Electrochemical Remedy and Analysis for the Environment Based on the New Polymer-DNA Composite Material.

Noor Feuza Hussain

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Electrochemical Remedy and Analysis for the Environment Based on the New Polymer–DNA Composite Material

A thesis
presented to
the faculty of the department of Chemistry
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of the requirements for the degree
Master of Science in Chemistry

by
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August 2005

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ABSTRACT

Electrochemical Remedy and Analysis for the Environment Based on the New Polymer–DNA Composite Material

by

Noor Feuza Hussain

In this work a new material, the conducting polymer–DNA composite, has been reported for the first time due to its promise in micro extraction, transfer, and release of cations under controlled potential conditions by using electrochemically assisted solid phase micro extraction (SPME). The Polypyrrole/DNA composite can be formed easily by oxidation of pyrrole monomers in the presence of chromosomal DNA by electropolymerization. Environmental significant pollutants such as Cd, Pb, Hg, Co, Zn, Cu, and Bi metal ions can be extracted from the aqueous solution and are able to be transferred to another medium defined as the release solution where the metals were detected by anodic stripping voltammetry. Using Cd$^{2+}$ as a model, this method has been examined to optimize its operational condition. Extraction efficiency and potential interference for this method were studied.
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CHAPTER 1
INTRODUCTION

Effects of Heavy Metals on the Environment

Heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), lead (Pb) and most of the transitional metal elements. They are natural components of the Earth’s crust. Heavy metals can not be degraded or destroyed. Heavy metal elements have relatively high density and at certain level are toxic to human beings. To a small extent heavy metals enter our body via food, drinking water, and air as trace elements. Although some heavy metal elements such as copper, selenium, and zinc are essential to maintain the metabolism of the human body, at higher concentration these elements can be harmful.

Heavy metals have posed a health-risk because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in biological organs over time, compared to its content in the environment. During this process, toxic chemicals are stored faster than they are broken down (metabolized) or excreted in the living organ at any given time.

Cadmium (Cd)

Elemental cadmium is bio-persistent. Once absorbed by an organism Cd will remain resident for many years (over decades in humans). In humans, long term exposure leads to renal dysfunction, lung cancer, and bone defects (osteomalacia, osteoporosis) \(^1\).

Cd metal is produced as a byproduct of zinc refining because these metals usually exist together within the raw ore. The application of Cd includes nickel/cadmium batteries,
corrosion resistance, pigments, stabilizers for PVC plastics, and electronic circuits. It is also present as an impurity in phosphate fertilizers, detergents, and refined petroleum products.

In general, for non smoking population the major Cd uptake pathway is through food, via the addition of cadmium compound to agricultural soil from various sources. Additional Cd exposures to humans arise from trace amount of cadmium in ambient air and drinking water\textsuperscript{(1)}.

**Lead (Pb)**

Humans’ exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. High levels of lead cause problem in the synthesis of hemoglobin, affect the kidneys, gastrointestinal tracts, joints, and reproductive systems, and cause acute or chronic damage to the nervous system. Lead poisoning can cause small, subtle, sub clinical effects, particularly neuro-physiological developments in children.

Although lead enters the human body mainly through food, for some specific groups lead can enter their system by other means such as the plumb solvent water, the lead pipe borne water, and others\textsuperscript{(1)}. Additionally, soil, dust, paint flakes in old buildings, gas stations and abandoned industrial or warehouse lands may contain lead compound residues. Another source is air near the emission points.

Environmental lead contamination comes from both natural and anthropogenic sources. In general for the non-smoking adult population the major exposure pathway of lead is from food and water. For infants and the young children, they are specially susceptible and vulnerable to lead–containing air, water, and dust.
The major application of lead and its compound are batteries, petroleum additives (no longer allowed in the world), rolled and extruded products, alloys, pigments and compounds, cable sheeting, shot, and ammunition.

**Mercury (Hg)**

Mercury is a toxic substance that has no known function in human biochemistry or physiology and does not occur naturally in living organisms. Inorganic mercury poisoning is associated with tremors, gingivitis, and/or minor psychological changes together with spontaneous abortion and congenital malformation \(^1\). Organic mercury such as monomethylmercury causes damage to the brain and the central nervous system, while fetal and postnatal exposure have given rise to abortion, congenital malformation, and developmental changes in young children \(^1\).

The major natural sources of mercury are emissions from volcanoes and evaporations from natural bodies of water \(^1\). Mercury is used in batteries, lamps, and thermometers. It is also used in dentistry as amalgam for fillings and by the pharmaceutical industry.

Natural biological process can convert methylated mercury into forms that can be bioaccumulated over a million-fold in living organisms (especially in fish) in comparison to inorganic mercury in the environment. These monomethylmercury and dimethylmercury are highly toxic, causing neurotoxicological disorders. The main pathway for mercury to humans is through the food chain and not by inhalation \(^1\).

Heavy metals can enter waterways by industrial and consumer wastes. Other pollution routes include weapon–storage sites, contaminated ground water, abandoned mining facility, etc.
Because of the widespread presence of heavy metals, their compounds, their long term environmental impact, and potential health risk, there is an urgent need to monitor and reduce heavy metal elements from the environment.

In this work the environmentally significant Cd, Pb, Hg, Co, Zn, Cu, and Bi metal ions have been studied for their trace measurement and possible remediation. We found that these metal ions can be extracted from the aqueous matrix solution and then transferred to another solution. Further, Cd$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, and Co$^{2+}$ can be extracted and detected by a mixture of solution at the same time.

To serve these monitoring/remediation purpose, a new material, polypyrrole/DNA composite, was presented to be used as an extraction medium. This new material, coupled with anodic stripping voltammetry (ASV), constitutes a new environmental remedy technique that we called as solid phase micro extraction (SPME). SPME in this work is mainly divided into two parts:

1. SPME extraction and release of environmental heavy metal ions;
2. Micro detection of metal ions by ASV.

**Polypyrrole (PPy)**

**Basic Properties of Polypyrrole**

Among the many conducting polymers, polypyrrole (PPy) and its derivatives have become one of the most widely used and intensively studied classes of conducting polymers for two decades. The reasons for these are

- they can be easily polymerized from organic or aqueous media at neutral pH by electrochemical or chemical methods,
- they are relatively stable in air and in solution,
pyrrole monomer and some of its derivatives are available commercially \(^{(2)}\).

As a conducting polymer, PPy can exist in many different forms. PPy made from chemically induced polymerization is insulating. Electrochemical oxidization is used to prepare the conductive polymers, which come in two forms, partially oxidized and fully oxidized \(^{(3)}\). Low externally applied potentials can create a partially oxidized PPy film where its conductivity is isolated and difficult to reproduce. Higher applied potentials will result in a fully oxidized PPy film where its electrochemical properties can be easily reproduced and characterized \(^{(4)}\). Before deposition of the fully oxidized polymer deposition of some partially oxidized polymer may occur but by holding at or sweeping to higher potentials all partially oxidized PPy will be converted to fully oxidized PPy \(^{(3, 4)}\). The three oxidation states of PPy are shown in Figure 1.
Figure 1. Three Oxidation States of Polypyrrole.

Figure 2 indicates the oxidative electropolymerization of pyrrole. As polymerization continues, anions from solution come into the film to balance the positive charge on the poly pyrrole backbone. Typically, polymerization of pyrrole occurs at positions 2 and 5 and is shown by the mechanism presented below.\(^\text{(5)}\).
Figure 2. Accepted Mechanism of the Oxidative Electropolymerization of Pyrrole.

This polymerization follows an oxidative coupling mechanism, more specifically the radical step growth mechanism. Each polymer studied in this thesis was created by the exclusive use of electrochemical means. Pyrrole is oxidized at the anode to form radical cations, which quickly couple and eliminate two protons to rearomatize \(^{(5)}\). The pyrrole dimmer is now more easily oxidized than the monomer, allowing reoxidation and further coupling to occur \(^{(3)}\).

**Primary Structure of DNA Molecule**

Deoxyribonucleic acids (DNAs) are a group of vital biomolecules that are crucial for many biological and biochemical processes. DNAs possess the following basic structural features:

- Two anti parallel and complementary strands.
- Hydrophilic polar external sugar-phosphate backbone.
- Hydrophobic core of bases: Adenine, Thymine, Guanine, and Cytosine.
- Significant secondary structure.
**Sugar-Phosphate Backbone**

The basic structure of DNA (as shown in Figure 3) can be divided into two portions: the external sugar-phosphate backbone and the internal bases. The sugar-phosphate backbone, as its name implies, is the major structural component of the DNA molecule. The backbone is constructed from alternating ribose sugar and phosphate molecules, which are highly polar. Because the backbone is polar, it is hydrophilic which means that it likes to be immersed in water\(^6\).

![Sugar-phosphate backbone](image)

**Figure 3. Primary Structure of DNA.**
Complementary Core of Bases

The interior portion of a DNA molecule is composed of a series of four nitrogenous bases with patterned or random arrangements: adenine (A), guanine (G), thymine (T), and cytosine (C). These bases specifically are non-polar and, therefore, hydrophobic. Inside a DNA molecule these bases pair up, A to T and C to G, by hydrogen bonds that stabilize the DNA molecule. Because of these specific pairings, the DNA double helix is a complementary structure. It is this sequence of bases inside the DNA double helix that we refer to as the genetic code.

The sugar-phosphate backbone of DNA is polar and, therefore, hydrophilic. The interior portion of DNA, the bases, is relatively non-polar and, therefore, hydrophobic. This duality has a very stabilizing effect on the overall structure of the DNA double helix: the hydrophobic core of the DNA molecule 'wants' to be hidden inside the sugar-phosphate backbone which acts to isolate it from the polar water molecules. Due to these hydrostatic forces, there is a strong pressure gluing the two strands of DNA together (6).
Anti Parallel Direction and Polarity in DNA

Anti parallel nature of DNA means that two strands of DNA have opposite chemical polarity, in other words, their sugar-phosphate backbones run in opposite directions. Direction in nucleic acids is specified by referring to the carbons of the ribose ring in the sugar-phosphate backbone of DNA. 5' specifies the 5\textsuperscript{th} carbon in the ribose ring, counting clockwise from the oxygen molecule, and 3' specifies the 3\textsuperscript{rd} carbon in the ring. Direction of DNA molecules is then specified relative to these carbons. For example, transcription, the act of transcribing DNA to RNA for eventual expression, always occurs in the 5' to 3' direction. Nucleic acid polymerization cannot occur in the opposite direction, 3' to 5' because of the difference in chemical properties between the 5' methyl group and the 3' ring-carbon with an attached hydroxyl group\((^6)\).

Figure 4. Anti Parallel Nature of DNA.
PPY–DNA

Preparation of 0.1M PPy/100 ppm dsDNA Film

This was prepared by using a solution containing 0.1 M Pyrrole and 100 ppm dsDNA. We applied a 0.7 V potential by bulk electrolysis to a glassy carbon electrode which is inserted in the above mentioned 0.1 M pyrrole and 100 ppm dsDNA solution. At this condition the pyrrole is oxidized and PPy is formed then the dsDNA gets adhere to the positive charges of the PPy. This is called co-polymerization. The film is formed on the surface of the glassy carbon electrode.

Ion Movement in PPy-DNA

A charged conducting polymer must be electric charge balanced at any time by incorporating an oppositely charge counter ion - the dopants into the polymer matrix. This incorporation is defined as “doping”. Under certain circumstance the redox doping processes are reversible and can be accomplished electrochemically. During electrochemical switching, ions move in and out of the polymers as charge-balancing species for the charge carriers in the polymer backbone. In the polymerization of pyrrole, the electrolyte (DNAs) and the monomer (pyrrole) are simultaneously deposited. DNAs behave as both polyelectrolyte and dopants. A characteristic of DNA being a polyelectrolyte is that it is a large doping anion when compared to typical small doping anions, i.e. C1\(^-\), NO\(_3^-\) or F\(^-\). As a result, movement of this large electrolyte is somewhat restricted\(^7\). Figures 5 and 6 shown below are schematic representations of counter ion movement associated with the polymer oxidation and reduction.
The small ions like Cl\(^-\) are electrostatically attracted to the (+) charge of PPy backbone.

Reduction of PPy in the presence of cations.

In reduction, the small ions move from the film as the polypyrrole reduces to its neutral state. Cations are not extracted.

Figure 5. Ion Transport in PPy/SA\(^-\) (Cl\(^-\)) Film in Different Potentials.
The large ions like DNA are electrostatically attracted to the (+) charge of PPy backbone.

Reduction of PPy in the presence of cations.

The large ions do not move from the film, so the cations get adsorbed to the (-) charge of the macro anions (DNA).

Figure 6. Ion Transport in PPy/MA⁻ (DNA) Film in Different Potentials.
The electrochemical behavior of these polypyrrole film electrodes depends on the mobility of the doping anions within the poly pyrrole layer and the interaction between the anionic and the oxidized polypyrrole. Thus electrochemical reduction and oxidization of polypyrrole films doped with small anions such as chloride ions usually involve the expulsion and re-incorporation of the small anion SA\(^{-}\) of the electrolyte according to equation 1.1 –

\[
PPy^+/SA^- (surface) + e^- \rightleftharpoons PPy^0 (surface) + SA^- (aq)
\]  

(1.1).

On the other hand, polypyrrole films doped with macro anions (MA\(^{-}\)) such as DNA can also be prepared in the same way. In this situation the dopants remain in the PPy matrix during the redox reaction due to their bulky, entrapped, and immobile properties. Thus the movement of the electrolyte cations M\(^{n+}\) preserves the charge neutrality of the film and the process can be represented by equation 1.2 \(^{(8)}\) –

\[
PPy^+/MA^- (surface) + M^{n+} (aq) + e^- \rightleftharpoons PPy^0 / MA^- / M^{n+} (surface)
\]  

(1.2)

**Different Methods of Metal Extractions**

**Solvent Extraction of Metals**

Solvent extraction has become one of the important separation techniques for metal cations \(^{(9)}\). In this technique the metal ion, through coordination chemistry, distributes from an aqueous phase into a water-immiscible organic phase. The separation can be accomplished in several ways. Uncharged organic molecules tend to dissolve in the organic layer while the charged anion from the ionized molecules remains in the polar aqueous layer. This is an example of “like dissolves like”. Metal ions do not tend to dissolve appreciably in the organic phase. To enhance their organic solubility their charges must be neutralized and complexing
ligands must be added to make them organic-like. This can be done through two major avenues as discussed below.

**Extraction of Ion-Pair Complexes.** In this protocol the metal ion is incorporated into a bulky molecule and then associates with another ion of the opposite charge to form an ion pair, or a metal ion associates with another ion of great size (organic-like). For example, it is well known that iron (III) can be quantitatively extracted from hydrochloric acid medium into diethyl ether (9). The mechanism is not completely understood, but evidence exists that the chloro complex of the iron is coordinated with the oxygen atom of the solvent and this ion associates with a solvent molecule that is coordinated with a proton:

\[ \{(C_2H_5)_2 O : H^+, \text{FeCl}_4\}^{2-} \]

Permanganate forms an ion pair with tetraphenylarsonium ion \([(C_6H_5)_4\text{As}^+, \text{MnO}_4^-]\), which makes it organic-like. This permanganate–containing pair is then extracted into methylene chloride (9).

**Extraction of Metal Chelates.** The most widely used method to extract metal ions is through the formation of a chelate molecule with an organic chelating agent.

A chelating agent contains two or more complexing groups. Many of these reagents form colored chelates with metal ions and form the basis of spectrophotometric methods for determining the metals. The chelates are often insoluble in water and will precipitate. They are, however, usually soluble in organic solvents such as methylene chloride.

**Extraction Process for Metal Chelates.** Most chelating agents are weak acids that disassociate in water. The ionizable proton is usually displaced by the metal ion when the chelate is formed, and the anionic charge on the organic base neutralizes the charge on the
metal ion. An example is diphenylthiocarbazone (dithizone), which forms a chelate with lead ion \(^{(9)}\).

![Chemical structures of diphenylthiocarbazone and its chelate with lead ion](image)

**Figure 7. A Chelate Formation of Diphenylthiocarbazone (Dithizone) Lead Ion.**

### Solid Phase Extraction

Liquid-liquid extractions are very useful but have certain limitations. The extracting solvents are limited to those that are water immiscible (for aqueous samples). Emulsions tend to form when the solvents are shaken, and relatively large volumes of solvents are used to generate a substantial waste disposal problem. The operations are often manually performed and may require a back extraction.

Many of these difficulties are avoided by solid phase extraction (SPE), which has become a widely used technique for sample cleanup and pre-concentration, prior to chromatographic analysis in particular \(^{(9)}\). In this technique, hydrophobic organic functional groups are chemically bonded to a solid surface, such as powdered silica. A common example is the bonding of C\(_{15}\) chains on silica. These groups will interact with hydrophobic organic compounds by Van Der Waals forces and extract them from an aqueous sample in contact with the solid surface. The same solid phases used in high-performance liquid chromatography are employed in the solid-phase extraction \(^{(9)}\). The powdered phase is generally placed in a small cartridge, similar to a plastic syringe. Sample is placed in the cartridge and is forced to go through it by means of a plunger (positive pressure) or a vacuum (negative pressure) or by
centrifugation (see Figure 8). Trace organic molecules are extracted, pre-concentrated on the column, and separated away from the sample matrix. Then they can be eluted with a solvent such as methanol and then analyzed, for example, by chromatography. They may be further pre-concentrated prior to analysis by evaporating the solvent (9).

The nature of extracting phase can be varied to allow extraction of different compounds. Figure 9 illustrates bonded phases based on Van Der Waals forces, hydrogen bonding (dipolar attraction), and electrostatic attraction.

When silica particles are bonded with a hydrophobic phase, they become “waterproof” and must be conditioned in order to interact with aqueous samples. This is accomplished by passing methanol or a similar solvent through the sorbent bed. This methanol penetrates into the bonded layer and permits water molecules and analyte to diffuse into the bonded phase. After conditioning, water is passed to remove the excess solvent prior to introducing the sample.
Figure 8. Solid Phase Cartridge and Syringe for Positive Pressure Elution. (Adapted from G.D. Christian, Analytical Chemistry 6th ed., 2003. p. 541)

Figure 10 illustrates a typical sequence in a solid phase extraction. Following conditioning, the analyte and other sample constituents are adsorbed on the sorbent extraction bed. A rinsing step removes some of the undesired constituents, while elution removes the desired analyte, perhaps leaving other constituents behind, depending on the relative strengths of interaction with the solid phase or solubility in the eluting solvent. Such a procedure is used for the determination of organic compounds in drinking water, as certified as an Environmental Protection Agency (EPA) standard method.

Figure 10: Principles of Solid Phase Extraction. (Adapted from N. Simpson, Am. Lab., August 1992, p. 37. Reproduced by Permission of American Laboratory. Inc.)
Solid Phase Micro Extraction (SPME)

Solid phase micro extraction (SPME) was first reported by Arthur and Pawliszyn in the late 1980s. SPME was initially introduced as an alternative to traditional extraction procedures such as liquid-liquid extraction, purge and trap, static headspace, and solid phase extraction (SPE) (10).

SPME is a solvent-less extraction technique usually used for analyte collection for determination by gas chromatography and is based on adsorption. A fused silica fiber is coated with the solid adsorbent, an immobilized polymer, or a combination of the two. Figure 11 illustrates an SPME fiber. Typical fiber dimensions are 1 cm x 110 µm. The fiber is inserted in a syringe needle device. Solid, liquid, or gaseous matrices can be sampled by SPME. The fiber is exposed to a gaseous or liquid sample or the headspace above a solid or liquid sample for a fixed time and temperature; samples are often agitated to increase efficiency of analyte adsorption. Following adsorption, the analyte is thermally desorbed, usually directly in the injection port of a gas chromatograph for introduction into the gas chromatograph column (9).

There are a limited number of adsorbents. A widely used one is poly(dimethylsiloxane), which is useful for screening of volatile flavor components in beverages and food (9). A 100 µm layer coating of polydimethysiloxane is applied for non-polar volatile compounds. Another example is an 85 µm layer of polyacrylate, which is relatively non-polar due to the presence of methyl group. However, this polyacrylate coating is more polar due to the presence of carbonyl groups and extracts polar semi volatile compounds.

In our research we produced a new film from bulk electrolysis as an adsorbent equivalent for heavy metals using PPy/DNA. The heavy metals are adsorbed by SPME. The
film is formed on the surface of a glassy carbon substrate electrode. This was used to extract the heavy metals with the integration of electrochemical workstation.


Basic Principle

The principle for SPME is based on equilibrium of analyte between the sample matrix and the extraction medium. The most common sample matrices are liquids and gases. By using this method, a small amount of extracting phase can be taken out from a large sample volume.
The extracting phase can be either high molecular weight polymeric liquid (similar to the SPE in chromatography) or it can be solid sorbent (typically of high porosity to increase the surface area available).

**Instrumentation**

SPME consists of basically two main steps.

1. Equilibration of the analyte(s) between the fiber coating (extracting phase) and the sample matrix.
2. Desorption of the concentrated analyte(s) into an analytical instrument.

**SPME Steps in More Details**<sup>(10)</sup>:

1. The fragile fiber is initially withdrawn into the steel syringe needle.
2. The sample septum is pierced and the coated fiber is extended into the sample solution for a set time, (typically 2-15 minutes for liquid samples) where the analytes are adsorbed by the fiber until equilibrium is reached.
3. Amount of analyte extracted by coating at equilibrium is determined by the magnitude of the partition coefficient of the analyte between the sample matrix and the coating material.
4. The fiber is drawn back into the protective needle and the needle is withdrawn from the sample container.
5. The needle is injected into the sample port of an analytical instrument, the fiber is extended, and the analytes are desorbed.
SPE vs. SPME

SPE (solid phase extraction) is a commonly used sorbent extraction technique.

- It is a total extraction technique: the total amount of analyte in the sample are transferred to a solid extraction phase.
- Analytes are extracted together with the interfering compounds by passing an aqueous matrix through a plastic cartridge containing dispersed sorbent on a particulate support.
- A selective organic solvent is used to remove interferences first, and then another solvent is chosen to wash out the target analytes.
- Attractive features: simple, inexpensive, can be used in the field, can be automated, and uses relatively little solvent.
- Limitations: low recovery - resulting from interaction between the sample matrix and analytes and need of solvent.

On the other hand, SPME preserves all of the advantages of SPE while eliminating the main disadvantages of low analyte recovery and solvent use.

SPME Theory

The principle behind SPME theory is the partitioning of analytes between the sample matrix and extraction medium.

To simplify the theory we can assume that the vial containing the sample is completely filled (no headspace is present). If a liquid polymer coating is used, we can use the following equation to relate the amount of analyte adsorbed by the coating at equilibrium to its concentration in the sample (10):
\[
m = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + V_s}
\]

- \(m\) = mass of the analyte adsorbed by coating
- \(V_f\) = volume of the fiber coating or the film
- \(V_s\) = volume of the sample
- \(K_{fs}\) = the distribution constant of the analyte between the coating and the sample matrix
- \(C_o\) = the initial concentration of the analyte in the sample

Coatings used in SPME typically have strong affinities for organic compounds and, therefore, have large \(K_{fs}\) values for targeted analytes. This means that SPME is selective and has a very high concentrating effect. However, many times the \(K_{fs}\) values are not large enough to exhaustively extract most analytes in the matrix and only through proper calibration can SPME be used to accurately determine concentration of target analytes. Calibration can be done by the external standard method in a relatively clean sample and by standard addition or internal standard in a more complex matrix. From equation 1.3,

If \(V_s\) is very large,

\[(V_s \gg K_{fs} V_f):\]

\[
n = K_{fs} V_f C_o
\]

This means that when the volume of the sample is very large, the amount of the analyte extracted by the fiber coating is not related to the sample volume. This feature, combined with its simple geometry, makes SPME ideally suited for the field sampling and analysis because the fiber can be exposed to air dipped directly into a lake or river without collecting a defined sample volume prior to analysis \(^{(10)}\).
SPME Sampling

Three basic modes of SPME sampling:

**Direct Extraction Mode**
- Coated fiber is inserted into sample and analytes are transported directly from the sample matrix to the extracting phase.
- Rapid extraction facilitated by agitation.
- For gaseous samples, convection is usually sufficient to facilitate rapid equilibration but for aqueous matrices more efficient agitation needed.

**Head Space Extraction Mode**
- Analytes extracted from the gas phase that is equilibrated with the sample.
- Protects fiber from adverse effects caused by sample matrix.
- Allows matrix modifications without affecting the fiber.
- Sensitivity is the same as direct extraction as long as sample and gaseous headspace volumes are the same.
- Extraction kinetics is different than in direct extraction mode.

**Membrane Protected Mode**
- Fiber is separated from the sample with a selective membrane, lets analytes pass through while blocking interferences.
- To protect fiber from adverse effects caused by sample matrix.
- To serves same purpose as headspace mode except that it can still analyze compounds having a low volatility.
- Extraction process substantially slower than conventional extraction.
SPME: Experimental Variables

Coating Materials

Choosing a coated fiber similar in chemical structure to the analyte can enhance selectivity.

- Poly(dimethyl) siloxane-used for alkyl benzenes, volatile halogenated compounds.
- Polyacrylate or mixture of polyacrylate with Carbowax and/or polydivinylbenzene-used for alcohols and small polar compound.

Increasing coating thickness increases $V_f$ and extracts a higher proportion of the analyte.

Agitation Methods

- To increase the agitation sonication is the best method to reduce $t_e$ (equilibrium time).

Salting out Effect

- Addition of an inorganic salt to the aqueous sample shifts the partition equilibrium so more analytes are extracted.

Effect of pH

- Unless ion exchange coatings are used, SPME can extract only neutral (non-ionic) species. To ensure that at least 99% of acidic compound is in the neutral form, pH should be at least two units lower than pKa of the analyte and the same goes for a basic compound, the pH should be at least two units higher than the pKb of the analyte.

Sample Heating

- Heating liquid samples gives faster diffusion rates of analytes (to coated surface) and reduces time needed for equilibrium. However, at high temperatures less analyte is extracted. The best method to reduce equilibrium time and still extract sufficient
analytes is to use an internally cooled fiber (via an inner capillary of liquid CO₂) while heating the solution.

**Derivatization**

- This experimental variable is necessary for extracting and separating polar compounds.

Derivatization can occur by:

1) Adding appropriate reagents to matrix, followed by extraction.
2) Doping fiber with the reagents, followed by extraction.
3) By extracting and then exposing the fiber to derivatizing reagent.
4) By derivatizing within GC inlet.

**SPME Advantages**

- It is an equilibrium technique (involving partition in two phases) and is, therefore, selective.
- Time required for analyte to reach equilibrium between the coated fiber and sample is relatively short.
- Ideal for field sampling: large volume sampling, direct sampling, portable apparatus.
- Solvent-less extraction and injection, eliminating solvent disposal.
- Smooth liquid coating can be used, eliminating the problem of plugging.
- By sampling from headspace, SPME can extract analytes from very complex matrices.
- All analytes collected on the solid phase can be injected into GC for further analysis.
- Method is simple, fast, inexpensive, and easily automated.
SPME Limitations

- Often only a small fraction of the sample analytes is extracted by the coated fiber.
- Quantification in SPME requires calibration.
- Carry over resulting from incomplete desorption.
- Fiber easily broken.
- Limited number of polymeric coatings for SPME – lack of fibers that are sufficiently polar \(^{10}\).

SPME Applications

Food and Pharmaceuticals

Advantage in this area is that SPME can extract substances without opening the package. Furthermore, an insignificant amount is extracted and composition of the product does not change.

Environmental

SPME can meet U.S. EPA method requirements with its low LOD’s. These low detection limits reflect that all of the extracted analytes are introduced into the analytical instrument.

Clinical and Forensic

The major advantage of SPME in this field is its portability: It allows for better monitoring of patients during treatment or therapy and better preservation of crime scenes (evidence does not need to be taken back to the central lab for analysis).
Electrochemistry Assisted Solid Phase Micro Extraction

Solid-phase micro extraction (SPME) combined with electrochemistry or electronically conducting polymers is a recent technique (11). The important goal of these investigations has been the development of electrochemically controlled micro extraction process by which the extraction and desorption can be controlled simply by adjusting the potential of an electrode. The attractive feature of using conducting polymers in SPME is that the charge of the coatings can be readily controlled by oxidation and reduction of polymers. These potential controlled ion exchanges can, therefore, be used to extract ions, and analytes that normally need to be derivatized prior to the traditional SPME extractions. Electrochemically assisted desorption is faster than the normal SPME desorption. This technique can be combined with miniaturized analytical systems with small volume detection to further challenge micro detection / remedy problems.
CHAPTER 2
ANALYTICAL METHOD

The electrochemical cell used in this study consists of three electrodes, a working electrode, a reference electrode, and a counter electrode. The purpose of an electrode is to provide a solid/solution interface across which charges can be transferred between the electrode and testing ions. When an electroactive species is oxidized or reduced at the surface of the working electrode upon application of the appropriate potential, electric current is produced because of the surface oxidation or reduction reaction\(^\text{(12,13)}\).

Many techniques exist in electroanalytical chemistry for the analysis of conducting polymers. The instrumental methods employed in our research include cyclic voltammetry/anodic stripping voltammetry and bulk electrolysis with coulometry. The following are their major features, respectively.

**Cyclic Voltammetry**

A simple potential waveform that is often used in electrochemical experiments is the linear scan, i.e., the potential is continuously changed as a linear function of time. The rate of change of potential with time is referred to as the scan rate (Vs\(^{-1}\)). The corresponding technique that uses this waveform is linear sweep voltammetry (LSV). In LSV, the potential range is scanned in one direction, starting at the initial potential and finishing at the final potential\(^\text{(14)}\). A more commonly used variation of this technique is cyclic voltammetry (CV), in which the direction of the potential is reversed at the end of the first scan. This has the advantage in the product of the electron transfer reaction that has occurred in the forward scan (can be probed if needed) as well as in the reverse scan. In addition, CV is a powerful tool for the determination
of formal redox potentials, detection of chemical reactions that precede or follow the
electrochemical reaction, and evaluation of electron transfer kinetics \(^{(15)}\).

![Figure 12. Typical CV of a Reversible System \((\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-})\).](image)

In CV, the electric potential is first held at the initial potential where no electrolysis
occurs and, hence, no Faraday current flows. As the potential is scanned in the positive-going
direction, the compound in its reduced state is oxidized at the electrode surface. At a particular
set value, the scan direction is reversed and the oxidized product is then re-reduced. Once the
potential is returned to the initial value, the experiment can be terminated \(^{(16)}\). The basic
reversible shape of the current response for the CV experiment of the ferricyanide/ferrocyanide
couple is shown in Figure 12. The important parameters of a cyclic voltammogram are the
peak potentials \((E_p^c, E_p^a)\) and peak currents \((i_p^c, i_p^a)\) of the cathodic and anodic peaks,
respectively. If the electron-transfer process is fast with respect to the diffusion of the
electroactive species, then the reaction is said to be reversible, such as the
ferricyanide/ferrocyanide redox reaction. For cases where electron transfer occurs only in one

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direction, the process is termed as irreversible. In other words, equilibrium requires that the surface concentrations of oxidized species and reduced species are maintained at the values required by the Nernst equation \(^{14-16}\).

CV gives rise to current signals that show characteristic features. While a characteristic peak potential will give qualitative information about the reactant, the signal itself can rarely be used to identify a species because peak position is affected by many variables. Thus, as a qualitative tool, it is best used to exclude electroactive species than to identify species. CV is a powerful means to differentiate reversible and irreversible electrochemical reactions. Other merits of cyclic voltammetric techniques are their good accuracy, excellent precision, and sensitivity \(^{17}\).

**Anodic Stripping Voltammetry**

Anodic stripping voltammetry (ASV) is a very sensitive, convenient, and cost-effective analytical method for detection and quantification of trace amount of metal ions. This method is used for analyzing Cu, Pb, Cd, Zn, Bi, Co, and Hg. Several metals can be analyzed simultaneously, such as Cu, Pb, Cd, and Zn metal ions, or Hg, Co, Pb, and Cd metals ions respectively. The metal ions are plated on to an electrode by applying a negative potential for a specific period of time and the potential is then scanned to positive potentials. Current peaks will appear at potentials corresponding to the oxidation of the metals as they are oxidized (stripped) from the electrode surface back into the solution. The peak height or area can be correlated with the concentration of the metals ions in the solution. It is necessary to calibrate the procedure with standard solutions containing known quantities of respective metal ions \(^{18}\).
Bulk Electrolysis with Coulometry

Another useful technique is bulk electrolysis with coulometry. Coulometry produces a plot of current versus time when a constant potential is applied to the working electrode. Likewise, bulk electrolysis yields a plot of charge versus time when a constant potential is applied to the working electrode. Both plots can be obtained from running a single trial.

Coulometry is based on completely converting analyte into product and measuring the amount of charge required to do so. Faraday Law shows the relationship between charges consumed and the amount of analyte (19):

\[ Q = nFVC = nFW/M_w \]  (2.1)

Where \( Q \) is the consumed charge (C), \( n \) is the number of equivalents of charge required per mole of reaction (equivalents/mole), \( F \) is Faraday’s constant (96,486 Coulomb/mol), \( V \) is the volume of the electrolytic solution (L), \( C \) is the concentration of the analyte (mole/L), \( W \) is the weight of the analyte, and \( M_w \) is its molecular weight. Coulometry can be carried out by exhaustive electrolysis at constant potential, offering some discrimination among analytes through choice of potential. The current will decrease exponentially, and the experiment is considered finished when current drops to an acceptably low fraction of the initial value.

Charge is obtained by electronic integration of the current. On the other hand, coulometry can also be done with constant current using a redox buffer to maintain the potential in the range required to ensure that all the current goes to the reaction of the analyte (20).
Figure 13 shows a typical electropolymerization of pyrrole at 0.7 V by bulk electrolysis with coulometry. Using a three-electrode system, the oxidizable components can be deposited on the working electrode surface at respective potentials. That is, selecting the correct potential allows the deposition of a desired electro active component on the electrode surface. Therefore, this consideration can be applied in preparation of conducting polymers and conducting polymer-DNA composites (20).

**Differential Pulse Voltammetry**

Differential pulse voltammetry (DPV) is a derived differential pulse polarography (DPP) when the working electrode is a solid substrate instead of a dropping mercury electrode. It uses a series of discrete potential steps rather than a linear potential ramp to obtain the experimental polarogram (21). Many of the experimental parameters for DPV are the same as with normal pulse voltammetry (NPV) (for example, accurately timed sampling potential step.
duration of 50-100 ms at the end of the sampling/dropping lifetime). Unlike NPV, however, each potential step has the same amplitude, and the return potential after each pulse is slightly negative of the potential prior to the step.

Figure 14: The Applied Potential vs. Time in Differential Pulse Voltammetry.

In this manner, the total waveform applied to the working electrode is very much like a combination of a linear ramp with a superimposed square wave. The differential pulse voltammogram is obtained by measuring the current immediately before the potential step and then again just before the end of the drop lifetime. The analytical current in this case is the difference between the current at the end of the step and the current before the step (the differential current). This differential current is then plotted against average potential (average of the potential before the step and the step potential) to obtain the differential pulse voltammogram. Because this is a differential current, the voltammogram in many respects is like the differential of the sigmoidal normal pulse voltammogram. As a result, the differential pulse voltogram for an electrochemical reaction is peak shaped.
DPP has even better ability to discriminate against capacitive current because it measures a difference current (helping to subtract any residual capacitive current that remains prior to each step). Limits of detection with DPP are $10^{-8} - 10^{-9}$ M (21).

**Proposed Research and Objectives**

PPy/DNA is a interesting new film. Our aim was to use PPy/DNA film to extract heavy metals such as Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, while extraction and detection of heavy metals has become a major environmental concern today.

The following protocols are proposed:

1. Preparation of 0.1 M PPy/100 ppm dsDNA composite film.
2. Extraction and detection of heavy metals by using this composite film.
3. Comparison of the extraction efficiency from different solid phases for Cd$^{2+}$. These include PPy/Cl-, Bare GC, PPy/TsO$^-$ (P-toluenesulfonate).
4. Extraction of Cd$^{2+}$ using PPy/DNA at open circuit condition.
5. Optimization of extraction/detection the using Cd$^{2+}$ as a model.
   a) Comparison of extraction efficiency of different media for Cd$^{2+}$ adsorption using PPy/DNA.
   b) Comparison of the release efficiency of extracted Cd$^{2+}$ into different media.
   c) Finding the optimal concentration of the extraction media.
   d) Finding the optimal concentration of the releasing media.
6. Enhancement of co-existing Hg$^{2+}$ for M$^{n+}$ (Cd$^{2+}$) detection.
7. Possibilities of extracting of other cations individually and simultaneous extraction of e coexisting cations.
8. Comparison of double standard and single standard DNA as composite dopants for extraction efficiency.

9. Interference from other cations and anions.

10. Effect of film thickness.

11. Linear range of $\text{Cd}^{2+}$ extracted concentration vs. their stripping oxidation current.


13. SPME detection with atomic absorption spectroscopy.

14. Finding the overall recovery.

15. Extraction of $\text{Pb}^{2+}$ and finding the linearity range for $\text{Pb}^{2+}$.

16. Reproducibility of this method.

17. Mechanism of this electrochemical solid phase micro extraction based on PPy/DNA composite.
CHAPTER 3
EXPERIMENTAL PROCEDURES

In this chapter, the procedures used to perform experiment to accomplish the research objectives are discussed. The instruments, reagents, and experimental procedures for the analysis are described. All measurements were carried out with the laboratory ambient temperature.

Instrumentation

The glassy carbon working electrode, Pt counter electrode, and Ag/AgCl reference electrode were obtained from CH Instruments, Inc. (Austin, TX).

The electrode polishing powder (0.05 micron) and a polished pad (73 mm diameter 12000 grit carbimet disk) were obtained from CH instruments; Inc. (Austin, TX).

The electrochemical analyzers/workstations model 400A, 608A, and 842B were obtained from CH instruments, Inc.

Using the electrochemical workstations mentioned above, all electrochemical experiments were performed in a three-electrode cell occupied with a reference electrode (Ag/AgCl), a counter electrode (Pt), and a PPy/DNA composite modified or bare glassy carbon.

The electrochemical parameters were recorded, processed, and output through different installed software and a combined PC machine.

Reagents

The following reagents were employed for the experiments performed to accomplish the research goals.

1. De-ionized water.
2. Pyrrole (98%) (Aldrich, Milwaukee, WI).
3. Double stranded (ds) and single stranded (ss) DNA (Sigma Aldrich, St. Louis, MO).
6. Cadmium, lead, mercury and cobalt were prepared by dissolving respective metals in acids and were diluted to appropriate concentration before use.
7. 6 M HNO₃ solution.
8. 6 M HCl solution.

**Preparation of PPy/DNA Film**

Pyrrole stock solution (0.5 M) was freshly made by using original 98% pyrrole and de-ionized water before each experiment. The pyrrole stock solution was then stored in the refrigerator.

The preparation solution for PPy/DNA was made by mixing 20 µL of 0.5 M pyrrole, 10 µL of 1000 ppm dsDNA, and 70 µL of distilled water to reach concentrations as 0.1 M pyrrole + 100 ppm DNA. The polymerization of pyrrole doped with DNA was done by bulk electrophoresis at an upside - down experimental setup as indicated in Figure 15. 35 µL of 0.1 M pyrrole/100 ppm dsDNA was placed in the gap sandwiched by the Ag/AgCl reference electrode and the glassy carbon working electrode. A counter electrode was attached with the reference electrode. A constant potential of 0.7 V was applied by using CH 400 potentiostat. The accumulation of charge vs. time was monitored to ensure identical film produced in each preparation. Under these condition, the PPy/DNA film was formed on the surface of the glassy carbon electrode.
Figure 15. Electrode setup for the preparation of PPy/DNA film.

Figure 16: Charge vs. Time Graph by Bulk Electrolysis When Preparing the PPy/DNA Film.
Figure 16 shows the accumulated charge on the film with time by bulk electrolysis. The electrolysis was terminated when appropriate charge value was achieved. The resultant film/electrode (PPy/DNA/GC) was then washed gently three times with distilled water before using for the extraction of heavy metals.

**Solid Phase Micro Extraction Procedure**

![Diagram of Solid Phase Micro Extraction Procedure]

Figure 17. Protocol for the Solid Phase Micro Extraction of Cd$^{2+}$ As a Model Heavy Metal Extraction.

**Extraction of Heavy Metals**

The three-electrode set up, including the PPy/DNA/GC as working electrode, the Ag/AgCl reference electrode, and the platinum counter electrode was placed in a 25 mL of cation containing solution as shown in the Figure 17 A. Then a -1.0 V potential was applied
for about 1000 s by bulk electrolysis. Under this condition the solution cations would approach
the working electrode because of the electrostatic force. At end of the 1000 s the three-
electrode setup was transferred to a solution containing 0.01 M HNO₃ (Figure 17 B). Then
under the same potential it was kept there for about 300 seconds by the bulk electrolysis.

**Release/Transfer of Heavy Metals from the Film into a Solution**

The electrode setup was now transferred to a 200 µL solution containing 0.01 M HCl.
Then a potential of 0.7 V was applied by bulk electrolysis. Under this condition, the deposited
Cd metal layer would be oxidized and resulting Cd²⁺ would leave the film surface, as indicated
in Figure 17 C.

**Detection of Cations in the Desorbed Solution**

Detection of cations in a desorbed solution was done by anodic stripping voltammetry
(ASV) on a new glassy carbon working electrode. A potential ranging from -1.0 V to 0.8 V
was applied with a standing time of 50 s, and the scan rate of 0.1 V/s. The corresponding
voltammogram was recorded. An anodic peak around -0.7 V is the indication of Cd presence in
the solution, which can be qualitatively verified by a Cd²⁺ control experiment, and
quantitatively measured by its peak height, respectively.

**Comparison of Extraction Efficiency from Different Types of Solid Phases for Cd²⁺**

The extraction efficiency of PPy/DNA was compared with other polypyrrole
composites including PPy/Cl⁻, PPy/TsO⁻ (P-toluenesulfonate) and bare glassy carbon as well.

**Preparation of 0.1 M PPy/0.5 M TsO⁻ Film**

The PPy/TsO⁻ film was prepared employing a solution containing 0.1 M pyrrole and
0.5 M TsO⁻ with a constant potential of 0.8 V for 20 minutes by bulk electrolysis⁽¹¹⁾. Then the
film was rinsed with distilled water before applied to the extraction procedure.
**Preparation of 0.1 M PPy/0.5 M Cl Film**

This film was made by electrolysis of the solution containing 0.1 M pyrrole and 0.5 M KCl with a constant potential of 0.8 V for 10 minutes. The resultant film was subject to wash several times before use.

A $4.4 \times 10^{-3}$ M Cd$^{2+}$ solution was used for the extraction, release, and detection in this SPME procedure. PPy/DNA, PPy/Cl$, PPy/TsO^-$, and the bare glassy carbon electrode was used as the working electrode, respectively, following the procedure described in Figure 16. The peak current given in the detection media was correlated with the amount of Cd$^{2+}$ extracted by each film. We took this for the purpose of comparison of the efficiencies of these composite films to extract Cd$^{2+}$ from solution.

**Comparison of Double and Single Stranded DNA as Composite Dopants for Extraction Efficiency**

All the previous work was done by using PPy/dsDNA. A PPy/ssDNA film was made by using 0.1 M pyrrole and 100 ppm ssDNA by the procedure explained earlier. Extraction was done by using these two films separately. The concentration of Cd$^{2+}$ solution used was $4.4 \times 10^{-4}$ M. The peak current was taken into the consideration to see the difference of the single stranded DNA and double stranded DNA.

**Extraction of Cd$^{2+}$ Using PPy/DNA at Open Circuit Condition**

We wanted to compare the results of electrochemically assisted SPME with the open circuit condition SPME. A film having 15.8 mC/cm$^2$ was prepared and used for extraction of $1.0 \times 10^{-5}$ M Cd$^{2+}$ by using electrochemically assisted SPME method mentioned before. 2 mL of extraction solution and 2000s extraction time was employed for this process. The amount of
Cd\(^{2+}\) extracted was estimated by the peak current obtained in the ASV detection process. This was done three times and the average was calculated. After this the same film was taken and used to extract 1.0 x 10\(^{-5}\) M Cd\(^{2+}\) solution without applying any potential (-1.0 V) to the PPy/DNA film. This is the extraction by open-circuit condition. Then electrochemically assisted release and detection was done. The peak current for Cd\(^{2+}\) in ASV was obtained. The detection was carried out triplicate and the average was calculated. The average peak currents for both methods were compared.

**Cadmium as a Model Target Optimizing the Condition**

**Comparison of the Extraction Efficiency of Different Media for Cd\(^{2+}\) Extraction by Using PPy/DNA**

Different solutions were used to compare their adsorption/extraction performance towards Cd\(^{2+}\). They are: 0.01 M \(\text{HNO}_3\), 0.01 M \(\text{HCl}\), 0.01 M \(\text{H}_2\text{SO}_4\), 1 M \(\text{KCl}\), and 0.01 M \(\text{NaAc-HAc}\) buffer (pH 4.7). 1.0 x 10\(^{-4}\) M Cd\(^{2+}\) was employed for this examination.

**Comparison of the Release Efficiency of Extracted Cd\(^{2+}\) from Different Media**

Different types of media were used to find the best solution for the releasing media or detection media for Cd\(^{2+}\). The following solutions and their concentrations were studied: 0.01 M \(\text{KCl}\), 0.01 M \(\text{NaAc-HAc}\) buffer (pH 4.7), 0.01 M \(\text{HNO}_3\), 0.01 M \(\text{Na}_2\text{SO}_4\) in 0.01 M \(\text{H}_2\text{SO}_4\), 0.01 M \(\text{H}_2\text{SO}_4\), and 0.01 M \(\text{HCl}\).

A 2.1 x 10\(^{-4}\) M Cd\(^{2+}\) in 1 M KCl was extracted and was then put into the above solution respectively to examine the best releasing performance upon different solutions.

**Optimization of the Concentrations of the Extraction Media and Release Media**

After selecting the optimal adsorption medium and releasing medium respectively, the next step was to find the best concentration of the respective solution. Extraction, desorption
and detection of $4.2 \times 10^{-5} \text{ M Cd}^{2+}$ in a series of solutions containing best adsorption and best releasing medium were carried out. The optimal concentration(s) for each medium lie in the highest Cd stripping peak current. The concentration series used for the adsorption medium was 0.01 M, 0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M, 0.4 M, 0.8 M, and 1.0 M. The concentration series used for the releasing medium was 0.005 M, 0.01 M, 0.02 M, 0.04 M, 0.08 M, and 1 M.

**Finding the Best Accumulated Charge on the Film**

When preparing the film by bulk electrophoresis, we can observe charge accumulated on the film with the time. At a certain desired charge, the film preparation was stopped to get the desired charge accumulated on the film. The films having following charges were prepared. Then the extraction, desorption, and detection was done by using those films separately. The best accumulated charge was decided by the highest cadmium peak given by the film. We used $8.89 \times 10^{-5} \text{ M Cd}^{2+}$ concentration for this purpose.

**Table 1. Different Types of Films with Different Types of Charges.**

<table>
<thead>
<tr>
<th>Film number</th>
<th>Charge accumulated on the film/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$8.8134 \times 10^{-4}$</td>
</tr>
<tr>
<td>2</td>
<td>$1.1176 \times 10^{-3}$</td>
</tr>
<tr>
<td>3</td>
<td>$1.7646 \times 10^{-3}$</td>
</tr>
<tr>
<td>4</td>
<td>$2.9629 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
Extraction of Multiple Coexisting Cations Like \( \text{Hg}^{2+}, \text{Pb}^{2+}, \text{Cd}^{2+} \text{ and } \text{Co}^{2+} \)

We used PPy/DNA film to extract a solution containing \( 1.5 \times 10^{-4} \text{ M} \text{ Cd}^{2+}, 4.0 \times 10^{-4} \text{ M} \text{ Pb}^{2+}, 5.0 \times 10^{-4} \text{ M} \text{ Hg}^{2+}, \text{ and } 5.0 \times 10^{-4} \text{ M} \text{ Co}^{2+} \). The extraction of these metals were determined by the peaks obtained by the detection process by getting the oxidation potential of appropriate metals.

Possibilities of Extracting of Other Cations

The following cation solutions and their concentrations with the presence of \((\text{Cd}^{2+})\) was used for the extraction, desorption, and detection. If a peak was seen at its appropriate oxidation potential of that cation, then we came to a conclusion that those cations were extracted from the solution.

I. \( [\text{Fe}^{3+}] = 8.8 \times 10^{-4} \text{ M} + [\text{Cd}^{2+}] = 8.8 \times 10^{-4} \text{ M} \)

II. \( [\text{Al}^{3+}] = 8.8 \times 10^{-4} \text{ M} + [\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \)

III. \( [\text{Cu}^{2+}] = 1.0 \times 10^{-3} \text{ M} + [\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \)

IV. \( [\text{Zn}^{2+}] = 8.0 \times 10^{-5} \text{ M} + [\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \)

V. \( [\text{Bi}^{3+}] = 1.0 \times 10^{-4} \text{ M} + [\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M}. \)

Interference from Other Cations and Anions

Cations

The following cation solutions and their concentration with the presence of \(\text{Cd}^{2+}\) were used to find the interference. A 20% decrease of the peak height from the control was considered as interference.

I. \( [\text{Cd}^{2+}] = 1.5 \times 10^{-4} \text{ M} \text{ and } [\text{Co}^{2+}] = 5.0 \times 10^{-4} \text{ M} \)

II. \( [\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \text{ and } [\text{Cu}^{2+}] = 1.0 \times 10^{-3} \text{ M} \)

\( [\text{Cd}^{2+}] = 5.0 \times 10^{-4} \text{ M} \text{ and } [\text{Cu}^{2+}] = 1.0 \times 10^{-4} \text{ M} \)
III. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Pb}^{2+}] = 8.8 \times 10^{-5} \text{ M}\)

IV. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Zn}^{2+}] = 8.8 \times 10^{-5} \text{ M}\)

V. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Bi}^{3+}] = 1.0 \times 10^{-4} \text{ M}\)
   \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Bi}^{3+}] = 8.0 \times 10^{-5} \text{ M}\)

VI. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Ni}^{2+}] = 1.7 \times 10^{-4} \text{ M}\)

VII. \([\text{Cd}^{2+}] = 8.8 \times 10^{-4} \text{ M} \) and \([\text{Fe}^{3+}] = 8.8 \times 10^{-4} \text{ M}\)

VIII. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Al}^{3+}] = 8.8 \times 10^{-4} \text{ M}\)

Anions

The following anions solutions and their concentrations with the presence of cadmium were used to find the interference. A 20% decrease of the peak current from the control was considered as interference.

I. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{CH}_3\text{CO}_2^-] = 8.0 \times 10^{-4} \text{ M}\)

II. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{SO}_4^{2-}] = 8.0 \times 10^{-3} \text{ M}\)

III. \([\text{Cd}^{2+}] = 8.0 \times 10^{-5} \text{ M} \) and \([\text{PO}_4^{3-}] = 8.0 \times 10^{-4} \text{ M}\)

IV. \([\text{Cd}^{2+}] = 8.8 \times 10^{-5} \text{ M} \) and \([\text{Cr}_2\text{O}_7^{2-}] = 8.0 \times 10^{-4} \text{ M}\)

Comparison of Linearity Range of \(\text{Cd}^{2+}\) with the Atomic Absorption Spectroscopy (AAS)

The concentration series used for to determine the linearity of cadmium was
\(1.0 \times 10^{-6} \text{ M}, 5.0 \times 10^{-6} \text{ M}, 1.0 \times 10^{-5} \text{ M}, 5.0 \times 10^{-5} \text{ M}, 10 \times 10^{-5} \text{ M}, 15 \times 10^{-5} \text{ M}\). We used the atomic absorption spectroscopy to compare the results of the linearity range for \(\text{Cd}^{2+}\) in this procedure. The absorbance at 288.8 nm was measured by AAS by using the same concentration series used to check the linearity range for \(\text{Cd}^{2+}\) of this SPME procedure.
Percent Overall Recovery

The extracted amount of mass from the film can be expressed by Equation 3.1.

\[
m = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + V_s}
\]  

(3.1)

\[
m = \text{mass of the analyte adsorbed by coating}
\]

\[
V_f = \text{volume of the fiber coating or the film (extracting phase)}
\]

\[
V_s = \text{volume of the sample}
\]

\[
K_{fs} = \text{the distribution constant of the analyte between the coating and the sample matrix}
\]

\[
C_o = \text{the initial concentration of the analyte in the sample}
\]

There exist linear relationship between the amount of analyte adsorbed and their initial concentration in the sample.

If \(V_s\) is very large,

\[
(V_s >> K_{fs} V_f):
\]

\[
m = K_{fs} V_f C_o
\]  

(3.2)

As described in the introductory chapter (Chapter 1), when the sample volume is large, the amount of the analyte extracted by the film or fiber coating is not related to the sample volume. But it is related to the concentration of the sample and the volume of the film.

If \(V_f\) is kept constant throughout different extraction process, then:

\[
m = k \times C_o
\]  

(3.3)

where \(k = K_{fs} \times V_f = \text{constant}\)

The uptake amount of the analyte is proportional to its concentration in the sample.
To verify if our electrochemically assisted SPME process follows the same extraction principle as in Equation 3.3 we used the PPy/DNA extracting phase and Cd$^{2+}$ model in different concentrations and volumes (Table 2) to examine the overall recovery.

The overall recovery was calculated by using Equation 3.4.

$$\text{Overall Recovery} \% = \frac{\text{absolute moles of the analyte}}{\text{in the detection media} \ (n_f)} \times 100 \quad (3.4)$$

$$\text{absolute moles in the extraction media} \ (n_i)$$

The amount of moles in the extraction media (not the extraction phase or film) or the sample solution was calculated using the Equation 3.5.

$$n_f = C_o \times V_s \quad (3.5)$$

$$C_o = \text{concentration of the sample solution (mol/L)}$$

$$V_s = \text{volume of the sample solution} \ / \ L$$

A calibration graph was drawn to find the number of moles released to the releasing solution (200 µL detection solution). This was done by the following procedure: A standard Cd$^{2+}$ concentration series (1.0 x 10$^{-5}$ M, 2.0 x 10$^{-5}$ M, 4.0 x 10$^{-5}$ M, 8.0 x 10$^{-5}$ M, 16.0 x 10$^{-5}$ M) was prepared in 200µL volume respectively. We used 0.05 M HCl as the medium for the above concentration series. In so doing, the detection volume for these concentrations were controlled to be the same for easy comparison purpose.

By using these standard solutions, we ran the ASV. The initial potential was -1.2 V for 60 seconds then scanned from -1.2 V to 0.8 V. The scanning rate was 0.1 V/s. 10µL of 0.002 M Hg$^{2+}$ was added to the standard solution before running the ASV. This was done because we used the same amount of Hg$^{2+}$ to enhance the detection of Cd$^{2+}$ peak in our SPME procedure. A calibration curve was drawn by using the peak height/current vs. Cd$^{2+}$
concentration (Figure 18). The Cd\textsuperscript{2+} concentration was taken as the X axis and peak height/current was defined as Y axis. In Figure 18, \(i_f\) represents the detected Cd\textsuperscript{2+} signal current after its extraction, washing, transfer, release, and detection steps. \(C_f\) on the other hand is detected Cd\textsuperscript{2+} concentration. This calibration curve was used to determine the amount of Cd\textsuperscript{2+} moles present in the detection media.

![Graph](image_url)

**Figure 18. Calibration of the Detected Signal vs. Cd\textsuperscript{2+} Concentration.**

By obtaining \(C_f\) we calculated the Cd\textsuperscript{2+} moles present in the releasing solution by using the Equation 3.6.

\[
n_f = C_f \times V_f
\]  

(3.6)

These mole numbers were used to calculate the percent overall recovery of this SPME procedure, which was discussed in Equation 3.4.
Table 2. The Concentrations and the Volumes Used to Find the Percent Overall Recovery of
PPy/DNA.

<table>
<thead>
<tr>
<th>Cd$^{2+}$ concentration in the sample solution/M</th>
<th>Volume of the sample solution/µL</th>
<th>Stirring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 x 10^{-5} M</td>
<td>200</td>
<td>no</td>
</tr>
<tr>
<td>1.0 x 10^{-5} M</td>
<td>500</td>
<td>no</td>
</tr>
<tr>
<td>1.0 x 10^{-5} M</td>
<td>2000</td>
<td>yes</td>
</tr>
<tr>
<td>1.0 x 10^{-5} M</td>
<td>25000</td>
<td>yes</td>
</tr>
<tr>
<td>5.0 x 10^{-6} M</td>
<td>2000</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 2 shows the concentrations of Cd$^{2+}$ solutions and their volumes used to find the
overall recovery for PPy/DNA. The same PPy/dsDNA film (15.8 mC/cm$^2$) was used for all of
the conditions. The release or detection solution was 200 µL (0.05 M HCl) in all of these
conditions as well.

The next step was to compare the percent overall recovery for PPy/dsDNA with other
extraction phases (PPy/Cl$^-$, PPy/TsO$^-$, GC). The preparation of these films were detailed
previously (preparation conditions: 0.1 M pyrrole + 0.5 M Cl$^-$, 0.1 M pyrrole + 0.5 M TsO$^-$,
0.1 M pyrrole + 100 ppm dsDNA, bare glassy carbon). The same SPME procedure was
followed for all of these films. Table 3 lists the concentration of Cd$^{2+}$ and their volumes used
for this purpose. The charge/area of all those films was 15.8 mC/cm$^2$. 

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Table 3. The Concentrations and the Volumes Used to Find the Overall Recovery of PPy/dsDNA, PPy/Cl⁻, PPy/TsO⁻, Glassy Carbon.

<table>
<thead>
<tr>
<th>Cd²⁺ concentration in the sample solution/M</th>
<th>Volume used for extraction/µL</th>
<th>Stirring</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 x 10⁻⁶</td>
<td>2000</td>
<td>Yes</td>
</tr>
<tr>
<td>1.0 x 10⁻⁵</td>
<td>200</td>
<td>No</td>
</tr>
<tr>
<td>1.0 x 10⁻³</td>
<td>25000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The results from this testing are tabulated and plotted to verify the validity of the extraction theory for our electrochemically assisted SPME. (See results and discussion).

**Extraction of Pb²⁺ and Finding the Linearity Range for Pb²⁺**

Though our research was in finding the optimum extraction condition for Cd²⁺, we were also able to find that this film has higher extraction for Pb²⁺. So our next step was to find the linearity range for Pb²⁺. For this we used a series of concentration of Pb²⁺ (1.0 x 10⁻⁶ M to 1.5 x 10⁻⁴ M). The concentration series was increased until we get to the saturation point. This was done three times and the standard deviation was calculated. A graph of peak current vs. adsorbed concentration was drawn and the linearity was found.
CHAPTER 4
RESULTS AND DISCUSSION

Extraction and Detection of Heavy Metals Using the PPy/DNA Film

Anodic stripping voltammetry (ASV) is a very sensitive method for various electro-active inorganic and organic substances. Cadmium is a heavy metal that has environmental significance because of its toxicity to living organism. Cd$^{2+}$ is electro active and possesses excellent ASV features\(^\text{(18)}\). We, therefore, chose Cd$^{2+}$ as a model target to test and to optimize our new PPy/DNA based SPME protocol for its potential in environment monitoring and remedy. Figure 19 and 20 show the voltammograms of the detection after SPME extraction of 6.0 x 10\(^{-5}\) M Cd$^{2+}$ and 8.0 x 10\(^{-5}\) M Cd$^{2+}$ using PPy/DNA respectively in 0.2 M KCl, by anodic stripping voltammetry. From the figure an anodic peak current appears at -0.7 V.

Figure 19. The Peak Current of Cd$^{2+}$ in the Detection Media (0.05 M HCl) After Adsorption of 6.0 x 10\(^{-5}\) M Cd$^{2+}$ in 0.20 M KCl.
As a control experiment, a complete extraction/transfer/release/detection multiple steps was conducted by using the same PPy/DNA film following the same protocol and medium conditions but without Cd$^{2+}$ presence generate no signal at -0.7 V (data not shown). Further, adding more Cd$^{2+}$ in the extraction medium resulting in an increase for the peak at -0.7 V (6.0 x 10$^{-5}$ M Cd$^{2+}$ in Figure 19 vs. 8.0 x 10$^{-5}$ M Cd$^{2+}$ in Figure 20). Finally, direct addition of standard Cd$^{2+}$ in the detection medium would produce oxidation peak at -0.7 V (data not shown). These observations demonstrate that the anodic peak at 0.7 V is related to Cd$^{2+}$, which can be ascribed as Cd stripping (oxidation) reaction on the detector (glassy carbon) surface:

\[
\text{Cd} - 2e \rightarrow \text{Cd}^{2+} \quad (4.1)
\]

Figure 20. The Peak Current of Cd$^{2+}$ in the Detection Media (0.05 M HCl) After Adsorption of 8.0 x 10$^{-5}$ M Cd$^{2+}$ in 0.20 M KCl.
Comparison of Extraction Efficiency from Different Solid Phases for Cd$^{2+}$ and Comparison of Single and Double Stranded DNA as Composite Dopants

Figure 21 shows the variations of Cd$^{2+}$ (4.4 x 10$^{-3}$ M) peak currents with PPy solid phase containing the dopants dsDNA (green line), chloride (black line), and TsO$^-$ (red line) respectively, as well as bare glassy carbon (orange line). The highest current was obtained by the PPy/DNA film alone. The PPy/Cl$^-$, bare glassy carbon and PPy/TsO$^-$ all gave poor currents. It was thus concluded that PPy/DNA was the best film for the cadmium extraction. This new PPy/DNA composite offers much higher extraction efficiency than the conventional PPy as well as the substrate electrode.
Figure 21. Comparison of Extraction Efficiency from Different Solid Phases for Cd$^{2+}$

(Extraction: -1.0 V in 0.2 M KCl; Release: +0.7 V in 0.05 M HCl; Detection: ASV with Bare Glassy Carbon electrode, Initial Potential -1.2 V for 50 s and Scanning -1.2 ~ +0.8 V at 0.1 V/s).

The next experiment was to find the effect of ssDNA and dsDNA as the composite dopants in PPy/DNA film for the extraction of Cd$^{2+}$. The extraction of $4.4 \times 10^{-4}$ M Cd$^{2+}$ was done by using PPy/dsDNA, and PPy/ssDNA separately.
Figure 22. Comparison of Double Stranded and Single Stranded DNA as Composite Dopants for Extraction Efficiency. (Extraction: -1.0 V in 0.2 M KCl; Release: +0.7 V in 0.05 M HCl; Detection: ASV with Bare Glassy Carbon Electrode Potential Ranging from -1.4 ~ +0.0 V at 0.1 V/s.)

Figure 22 exhibits the Cd$^{2+}$ response using PPy/ssDNA, and PPy/dsDNA extraction respectively. While Cd$^{2+}$ current peaks show little difference in the two films, PPy/dsDNA contributes a slightly higher extraction. We, therefore, chose PPy/dsDNA as the SPME materiel in our study.

**Extraction of Cd$^{2+}$ Using PPy/DNA at Open Circuit Condition**

Table 4 shows the average of the peak currents obtained in the extraction 1.0 x 10$^{-5}$ M Cd$^{2+}$ solution by using electrochemically assisted SPME and open-circuit condition SPME,
respectively. We obtained $1.6 \times 10^{-5}$ A current for Cd$^{2+}$ from electrochemically assisted SPME whereas no current was obtained from open circuit condition SPME. This indicates that electrochemical assistance is necessary to achieve appreciable extraction of Cd$^{2+}$ by the new SPME material that is PPy/DNA composite. On the other hand, the open-circuit condition SPME might still attract cationic Cd$^{2+}$ towards its anionic DNA dopant. However, in comparison to the externally applied electric field, this spontaneous electrostatic force is negligible and, therefore, produced undetectable Cd$^{2+}$ extraction. We thus employed the electrochemically assisted SPME for environmental monitoring and remediation throughout the whole thesis work.

Table 4. Average of the Peak Currents Obtained from Different Types of Extraction.

<table>
<thead>
<tr>
<th>Method of extraction</th>
<th>Peak current/(-) $1.0 \times 10^{-5}$ A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemically assisted</td>
<td>1.60</td>
</tr>
<tr>
<td>Open circuit condition</td>
<td>No peak found</td>
</tr>
</tbody>
</table>

Optimization of the SPME Process

The SPME protocol for the Cd$^{2+}$ consists of extraction, rinse/transfer, release, and detection. The optimal operational parameters for each of these steps were carefully examined and discussed below by using the final detected Cd$^{2+}$ response as a comparable criterion.

The Extraction Media

Figure 23 shows that different peak currents were obtained from different adsorption media. The film was prepared three times and the extraction of Cd$^{2+}$ using each medium was done in triplicates. But the order of extracting each media by using the film was changed at each time.
Figure 23. Comparison of the Extraction Efficiency of Different Media for Cd$^{2+}$ by Using PPy/DNA. (Extraction: -1.0 V Using PPy (DNA) for 3000 s; Release: +0.7 V; Detection: ASV with Bare Glassy Carbon Electrode, Initial Potential -1.2 V for 50 s and Scanning -1.2 ~ +0.8 V at 0.1 V/s.)

The highest peak was obtained by 1 M KCl. So, it was concluded that 1 M KCl was the best extraction media for cadmium. This may be due to the presence of chloride in KCl. The HCl did not give higher peak current due to the acidic condition. So we can conclude that chloride is significant for extraction of Cd$^{2+}$ in the extraction media.
The Concentration of the Extraction Media (KCl)

After concluding that 1 M KCl was the best extraction medium, the next step was to find the best concentration of KCl. The Figure 24 shows the graph of the peak current vs. concentration of KCl. These peak currents were obtained by using different concentration of KCl for the extraction medium for Cd$^{2+}$.

Figure 24. The Graph of Peak Current vs. Molar Concentration of KCl.

Figure 24 shows that the peak current increases with increasing concentration. But after 0.2 M KCl, the peak current decreases. So, from the experiment we conclude that 0.2 M is the best adsorption concentration for Cd$^{2+}$. 
The Best Releasing Media

Figure 25 shows the different peak currents obtained from different releasing or detection media. The HCl gave the highest current. Here too the Cl⁻ plays a major role.

Figure 25. Comparison of the Releasing Media.
The Concentration of the Releasing Media

Figure 26. The Graph of Peak Current vs. Molar Concentration of HCl Concentration.

Figure 26 shows that with an increase of HCl concentration the peak current of Cd\textsuperscript{2+} increases and then plateau before starting to decrease gradually. So, the optimum concentrations for the best desorption and detection media is 0.05 M HCl.

Effect of the Film Thickness/Charge on Cd\textsuperscript{2+} Extraction

Table 5. Cd\textsuperscript{2+} Peak Currents Obtained from PPy/dsDNA Films with Different Charges.

<table>
<thead>
<tr>
<th>Charge on the film/C</th>
<th>Peak current/1.0 x 10\textsuperscript{-5} A</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.8134 x 10\textsuperscript{-4}</td>
<td>- 1.8</td>
</tr>
<tr>
<td>1.1176 x 10\textsuperscript{-3}</td>
<td>- 4.0</td>
</tr>
<tr>
<td>1.7646 x 10\textsuperscript{-3}</td>
<td>- 1.4</td>
</tr>
<tr>
<td>2.9629 x 10\textsuperscript{-3}</td>
<td>No peak found</td>
</tr>
</tbody>
</table>
Table 5 lists different Cd$^{2+}$ stripping peak currents obtained from PPy/DNA films with different charges accumulated on them from their respective preparations. These different charges represent different film thickness because all of the films share the same substrate area/diameter ($7.06 \times 10^{-2}$ cm$^2$/Φ = 3 mm) of the glassy carbon electrode. As can be seen from Table 5, the highest extraction occurs at the median value charge ($1.1176 \times 10^{-3}$ C) while no peak is found when a very thick film was used. Therefore, the $1.1176 \times 10^{-3}$ C film was chosen for our study. The charge/area for this film is 15.8 mC.cm$^{-2}$. The thickness of the film was estimated as 0.0395 µm based on the formula that 400 mC.cm$^{-2}$ corresponds to 1 µm$^{(22)}$. This is the optimal film thickness for the extraction of Cd$^{2+}$.

The Enhancement of Coexisting Mercury for M$^{n+}$(Cd$^{2+}$) Detection

To enhance the anodic peaks of cations in ASV, mercury has been used as reported in the literature$^{(22)}$. Our aim was to find how mercury affects our electrochemical SPME procedure. In our proposed procedure there are extraction, release, and detection. We had to add mercury at different steps of the procedure to find at which stage it impacts and how it affects.

The following three conditions were used for this purpose and the results were shown in Figure 27.

a. The original extraction sample containing $1.0 \times 10^{-5}$ M Cd$^{2+}$ and $1.0 \times 10^{-4}$ M Hg$^{2+}$.

b. The original extraction sample contains $1 \times 10^{-5}$ M Cd$^{2+}$ only. Hg$^{2+}$ ($1 \times 10^{-4}$ M) was added into the detection medium (0.05 M HCl) after Cd$^{2+}$ has been released.
c. The original extraction sample containing $1 \times 10^{-5}$ M Cd$^{2+}$ only. No Hg$^{2+}$ exists throughout the process.

According to Figure 27 the higher current was obtained when Hg$^{2+}$ was added to the detection step (orange line). This shows that there is enhancement of the peak of Cd when we add Hg at the detection step. On the other hand it shows that Hg has no effect in the adsorption step. This can be explained as follows:-

![Graph showing current vs potential for different Cd$^{2+}$ and Hg$^{2+}$ concentrations](image.png)
\[ \text{Hg}^{2+} + \text{Cd}^{2+} \rightarrow [\text{Hg}]\text{Cd} \text{ (Amalgan)} \quad (4.2) \]

Reduction at -1.0 V for 50 s.

\[ [\text{Hg}]\text{Cd} \rightarrow \text{Hg} + \text{Cd}^{2+} \quad (4.3) \]

Oxidation at 0.7 V.

When ASV was run we gave a standing potential of -1.0 V for 50 s. Then Hg\(^{2+}\) and Cd\(^{2+}\) in the solution gets reduced and form [Hg]Cd–amalgan (Equation 4.2), which increases the concentration of cadmium on the electroplate (glassy carbon electrode). Because of this there will be higher concentration of cadmium on the electrode plate, so we can see a higher current upon its oxidation at -0.7 V, when compared with the absence of Hg\(^{2+}\). This effect of Hg\(^{2+}\) was used to improve the sensitivity of our method.

**Extraction of Multiple Coexisting Cations Hg\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), and Co\(^{2+}\)**

After extracting Cd\(^{2+}\) alone, our next target was to find whether we can extract and detect multiple coexisting cations together. So, we used solutions containing different cations with different concentrations in our extraction procedure. Figure 28 shows the plot of results obtained for these solutions by the ASV in the detection process. Original concentrations before extraction of Hg\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), and Co\(^{2+}\) are shown in Figure 28.
Figure 28. Detection of Heavy Metal Ions After Extraction by the PPy/DNA. (Detection Medium, 0.05 M HCl; Standing Potential, -1.2 V; Standing Time, 50 s; Scan rate, 0.1 V/s.)

We were able to extract and detect mercury, cobalt, lead, and cadmium together as shown in Figure 28. This was confirmed by their respective anodic peaks found at their respective oxidation potentials. These potentials were figured by running ASV in respective standard solutions.

According to the peak current we assume that Pb$^{2+}$ and Cd$^{2+}$ have higher sensitivity under these conditions than Hg$^{2+}$ and Co$^{2+}$. However, it may be possible to change some of the conditions of this procedure to enhance the sensitivity of Hg$^{2+}$ and Co$^{2+}$.

This is a unique method for extraction of several metals and detect them simultaneously. Most of other equipments like ICP (Inductive couple plasma) can analyze
several metals together but it can not do the extraction. This method is an integrated method.

**Extraction Other Individual Cations**

In this step we were able to find whether we can extract other cations (other than mercury, lead, cobalt) individually in the presence of Cd\(^{2+}\). We did this as mentioned in the procedure (Chapter 3). There were no peaks found from the extractions Fe\(^{3+}\) and Al\(^{3+}\). But we were able to see peaks at their oxidation potentials for Cu\(^{2+}\), Zn\(^{2+}\), and Bi\(^{3+}\). From this we assumed that by using this method we can extract Cu\(^{2+}\), Zn\(^{2+}\), and Bi\(^{3+}\) individually. These metals can not be extracted with Hg\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), and Co\(^{2+}\) because their oxidation potentials overlap.

**Interference**

**Cations**

For \(1 \times 10^{-4} \text{ M Cd}^{2+}\), five-fold Co\(^{2+}\), ten-fold Cu\(^{2+}\), one-fold Pb\(^{2+}\), one-fold Zn\(^{2+}\), ten-fold Bi\(^{3+}\), one-fold Fe\(^{3+}\), ten-fold Al\(^{3+}\), one-fold Hg\(^{2+}\), and ten-fold Ni\(^{2+}\) showed no interference during the whole extraction/transfer/release/detection process.

Interference become significant for \(8.8 \times 10^{-5} \text{ M Cd}^{2+}\) in the presence of one hundred-fold Cu\(^{2+}\), and ten-fold Bi\(^{3+}\). The recovering was less than 70%.

**Anions**

For \(1.0 \times 10^{-4} \text{ M Cd}^{2+}\), ten-fold acetate, a hundred-fold sulphate, ten-fold phosphate have demonstrated no interference.

For \(8.8 \times 10^{-5} \text{ M Cd}^{2+}\), ten-fold dichromate start to interfere.
The Linearity Range for Cadmium

We did the linearity for this procedure by using a series of concentrations of Cd$^{2+}$. Those were (1.0 x $10^{-6}$ M, 5.0 x $10^{-6}$ M, 1.0 x $10^{-5}$ M, 5.0 x $10^{-5}$ M, 10 x $10^{-5}$ M, 15 x $10^{-5}$ M). We did the extraction, desorption, and detection by using these concentration series. The concentration series was increased until we get to the saturation point. Then a graph of the peak current vs. the concentration of Cd$^{2+}$ was plotted. This was done in triplicates. The standard deviation also was calculated.

Table 6. Peak Currents Obtained for Different Concentrations of Cd$^{2+}$.

<table>
<thead>
<tr>
<th>Concentration of Cd$^{2+}$ in the sample solution/M</th>
<th>Peak current/(-)1.0 x $10^{-5}$ A</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 x $10^{-6}$</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>5.0 x $10^{-6}$</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>1.0 x $10^{-5}$</td>
<td>0.8</td>
<td>1.02</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5.0 x $10^{-5}$</td>
<td>3.6</td>
<td>5.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>10.0 x $10^{-5}$</td>
<td>7.5</td>
<td>10.0</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>15.0 x $10^{-5}$</td>
<td>2.7</td>
<td>5.1</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 shows the results obtained for the study on the linearity range of Cd$^{2+}$. At the concentration level of 15 x $10^{-5}$ M Cd$^{2+}$ the peak current began to decrease. So, higher concentrations of Cd$^{2+}$ were not used in our subsequent experiments.

The graph in Figure 29 was drawn from the results obtained by the mean value of peak current vs. molar concentration of Cd$^{2+}$. By seeing the graph we can conclude that the linearity range for Cd$^{2+}$ is from 1.0x$10^{-6}$ M Cd$^{2+}$ to 10x$10^{-5}$ M Cd$^{2+}$.  

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The Linearity Range for Lead

Table 7 shows the results obtained for the linearity of Pb$^{2+}$. The saturated point was 30 x 10^{-5} M Pb$^{2+}$. At this concentration the peak current decreased.
Table 7. Different Peak Currents for Different Concentrations of Pb\(^{2+}\).

<table>
<thead>
<tr>
<th>Concentration of Pb(^{2+}) in the sample solution/M</th>
<th>Peak current(/-)1.0 x 10(^{-5})A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>1.0 x 10(^{-6})</td>
<td>0.2</td>
</tr>
<tr>
<td>5.0 x 10(^{-6})</td>
<td>1.65</td>
</tr>
<tr>
<td>1.0 x 10(^{-5})</td>
<td>2.5</td>
</tr>
<tr>
<td>5.0 x 10(^{-5})</td>
<td>9.0</td>
</tr>
<tr>
<td>10.0 x 10(^{-5})</td>
<td>16.0</td>
</tr>
<tr>
<td>15.0 x 10(^{-5})</td>
<td>27.0</td>
</tr>
<tr>
<td>30.0 x 10(^{-5})</td>
<td>7.0</td>
</tr>
</tbody>
</table>

From the results of Table 7 a graph of peak current vs. concentration of Pb\(^{2+}\) was drawn (Figure 30). The standard deviation also was calculated. It shows that the linearity range for Pb\(^{2+}\) is from 1.0 x 10\(^{-6}\) M to 15 x 10\(^{-5}\) M Pb\(^{2+}\). The linearity range for Pb\(^{2+}\) is wider than the Cd\(^{2+}\).

Figure 30. The Graph of Peak Current vs. Molar Concentration of Pb\(^{2+}\).
Comparison of Linearity of This Method to Atomic Absorption Spectroscopy (AAS)

To compare the presented electrochemically assisted SPME with a non-electrochemical detection the atomic absorption spectroscopy (AAS) was employed for Cd\textsuperscript{2+} detection. The graph in Figure 31 was drawn from the results obtained by AAS. The concentration series we used from 1.0 \times 10^{-6} \text{ M} \text{ Cd}^{2+} to 1.5 \times 10^{-4} \text{ M} \text{ Cd}^{2+}. It shows linearity from 1.0 \times 10^{-6} \text{ M} to 5.0 \times 10^{-5} \text{ M}. The electrochemically assisted SPME extraction detection linearity range is wider than the non-electrochemical detection atomic absorption spectroscopy (AAS).

![Figure 31. Absorbance at 228.8 nm vs. Molar Concentration Cd\textsuperscript{2+}.](image)

Percent Overall Recovery

The extraction performance is the core part of SPE. Likewise, it is probably the key for electrochemically assisted SPME as well. It should be related to factors including the extraction capacity of the solid phase, nature of the sample, volume of the sample, analyte
concentration, static or flowing transport, applied potential, etc. In this section we will discuss these factors and their impacts on the extraction efficiency of SPME. The theoretical treatment and the operational details were given in experimental procedure section.

The Figure 32 shows the calibration graph obtained for determination of moles of Cd\textsuperscript{2+} present in the detection media.

![Figure 32. Graph of Peak Current vs. Molar Concentration of Cd\textsuperscript{2+}.

Table 8 shows the results obtained for overall recovery of PPy/dsDNA under different conditions. The highest overall recovery (35.2\%) was obtained when we used 2 mL 1.0 x 10\textsuperscript{-5} M Cd\textsuperscript{2+} concentration with stirring. When we employed the same Cd\textsuperscript{2+} concentration with small volumes and without stirring, the overall recovery was low, e.g. 1.0 x 10\textsuperscript{-5} M Cd\textsuperscript{2+} concentration with 200 µL and 500 µL sample volume, without stirring, give overall recoveries of 15.4\% and 8\% respectively. Under quiescent (no stirring) condition, smaller sample volume (200 µL) have better extraction (15.4\%). In comparison, 500 µL sample volume delivers 8\% overall recovery. This can be explained based on the shortened mass transport path in the small sample volume in a fixed period of extraction time. On the other hand, stirring will greatly
promote mass transport that results in higher efficiency, which is evidenced by 35.2% overall recovery with 2000 µL volume. In this experiment we were unable to stir 200 µL and 500 µL volumes because of the limited experimental setting. However, we predict a stirring, dynamic, or flowing system will generate higher efficiency even for small size samples.

Table 8. Percent Overall Recovery of the PPy/dsDNA Film Under Different Cd$^{2+}$ Concentrations and Volumes. The Charge/Area of the Film was 15.8 mC/cm$^2$. The Desorbed Solution was 200 µL.

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration of Cd$^{2+}$ in the sample solution/M</th>
<th>Volume of the Cd$^{2+}$ sample solution/µL</th>
<th>Stirring while extraction - yes/no</th>
<th>Overall recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 x 10^{-5}</td>
<td>200</td>
<td>No</td>
<td>15.40%</td>
</tr>
<tr>
<td>2</td>
<td>1.0 x 10^{-5}</td>
<td>500</td>
<td>No</td>
<td>8.00%</td>
</tr>
<tr>
<td>3</td>
<td>1.0 x 10^{-5}</td>
<td>2000</td>
<td>Yes</td>
<td>35.20%</td>
</tr>
<tr>
<td>4</td>
<td>1.0 x 10^{-5}</td>
<td>25000</td>
<td>Yes</td>
<td>5.80%</td>
</tr>
<tr>
<td>5</td>
<td>5.0 x 10^{-6}</td>
<td>2000</td>
<td>Yes</td>
<td>13.20%</td>
</tr>
</tbody>
</table>

Conditions No. 3 and 5 (Table 8) are the same except for Cd$^{2+}$ concentrations. The overall recovery for 1.0 x 10^{-5} M Cd$^{2+}$ is 35.20% and for 5.0 x 10^{-6} M Cd$^{2+}$ it is 13.20%. The increased overall recovery upon higher Cd$^{2+}$ concentration in the samples can be explained by the higher uptake on the extraction phase, which also is predicted in Equation 3.1.

The sample No. 3 and 4 in Table 8 have the same concentration and both were stirred. The only difference is their volumes. When the volume is high (25,000 µL), the overall recovery is low (5.8%). When the volume is low (2,000 µL), the overall recovery is high (35.2%). In this comparison, higher sample volume (i.e. more analyte if concentration is the same) would not produce greater recovery. This is because we only increased the volume of
the sample but did not change the volume of the film. The film can adsorb a certain amount of analyte, after that it will be saturated. If we want to have higher overall recovery for larger volumes we must increase the volume of the film. The results from the comparison of No. 3 and 4 are also in agreement with Equation 1.4.

The amount of analyte adsorbed to a film depends on the volume of the film and the initial sample concentration (predicted in Equation 1.4). With the same initial analyte concentration the amount of analyte adsorbed depends only on the film volume (the amount of extraction phase).

In our experiment, however, the same film (15.8 mC/cm²) was used for all of the experiments; therefore, $V_f$ is constant. To consider all these factors, conditions No.3 and 4 would produce the same uploading in their extraction phase. The “lower” overall recovery value in No. 4 is caused by the bigger sample volume. Under this condition the amount of analyte extracted depends on $C_0$, $K_{fs}$, and $V_f$ according to Equation 3.2. Here these values are the same in both conditions. The overall recovery should be same. But there is a difference in the results. The higher/lower sample volume has lower/higher percent overall recovery. This difference is due to the presence of larger amount of analyte in larger volumes and also because we consider the absolute moles extracted to find the overall recovery. To get higher overall recovery, we have to have a higher sample concentration ($C_0$) or higher film volume ($V_f$). Equation 3.2 prevails here.

The next step was to compare results of overall recovery of PPy/dsDNA with other films produced (PPy/TsO− and PPy/Cl−). As a control we also compared them with the bare Glassy Carbon.
Table 9 displays the results obtained for the overall recovery of different films. Three experimental conditions were used. Each time the film was prepared and used for triplicate measurements. Under each condition the overall recovery was determined in triplicates and the average value was given in the table.

Table 9. The Values of the Overall Recoveries from Different Types of Films.

<table>
<thead>
<tr>
<th>Condition type</th>
<th>0.1 M Ppy/100 ppm DNA – film # 1</th>
<th>0.1 M Ppy/0.5 M Cl(^{-}) – film # 2</th>
<th>0.1 M Ppy/0.5 M TSO(^{-}) – film # 3</th>
<th>Glassy Carbon – # 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 x 10(^{-6}) M Cd(^{2+}) (2 mL with stirring)</td>
<td>13.20%</td>
<td>0.44%</td>
<td>0.88%</td>
<td>5.28%</td>
</tr>
<tr>
<td>1.0 x 10(^{-5}) M Cd(^{2+}) (200 µL without stirring)</td>
<td>15.40%</td>
<td>15.40%</td>
<td>4.40%</td>
<td>0.00%</td>
</tr>
<tr>
<td>1.0 x 10(^{-5}) M Cd(^{2+}) (25 mL with stirring)</td>
<td>5.80%</td>
<td>2.72%</td>
<td>1.84%</td>
<td>1.58%</td>
</tr>
</tbody>
</table>

The overall recoveries in Table 9 exhibit the same trends of the concentration, volume effects for individual film under all condition as those of Table 8 (see previous discussion). We can conclude that under all types of experimental conditions PPy/dsDNA is the best for extraction of Cd\(^{2+}\). It has the higher values in all three conditions. Only when we used 1.0 x 10\(^{-5}\) M Cd\(^{2+}\) concentration (200 µL) did the PPy/dsDNA and PPy/Cl\(^{-}\) give the same results. This may be due to the lower dsDNA dopant concentration (100 ppm) than the Cl\(^{-}\) (0.5 M) used to prepare the appropriate film. A higher dopant concentration of dsDNA when preparing the extraction phase may achieve higher overall recovery.
Also from Table 9 higher concentrations of Cd\(^{2+}\) always results in greater overall recovery, which is true for all films under all conditions. On the other hand, higher sample volume generates lower overall recovery, which is obvious for all the films.

As another control, the bare glassy carbon also revealed some extraction feature although its overall recovery is small under the conditions studied. This is because the carbon substrate itself can be electrochemically coated with cadmium metal under -1.0 V condition, which serves as an “extraction” step.

A distinct conclusion that can be drawn from Table 9 is that the PPy/dsDNA composite shows the best extraction performance towards Cd\(^{2+}\) among all of the films applied under all of the conditions studied.

Table 10 shows the amount of Cd\(^{2+}\) adsorbed and released by the PPy/DNA film from each Cd\(^{2+}\) initial sample concentrations.

<table>
<thead>
<tr>
<th>Cd(^{2+}) initial sample concentration/1.0 \times 10^{-4} M</th>
<th>Mass of Cd(^{2+}) adsorbed and released to the medium/1.0 \times 10^{-6} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.098</td>
</tr>
<tr>
<td>0.05</td>
<td>0.200</td>
</tr>
<tr>
<td>0.10</td>
<td>0.503</td>
</tr>
<tr>
<td>0.50</td>
<td>2.520</td>
</tr>
<tr>
<td>1.00</td>
<td>4.950</td>
</tr>
<tr>
<td>1.50</td>
<td>2.560</td>
</tr>
</tbody>
</table>
Figure 33. The Graph of Cd\textsuperscript{2+} Mass Extracted vs. Molar Concentration of Cd\textsuperscript{2+} in the Initial Sample Solution.

The graph based on Table 10 is given in Figure 33 that plotted the amount of analyte adsorbed vs. Cd\textsuperscript{2+} initial sample concentration. We were able to get a linear plot. This is in agreement with Equation 4.4. This relationship can be expressed by using a constant (k) for the Equation 3.3 mentioned in Chapter 3.

\[ m \propto C_0 \]  \hspace{1cm} (4.4)

\[ m = k \times C_0 \]  \hspace{1cm} (3.3)

\[ m = K_{fs} V_f C_0 \]  \hspace{1cm} (3.2)

The constant “k” which includes \( K_{fs} \) (the distribution constant of the analyte between the coating and the sample matrix) and \( V_f \) (volume of the film). So, from the graph in Figure 33 we can find the slope that gives the value of “k”. If we know the volume of the film we can...
calculate the value of $K_f$. The calculated value for $K_f$ for PPy/DNA film for the extraction of $5.0 \times 10^{-5}$ M Cd$^{2+}$ is $3.56 \times 10^{11}$. This value is important to show the efficiency of the film.

**Reproducibility**

For extraction, desorption, and detection we took $1.0 \times 10^{-5}$ M Cd$^{2+}$ solution. We did this five times and obtained the cadmium peak current in each occasion. The relative standard deviation was calculated.

Table 11. Peak Currents for Cd$^{2+}$ In Different Reproducibility Trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Peak current for the extraction of $1.0 \times 10^{-5}$ M Cd$^{2+}$/(-) $1.0 \times 10^{-5}$ A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The relative standard deviation is 14.87 %. The relative standard deviation should be less than 8 %, but our result is higher than that. This may be due to error occurring in the process when transferring electrodes from one solution to another and using a very small amount of desorption solution-200 µL.

**Differential Pulse Voltammetry (DPV)**

To compare our results with conventional detection mode of ASV, differential pulse voltammetry (DPV) technique was used in the detection step, following the same protocol of SPME as discussed before.
Table 12 shows the peak currents obtained by the above mentioned procedures.

Table 12. Peak Currents Obtained for $1.0 \times 10^{-5}$ M Cd$^{2+}$ Extraction by Different Detection Methods.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Peak current/(-)1.0 x $10^{-5}$ A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection with ASV in the presence of Hg$^{2+}$</td>
<td>0.25</td>
</tr>
<tr>
<td>Detection with DPV</td>
<td>0.40</td>
</tr>
</tbody>
</table>

So, there is a current increase if we used DPV. So, by using DPV method we were able to lower our detection limit up to $5.0 \times 10^{-7}$ M Cd$^{2+}$ and with extraction time 3000 s.

As we discussed in the introduction part of the differential pulse voltammetry, the potential starts from zero after short time. But in the cyclic voltammetry the potential increases from initial to final stage. So, we conclude saying that DPV gives a higher current when comparing with CVV or ASV.

**Mechanism of Electrochemical Solid Phase Micro Extraction Based on PPy/DNA Composite**

The mechanism behind this SPME of heavy metals from aqueous solutions has been discussed below. It is explained in three steps.

**Extraction**

When the PPy/DNA composite film is dipped into a solution containing heavy metal cations, the cations will approach the SPME material by electrostatic force through anionic DNA and by a coordinating force through the nitrogen and oxygen binding sites on pyrrole rings and DNA base moieties:
Mn\(^{n+}\): Cd\(^{2+}\) or other heavy metal ions

\[
\text{(DNA}^{n-}\text{)} \text{PPy surface} + \text{M}^{n+} (\text{aq}) = \text{M}^{n+}(\text{DNA}^{n-}) \text{PPy surface} \quad (4.5)
\]

**Accumulation**

When we apply a \(-1.0\) V (vs. Ag/AgCl) on the extraction phase (PPy/DNA), more solution \(\text{M}^{n+}\) will be attracted and the surface \(\text{M}^{n+}\) will be reduced and thus the \(\text{M}^{n+}\) ions are remarkably pre concentrated and accumulated.

\[
\text{M}^{n+} \text{(DNA}^{n-}\text{)} \text{PPy surface} + \text{ne}^- = \text{M}^{0} \text{PPy surface} + \text{(DNA}^{n-}\text{)} \text{PPy surface}
\]

**Transfer/Release**

When we take the film out from the extraction solution and place it in the desorption or releasing solution (0.05 M HCl), and apply a 0.7 V (vs. Ag/AgCl) then the reduced cation \((\text{M}^{0})\) on the PPy surface gets oxidized into \(\text{M}^{n+}\) (aq) and will be released to the aqueous solution.

\[
\text{M}^{0} \text{PPy surface} - \text{ne}^- = \text{M}^{n+} (\text{aq}) \quad (4.6)
\]

ASV was used to detect the released cations. For this purpose a new glassy carbon electrode was used. When we applied \(-1.0\) V for 50 s, all cations \((\text{M}^{n+})\) in the solution become plated on to the glassy carbon electrode \((\text{M}^{0}-\text{plate})\). Then we scan a potential of \(-1.2\) V to 0.8 V, the \(\text{M}^{0}\) on the plate becomes oxidized to \(\text{M}^{n+}\) and released to the medium, releasing a current at their oxidation potential. This current height is a measure of the concentration of cations present in the solution. So, this is the proposed mechanism for our experimental and results obtained. This mechanism is explained schematically in Figure 34.
Figure 34: The Schematic Diagram Showing the Mechanism of the Electrochemically Assisted SPME.

Extraction -
Heavy metal ions approach the composite film due to the electrostatic attraction from anionic DNA moieties and coordination from N, O-containing pyrrole rings and doped DNAs.

Accumulation -
At -1.0 V, the surface metal ions are reduced to their atomic state. More metal ions in the bulk solution are attracted to the film.

Release -
At 0.7 V, the extracted and accumulated metal layers are oxidized and released into a medium other than the sample matrix for detection. The film is regenerated.
CHAPTER 5
CONCLUSION

Solid phase microelectretaction is a newly developing technique for environmental monitoring and remedy. In this work a new SPME material PPy/DNA composite film has been shown for the first time for its promise in extraction, transfer, and release of environmental heavy metal cations under controlled potential conditions. This film was constructed by bulk electrolysis of pyrrole in the presence of DNA molecules at 0.7 V (vs. Ag/AgCl). By using this film we were able to extract Cd$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and Bi$^{3+}$. This film exhibits higher extraction efficiency than conventional PPy/Cl$^-$, PPy/TsO$^-1$ and the bare carbon material.

Using Cd$^{2+}$ as a model target we were able to get the following optimal conditions.

- Film preparation: 100 ppm ds DNA + 0.1 M pyrrole, 0.7 V for 100 s on glassy carbon electrode or other substrates.
- Extraction: At -1.0 V in 0.2 M KCl using PPy/ dsDNA with stirring.
- Release: At 0.7 V in 0.05 M HCl.
- Detection: ASV with initial potential -1.2 V for 50 s and scanning from -1.2 V to 0.8 V at 0.1 V/s.
- Linearity: 1.0 x 10$^{-6}$ M to 1.0 x 10$^{-4}$ M.
- Extraction/detection limit: 5.0 x 10$^{-7}$ M for 3000 s extraction time and using DPV.
- Reproducibility: Relative standard deviation of 14.87% for 1.0 x 10$^{-5}$ M Cd$^{2+}$.
- The optimal charge density of the film: 15.8 mC/cm$^2$. This corresponds to the film thickness of 39.5 nm.
Interference: No interference from other cations and anions when they are in equal or lower concentration than Cd$^{2+}$ except for dichromate.

Hg$^{2+}$ was found to enhance Cd$^{2+}$ detection sensitivity.

Using polypyrrole network, dopants such as dsDNA and ssDNA exhibit almost the same Cd$^{2+}$ extraction efficiency.

The mechanism of this new SPME material in response to heavy metal ions is proposed as follows:

- **Extraction**
  \[
  \text{M}^{n+} : \text{Cd}^{2+} \text{ or other heavy metal ions} \\
  \text{(DNA}^{n-}\text{)} \text{ PPy surface} + \text{M}^{n+} (\text{aq}) = \text{M}^{n+}\text{(DNA}^{n-}\text{)} \text{ PPy surface}
  \]

- **Accumulation**
  At applied voltage -1.0V (vs. Ag/AgCl)
  \[
  \text{M}^{n+} \text{(DNA}^{n-}\text{)} \text{ PPy surface} + n \text{e}^- = \text{M}^0 \text{ PPy surface} + \text{(DNA}^{n-}\text{)} \text{ PPy surface}
  \]

- **Transfer/Release**
  At 0.7 V (vs. Ag/AgCl); Medium: 0.05M HCl.
  \[
  \text{M}^0 \text{ PPy surface} - n \text{e}^- = \text{M}^{n+} \text{ (aq)}
  \]

For future application, an integrated experimental set is possible by combining the electrochemical detection with the electrochemically assisted extraction with the ease of regeneration / recycling of the used SPME material.


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Best student award at All Saints College, Sri Lanka in 1990.

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