limit of 0.56 μM has been ob-

tained. The fast-scan flow-injection system typically consumes a few μL of sample and can detect less than a nanomole of methylmercury. Since the oxidation potential of inorganic mercury is very different than that of the methylmercury radical, the presence of large amounts of inorganic mercury does not cause interference. Through the analysis of an elevated dogfish muscle sample, this approach was demonstrated to be a viable analytical procedure for methylmercury determination in complex sample media.

5. Acknowledgements

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