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Cloud Point Extraction for Electroanalysis: Anodic Stripping Voltammetry of Cadmium

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Abstract

Cloud point extraction (CPE) is a well-established technique for the pre-concentration of hydrophobic species from water without the use of organic solvents. Subsequent analysis is then typically performed via atomic absorption spectroscopy (AAS), UV-Vis spectroscopy, or high performance liquid chromatography (HPLC). However, the suitability of CPE for electroanalytical methods such as stripping voltammetry has not been reported. We demonstrate the use of CPE for electroanalysis using the determination of cadmium (Cd^{2+}) by anodic stripping voltammetry (ASV) as a representative example. Rather than using the chelating agents which are commonly used in CPE to form a hydrophobic, extractable metal complex, we used iodide and sulfuric acid to neutralize the charge on Cd^{2+} to form an extractable ion pair. Triton X-114 was chosen as the surfactant for the extraction because its cloud point temperature is near room temperature (22–25° C). Bare glassy carbon (GC), bismuth-coated glassy carbon (Bi-GC), and mercury-coated glassy carbon (Hg-GC) electrodes were compared for the CPE-ASV. A detection limit for Cd^{2+} of 1.7 nM (0.2 ppb) was obtained with the Hg-GC electrode. Comparison of ASV analysis without CPE was also investigated and a 20x decrease (4.0 ppb) in the detection limit was observed. The suitability of this procedure for the analysis of tap and river water samples was also demonstrated. This simple, versatile, environmentally friendly and cost-effective extraction method is potentially applicable to a wide variety of transition metals and organic compounds that are amenable to detection by electroanalytical methods.

Keywords

Cloud point extraction; electroanalysis; cadmium; stripping voltammetry; glassy carbon electrode

Cloud point extraction (CPE) is used for the extraction of hydrophobic species from a variety of media without using an organic solvent.^{1–3} The ability to extract analyte from a complicated sample matrix and also concentrate it in a smaller volume for analysis without

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Notes

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the use of harmful organic solvents that require proper disposal has driven the considerable interest in this method.¹⁻⁶ Analysis is typically performed by atomic absorption spectroscopy (AAS), UV-Vis spectroscopy, or high performance liquid chromatography (HPLC).¹⁻⁸ However, the suitability of cloud point extraction for electroanalytical methods, such as stripping voltammetry, has not been reported.

CPE incorporates a surfactant molecule that when above its critical micelle concentration and cloud point temperature, forms micelles in solution. Within these conditions, when the solution is heated above the cloud point temperature it becomes turbid and a micellar phase begins to form either on top or bottom of the sample solution (orientation depends on density of surfactant and components of the solution). The micelles encapsulate the target analyte molecules which are extracted and pre-concentrated into the micellar phase.¹⁻⁸ This results in an improvement in sensitivity that lowers the detection limit. In the case of metal ions, a ligand or chelating agent is used to form a hydrophobic metal ion complex that is extractable.

Electrochemical methods are attractive for trace metal detection due to low cost, excellent sensitivity and selectivity, and suitability for miniaturization.⁹ Square-wave stripping voltammetry (SWSV) is a common electroanalytical technique used for trace metal analysis with detection limits down to the 10^{-10} M level.^{10,11} SWSV is typically more sensitive than other voltammetric methods due to the incorporation of a pre-concentration or deposition step.¹⁰⁻¹² In stripping voltammetry, the working electrode is held at a deposition potential sufficient enough to reduce (anodic stripping voltammetry, ASV) or oxidize (cathodic stripping voltammetry, CSV) the analyte onto the electrode surface.¹⁰⁻¹⁴ Longer deposition times can result in lower limits of detection. The potential is then swept in a positive direction (ASV) or a negative direction (CSV) in order to re-oxidize or re-reduce the deposited analyte back into solution and the resulting current is measured.¹⁰⁻¹⁴ Stripping voltammetry is fully capable of multi-element detection, making it an efficient mode of detection as well.

Cadmium (Cd) is a toxic transition metal known to cause cancer in humans.¹⁵⁻¹⁸ Furthermore, it is capable of causing damage to a number of organ systems leading to kidney failure, respiratory issues, gastro-intestinal problems, and neurological complications.^{15,17} The Occupational Safety and Health Administration (OSHA) estimates that 300,000 workers are exposed to cadmium each year, mostly in industry sectors.¹⁶ Other modes of exposure include via drinking water where the United States Environmental Protection Agency's (US EPA) maximum contaminant level (MCL) for Cd is 44 nM (5 ppb).¹⁹ Thus, its trace detection is important to human health.

In this paper, we introduce cloud point extraction with electrochemical detection. We chose ASV as the electrochemical method because of its exceptionally low detection limit and our interest in creating disposable point-of-care sensors for detection of heavy metals.²⁵ Cd^{2+} was used as a representative analyte because of its ideal properties for ASV and its importance to health as discussed above. The issues involved in adapting CPE to an electrochemical technique such as ASV, an evaluation of three different electrodes for the ASV step, and a comparison of results with AAS are described.

Experimental

Chemicals and Materials

Potassium iodide (KI), sulfuric acid (H₂SO₄), sodium carbonate Na₂CO₃, and Triton X-114 were purchased from Fisher Scientific and used without further purification. House distilled water was further purified with a D2798 Barnstead water purification system and was used for all standard solutions. A 1000 mg/L Cd²⁺ atomic absorption standard solution was purchased from Acros Organics and diluted to desired concentrations. Acetate buffer (pH 4.65, 0.2 M) was purchased from Sigma Aldrich. Mercury and bismuth solutions were made by diluting mercuric nitrate (HgNO₃, J.T Baker Chemical Co.) and a 1000 mg/L bismuth (Bi³⁺) atomic absorption standard solution (Sigma) in deionized water. Tap water samples were collected in Crosley Tower at the University of Cincinnati on July 18th, 2014. River water samples were collected from the Little Miami River (Cincinnati, Ohio) and Burnet Woods Pond (Cincinnati, Ohio) on July 18th, 2014. No further treatment of the water samples was done and they were immediately used. All water samples were spiked with various concentrations of Cd²⁺ standard.

Instrumentation

ASV measurements were executed in a 20 mL conventional three electrode cell consisting of a glassy carbon working electrode (bare or modified) (BASi), a Ag/AgCl reference electrode (3.0 M KCl solution), and a platinum (Pt) wire auxiliary electrode. Between measurements, the working electrode was polished with an alumina slurry and polishing pad before being rinsed and dried with a Kimwipe. Solution volumes varied from 2–10 mL and all vessels used for trace analysis were soaked in 10–15% nitric acid for 24 h prior to use. The potentiostat was a BASi 100B Electrochemical Analyzer. The basic parameters for Osteryoung square wave voltammetry were: square wave amplitude = 25 mV, step potential = 5 mV and frequency = 25 Hz. The extrapolated baseline current method described by Kissinger and Heineman was used to measure peak currents (*i_p*).¹¹ AAS measurements were performed using a Varian 2240FS Atomic Absorption Spectrometer equipped with a 228.8 nm Cd hollow cathode lamp. Lamp current was 4 mA and a 13.50:2.00 L/min flow rate ratio of air:acetylene was used.

Procedure

Figure 1 shows the scheme for CPE. 25 mL solutions containing 0.04–4.0 μM Cd²⁺ ion were added to 50 mL graduated plastic centrifuge tubes. The iodide, sulfuric acid, and Triton X-114 were then added and diluted to 25 mL with deionized water. The tubes were immediately vortexed for 10 s until the solutions appeared turbid and were mixed well. Triton X-114 was heated to 60° C before addition to the solution to improve reproducibility in the micro-pipetting by decreasing viscosity.

The solutions were then placed in a water bath at 55°C for 30–45 min. Once phase separation was apparent, the solutions were removed from the water bath and centrifuged at 3500 rpm for 12 min. Then, the solutions were placed in an ice bath for 5 min causing the micellar phase to become very viscous and the aqueous phase could be easily poured off the top. Three different electrodes that are commonly used for ASV were used for the final

detection step: bare glassy carbon (GC), bismuth-coated glassy carbon (Bi-GC), and mercury coated glassy carbon (Hg-GC). For ASV with GC and Bi-GC, 2.5 mL of ethanol and 3.5 mL of pH 4.65 acetate buffer were added to the micellar extract for a final solution volume of 6.5 mL. For ASV with Hg-GC, the micellar phase extract was then diluted with 1.0 mL of ethanol and 1.0 mL of Na₂CO₃ for a final solution volume of 2.25 mL. For AAS, the extract was diluted with 0.750 mL of ethanol and 1.5 mL of pH 4.65 acetate buffer. Approximately 3.0 mL of solution was needed for AAS measurements so that 3 replicates could be measured.

Mercury and bismuth films on GC were prepared by properly diluting the concentration of a Hg²⁺ or Bi³⁺ stock solution to 0.5 mM in the post-extraction analysis solution. The potential was held at -500 mV for 2 min to form a base bismuth or mercury film before shifting the potential to -1100 mV for a variety of deposition times (2–20 min) for cadmium deposition. When using the bare glassy carbon electrode, the potential was held at -1100 mV for 5–10 min unless otherwise noted. Stirring was then stopped and 15 s was allowed to pass before the potential was swept from -1100 mV to -350 mV. To clean the electrode following each trial, the potential was held at +500 mV to oxidize any remaining Cd on the electrode surface. The potential was then scanned with no deposition to verify that all Cd was removed. If any Cd remained on the electrode surface after these two steps, the electrode was polished again before use in subsequent analyses.

Results and Discussion

Choice of Complexing Agent

To extract heavy metals with CPE, a chelating agent is typically used to neutralize the charge on the metal to form a sufficiently hydrophobic species that can be encapsulated in the micelles. Some complexing agents reported in literature include but are not limited to 1-pyridylazo-2-naphthol, dithizone, and diethyldithiocarbamate.^{26–28} Chelating agents like these are suitable for techniques such as AAS because the compound is burned and ionized in a flame or ashed with a graphite furnace. However, many of these ligands form complexes with Cd²⁺ that are too stable to analyze via ASV because the reduction potential is shifted too negative for the complexed metal ion to be reduced to the metal.²⁹ For example, 1-pyridylazo-2-naphthol was investigated for its use with CPE-ASV. No Cd²⁺ peaks were observed in the voltammograms even at the most negative deposition potential possible without extensive solvent reduction, validating that the compound was too stable to deposit any Cd onto the electrode surface. Without the ligand, Cd²⁺ alone will not extract because of its 2+ charge, increasing its hydrophilicity.

On the other hand, Cd²⁺ forms complexes with halides that neutralize the charge while being sufficiently weak to not prevent metal deposition in the preconcentration step.²⁹ I⁻ was chosen due to its greater hydrophobicity relative to that of the other halides because of its larger atomic radius. Cadmium and iodide are known to form the following complexes in aqueous media: CdI⁺, CdI₂, CdI₃⁻, and CdI₄²⁻ with CdI₄²⁻ being the most stable.^{29,30} This is shown in Figure S1, a distribution diagram of the Cd-I complexes with increasing I⁻ concentration. In fact, 99% of the Cd²⁺ complexes formed in these experiments is CdI₄²⁻ and very little is in the neutral, extractable CdI₂ form.³⁰ Because CdI₄²⁻ is the main Cd-I

complex formed, its charge must be neutralized so that it will be extracted into the micellar phase. This is where the use of sulfuric acid becomes crucial. We and others observed that these cadmium complexes can be extracted using CPE as ion pairs $[2\text{H}^+, \text{CdI}_4^{2-}]$ as well as the neutral CdI_2 species.³¹

Surfactant

Electrochemical detection for CPE can be an ideal alternative to other analysis methods because most surfactants (including Triton X-114) are electroinactive. There are a variety of surfactants that can be used in CPE, all of which yield excellent extraction efficiencies.²⁻⁴ Triton X-114 was used in this extraction because its cloud point temperature is near room temperature (22–25° C). This allows for shorter extraction times because the phases separate more readily. Triton X-100 was explored as well and similar results were obtained but its cloud point temperature is around 65° C, requiring longer incubation time and higher incubation temperature.

Extraction Parameter Optimizations

In order to develop CPE for use with ASV, the concentrations of each of the components in the extraction (iodide, sulfuric acid, Triton X-114) were optimized for their use in the subsequent experiments. The CPE procedure described above was followed for each optimization with different concentrations of the parameter to be optimized. All optimizations were initially done with a GC electrode. However, the Hg-GC subsequently proved to be most suitable for real water sample analysis, so all parameter optimizations were repeated with this electrode material. Only results for the Hg-GC electrode are presented here.

Extraction efficiency is directly dependent on the concentration of iodide that is needed to convert Cd^{2+} into an extractable form. The effect of iodide concentration over a range of 5–45 mM is shown in Figure 2A using both ASV and AAS. Complexation of the iodide with cadmium causes the compound to become more hydrophobic and the peak current increases with I^- concentration until the Cd^{2+} is essentially all extracted. As expected, the trends for both analysis techniques were similar in that the signal increased until the concentration reached 30 mM, where the current/absorbance readings began to level off as all of the Cd^{2+} is efficiently converted into an extractable form. However, at higher iodide concentrations (40–45 mM), oxidation of iodide (I^-) to iodine (I_2) was observed on the counter electrode (Pt wire) during ASV analysis. This did not affect current response but may require cleaning of the Pt wire after each trial. Thus, an iodide concentration of 30–35 mM was chosen as the optimal condition for extraction and analysis techniques.

Because the primary metal-ligand complex formed is CdI_4^{2-} as shown in Figure S1, sulfuric acid directly influences extraction efficiency by donating protons to form a neutral, extractable ion pair, $[2\text{H}^+, \text{CdI}_4^{2-}]$. A range of 0.1–1.25 M sulfuric acid was explored with both ASV and AAS. As shown in Figure 2B, the response increased up to 1.0 M for both analytical methods as increasingly larger fractions of cadmium were extracted. Lower acid concentrations decreased extraction efficiency due to the reasons explained previously and no Cd^{2+} extraction was observed for the 0.1 M H_2SO_4 samples. Acid concentrations higher

than 1.0 M were more difficult to extract because the micellar phase would appear on top rather than the bottom of the solution due to the increased density of the aqueous phase. Consequently, 1.0 M was chosen in order to maximize the extraction efficiency.

Acid concentration is also a critical parameter in the ASV detection step following the extraction because higher acid concentrations can become an interference by causing issues with the electrode hydrogen overpotential. This problem was experienced with ASV at higher acid concentrations (0.6, 0.75, 0.85, 1.0 M) where repeatability of the current response was worse than at lower acid concentrations. Reduction of H^+ at the electrode surface becomes more prevalent and hydrogen bubbles formed on the electrode surface, interfering with the deposition. In addition, the hydrogen reduction wave becomes much more dominant, making it more difficult to establish a reproducible baseline for accurately measuring the peak current. To address this issue, post-extraction adjustment of solution pHs prior to the ASV measurement was investigated. Solution volume needs to be kept to a minimum so as not to adversely affect the pre-concentration and improvement factors, which are discussed below. After the addition of ethanol to the 1.0 M sulfuric acid samples, the pH observed was ≈ 0.9 . Addition of 1.0–2.0 mL of pH 4.65, 5.0, 5.5, 6.0, and 6.5 acetate buffer was investigated and pH was found to increase up to 1.4 when adding pH 6.5 buffer. This pH still caused significant bubbles, even on the Hg-GC surface, which has the best hydrogen overpotential of the three electrodes compared. This pH trend continued with the addition of more acetate buffer until the solution reached pH 2 where the bubbles and hydrogen overpotential were no longer an issue and reproducible current responses were observed. At this point however, because the solution volume had increased to 7.0 mL, the pre-concentration factor had decreased to a point where the extraction could be considered almost negligible. To solve this problem, use of a concentrated Na_2CO_3 solution was explored to increase the extract solution pH while not significantly increasing overall analysis solution volume. Addition of 0.85–1.0 mL increased the pH to a workable range of 1.9–2.0 while keeping the solution volume to 2.25 mL and thereby retaining the preconcentration factor.

Figure 2C shows the Triton X-114 concentration optimization, an important parameter for both the extraction and ASV procedures. The addition of more surfactant increases the volume of the micellar phase, which in turn increases the viscosity of the final analysis solutions. The more viscous the solutions become, the slower the mass transport of Cd^{2+} ions to the electrode surface during the deposition step. Surfactant concentrations of 0.1%–3% (v/v) were investigated. Current is essentially zero for the 0.1 % samples and increases until it levels around 0.5 % where the micellar phase was only about 0.5 mL. However, as more surfactant is added, more ethanol is needed to dissolve the micellar extract, also decreasing the pre-concentration and improvement factors as 1.0 % (v/v) yields a micellar phase volume of 1.0 mL. Therefore, 0.5% (v/v) Triton X-114 was chosen to be optimal due to these reasons.

Ethanol helps dissolve the surfactant molecules and break up the micelles formed during extraction. If this does not happen, some of the cadmium remains within the micelle where it is essentially sequestered from the electrode process needed for ASV. As a result, current readings will be low because those ions will not reach the electrode surface and undergo

reduction during the deposition step. This trend is observed in Figure 3D where the ASV current increases from essentially zero at 5% ethanol, where most of the Cd^{2+} is trapped in the micelles, to a maximum at about 50 % (v/v) where the micelles are broken up, releasing the Cd^{2+} for electrochemical deposition. 50% (v/v) of ethanol with water was chosen since it gives the best sensitivity.

Stripping Voltammetry Parameter Optimizations

Osteryoung square wave mode was used for the stripping step in the ASV analyses; deposition potential, and deposition time were the parameters examined. Osteryoung square wave was chosen due to its ability to minimize non-faradaic current.¹⁵

The deposition potential is a critical parameter in stripping voltammetry. In ASV, the deposition potential is typically a potential negative relative to the standard reduction potential to sufficiently reduce ions to atoms on the electrode surface. Deposition can become an issue when moving to very negative potentials where the hydrogen overpotential can affect different electrode materials. Different electrodes have different potential windows, limiting how far negative or positive they can extend. Mercury electrodes have an advantage over other electrode materials because they have an excellent negative potential window. Figure 3A shows the deposition potential optimization on Hg-GC; a range of -800 to -1250 mV was measured. Deposition potentials more positive than -950 mV gave no current response because no metal was reduced. From that point, current readings began to increase and reached a maximum at -1100 mV. At more negative deposition potentials, the reduction wave of H^+ ion began to become an issue and hydrogen bubbles began to form on the electrode surface. As a result, current decreased because the electrogenerated bubbles interfered with the deposition process by decreasing the effective electrode surface for electrolysis.

Deposition times can affect current response significantly. As the deposition time increases, more analyte molecules deposit on the electrode surface. This time can be increased to a point where analyte is effectively depleted from the solution onto the electrode surface. Often, these longer times are not needed to achieve sufficient detection limits, but it can be an important aspect of this analysis method when the lowest possible detection limit is required. Figure 3B shows the deposition time optimization on the Hg-GC. It can be observed from the graph that current increased up to a deposition time of 15 min where it began to level off because saturation is being reached within the mercury film or depletion of Cd^{2+} in the solution. A deposition time of 5 min was chosen for Hg-GC because it gave the best linear response over the widest range when doing calibration experiments for finished water sample analysis. Deposition times of 10 and 20 min gave lower detection limits but the calibration curves were linear over a much smaller range than a deposition time of 5 min. A deposition time of 5 min was chosen for the GC and Bi-GC electrodes.

Glassy Carbon Electrode Modifications

Mercury and bismuth modified glassy carbon electrodes have been extensively investigated in literature.^{32,33} Mercury is an attractive electrode material because of its superb negative potential window and its ability to form an amalgam with target metal analytes rather than a

separate phase, which is observed with solid electrode surfaces such as glassy carbon.^{32,33} Formation of an amalgam in mercury electrodes increases sensitivity because of its ability to eliminate background interferences.³²⁻³⁷ Bismuth offers a similarly high hydrogen over-potential to that of mercury but does not offer the liquid electrode interface advantages that mercury does. Bismuth film electrodes are also less likely to experience interferences from dissolved oxygen.³²⁻³⁷ With this, due to its non-toxicity, bismuth has been an enticing replacement for mercury electrodes.³²⁻³⁷ Thus, these three electrode materials were investigated for their applicability to cloud point extraction for trace Cd^{2+} determination.

Figure 4 compares performance of the mercury-coated (Hg-GC), bismuth-coated (Bi-GC), and bare glassy carbon (GC) electrodes. It can be seen that at this low level of Cd ($[\text{Cd}^{2+}] = 500 \text{ nM}$, 50 ppb) almost negligible current response was obtained from GC. The Bi-GC gave a larger current response while the Hg-GC showed a significant increase in peak current. It can also be seen in Figure 4 that the peak potential shifts when a mercury film is used. The amalgam formation described previously makes Cd^{2+} easier to reduce because the reduction product, metallic Cd, is stabilized by its amalgamation with mercury. Although GC shows easily measured current response for higher Cd^{2+} concentrations, Figure 4 shows that it may not be sensitive enough at this deposition time for trace metal analysis, depending on the concentration of the sample. Because of its significantly greater sensitivity, the Hg-GC electrode was further evaluated.

Foreign Ion Effects

Two types of interferences by other ions were investigated: interference with the efficiency of extraction by CPE and interference with the ASV detection. Major cations and anions in tap water and water from environmental sources are Ca^{2+} , Mg^{2+} , K^+ , Na^+ , CO_3^{2-} , SO_4^{2-} , and Cl^- . Concentrations of these ions can range from 0.1–10 ppm (mg/L) depending on the hardness and source of the water.^{38,39} Each of these ions were added at concentrations up to 2.5 ppm (mg/L) to observe the effects on the extraction efficiency of trace Cd^{2+} . ASV can experience interferences from foreign metal ions that may reduce onto the electrode surface when using such negative deposition potentials (–1100 mV). Pb^{2+} , Zn^{2+} , Bi^{3+} , Fe^{3+} , Cu^{2+} , Co^{3+} , and Sn^{2+} (50 ppb each) were also added with the ions mentioned previously to observe their effects as interferences on extraction and ASV analysis. Sn^{2+} was added due to the fact that its stability constants with iodide are similar to that of Cd^{2+} . No issues were observed when analyzing 35 ppb ($\mu\text{g/L}$) Cd^{2+} samples in the presence of any of these ions as shown in Figure S2.

Calibration and Electrode Material Comparison

To evaluate CPE-ASV as a method to quantitatively pre-concentrate and determine Cd^{2+} concentration in water, a series of Cd^{2+} standards was studied under the optimized conditions described above. The performance of the three electrode materials was also evaluated for CPE-ASV. For the GC and Bi-GC electrodes, 2.5 mL of ethanol and 3.0 mL of pH 4.65 acetate buffer was added to the micellar phase extract after CPE was completed and a deposition time of 5 min was used. The addition of Na_2CO_3 that was done with the Hg-GC electrode gave poorer sensitivity and detection limit on GC and Bi-GC than the addition of higher volumes of buffer. For GC, a linear response was obtained from 0.667 to 3.11 μM [Y

$= (0.021 \pm 0.005)x \text{ (}\mu\text{A/nM)} + (4.7 \pm 0.5) \text{ (}\mu\text{A)}$, $R^2 = 0.989$] with a calculated ($3\sigma/m$) detection limit of 174 nM (20 ppb). However, no current response was observed for samples below 667 nM (75 ppb). These results signify that a GC electrode is a poor choice for trace determination of Cd^{2+} because the detection limit is rather poor with a deposition time of 5 min. The Bi-GC electrode gave slightly better sensitivity and yielded a better detection limit. A linear response was obtained from 0.178 to 1.34 μM [$Y = (0.029 \pm 0.001)x \text{ (}\mu\text{A/nM)} - (4.9 \pm 0.8) \text{ (}\mu\text{A)}$, $R^2 = 0.994$] with a calculated ($3\sigma/m$) detection limit of 96 nM (11 ppb). However, no current response was seen for any concentrations lower than 174 nM (20 ppb). Though the detection limit is lower, it would also be difficult to use a bismuth film electrode at 5 min deposition for trace determination of Cd^{2+} . This small improvement from the bare glassy carbon electrode can be attributed to the advantages described earlier. The increase in hydrogen over potential from the bare glassy carbon caused a twofold improvement in detection limit. As expected based on the voltammograms in Figure 4, the mercury film electrode exhibited the lowest detection limit, best sensitivity, as well as the greatest linearity. A linear range was achieved from 9–889 nM [$Y = (0.136 \pm 0.004)x \text{ (}\mu\text{A/nM)} - (3.2 \pm 1.5) \text{ (}\mu\text{A)}$, $R^2 = 0.996$] with a calculated detection limit of 1.7 nM (0.2 ppb) but no signal could be distinguished from baseline below 4.0 nM (0.5 ppb). Figure 5A shows voltammograms for each standard concentration on Hg-GC and Figure 5B shows an expanded current scale for better viewing of the lower Cd^{2+} concentrations. These results confirm that a mercury film electrode is best for precise determination of Cd^{2+} in the water samples after CPE as it offers a 10x improvement in detection limit over Bi-GC at the same deposition time.

To compare ASV and AAS, CPE with the same set of standard concentrations used for the ASV measurements at Hg-GC was analyzed with AAS. A linear response was obtained [$R^2 = 0.994$] and a detection limit of 7.8 nM (0.8 ppb) was obtained. This shows that Hg-GC can yield an even better detection limit than AAS, a common analysis method for many CPE procedures, with a deposition time of just 5 min. This 4x improvement in detection limit could be further improved with longer deposition times.

As stated previously, the detection limit can be further improved with Hg-GC at longer deposition times but with a smaller linear concentration ranges, and this concept was also demonstrated. Solutions containing 4–44 nM Cd^{2+} were extracted using the CPE procedure described previously. By increasing the deposition time to 10 min, a linear response was obtained over this concentration range [$Y = (0.38 \pm 0.01)x \text{ (}\mu\text{A/nM)} - (0.39 \pm .335) \text{ (}\mu\text{A)}$, $R^2 = 0.998$] with a calculated detection limit of 0.1 nM (0.01 ppb, 11 ppt). This is a 17x decrease in detection limit compared to the 5 min deposition time. At concentrations higher than 44 nM, linearity is lost. Further increasing the deposition time to 20 min results in even better sensitivity and even lower detection limit, but the magnitude is smaller. A linear response was obtained [$Y = (1.04 \pm 0.03)x \text{ (}\mu\text{A/nM)} - (1.54 \pm 0.72) \text{ (}\mu\text{A)}$, $R^2 = 0.997$] with a calculated detection limit of 0.06 nM (0.007 ppb, 7 ppt), a 28x and 1.7x improvement in detection limit over 5 min and 10 min deposition times, respectively. Similar to the 10 min deposition time, linearity is lost when higher Cd^{2+} concentrations than 44 nM are used.

This is another potential advantage of CPE-ASV because it adds an adjustable parameter depending on the required detection limit. Techniques like AAS and HPLC can only get pre-

concentration from the extraction whereas CPE-ASV offers additional pre-concentration from the deposition step. However, it must be noted that mercury samples require extra care when handling and the cost to dispose of mercury waste is higher than that of other heavy metals.

Natural Matrix and Finished Water Sample Analysis

To further assess the use of each electrode material, Cd^{2+} detection in more complex matrices was evaluated and results compared with AAS. The CPE procedure shown in Figure 1 was applied to three water samples – one sample of finished water and two samples of natural water. Cincinnati tap water (Cincinnati, OH) was collected and analyzed with all three electrode materials as well as AAS. Burnet Woods (Cincinnati, OH) Pond water and Little Miami River (Cincinnati, OH) water were collected from shore and analyzed using the Hg-GC electrode and AAS. No Cd^{2+} was detectable in any of the water samples by CPE-ASV with the Hg-GC electrode or AAS. Consequently, known amounts of Cd^{2+} were spiked into the water samples for analysis to determine if these sample matrices provided any interference for CPE-ASV. Different levels of Cd^{2+} were spiked into the samples depending on the sensitivities of the three electrodes. For the GC electrode, the least sensitive electrode, 889 nM (100 ppb) of Cd^{2+} was spiked into the tap water sample while 444 nM (50 ppb) was spiked into the sample analyzed with the Bi-GC electrode. For the Hg-GC electrode and AAS, 130 nM (15 ppb) of Cd^{2+} was spiked into the finished, pond, and river water samples. These concentrations of Cd^{2+} were chosen for spiking because a standard was run at these concentrations as well when completing the calibration curve to simplify data analysis. Because uncertainties in the finished water samples were so great with the GC and Bi-GC electrodes, their applicability to the pond and river water samples was not investigated. No further treatment to the pond or river water was done and they were extracted immediately after collection. The results for all water samples are shown below in Table 1. At ± 50 and ± 31 nM respectively, it can be seen that the uncertainties in the BGC and Bi-GC are too great to be quantitatively acceptable, validating the point that these electrodes are not sensitive enough to be used for finished or natural water sample analysis at such low levels of Cd^{2+} . The uncertainties for both analysis methods are similar, ± 10 nM for Hg-GC and ± 14 nM for AAS, further demonstrating that CPE-ASV with Hg-GC is just as reliable and accurate as CPE-Flame AAS for determination of trace Cd^{2+} .

Statistical Parameters

Under the optimized conditions given, a variety of analytical parameters were determined to show the performance characteristics of CPE-ASV. Accuracy, pre-concentration factors, and improvement factors were determined for ASV using the Hg-GC electrode and the results were compared with AAS measurements made on the same samples. To assess the accuracy, precision, and repeatability of the method, three 222 nM (25 ppb) and 448 nM (50 ppb) Cd^{2+} standard solutions were extracted and analyzed. For ASV, the relative error in Cd^{2+} current response ranged from 4.0–6.0% and the relative standard deviation was 5%.

The pre-concentration factor for the extraction procedure is defined as the ratio of the concentration of Cd^{2+} in the surfactant rich phase to that in the bulk phase, initially. For CPE-ASV, the pre-concentration factor was 10.1. For the AAS conditions used in this work,

the pre-concentration factor was 16.7. This does not include the addition of acetate buffer. Under different AAS conditions, pre-concentration factors can be even higher where less surfactant can be used.³¹ The improvement factor is defined as the ratio of slope of the calibration curve in the extracted samples to that of the pre-extraction solution in the same medium. The same volume of Triton X-114 was pipetted into each set of solutions. To calculate this, a series of Cd^{2+} standards equal to the CPE standards described above were evaluated in pH 4.65 acetate buffer with an Hg-GC with no CPE. The same electrochemical deposition used for CPE-ASV at Hg-GC was followed in the buffer solution as well. A linear response was obtained. $[Y (\mu\text{A}) = (0.016 \pm 0.001)x (\mu\text{A/nM}) + (1.4 \pm 0.2), R^2 = 0.992]$ with a calculated detection limit of 44 nM (5.0 ppb). Comparing this to the slope of the Hg-GC samples after CPE, an improvement factor of 8.4x is obtained. Thus, CPE-ASV yields a 10x increase in pre-concentration, and over 8x increase in sensitivity. The greatest advantage however, is the 13.5x improvement in detection limit when using CPE, signifying a large advantage over traditional experiments done in buffer solutions.

Comparison of Analysis without Cloud Point Extraction

To assess the efficacy of CPE-ASV, a comparison of analyses of a buffer solution without CPE was investigated concurrently with the improvement factor assessment completed previously. The electrode was prepared in the same manner as in CPE, however, no surfactant was added to the solutions used here.

A calibration curve was constructed in the same manner as the CPE samples and a 20x increase in the detection limit was observed (38 nM, 4.0 ppb) with a 5 min deposition time. A deposition time of 30 min was needed to reach a similar detection limit obtained with CPE-ASV (1.9 nM, 0.1 ppb). With this, it can be seen that executing the CPE process (1 hr), results can be obtained much faster because a much shorter deposition time can be used.

Conclusion

A CPE procedure with ASV determination of trace Cd^{2+} was demonstrated. CPE-ASV yielded a 13.5x in improvement in the detection limit compared to traditional ASV done in buffer solutions with no extraction. It was also shown that when using Hg-GC, the detection limit and sensitivity can even be better than those obtained with AAS. GC and Bi-GC are able to perform analysis of Cd^{2+} in water, but may not be suitable for quantitative determination of extremely low levels. An Hg-GC electrode greatly increases the precision, accuracy, and sensitivity method. By optimizing the extraction and analysis methods, a detection limit of 1.7 nM (0.2 ppb) was achieved. With longer deposition times of 10 and 20 min, detection limits of 0.1 nM (.01 ppb, 11 ppt) and 0.06 nM (0.007 ppb, 7 ppt), respectively, were achieved. Techniques like AAS and HPLC do not offer this adjustable parameter.

The ease of execution and elimination of harmful organic solvents makes the extraction method an attractive one. Stripping voltammetry brings more advantages to this process as well with the benefits of low-cost instrumentation and its ability to be miniaturized. HPLC and AAS instruments cost a substantial amount more than potentiostats used for electroanalytical methods. Electroanalytical techniques can be easily miniaturized as well.

Recently, there have been a number of companies manufacturing mini-portable potentiostats with the capability of putting out a number of potential waveforms. Combination of these advantages brings applications to field, point of care, or any real time analysis procedure. This novel combination further exemplifies the versatility and significance of cloud point extraction and anodic stripping voltammetry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cloud point extraction schematic with anodic stripping voltammetry analysis. Working electrode- glassy carbon (bare or modified), reference electrode- Ag/AgCl, counter electrode- Pt wire.

Figure 2.

Concentration optimizations on Hg-GC: (A) iodide with ASV and AAS ($[\text{Cd}^{2+}] = 50$ ppb), (B) sulfuric acid ($[\text{Cd}^{2+}] = 50$ ppb, $[\text{I}^-] = 35$ mM), (C) Triton X-114 ($[\text{Cd}^{2+}] = 50$ ppb, $[\text{I}^-] = 35$ mM), and (D) ethanol ($[\text{Cd}^{2+}] = 25$ ppb, $[\text{I}^-] = 35$ mM). Deposition potential: -1100 mV. Deposition time: 5 minutes.

Figure 3.

(A) Deposition potential optimization on Hg-GC. (B) Deposition time optimization on Hg-GC. $[\text{Cd}^{2+}] = 125 \text{ ppb}$

Figure 4. Voltammogram of Cd CPE extract using mercury-coated, bismuth-coated, and bare glassy carbon electrodes in pH 4.65 acetate buffer. Deposition potential- -1100 mV. Deposition time- 3 minutes. $[Cd^{2+}] = 0.4 \mu M$ (50 ppb).

Figure 5.

(A) CPE-ASV for different concentrations of Cd²⁺ on Hg-GC. (B) Voltammograms of lower concentrations (1, 5 ppb) Deposition potential: -1100 mV. Deposition time: 5 min.

Table 1

Real/Finished water sample analysis with different electrode materials and compared to AAS. BW- Burnet Woods pond water, LMR- Little Miami River water.

Technique	Sample	[Cd ²⁺] Added, nM (ppb)	[Cd ²⁺] Measured, nM (ppb)	% Rec
CPE-ASV-GC	Tap Water	889 (100)	102 ± 5.7	102%
CPE-ASV-Bi GC	Tap Water	445 (50)	51 ± 3.5	101%
CPE-ASV-Hg GC	Tap Water	133 (15)	15 ± 1.1	99%
	BW	133 (15)	15 ± 1.1	98%
	LMR	133 (15)	15 ± 1.1	100%
CPE-AAS	Tap Water	133 (15)	15 ± 1.6	100%
	BW	133 (15)	15 ± 1.6	100%
	LMR	133 (15)	15 ± 1.6	100%