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A Selectivity Study on the Use of Caffeine and Theobromine Imprinted Polypyrrole Surface Electrodes

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A SELECTIVITY STUDY ON THE USE OF CAFFEINE AND THEOBROMINE IMPRINTED POLYPYRROLE SURFACE ELECTRODES

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A SELECTIVITY STUDY ON THE USE OF CAFFEINE AND THEOBROMINE IMPRINTED POLYPYRROLE SURFACE ELECTRODES

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(Dr. Darwin B. Dahl)

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Dean, Graduate Studies and Research Date

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List of Abbreviations and Symbols

General

A	ampere
AFM	atomic force microscopy
cm	centimeter
CIE	caffeine imprinted electrode
CP	conducting polymer
EIS	electric impedance spectroscopy
EDX	energy dispersive x-ray spectrum
I	current
mM	millimolar
mAU	milli absorbance units
mg	milligrams
min	minutes
mL	milliliters
µm	micrometer
MIP	molecular imprinting polymer
mPpy	molecular imprinting polypyrrole
PAD	pulsed amperometric detection
Q	charge
SEM	scanning electron microscope
s	seconds
S	Siemens
t	time
TIE	theobromine imprinted electrode

A SELECTIVITY STUDY ON THE USE OF CAFFEINE AND THEOBROMINE IMPRINTED POLYPYRROLE SURFACE ELECTRODES

Anil Kiran Kumar Vinjamuri August 2008 60 pages

Directed by: Dr. Darwin B. Dahl

Department of Chemistry Western Kentucky University

Molecularly imprinted polymers (MIPs) are proving to be very effective in development of synthetic recognition systems and are of great interest to those interested in the field of sensor technology. The use of MIPs is receiving considerable interest due to the ability to prepare recognition matrices that possess high substrate selectivity and specificity. Conducting polymers (CP) have proved to be an excellent tool for the preparation of nano-structured biologically selective systems. Polypyrrole (Ppy) is one such CP that is extensively used for the construction of bioanalytical sensors. Ppy has shown great promise primarily due to its biocompatibility and thermal stability under a variety of environmental conditions. In this study, caffeine imprinted electrodes (CIE) and theobromine imprinted electrodes (TIE) were prepared. This research project subsequently focused on three main aspects: 1) to determine the selectivity of a caffeine and theobromine imprinted MIP using Ppy as the conducting polymer matrix, 2) comparing pulsed amperometric detection (PAD) and electrical impedance spectroscopy (EIS), for their value as potential detection schemes and 3) to determined the applicability of the molecularly imprinted polypyrrole by analyzing commercial

samples of instant coffee and tea and comparing results to that obtained from established HPLC procedures.

In summary, the following conclusions are stated:

- Both PAD and EIS measurements taken from CIE and TIE MIPs showed no statistical difference in response at the 95% confidence level using a standard paired t-test.
- Reproducibility for both MIPs was estimated by calculating an average percent relative standard deviation (%RSD) for the corresponding MIPs and was determined to be less than 3%.
- The degree of selectivity was estimated by calculating a % relative error for the CIE and TIE electrodes using both PAD and EIS analysis. These results revealed percent relative errors typically less than 5% for equimolar amounts of "interfering" analyte.
- A ruggedness revealed that over the concentration range and time interval tested (1-20 mM and 5 days), the average percent relative standard deviation was determined to be less that 7%.
- The caffeine content in the coffee sample analyzed, as determined by PAD and EIS, was consistent with results obtained by HPLC analysis however, the theobromine content determined in tea using PAD and EIS was significantly different from that determined by HPLC at the 95% confidence level.

I. INTRODUCTION

A. Background

Nanotechnology is a rapidly evolving field that constantly requires new combinations of techniques and methods that can aid in resolving challenging chemical issues. Scientists interested in solving complex analytical challenges require at a minimum, techniques that possess specificity, stability and sensitivity. Conducting polymers (CP) have proved to be an excellent tool for the preparation of nano-structured biologically selective systems and have as such, received considerable attention in analytical applications.[1]

Polymers are macromolecules that often consist of repeating monomeric units connected by covalent chemical bonds. Many natural polymers exist such as cellulose, DNA, RNA, polysaccharides and starch. There is a wide array of useful synthetic polymers such as Bakelite, neoprene, and nylon to name but a few.[2] Polypyrrole is an interesting class of synthetic polymers that exhibit high electric conductivities and hence fall into the category of conducting polymers. The ability to easily prepare analogs of polypyrrole makes it an attractive material for analytical use.[3]

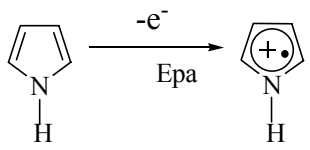
Polypyrrole (Ppy) was first synthesized in 1912 and are included in the rigid-rod shape family of polymers. Polypyrrole was the first of this key family of compounds to show high conductivity. It has found widespread use as a conducting polymer in the design of bio-analytical sensors.[4,5] Versatility of this polymer is determined by a number of properties such as its redox activity and ability to form nanowires with room temperature conductivity ranging from 10^{-4} to 10^{-2} Scm^{-1}. Two major routes are used to synthesize polypyrrole. The first is based on induction of polymerization by either

chemical initiation using oxidative agents or by photo-induced polymerization. Second polymerization can be brought about by electrochemical processes.[1] Diaz et al. proposed the mechanism of polymerization of pyrrole which includes oxidation, coupling, and deprotonation until the final polymer product is obtained and is depicted in Figure 1.1.[6]

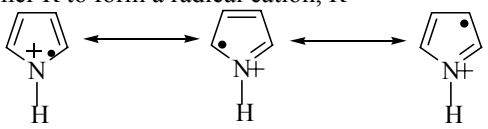
B. Conductivity of polypyrrole

Polypyrrole contains an extended system of delocalized bonds. When charge carriers (from the addition or removal of electrons) are introduced into the conduction or valence band of this material, electrical conductivity will increase[7]. Conductive polymers, such as polypyrrole, can be likened to semiconductors due to the small band gaps and low electronic mobility.[8] Notable differences between conductive polymers and inorganic semiconductors is that the former have inherently lower mobilities. Recent advancements in molecular self–assembly however, are closing that gap. Rationale is that delocalized can be accomplished by forming a conjugated backbone of continuous overlapping orbitals. For example, alternating single and double carbon-carbon bonds can form a continuous path of overlapping p-orbitals.

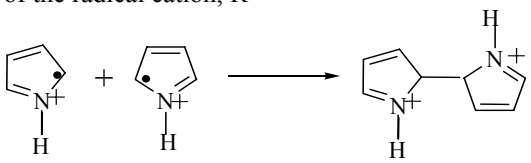
The introduction of charge carriers (usually electrons or holes) will also cause an increase in conduction. Charge carriers are formed by doping i.e., oxidation (p-type) or reduction (n-type) reaction of the polymer chain.[9, 10]



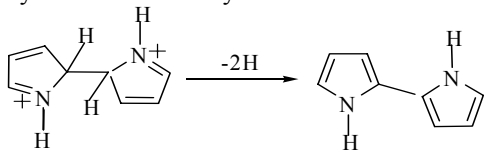
Scheme 1: Oxidation of monomer R to form a radical cation, $R^{+\cdot}$



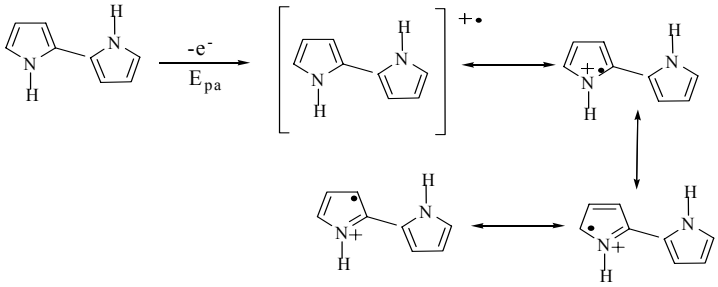
Scheme 2: Resonance forms of the radical cation, $R^{+\cdot}$



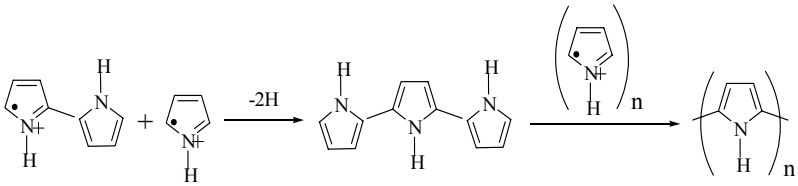
Scheme 3: Formation of the dihydromer dication by dimerization.



Scheme 4: Deprotonation of dimer.



Scheme 5: Polymerization reactions follow the oxidation of the dimer into the radical cation.



Scheme 6: Resonance form of the above reacts by position 5 or 5' with a monomer radical cation to give a neutral trimer and then forms a polymer polypyrrole.

Figure 1.1 Proposed mechanism of polymerization of pyrrole.[6]

Oxidation or p-type polymer charge carriers, leave a vacancy that does not delocalize completely. This vacancy is a hole or a radical cation that partially delocalizes over several monomeric units resulting in structural deformity. Energy will be greater in the newly deformed structure than for the undoped polymer. The radical cation destabilizes the bonding orbital that has a higher energy than the valence band. The band gap is the only difference between the destabilized bonding orbitals and the valence band.

As stated previously, polypyrrole has found application in the construction of bioanalytical sensors. This is largely due to its conductivity, biocompatibility and thermal stability under a variety of environmental conditions.[11] A further advantage of Ppy is that it can be easily modified by covalently attached functionalities. The ability to attach even macromolecules such as proteins is possible. This specific type of modification results in a class of sensors known as affinity sensors. Affinity sensors mainly rely on bio-molecules providing a recognition site on the polymer.[9] However, bio-molecules tend to have poor chemical and physical stability after immobilization. Artificial receptors attached to the polymer have been gaining importance as possible alternatives to bio-molecules.

C. Biosensors

A bio sensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component.[11] Important considerations for the creation of any type of electrochemical biosensors are: i) the immobilization of a bio-catalyst; ii) application of an appropriate electrochemical technique (e.g. potentiometric, amperometric and impedimetric techniques) iii) establishment of efficient electron transfer.[1] The immobilization of biologically active material is of pivotal importance in

the creation of effective biosensors if a number of analysis cycles are sought from the biologically active material. Requirements for successful biomaterial immobilization include: i) biological recognition properties and/or catalytic properties of the biomaterial should remain after immobilization ii) the biomaterial should adhere well to the substrate otherwise, a loss of activity will occur iii) display little to no change in selectivity. Conducting polymers have found utility in satisfying a majority of these requirements and are seen as very effective substrates for biomaterial immobilization.[11]

Among conducting polymers, polyaniline has often been used as an immobilizing substrate for biomolecules and sometimes also as efficient electro catalysts. However, the necessity to detect bio-analytes at or near neutral pH leads to the electro-inactivity of the deposited film, thereby negating the use of polyaniline or polythiophene as useful biosensing materials. In contrast to these polymers, polypyrrole is easily deposited from a neutral pH aqueous solution. This property has made Ppy very attractive as a biosensing material and at present is one of the most extensively studied materials for immobilization of different biomolecules and even extended to immobilizing living cells. Additionally, Ppy is often used in catalysis and in the construction of affinity biosensors because of its exceptional biocompatibility and the ease of immobilization of biomolecules.

Biomaterials can be immobilized by various methods: i) adsorption on electrochemically or chemically formed Ppy surface, ii) entrapment during electrochemical deposition of polypyrrole and iii) self entrapment, if the biomaterial is able to initiate polymerization of pyrrole.[12] As stated previously, chemical initiation can also be used to produce polypyrrole modified with biomaterials, however this method is

not suitable for the formation of well defined layered structures that are desired for sensor design. Contrary to chemical initiation, electrochemical polymerization of pyrrole allows for the formation of uniform films. The thickness and morphology of these films are easily controlled by regulation of current and/or applied potential.

Application of pulsed potential techniques allows for the pre-concentration of biologically active molecules near the electrode surface by applying the proper potential between pulses that initiate polymerization of polypyrrole. This technique greatly enhances the amount of biologically active compound incorporated inside the film when compared to steady-state polymerization. Ppy has the added ability of protecting electrodes from becoming fouled by proteins and/or other biological substances present in the samples such as blood serum or urine.[1]

The stability of Ppy-based biosensors is typically dictated by the rate of degradation of Ppy in aqueous solutions resulting from a continually applied voltage. Overall, Ppy has a number of very attractive characteristics: i) it can be synthesized electrochemically and modified by enzymes in various ways that generate unique analytical characteristics for the constructed biosensor ii) it extends the lifetime of a biosensor by protecting electrodes from fouling iii) it is biocompatible and hence, causes minimal and reversible disturbance to the working environment iv) in certain cases, it can even be exploited as a redox mediator able to transfer electrons from redox enzymes towards electrodes. [1]

D. Molecularly imprinted polymers (MIP)

Molecularly imprinted polymers (MIP) are highly stable synthetic polymers that possess molecular recognition properties due to cavities created in the polymer matrix

that are complementary to an analyte both in shape and in positioning of functional groups.[13] Historically, the first imprinted material was a silicon dioxide-based system and the first experimental use of this material was for the separation of dyes dating back to the 1940s.[14]

MIPs are proving to be very effective in development of synthetic recognition systems and are of great interest to those working in the field of sensor technology. Some of these polymers have shown very high selectivity, actually possessing affinity constants comparable to naturally occurring recognition systems. The preparation of molecularly imprinted polymers requires polymerization around the print species, using monomers that are selected for their capacity to form specific and definable interactions with the imprinted species. Chemical functionalities of the monomer residues become spatially positioned around the cavity in a pattern which is complementary to the chemical structure of the print molecule.[12, 13] These imprints constitute a permanent memory for the print species and enable the imprinted polymer to rebind the print molecule from a mixture of closely related compounds. This significant property is what leads to the selectivity of the process.

Molecularly imprinted Ppy exhibits both pre-determined selective molecular recognition and inherent electrical conductivity; it is this fact that makes this type of MIP a very attractive analytical tool for use in the field of sensor technology.[14, 15] Due to the electrical conductivity of Ppy, the detection of print molecules becomes possible by various electrochemical techniques such as pulsed amperometric detection (PAD). PAD is recognized as a more useful electrochemical detection technique when compared with constant potential-based detection methods. In this method, the changes in charge

densities or conductivities, are used for the detection of an analyte and therefore does not require any auxiliary reactions to facilitate detection.[12]

A MIP is formed in the presence of a molecule that is extracted afterwards, thus leaving complementary cavities behind. This process results in a chemical affinity for the original molecule. In recent times, the molecular imprinting technique has been developed to fabricate sensors, facilitate catalysis and for use in separations.[16] The functional mechanism mimics that used by enzymes for substrate recognition i.e., the "lock and key" model.[17, 18] In preparing a MIP, the target molecule should have one or more functional groups and should not be polymerizable or act to retard the polymerization process. [19]

E. Electrochemical Measurements

Electrochemical measurements often require a basic three electrode system: a working electrode, reference electrode and an auxiliary electrode.[11]

An electrode is an electrical conductor used to make contact with a non metallic part of a circuit. An electrode in an electrochemical cell will behave as either an anode or a cathode. The anode is defined as the electrode at which electrons leave the cell and oxidation occurs, and the cathode as the electrode at which the electrons enter the cell and allows reduction to occur. An electrode may become either the anode or cathode depending on the voltage applied to the cell. A bipolar electrode can exist whereby it can function as the anode of one cell and the cathode of another cell. [20 , 21]

E.1. Working electrode

The working electrode in an electrochemical system is one on which the reaction of interest is occurring. The working electrode is used in conjunction with an

auxiliary electrode and a reference in a three-electrode system. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode can be referred to as either cathodic or anodic. Common working electrode often consist of inert metals such as gold, silver or platinum, inert carbon such as glassy carbon or pyrolytic carbon and mercury drop or film electrodes.[22]

A working electrode acts as a source or sink of electrons for exchange with molecules in the interfacial region (the solution adjacent to the electrode surface), and must be an electronic conductor. It must also be electrochemically inert (i.e., does not generate a current in response to an applied potential) over a wide potential range (the potential window). Platinum, gold, mercury, and glassy carbon are commonly used working electrode materials for cyclic voltammetry. [22]

If material adsorbs on the surface of a working electrode, the current response will degrade and the electrode surface requires cleaning. Adsorption, or fouling, occurs more readily for some analytes than for others and hence, required cleaning frequency varies. In many cases, the only cleaning required is light polishing with a fine material such as 1mm diamond, or 0.05mm alumina. A few drops of polish are placed on a polishing pad and the electrode is held vertically and the electrode polished by rubbing in a figure-eight pattern for a brief period, typically 30 seconds to a few minutes depending upon the condition of the electrode surface.

After polishing, the electrode surface is rinsed thoroughly with water (for alumina) or methanol (for diamond), and allowed to air dry. (Electrodes polished with alumina may also need to be placed in an ultrasonic bath containing distilled water for a few minutes to remove any residual alumina particles. More pronounced surface defects,

such as scratches, may need to be polished with a coarser polish. Once the defect has been removed, the electrode should then be polished with successively finer polishes to obtain a mirror-like surface. Electrochemical cleaning (applying large anodic or cathodic potentials to the electrode) has also been shown to be effective in certain instances. [12, 23]

E.2. Reference electrode

The reference electrode requires an electrode that will possess a stable and well-defined potential. The stability of the reference electrode potential is reached by employing a redox system with a fixed concentration of participants found in the redox reaction.[24] Reference electrodes are necessary when measuring electrochemical cell potentials whereby the contents of one half-cell is unknown.

Two common reference electrodes and their potentials with respect to the standard hydrogen electrode are listed below.

- Saturated calomel electrode: E = 0.242 V (saturated)
- Silver-silver chloride electrode: E = 0.225 V (saturated)

E.3. Auxiliary electrode

The auxiliary electrode or counter electrode, exists to ensure that current does not run through the reference electrode and thereby allow the potential to vary. It often has a surface area much larger than that of the working electrode so that reactions occurring on the working electrode are not surface area limited. [12] The working electrode is the electrode on which the reaction of interest occurs, and on which the measurements are taken. The counter electrode changes in polarity opposite to that of the working electrode, but its current and polarity are not measured.

F. Electrochemical detection modes

There are many different types of electrochemical detection modes. However, pulsed amperometric detection (PAD) and electrical impedance spectroscopy (EIS) serve as exceptional electrochemical detection techniques for MIPs.[12]

F.1. Pulsed Amperometric detection (PAD)

PAD is an electrochemical detection technique which has shown to be useful for the detection of analyte during operation of a MIP-based affinity sensor. PAD in this mode measures the changes in charge densities or conductivities (current) of the electrode surface as a function of concentration.[25] For the measurement of conductivity, the polymer should have an inherently high conductivity. [1, 12]

F.2. Electrical Impedance spectroscopy (EIS)

Impedance is a measure of the overall opposition of a circuit to current, in other words: how much the circuit impedes the flow of current. It is like resistance, but it also takes into account the effects of capacitance and inductance. Impedance, symbolized by the letter Z, is measured in ohms and represents the relationship between voltage and current of which a device is capable of accepting or delivering. This mode of detection is thus achieved by measuring the impedance induced by changes in the electrode surface.[26]

$$\text{Impedance } (Z) = V / I$$

Where: I and V are the rms or "effective" values.

G. Purpose of the Study

The purpose of this study is three fold:

(i) To determine the selectivity of a caffeine-imprinted and theobromine-imprinted MIPs using Ppy as the conducting polymer matrix.

(ii) To examine the PAD technique and to test electrical impedance spectroscopy in comparison to PAD.

(iii) To determine the integrity of the data obtained from analyses of a commercial instant coffee and a tea sample using both PAD and EIS techniques and comparing results to that obtained from an established HPLC procedure.

H. Analytes in the Study

H.1. Caffeine:

Caffeine is a bitter white crystalline xanthine alkaloid which acts as a central nervous system (CNS) stimulant drug and mild diuretic.[27] Caffeine was discovered by the German chemist, Friedrich Ferdin and Runge, in 1819. Beverages containing caffeine, such as coffee, tea, soft drinks and energy drinks enjoy great popularity. Caffeine is the world's most widely consumed psychoactive substance, but unlike most other psychoactive substances, it is legal and unregulated in nearly all jurisdictions. Coffee is undoubtedly one of the most popular beverages across the world presumably due in part, to its effect as a stimulant. The concentration of caffeine ranges from 0.5 mg - 60 mg per gram of coffee.[28] The structure of caffeine is shown in Figure 1.2.

H.2. Theobromine:

Theobromine, also known as xantheose, is a bitter alkaloid of the cocoa plant, and can mainly be found in chocolate or chocolate containing products. Theobromine can also be found in certain teas having concentrations ranging 1.2- 4.4 mg/ cup.[29] Theobromine is a water insoluble (sparingly soluble in boiling water) crystalline, bitter tasting white powder. It is in the methylxanthine class of chemical compounds and is structural analog of caffeine. It therefore has a similar but lesser, effect to caffeine.

Theobromine is mildly diuretic, is a mild stimulant, and relaxes the smooth muscles of the bronchi in the lungs.[30] The structure of theobromine is shown in Figure 1.2.

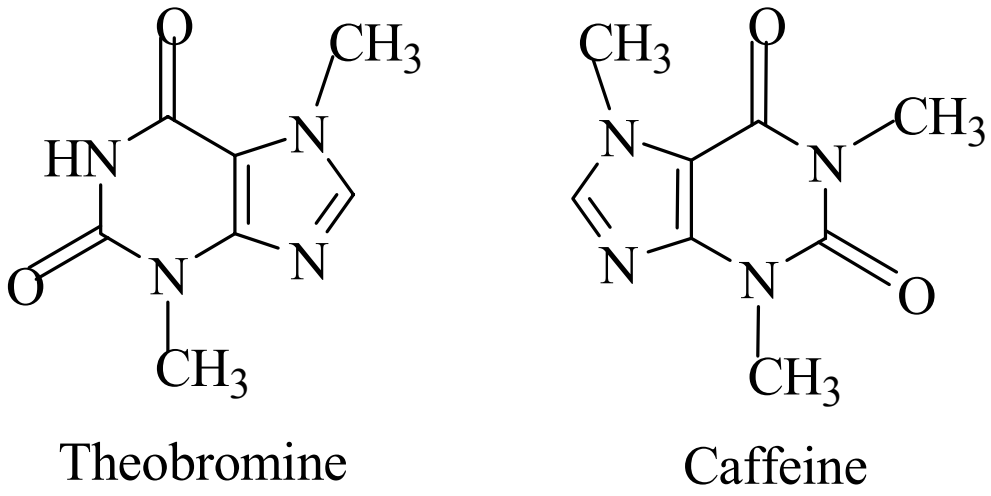


Figure 1.2 Structures of caffeine and theobromine

II. EXPERIMENTAL

A. Chemicals and Materials:

All chemicals used were of ACS reagent grade. All solutions were prepared using nanopure water (Barnstead, USA). Argon gas (ultra high purity) was purchased from Air Gas (GA, USA). Caffeine was purchased from MCB chemicals (CA, USA). Theobromine was purchased from Alfa Aesar (Ward Hill, MA). H_2PtCl_6, Pyrrole (98%), neutral alumina (Al_2O_3) and other basic chemicals were purchased from Sigma-Aldrich chemicals. Platinum electrodes (BAS- MF-2013) and polishing kit (BAS PK-4) were purchased from BAS, USA.

B. Instrumentation:

B.1 Potentiostat:

Electrochemical measurements were made using Princeton Applied Research (PAR) PARSTAT 2263 Advanced Workstation equipped with Electrochemistry PowerSUITE.

The PARSTAT® 2263 is a potentiostat capable of $\pm$ 10 V scan ranges, 200 mA current and EIS measurements up to 1 MHz. The PARSTAT 2263 is DC powered and can be operated with an AC/DC converter. The PARSTAT family is operated by PowerSUITE and offers the user the ability to perform many of the standard techniques (such as CV, LSV, Tafel Plots, EIS, etc.). Circuit analysis was accomplished using ZSimpWin.

B.2 High performance Liquid chromatography (HPLC):

A Varian™ HPLC was used for the determination of caffeine and theobromine content in coffee and tea samples. Samples were injected (25 μL) via a Varian Prostar Auto sampler (Model 410). The column used was a Varian Microsorb MV C_{18}, 100 mm x 4.6 mm (5 μm packing) reverse phase column. The detector employed was a Varian photodiode array (PDA) detector (Model 330) operated at 254 nm. The mobile phase was operated using a gradient mode: reservoir A; 1% acetic acid in nano pure water and reservoir B; 1% acetic acid in methanol. The gradient consisted of A:B, 90:10 to 0:100 in 25 min. and then held at 0:100 for 30 min. The flow rate was maintained at 1.0 mL/min and controlled by a Varian Reciprocating Pump (Model 9012).

B.3. Scanning Electron Microscope (SEM):

Images of the platinum electrode surface were taken using a JEOL-JSM -5400LV Scanning Electron Microscope (Tokyo, Japan).

B.4. Atomic Force Microscope (AFM):

The Z-axis image of the platinum electrode surface was taken using an Agilent 5500 AFM/SPM (CA, USA).

B.5. Light microscope:

The thickness of the polypyrrole polymer film deposited on a platinum electrode was measured using a JVC, KY-F75U (10X x 2.0X) light microscope with auto montage software (USA).

C. Preparation of Artificial receptors:

C.1. Electrodes

A working platinum electrode with a diameter of 1.6 mm and a Ag/AgCl

reference electrode in 3.0 M KCl was used for all described electrochemical procedures.

C.2. Electrode pretreatment

C.2.a. Ultrasonic and Mechanical Pretreatment:

Prior to use, all Pt electrodes were immersed in concentrated HNO_3 and treated for 10 minutes in an ultrasonic bath, rinsed with water, and then polished on a polishing cloth containing 0.05 μm alumina paste. The electrodes were then rinsed with water, and ultrasonically treated in 10 M NaOH then in 5 M H_2SO_4 for 10 minutes each. SEM images were taken before and after polishing of the electrodes and are shown in Figures 2.1 and 2.2.

C.2.b. Electrochemical Pretreatment and Platinization:

After mechanical and ultrasonic pre-treatment, potential cycling in 0.1 M H_2SO_4 using a scan rate of 100 mV s^{-1} from -300 to +1200 mV, was repeated until the cyclic voltammogram displayed the characteristic features of bare platinum. Oxygen-free solutions were used during electrochemical electrode pretreatment and platinization and achieved by degassing with Argon. Once the solution was determined to be free of oxygen, a series of voltammograms were then recorded. Potential cycling was continued until no differences in the voltammograms were detected. Platinization (electrochemical deposition of platinum, so-called 'platinum black') is recommended to improve the adhesion of the conducting-polymer film and simultaneously to increase the number of catalytic active sites on the electrode. The Pt electrode is platinized by placing the electrode in an oxygen-free solution of 0.1 M KCl and 0.8 mM H_2PtCl_6 and applying five potential cycles between +500 and -400 mV vs. Ag/AgCl (3.5 M KCl) at a scan rate of 10 mV s^{-1}. During the Platinization process, the Pt reduction peak is seen to slightly

increase as an enhanced coverage of platinum clusters occurs. These representative results are shown in the Figures 2.3 and 2.4. SEM images were taken to visualize platinization of the electrode and are shown in Figure 2.5. The corresponding EDX spectrum of the surface was taken and the results are shown in Figures 2.6.a and 2.6.b.

C.2.c. Deposition of Molecularly Imprinted Polypyrrole (mPpy):

Pyrrole was purified by passing 0.5 mL aliquots through a neutral Al_2O_3 packed column (5 cm length and 0.4 cm diameter) to remove all colored components. A 50 mM solution of pyrrole containing 100 mM KCl and 5 mM of caffeine was used for the electrochemical formation of the imprinted polypyrrole film. The immobilization of caffeine within polypyrrole film was performed following an in-situ entrapment method during electrochemical formation of the conducting-polymer film on the electrode surface. SEM and AFM images of the polypyrrole surface electrode are shown in Figures 2.7 and 2.8. A potentiostatic pulse profile was applied to the working electrode with the height of the potential pulses defined by the oxidation potential of the monomer used. The duration was determined by the diffusion properties of the monomers. Thus, the electrochemical formation of the caffeine containing polymer film was carried out by application of 30 potential pulses between +950 mV (1 s) and +350 mV (for 10 s) vs. Ag/AgCl to allow the caffeine and the pyrrole monomer to equilibrate in the neighborhood of the electrode. Electrodes modified with blank polypyrrole were prepared using the same deposition conditions in the absence of caffeine during the film-formation procedure. During the final step of mPpy preparation, caffeine is extracted from the polymeric backbone by 0.1M, pH 7 phosphate buffer. Caffeine, in this case serves as the molecular template during the preparation of the molecularly imprinted polypyrrole.

After the elution of the template, complementary binding sites are revealed allowing specific rebinding of analyte. For deposition of mPpy, an oxygen-free solution of caffeine and pyrrole is used for polymerization. The lack of oxygen concentration in the solution is crucial for pyrrole polymerization. After polymerization a distinct, homogenous, black or brown polymeric layer of over-oxidized Ppy is seen to cover the electrode surface. The appearance of black (over oxidized) polypyrrole is a more reliable indicator of a successful polymerization. An identical procedure was used in the preparation of a molecularly imprinted theobromine electrode. Using the described procedure, the preparation of a caffeine imprinted electrode (CIE) and theobromine imprinted electrode (TIE) was accomplished. SEM and AFM images were taken after polymerization of the electrodes. The thickness of the polypyrrole film on the electrode was determined using a AFM. The thickness was determined for three electrodes and it was in the range of 0.5 -1.5 μm. Figure 2.9 shows this surface as taken by a light microscope. Figure 2.10 shows the back-scattered electronic image of the half-coated electrode surface taken using SEM. Figures 2.11 and 2.12 , show the AFM image and corresponding height or Z-profile of the half-coated polymer electrode respectively.

D. Investigation of Molecularly Imprinted Polypyrrole:

Caffeine and theobromine standards were prepared in 0.1M phosphate buffer, pH 7.0. and subsequently analyzed using the respective caffeine and theobromine imprinted electrodes using the PAD technique. The resulting current response from the caffeine and theobromine standards using the imprinted electrodes in buffer is shown in Figures 2.13 and 2.14. After elution of the template molecules by soaking in a phosphate buffer, complementary binding sites are revealed allowing for the specific rebinding of analyte.

The recognition sites obtained should be highly selective and therefore possess sufficient binding affinity for the analyte. Pulsed amperometric detection (PAD) was applied to investigate elution of caffeine and theobromine from the polypyrrole matrix (dedoping) and to detect binding of analyte to the molecularly imprinted polypyrrole (redoping). All electrochemical experiments were performed using a conventional three-electrode system. All potentials are referenced to a Ag/AgCl reference electrode. Caffeine and theobromine concentrations of 1-20 mM were prepared for the construction of a calibration curve. The current of the Ppy modified electrode was recorded during a sequence of 5 potential pulses consisting of: 1 s, 0 mV and 1 s, +600 mV vs. Ag/AgCl. The mean differences between registered anodic and cathodic currents (ΔI) were measured. All voltammetric data were stored in ASCII format and downloaded into Microsoft Excel for analysis.

PAD was applied for the periods before elution, during elution and during incubation in solution containing the analyte. The differences in electrochemical signals registered were calculated and used as the value indicating redoping/dedoping of mPpy. To facilitate the evaluation of analytical signal the differences between anodic and cathodic peak-currents in potential pulse amperograms were calculated. Representative data for a CIE obtained by the potentiostatic pulse of PAD is illustrated in Figure 2.15. The resulting graphs of caffeine and theobromine standards (1-20 mM) are shown in Figure 2.16.

EIS measurements were also taken using the same potentiostat and associated equipment as described for the PAD work. The beginning and ending frequencies were 100. kHz and 1.00 Hz ,respectively. Ten points per frequency decade were collected,

using logarithmic point spacing. 10.0 mV_{rms} and a DC potential of zero V vs. the Ag/AgCl reference electrode was used with an ac amplitude of 10.0 mV_{rms}. A representative scanned EI spectrum for a CIE is shown in Figure 2.17. The resulting caffeine and theobromine standard graphs (1-20mM) are shown in Figure 2.18.

E. Determination of Caffeine and Theobromine Concentration in Coffee and Tea Samples using the Corresponding MIP:

To test the integrity of the prepared MIP, the caffeine content was determined in a sample of "Maxwell™ instant coffee." Theobromine content was not detected in the coffee sample therefore a tea sample (Great Value ™) was analyzed for its theobromine content. Samples, 2.0 g coffee and 3 bags of tea, were placed in 150 mL boiling demonized water respectively. After 5 minutes, the extract was filtered using a 0.45 μm Whatmann filter and was ready for the analysis. The caffeine and theobromine content in the respective test samples were determined by both PAD and EIS measurements using the method of standard addition. ΔI vs. concentration and Q vs. concentration plots were constructed for PAD and EIS data for caffeine and theobromine respectively and are shown in Figures 2.19, 2.20, 2.21, and 2.22. HPLC analysis was performed directly on the filtered extract and was used as a comparison technique for both PAD and EIS. The caffeine and theobromine standards for HPLC are shown in Figures 2.23 and 2.24. respectively. The resulting HPLC chromatograms for the coffee and tea samples are shown in Figures 2.25 and 2.26. The resultant HPLC data is summarized in Table 2.1.

To determine the caffeine and theobromine content using the MIP electrodes, the method of standard additions was used. Additions of 2.0, 4.0, 6.0, 8.0 and 10.0 mL of

the respective standard were added to 10.0 mL of sample and then diluted to 50.00 mL with phosphate buffer. After incubation, PAD and EIS measurements were performed.

F. Data Analysis:

F.1.PAD Determination:

Mathematica™ was used to calculate the amount of caffeine and theobromine present by first determining the constants a, b, and c from the following hyperbolic equation and then solving for x in the following equation:

where:

$$y = \frac{c + ab}{b + x}$$

a –maximum amplitude for changes in analytical signal (ΔI or Q)

b –adjustable parameter related decay rate in curve

c –component of analytical signal not influenced by analyte

y –analyte signal for regular calibration and blank signal for standard addition.

x– analyte concentration for regular calibrations and negative of analyte concentration in un-spiked sample for standard additions.

The concentration of caffeine and theobromine found in coffee and tea is summarized in Table 2.1.

F.2. EIS Determination:

An R (QR) model was used to fit the impedance data by determining the same variables as above.

F.3 Reproducibility:

A reproducibility study was implemented to determine the degree of precision the fabricated MIPs displayed employing the PAD and EIS procedure described. To facilitate this study, caffeine and theobromine contents of 6 and 8 mM were analyzed repeatedly six times, both by PAD and EIS, and resultant standard deviations were determined. Table 2.2 lists the results.

F.4 Selectivity:

An interference or selectivity test was performed using mixtures of 4, 6, 8, 10 and 12 mM caffeine and theobromine standards and analyzed by both PAD and EIS detection methods using both the CIE and TIE.

The relative percent error found for the CIE and TIE electrodes by PAD and EIS analysis are summarized in Tables 2.3 and 2.4.

Additionally, CIE and TIE electrodes were tested for their response to 10 mM caffeine and 10 mM theobromine solutions respectively in differing concentrations of interfering analyte. For the CIE, varying concentrations of theobromine were added to the 10 mM caffeine solution and the response generated by PAD and EIS were recorded. Likewise, varying concentrations of caffeine were added to the 10 mM theobromine solution and again, the response by PAD and EIS was recorded. These selectivity results are shown in the Figures 2.27 and 2.28.

F.5 Ruggedness study:

A ruggedness study was designed to determine how long the CIE and TIE electrodes would give useful and reproducible data. Caffeine and theobromine standards

(1-20 mM) were prepared and analyzed repeatedly for 5 days to aid in establishing the ruggedness of both CIE and TIE. The data is summarized in Figures 2.29 and 2.30.

Table 2.1

PAD, EIS and HPLC results of caffeine and theobromine content in coffee and tea

Analyte	PAD	EIS	HPLC
Caffeine (mg/g)	**1.12 ± 0.1**	**0.80 ± 0.09**	**1.23**
Theobromine (mg/bag)	**2.23 ± 0.08**	**2.37 ± 0.12**	**1.80**

Table 2.2

Standard deviations of caffeine and theobromine standards

Standards	PAD		EIS	
	6mM	8mM	6mM	8mM
Caffeine	0.13	0.19	0.17	0.14
Theobromine	0.16	0.23	0.14	0.21

Table 2.3
Percent Relative Error using PAD Analysis

Concentration	CIE	TIE
4 mM Caffeine + 4 mM Theobromine	1.12	1.89
6 mM Caffeine + 6 mM Theobromine	2.09	2.77
8 mM Caffeine + 8 mM Theobromine	2.91	3.30
10 mM Caffeine + 10 mM Theobromine	3.62	4.23
12 mM Caffeine + 12 mM Theobromine	4.87	5.16

Table 2.4
Percent Relative Error using EIS Analysis

Concentration	CIE	TIE
4 mM Caffeine + 4 mM Theobromine	0.93	1.36
6 mM Caffeine + 6 mM Theobromine	2.11	2.52
8 mM Caffeine + 8 mM Theobromine	3.03	3.92
10 mM Caffeine + 10 mM Theobromine	3.78	4.87
12 mM Caffeine + 12 mM Theobromine	4.63	6.14

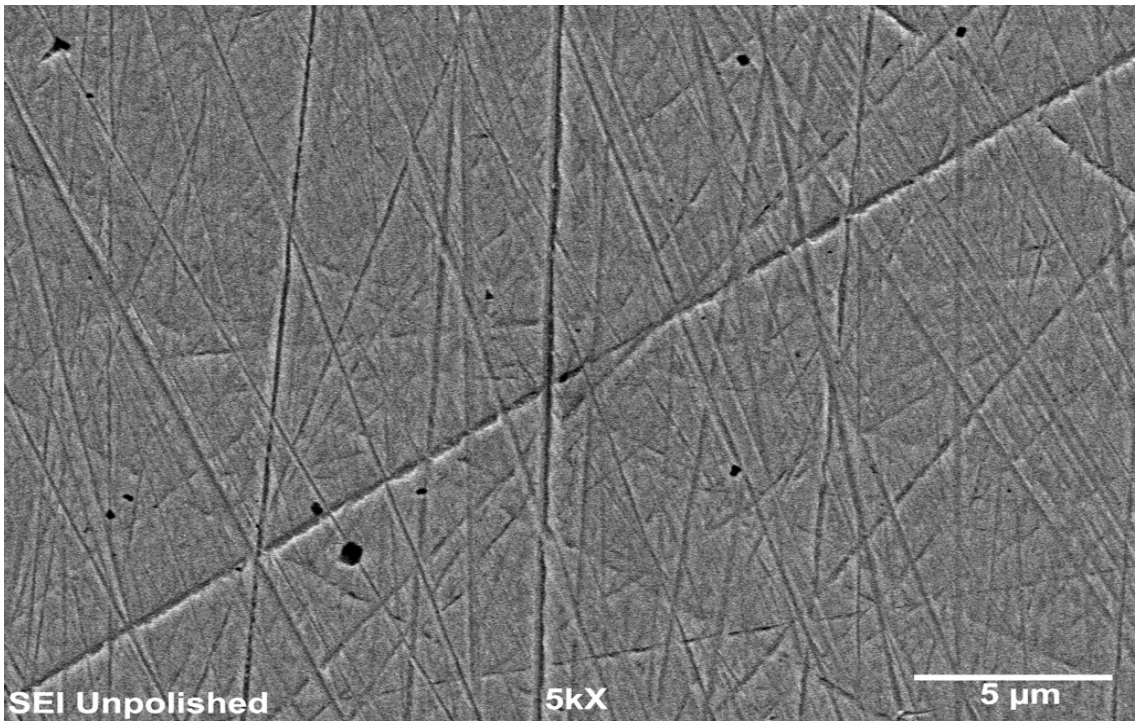


Figure 2.1 SEM of an unpolished Pt electrode

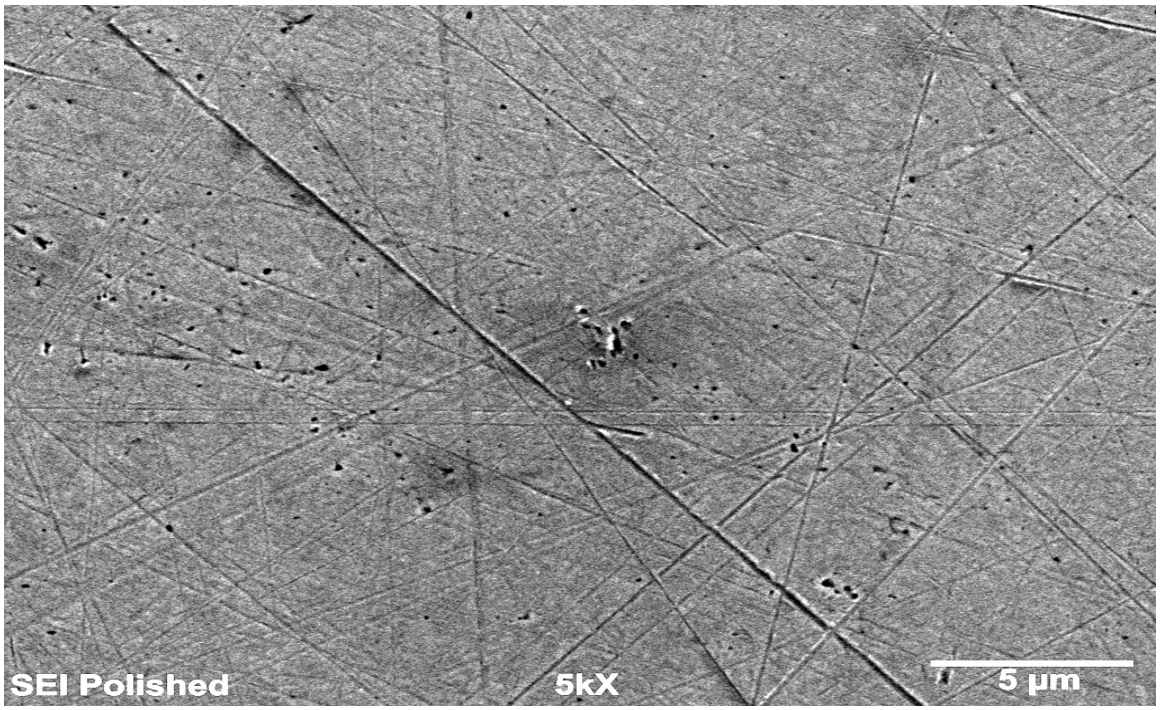


Figure 2.2 SEM of a polished Pt electrode.

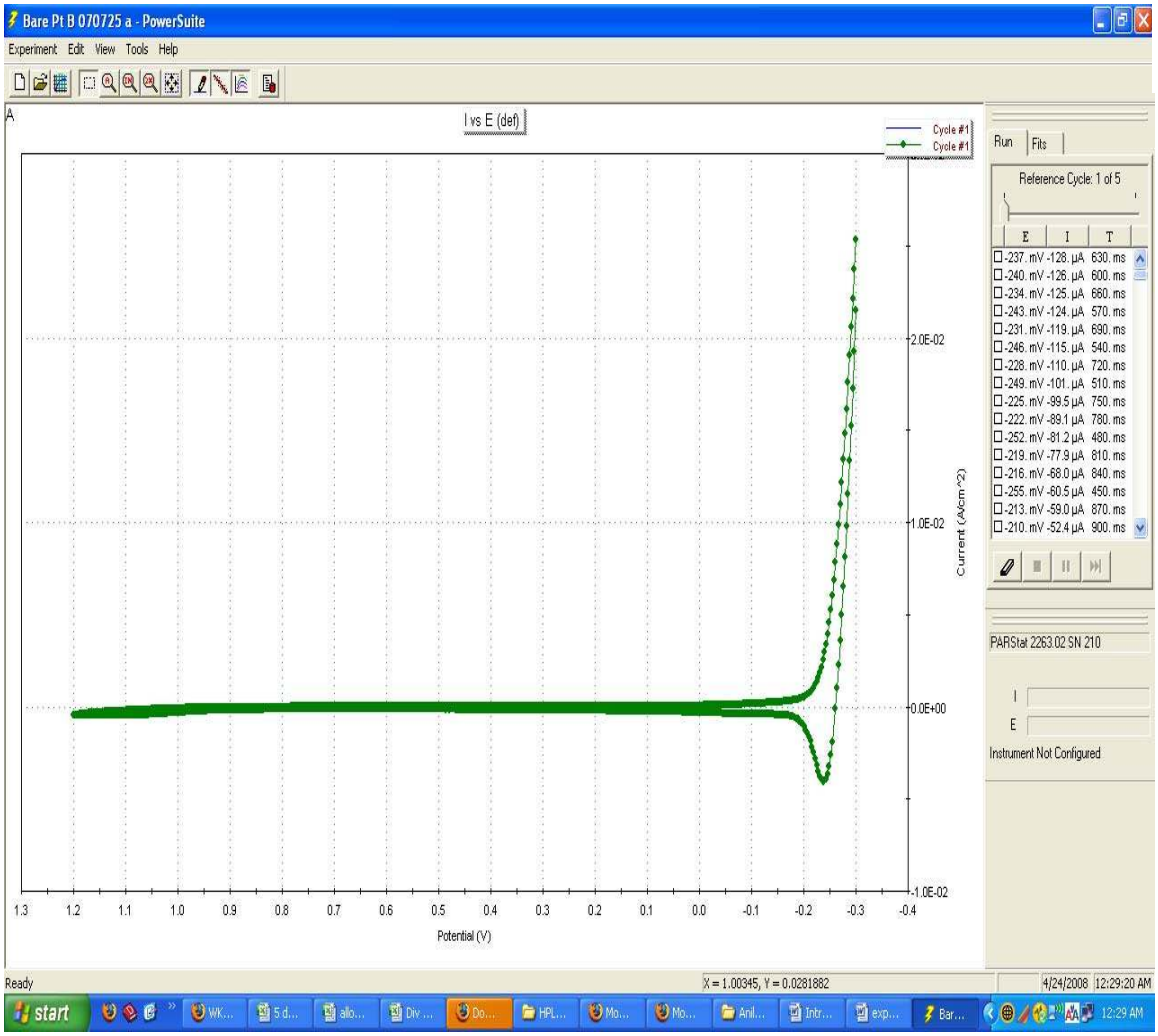


Figure 2.3 Cyclic voltammogram of untreated platinum electrode.

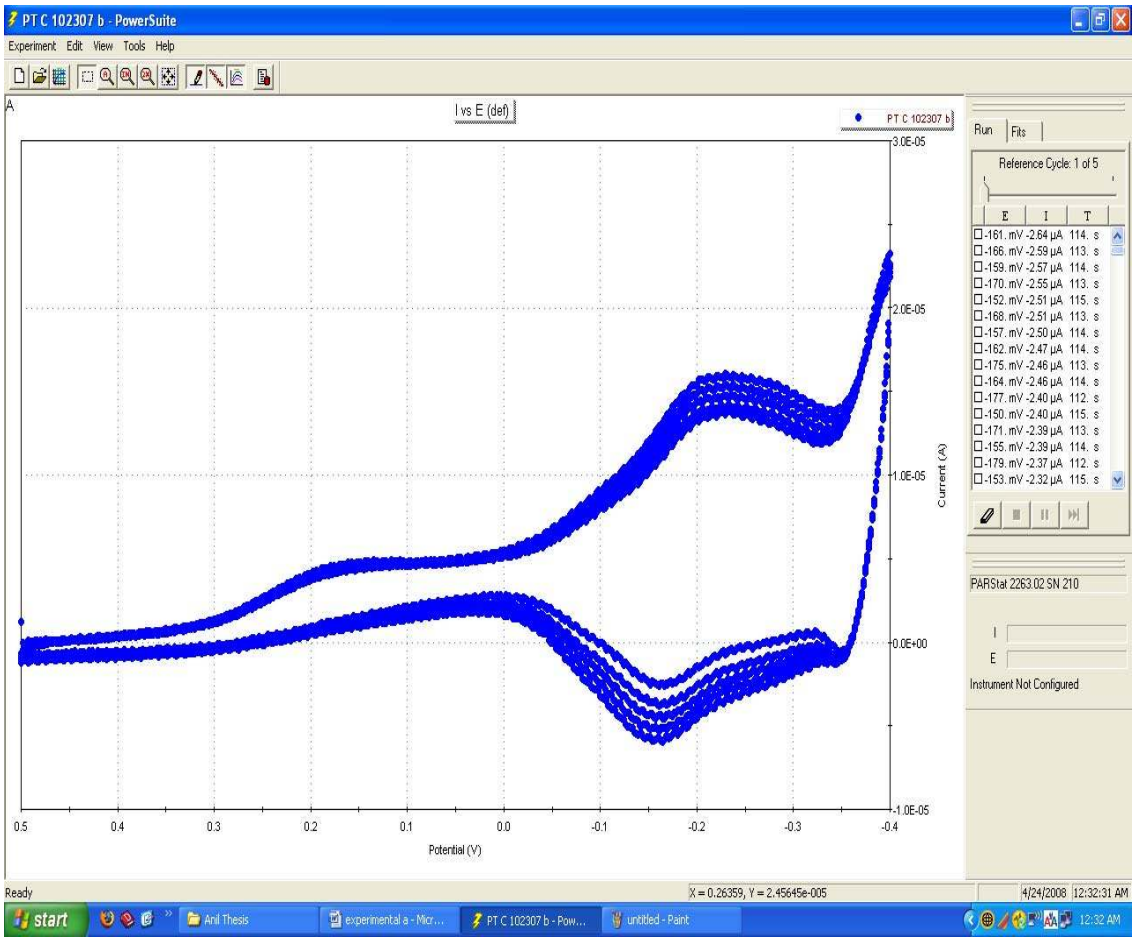


Figure 2.4 Cyclic voltammogram of platinized platinum electrode.

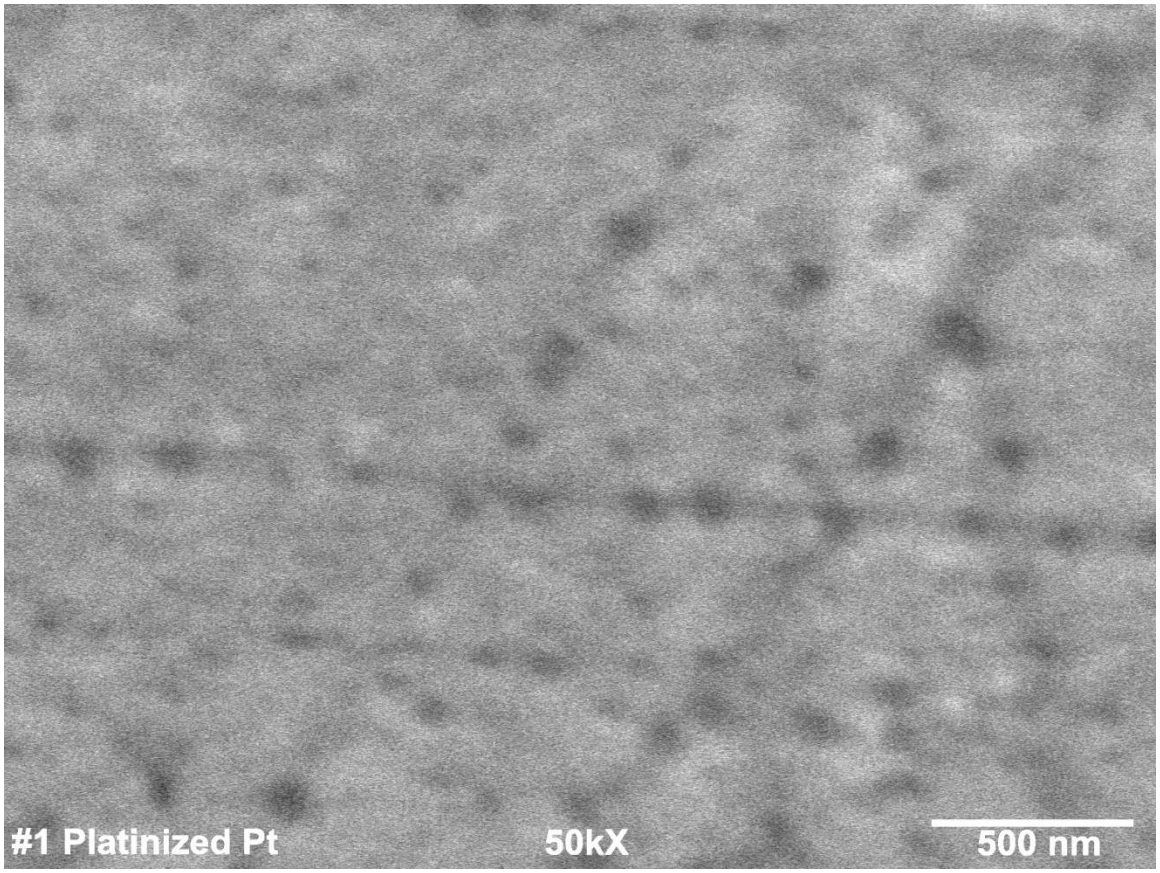
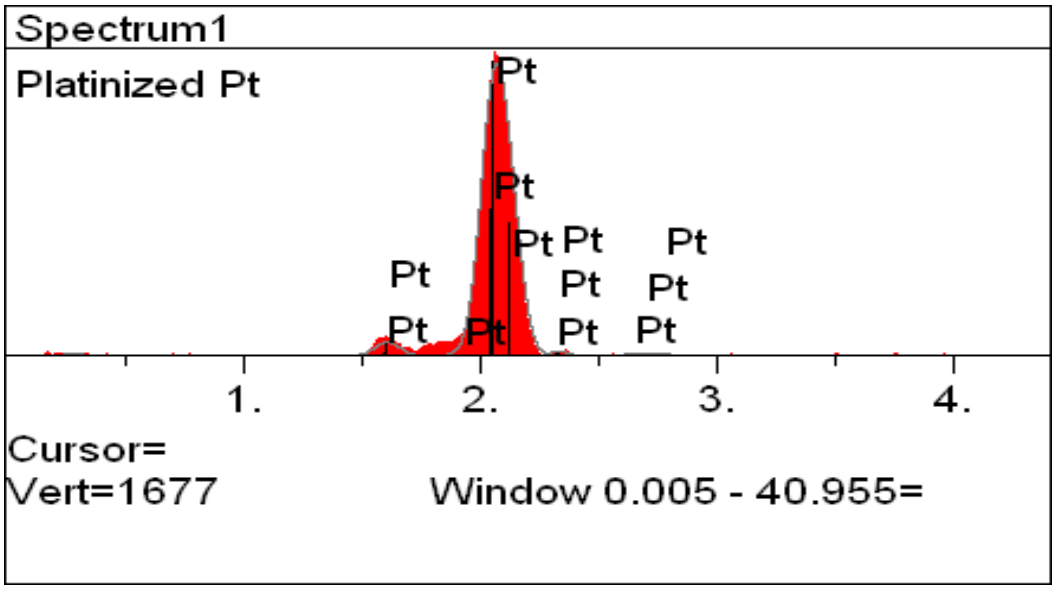


Figure 2.5 SEM image of platinized polished electrode



Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc	Units	
C	Ka	5.36	0.846	0.14	0.524	wt.%	
Pt	Ka	1,054.35	11.856	99.86	99.476	wt.%	
				100.000	100.000	wt.%	Total

Figure 2.6 a) EDX spectrum of a platinized electrode. b) EDX elemental results of platinized electrode.

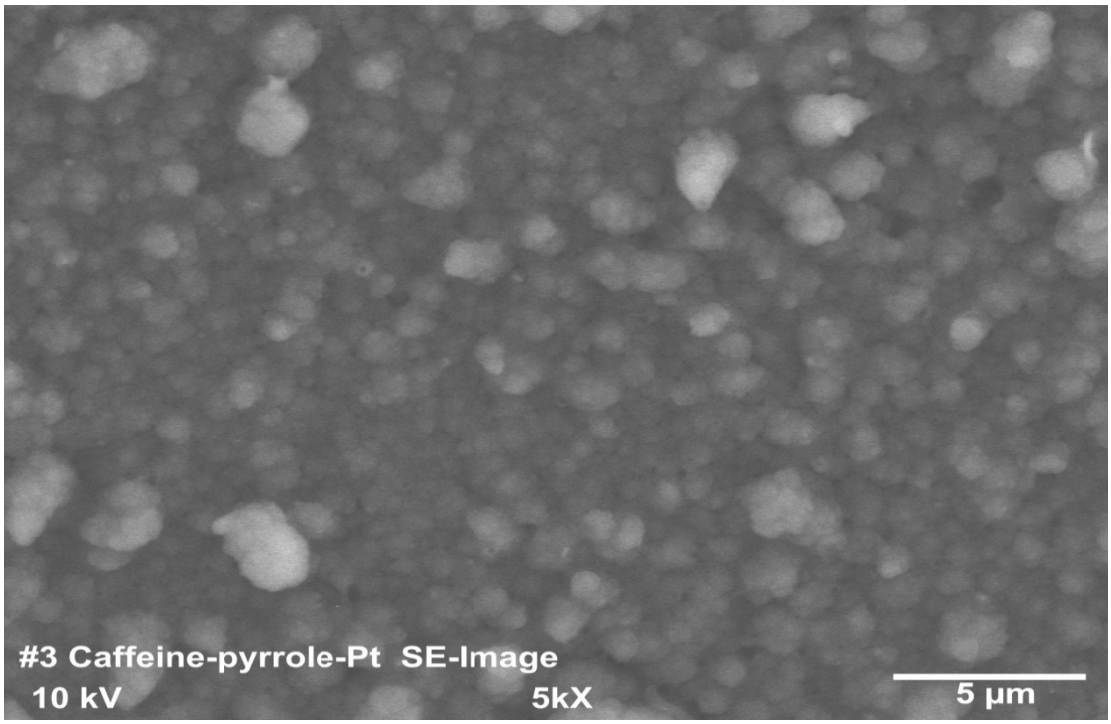


Figure 2.7 SEM of a caffeine imprinted polypyrrole electrode.

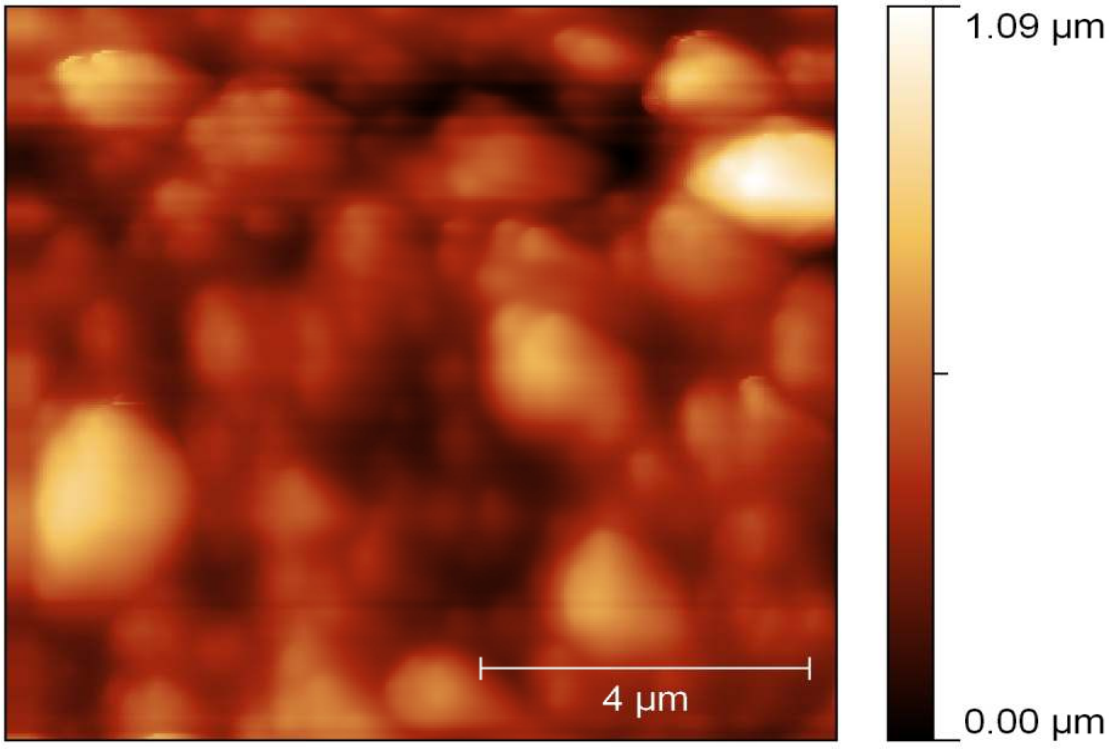


Figure 2.8 AFM image of caffeine imprinted polypyrrole electrode.

Figure 2.9 Light microscope image of Pt electrode, half-coated with polymer film.

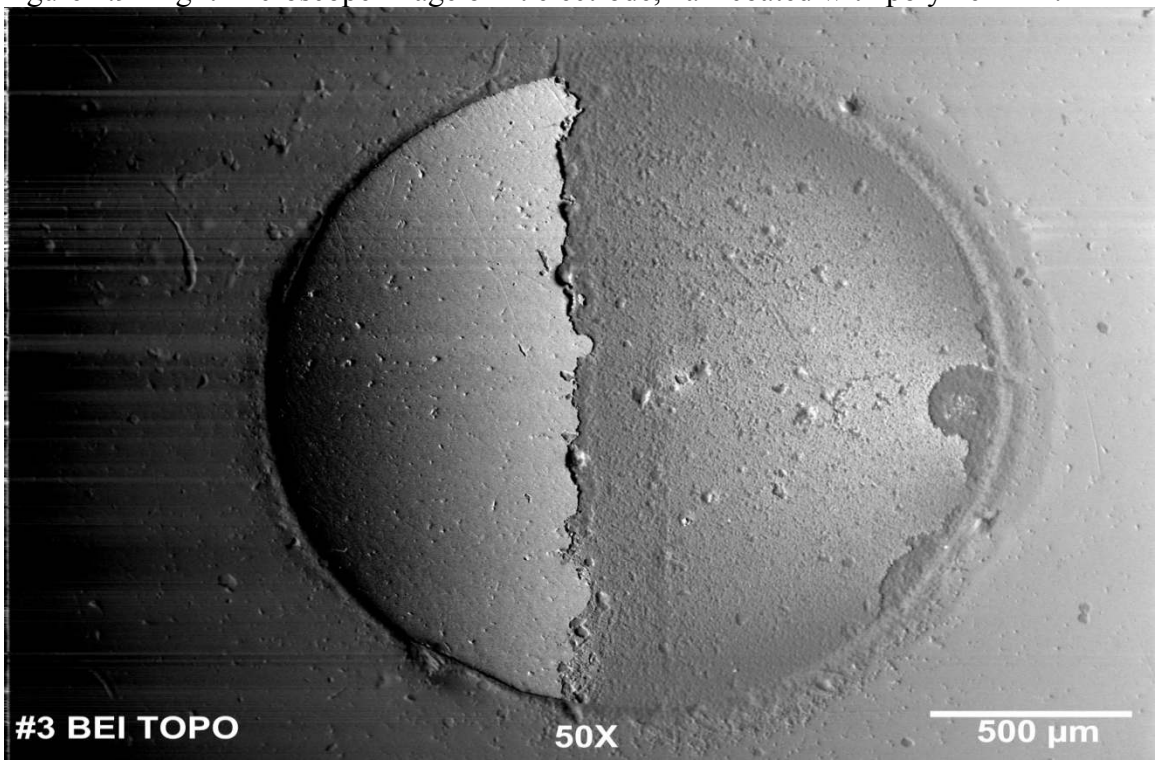


Figure 2.10 SEM (BEI) image of Pt electrode, half-coated with polymer.

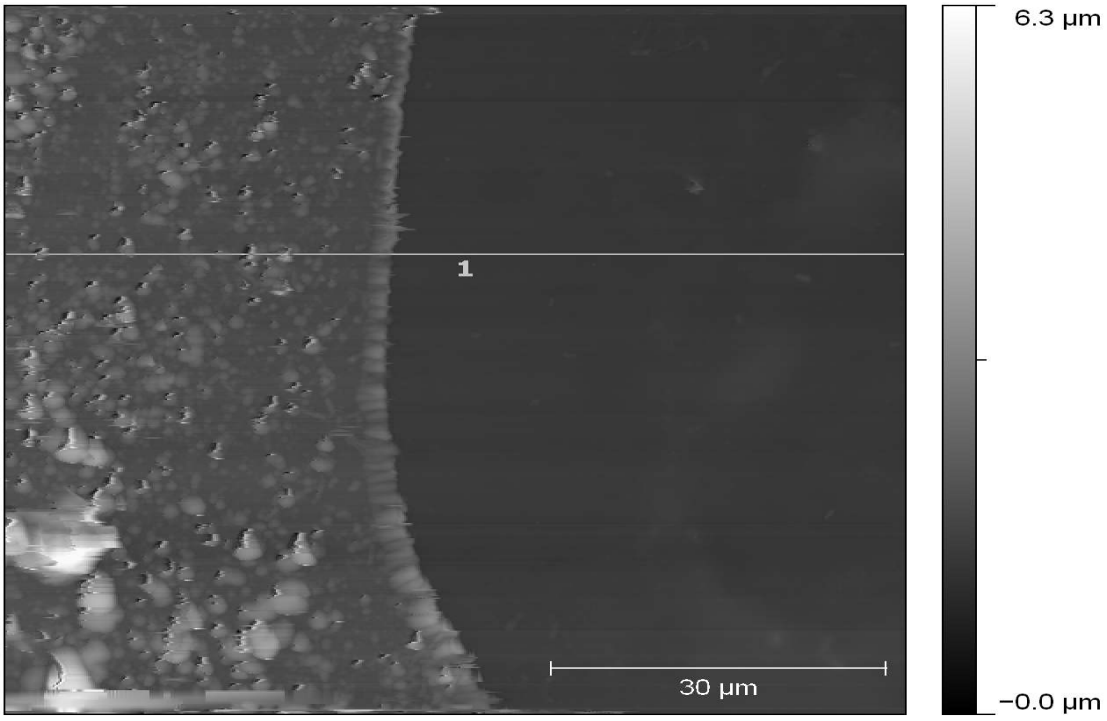


Figure 2.11 AFM image of Pt electrode, half-coated with polymer film.

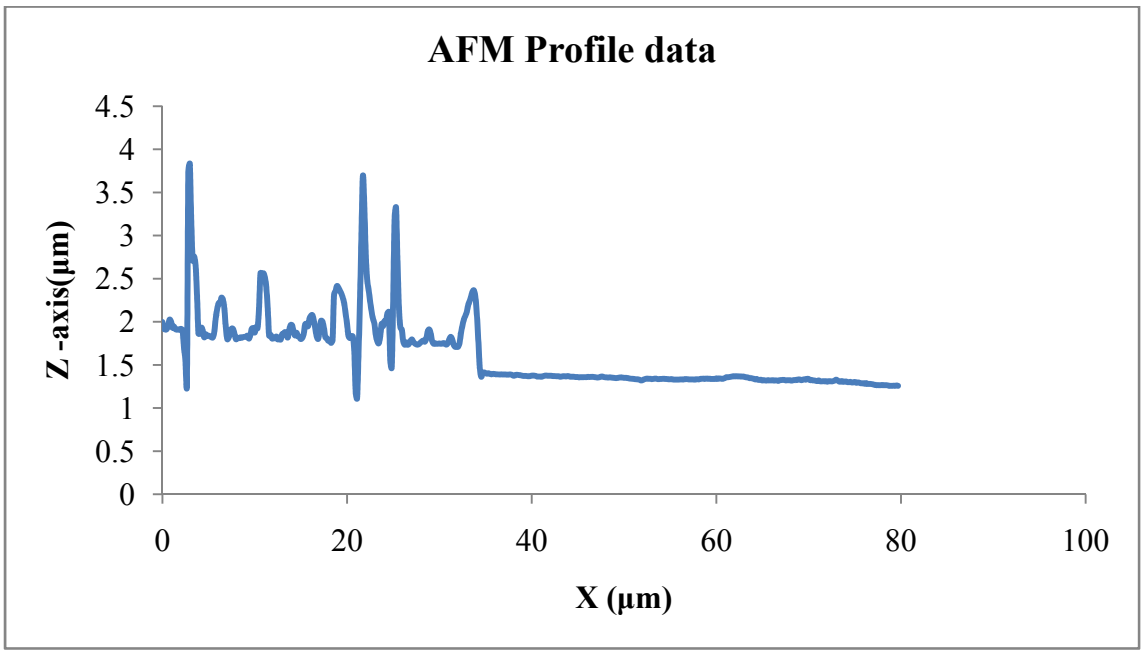


Figure 2.12 AFM image profile of Pt electrode, half-coated with polymer film.

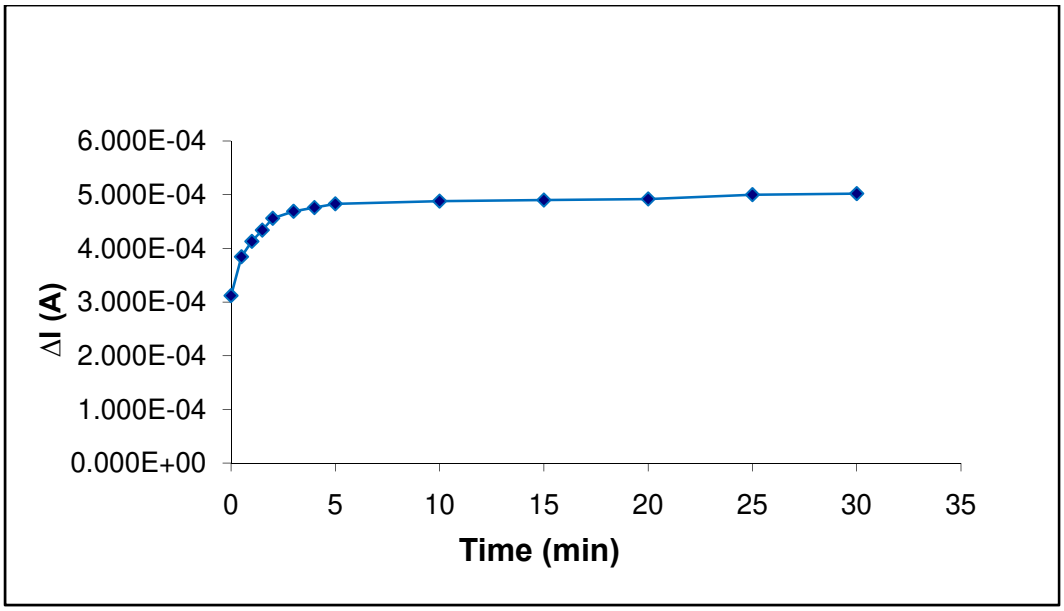


Figure 2.13 PAD of blank solution (0.1M phosphate buffer, pH= 7.0) on caffeine imprinted electrode.

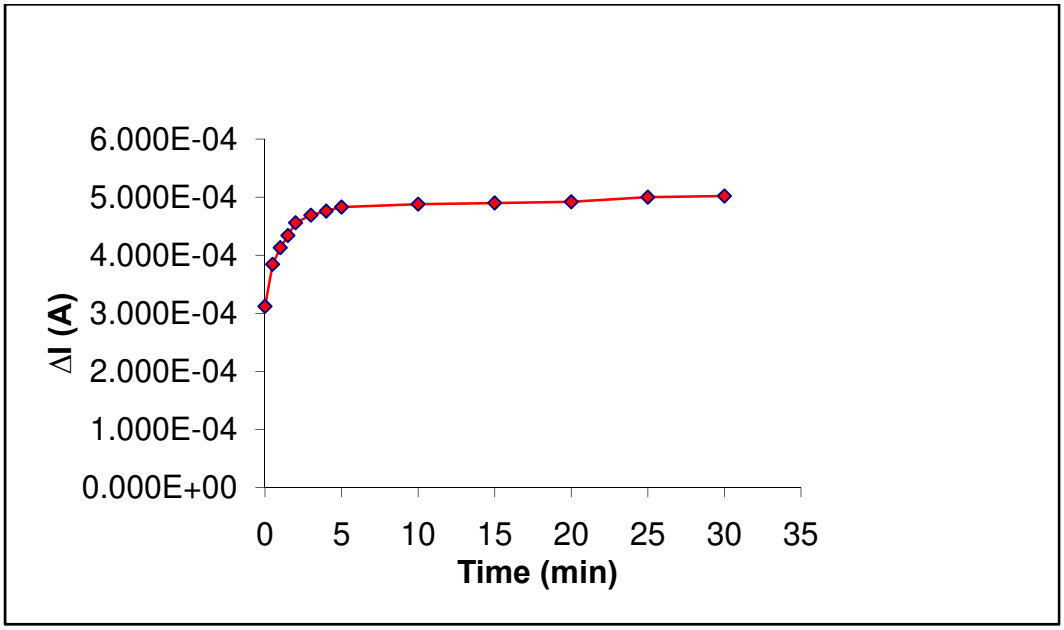


Figure 2.14 PAD of blank solution (0.1M phosphate buffer, pH= 7.0) on theobromine imprinted electrode.

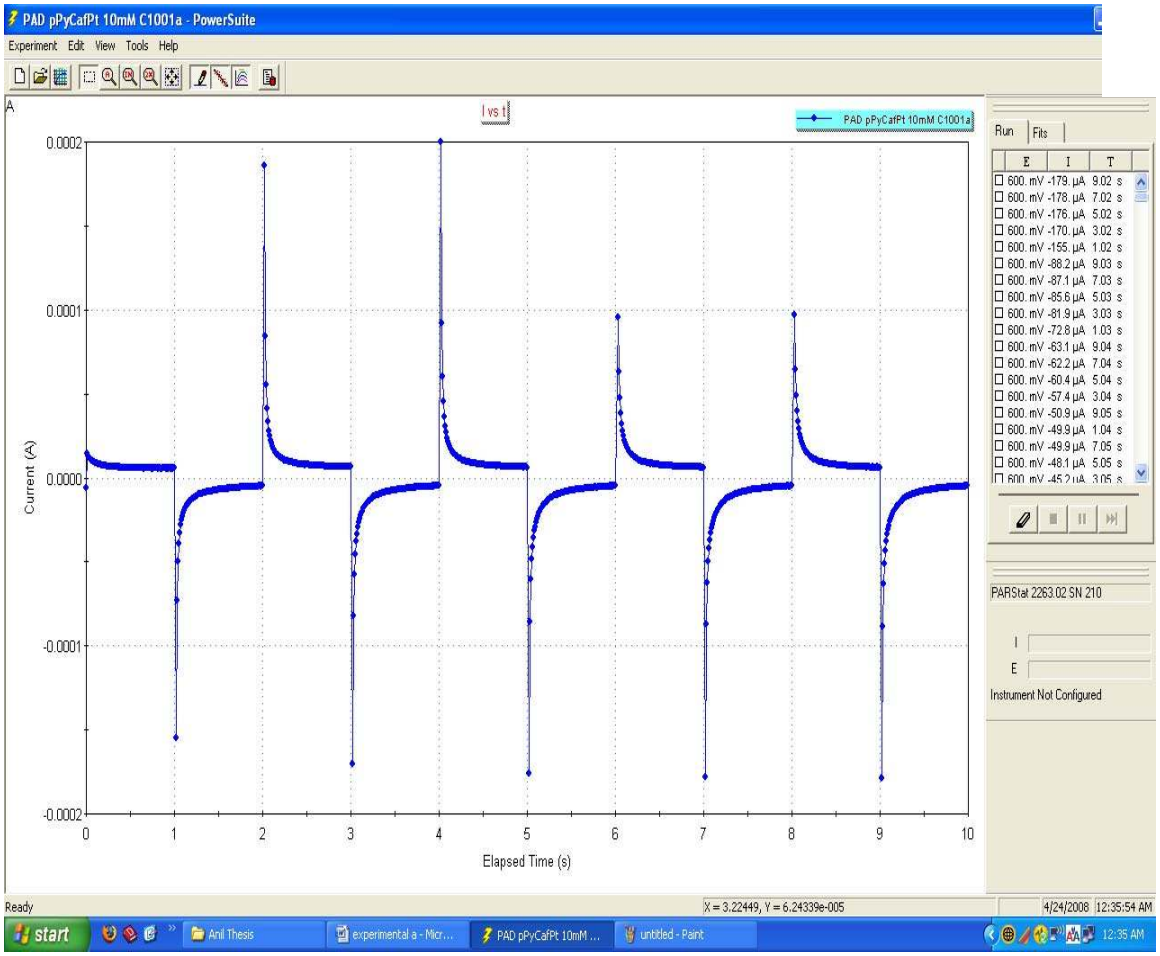


Figure 2.15 PAD of caffeine standards on caffeine imprinted electrode.

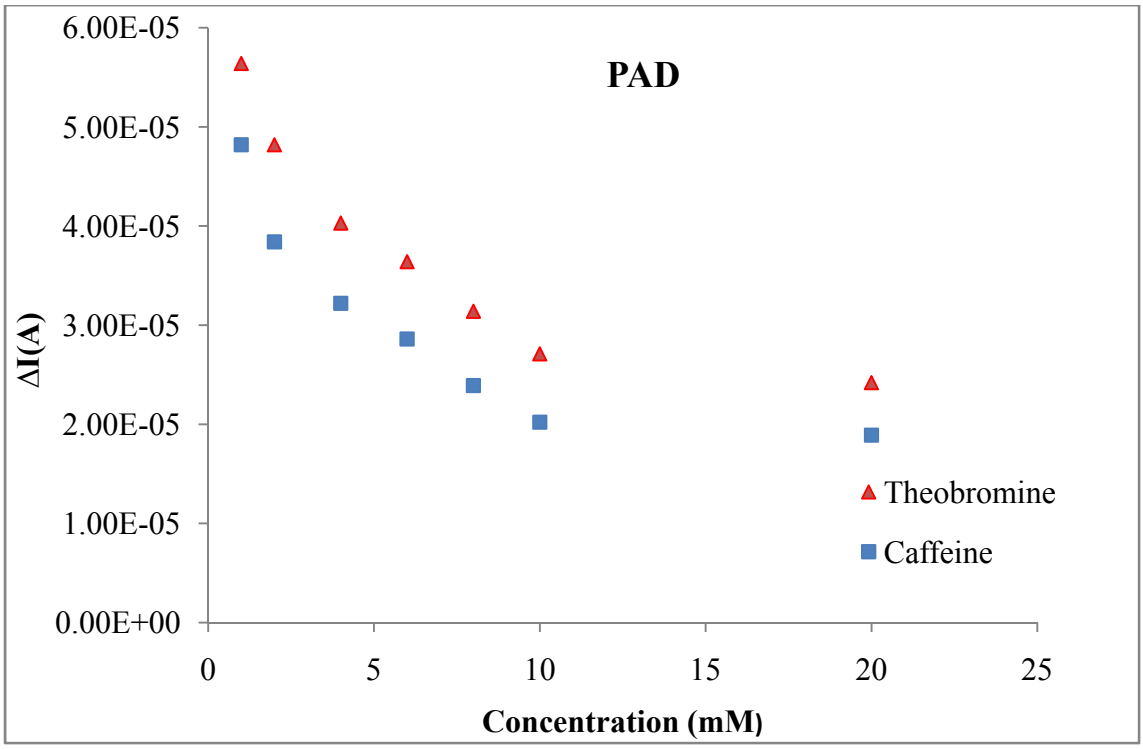


Figure 2.16 PAD of caffeine and theobromine standards from 1-20 mM.

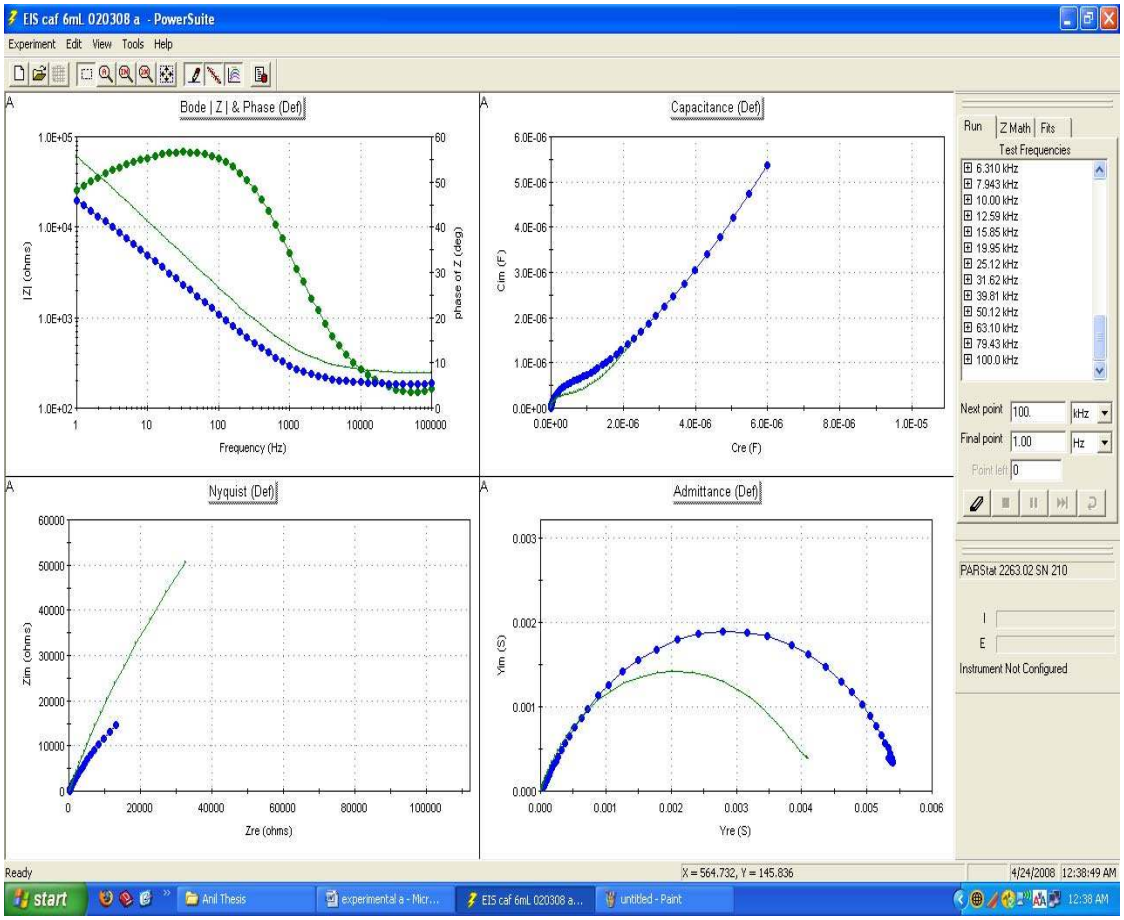


Figure 2.17 EIS of caffeine standard on caffeine imprinted electrode.

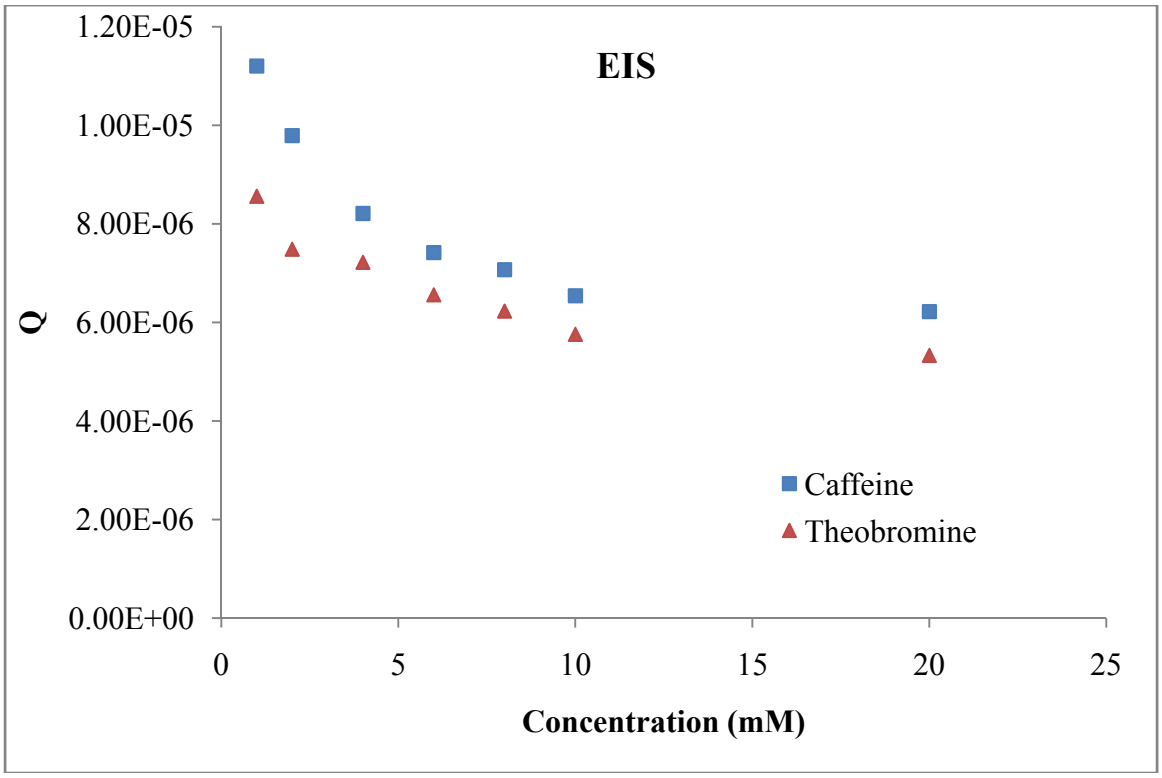


Figure 2.18 EIS of caffeine and theobromine standards from 1-20 mM.

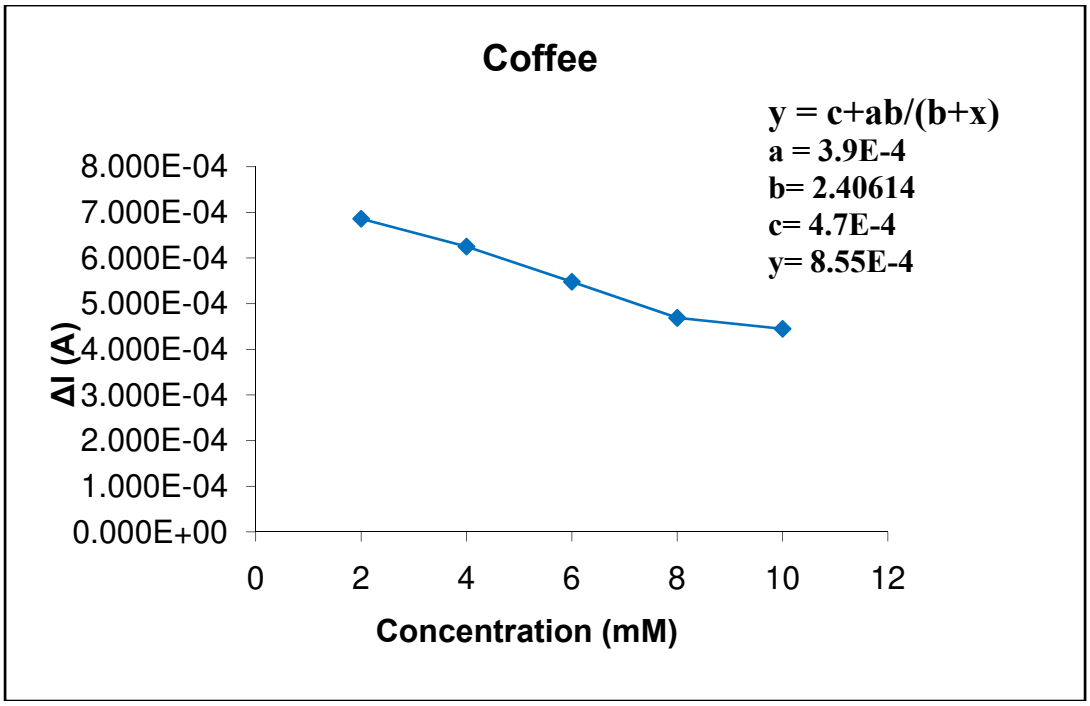


Figure 2.19 PAD of caffeine in coffee.

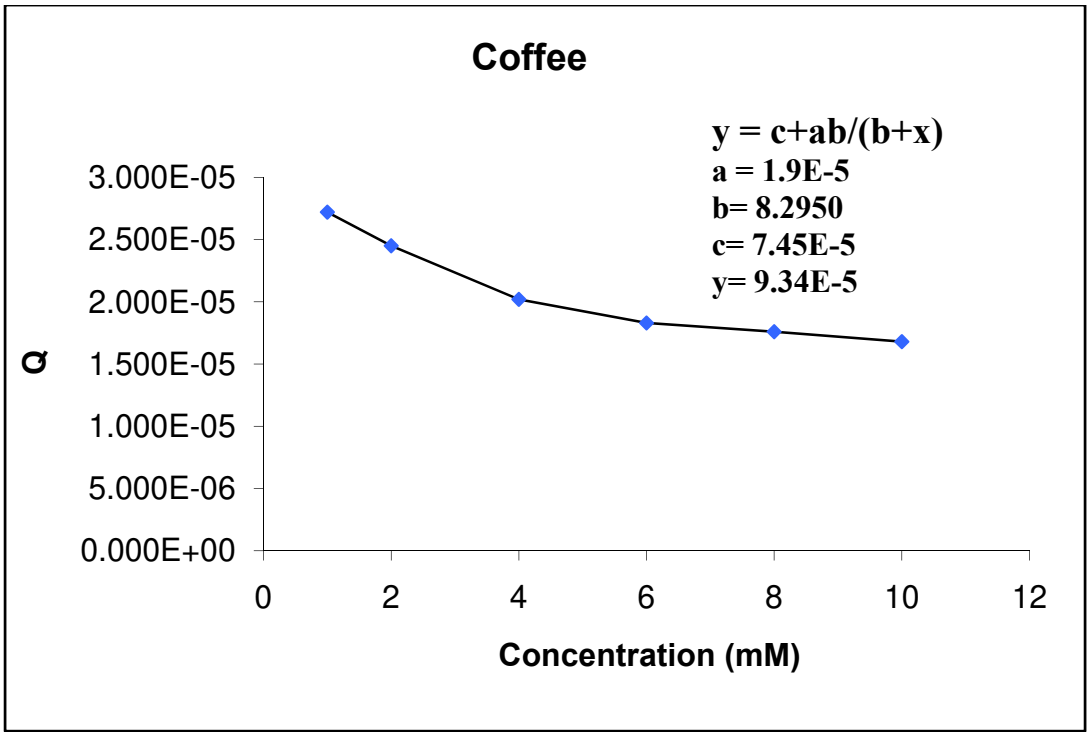


Figure 2.20 EIS of caffeine in coffee

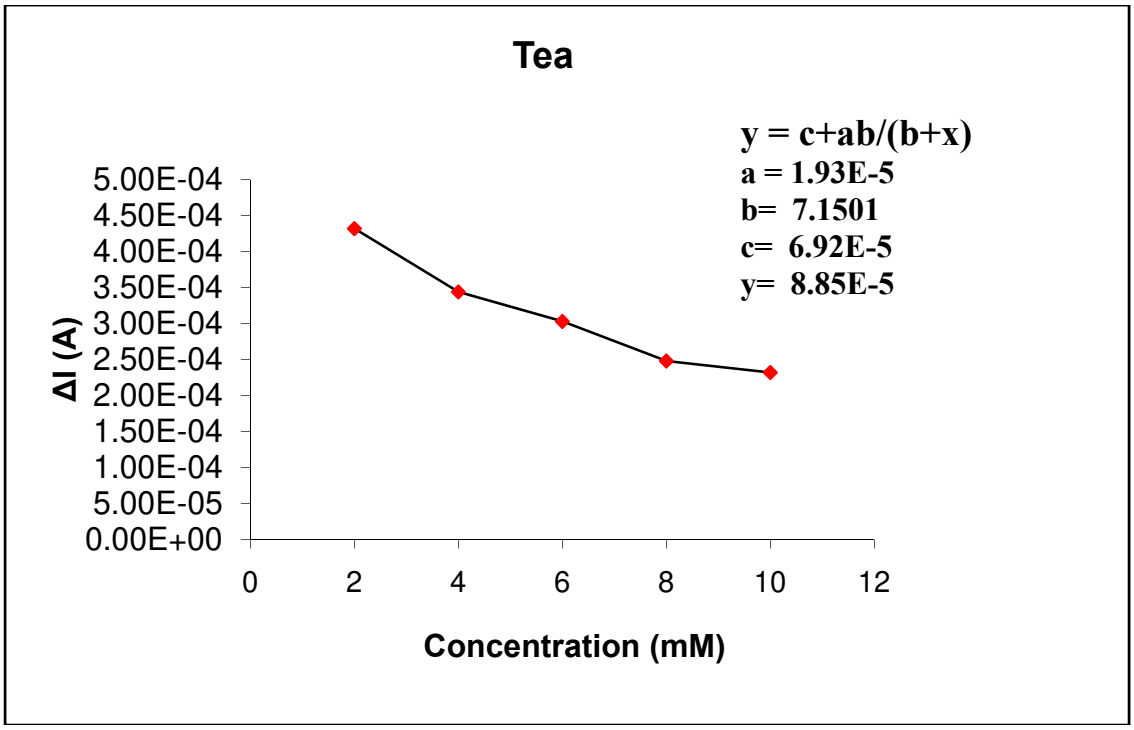


Figure 2.21 PAD of theobromine in tea.

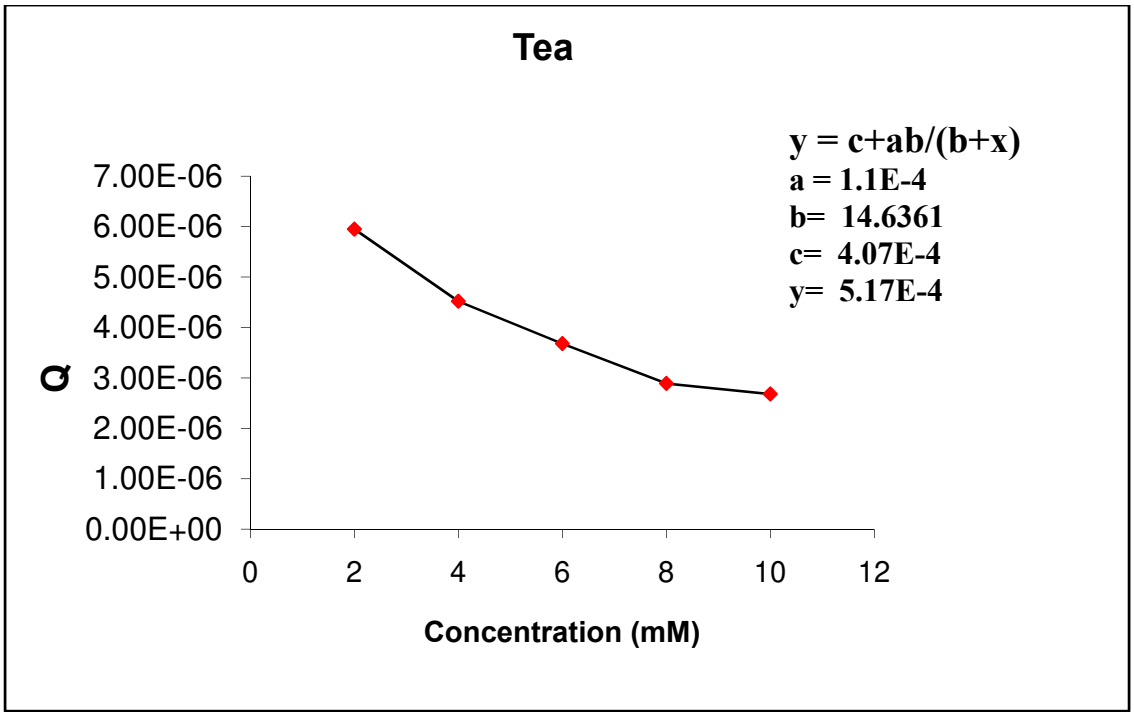


Figure 2.22 EIS of theobromine in tea.

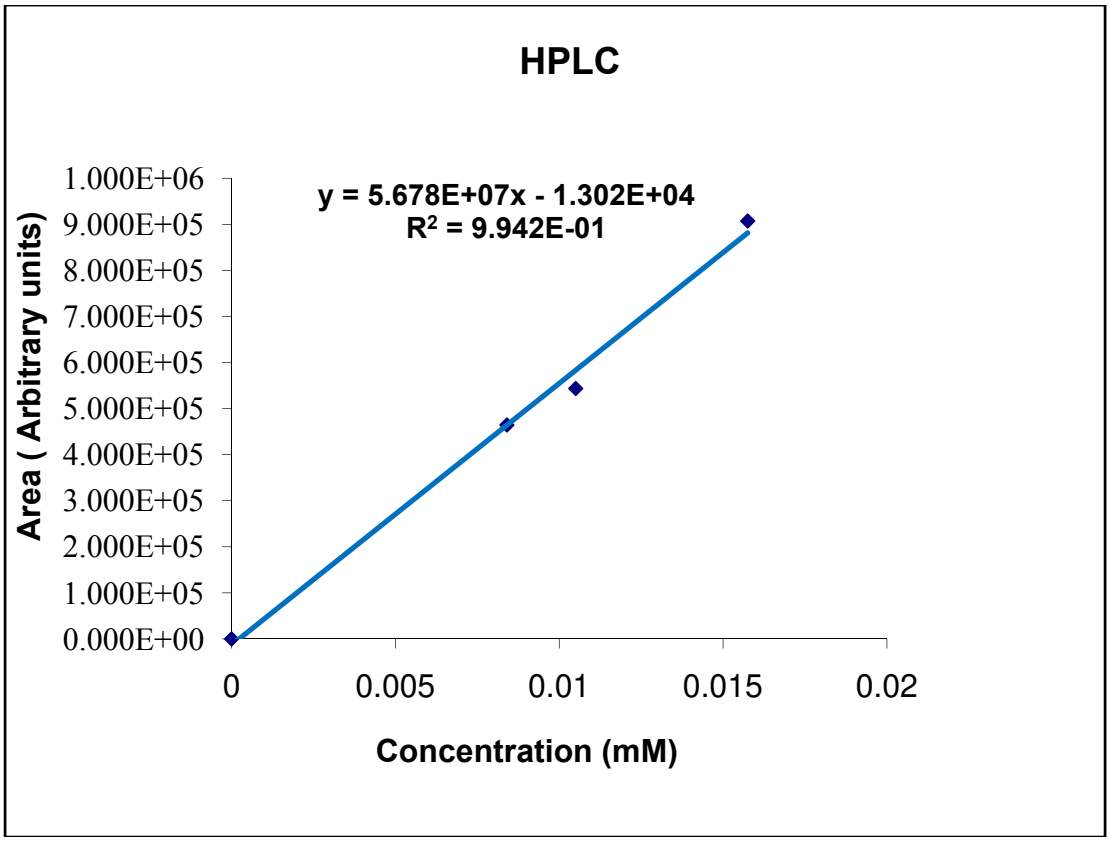


Figure: 2.23 HPLC calibration curve for caffeine.

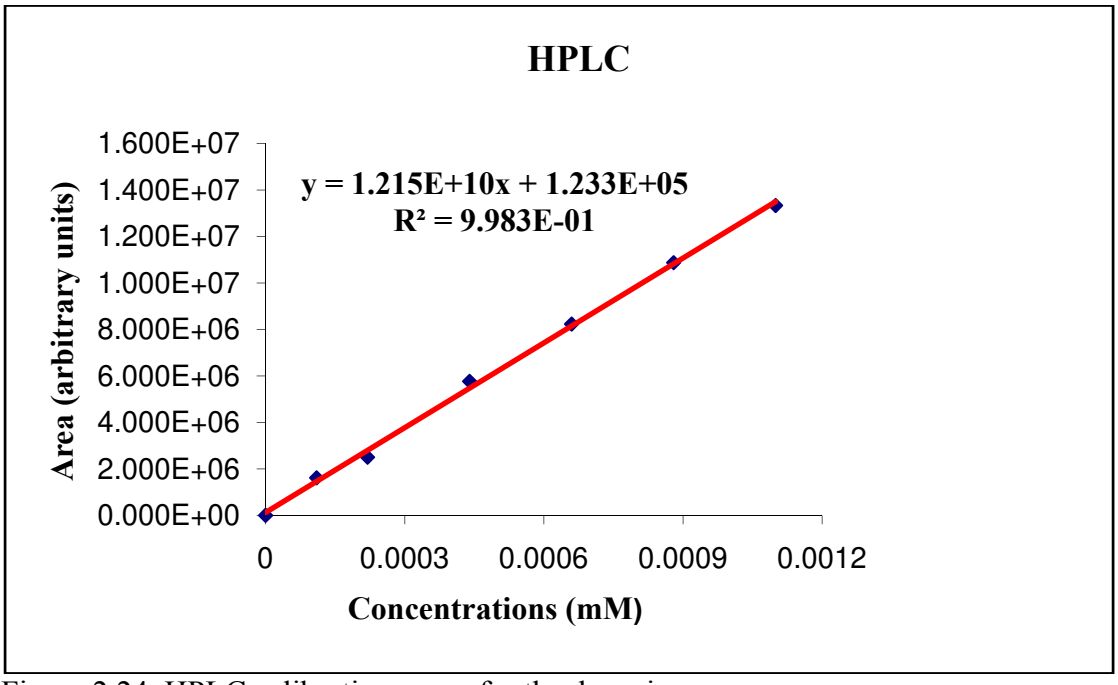


Figure 2.24 HPLC calibration curve for theobromine.

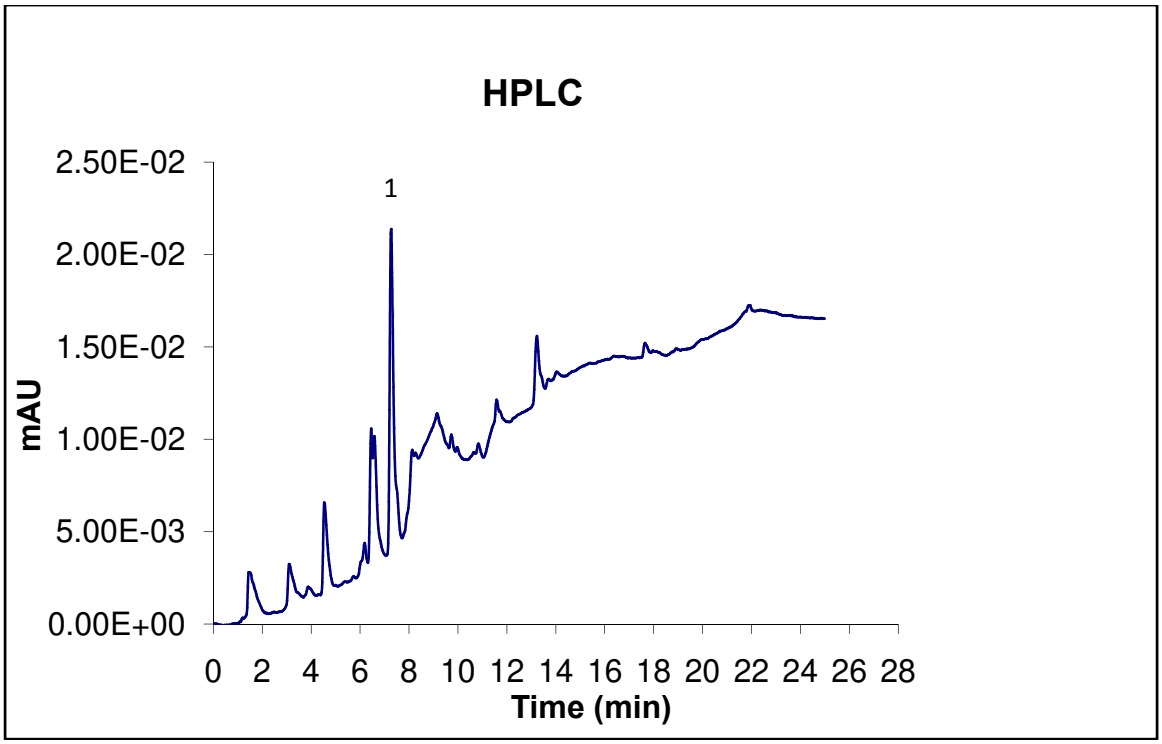


Figure 2.25 Representative chromatogram coffee sample (1 illustrates the caffeine peak.)

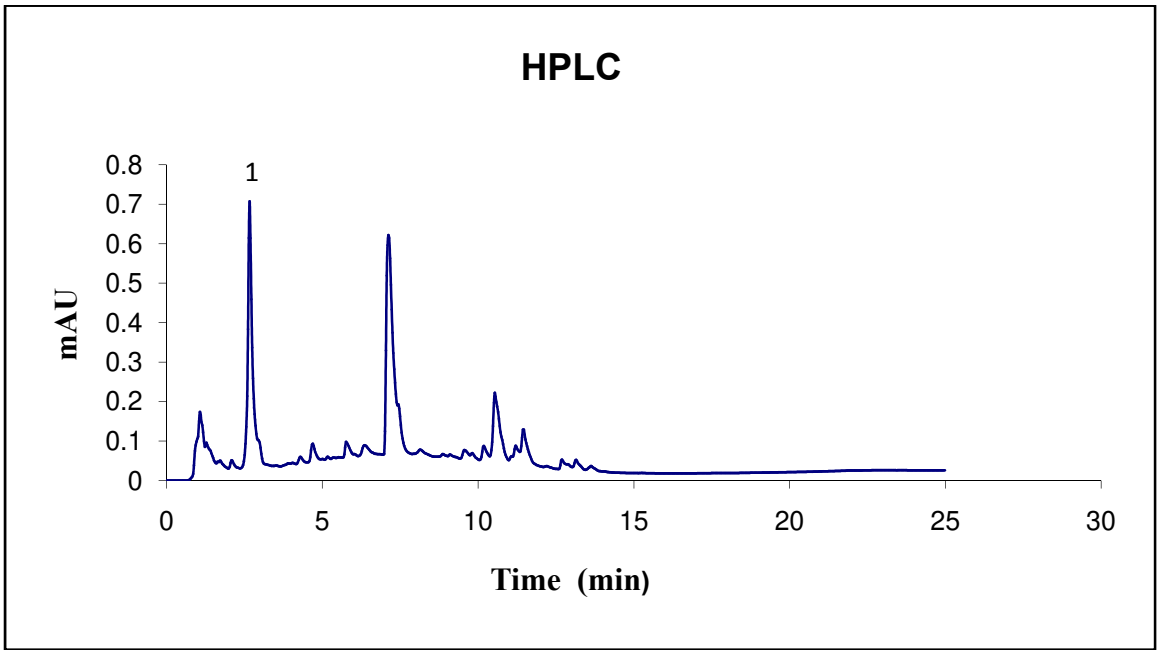


Figure 2.26 Representative chromatogram tea sample (1 illustrates the theobromine peak).

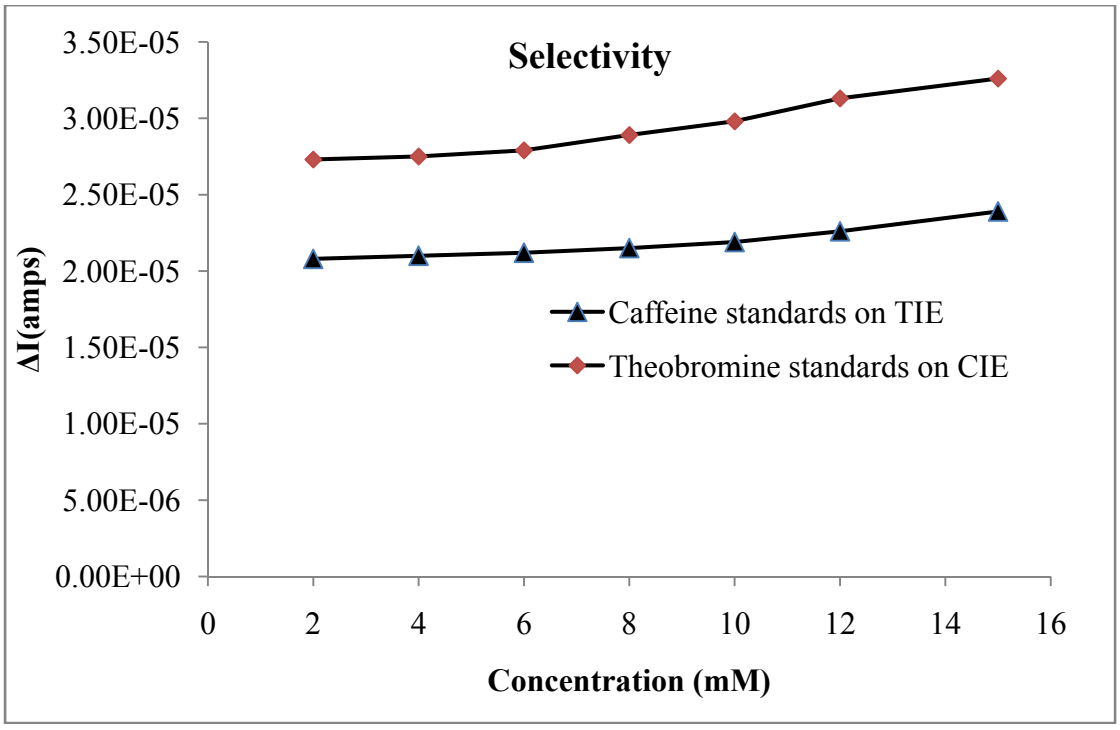


Figure 2.27 Selectivity of caffeine and theobromine on CIE and TIE by using PAD.

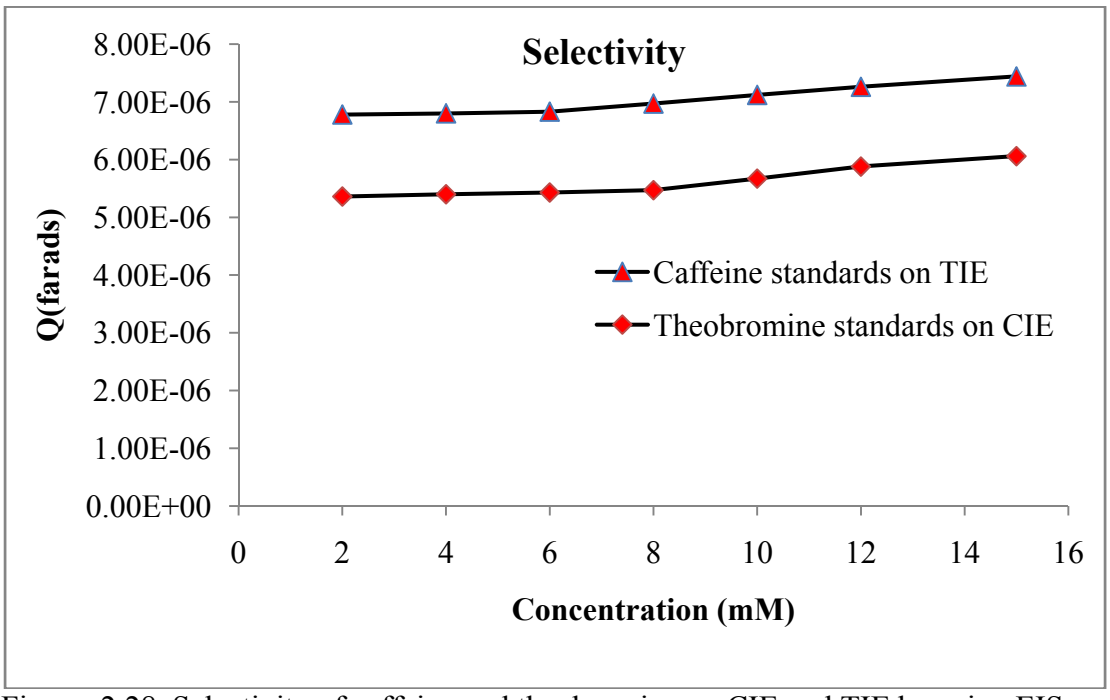


Figure 2.28 Selectivity of caffeine and theobromine on CIE and TIE by using EIS.

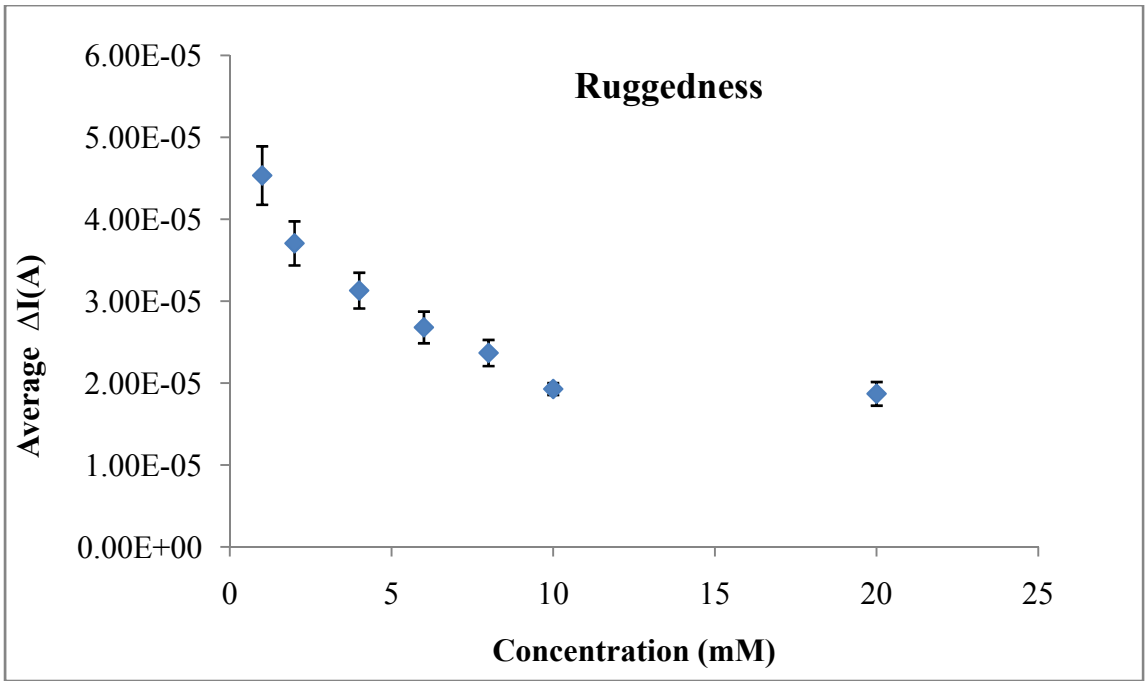


Figure 2.29 Five-day study of caffeine standards on CIE by using PAD.

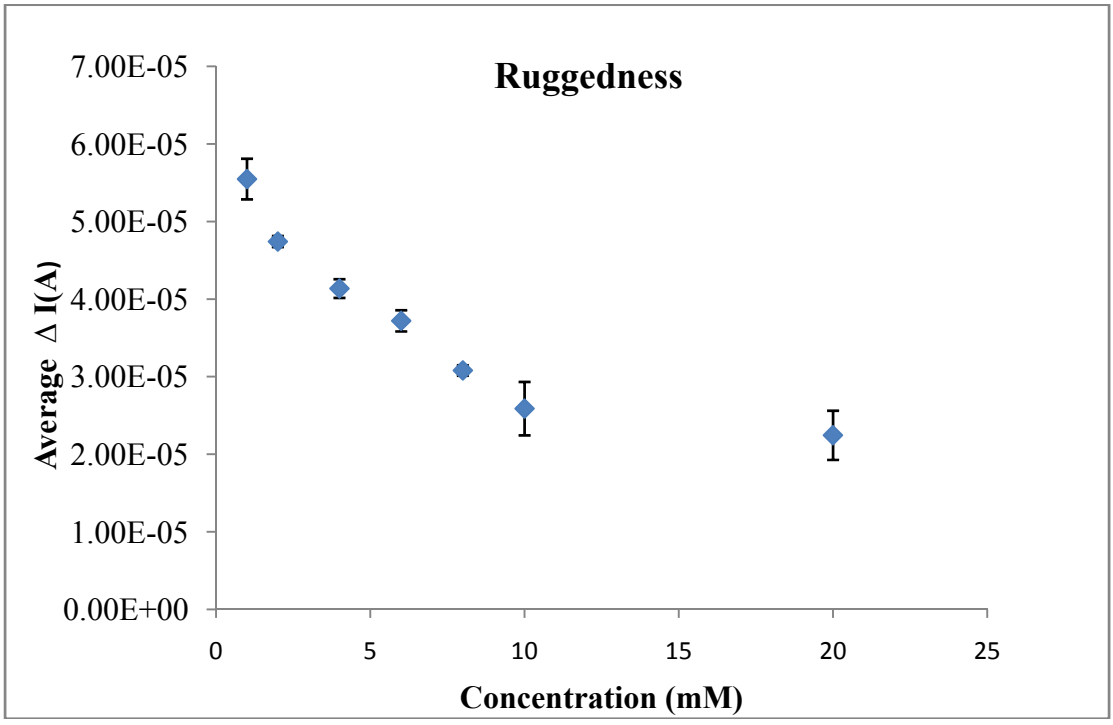


Figure 2.30 Five-day study of theobromine standards on TIE by using PAD

III. RESULTS AND DISCUSSION

A. Objectives:

The main objectives of this study were: i) to determine the selectivity of a caffeine-imprinted and theobromine- imprinted MIPs using Ppy as the conducting polymer matrix. ii) to examine a PAD technique and to test a electrical impedance spectroscopy in comparison to PAD and iii) to determine the integrity of the data obtained from analyses of a commercial instant coffee and a tea sample using both PAD and EIS techniques and comparing results to that obtained from an established HPLC procedure.

B. Platinum electrode preparation

B.1. Polishing:

Prior to use, platinum electrodes were immersed in concentrated HNO_3 and treated for 10 minutes in an ultrasonic bath, then rinsed with water to remove any residual matter remaining on the surface of the electrode. The surface of the electrode was examined by SEM and was seen to be characteristically rough with numerous scratches. The SEM micrograph of this surface is shown in Figure 2.1. The electrode was polished using a polishing cloth containing 0.05 μm alumina paste to achieve a more uniform smooth surface. Figure 2.2 shows the polished electrode.

B.2. Platinization process

Platinization is recommended to improve the adhesion of a conducting-polymer film and simultaneously to increase the number of catalytic active sites in the electrode. The Pt electrode was platinized as described in the experimental section. During the Platinization process, the Pt reduction peak is seen to slightly increase during cyclic

voltammetry, as an enhanced coverage of platinum clusters occurs. These representative results are shown in Figures 2.3 and 2.4. SEM images were taken to visualize platinization of the electrode and are shown in Figure 2.5. The corresponding EDX spectrum of the surface was taken and the results are shown in Figures 2.6.a and 2.6.b. This EDX spectrum verified the deposition of platinum and the surface concentration of Pt was determined to be 99.86 %.

C. Polymerization:

Before polymerization, pyrrole was purified by passing through a neutral Al_2O_3 packed column. Increased purity was indicated by the visual removal of all colored components. A prior literature survey indicated that a 50 mM solution of pyrrole containing 100 mM KCl and 5 mM of caffeine was optimal for the electrochemical formation of the imprinted polypyrrole film. The imprinting of caffeine within polypyrrole film was performed following an in-situ entrapment method during electrochemical formation of the conducting-polymer film on the electrode surface. A uniform polymerization of mPpy requires an oxygen-free solution of caffeine and pyrrole. The lack of oxygen concentration in the solution is crucial for pyrrole polymerization and is achieved by degassing the solution with argon gas. After polymerization a distinct, homogenous, black or brown polymeric layer of over-oxidized Ppy is seen to cover the electrode surface. SEM and AFM images were taken after polymerization of an electrode and are shown in Figures 2.7 and 2.8. The appearance of black (over oxidized) polypyrrole is a reliable indicator of a successful polymerization as indicated in literature. An identical procedure was used in the preparation of a molecularly imprinted theobromine electrode. Using the described procedure, the

preparation of a caffeine imprinted electrode (CIE) and theobromine imprinted electrode (TIE) was accomplished. To check the uniformity of the coating, the thickness of the polypyrrole film on the electrode was determined using the AFM. One half of a platinum electrode surface was covered with vinyl tape while leaving the other half exposed for coating. The thickness of the deposited film was determined for three electrodes and results indicated a range of 0.5 -1.5 μm. Figure 2.9 shows this surface as taken by a light microscope. Figure 2.10 shows the back-scattered electronic image of the half-coated electrode surface taken using SEM. Figures 2.11 and 2.12, show the AFM image and corresponding height or Z-profile of the half-coated polymer electrode respectively.

D. Caffeine and Theobromine Imprinted Electrodes

D.1 Pulsed Amperometric Detection (PAD) analysis:

PAD was used to analyze the current response on a caffeine imprinted electrode (CIE) and a theobromine imprinted electrode (TIE). The mPpy electrode was incubated in a blank solution containing 0.1M phosphate buffer at pH 7.0. The incubation time intervals were: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes. The results are shown in Figures 2.13 and 2.14. These results indicate that the response of both imprinted electrodes displays significant ΔI increases up to 5 minutes then remains relatively constant up to the 30 minute time interval taken. PAD was then used to analyze the current response generated from the CIE and TIE when exposed to a range of concentrations of their respective standards contained in a solution of 0.1 M Phosphate buffered at pH 7.0. Response curves resulting from the caffeine imprinted electrode were generated by measuring the change in current ΔI, after incubation of the mPpy modified electrode in samples containing different caffeine standards of 1, 2, 4, 6, 8, 10, and 20

mM for five minutes each. Representative data for a CIE obtained by the potentiostatic pulse of PAD is illustrated in Figure 2.15. The current change (ΔI) is plotted as a function of concentration. ΔI is first determined by sorting the collected current versus time data (Figure 2.15) from high to low and then averaging the three highest and three lowest current values, then calculate ΔI as the difference between the two values. The resultant data obtained for caffeine and theobromine is illustrated in Figure 2.16 and is shown to be well approximated by a hyperbola. The concentration increase from 1-20 mM, reveals a decrease in Δ I up to 10 mM and then Δ I becomes relatively steady or the electrode can be said to be "saturated" throughout the remaining concentration region. PAD is indirectly a measurement of conductivity and data clearly indicate that as the concentration increases, conductivity decreases presumably due to cavities in CIE and TIE being occupied by respective analyte molecules.

D.2. Electrical impedance Spectroscopy (EIS) analysis:

EIS was used to analyze charge densities of the MIP using the same experimental procedure as for the PAD measurements. A representative scanned EI spectrum for a CIE is shown in Figure 2.17. An R (QR) model was used to fit all impedance data.

The results shown in Figure 2.18 are approximated by a hyperbola and as concentrations of analyte increase from 1-20 mM, the corresponding Q value is seen to decrease until a concentration of 10 mM is reached and then remains unchanged or becomes saturated throughout the remaining concentration region as was also seen in the PAD scheme. The Q parameter estimates the capacitance, or charge building capacity, of the MIP surface. It is clearly seen that as concentration increases capacitance decreases due to cavities in the imprinted electrode being occupied by the respective analyte.

E. MIP Applicability

E.1. Coffee analysis:

To test for the applicability of the CIE, the caffeine content was determined in a commercial sample of coffee. Both quantitative techniques i.e., PAD and EIS were used to evaluate the CIE response. It is presumable that the coffee sample is a complex mixture and therefore the method of standard additions was applied whereby a known amount standard (caffeine) is added into the diluted sample and analytical signal measured after incubation of five minutes. The resultant PAD data is presented graphically in Figure 2.19. The caffeine content was determined using the hyperbola generated by Mathematica™. The caffeine content was determined to be 1.12 ± 0.10 mg/g coffee. Graphical results using EIS are shown in Figure 2.20 and the caffeine content was found to be 0.80 ± 0.09 mg/g coffee. Statistically, there is no difference found between the two values at the 95% confidence level using the standard t-test.

E.2. Tea analysis:

The same procedure was implemented for the tea sample as that for the coffee sample. PAD and EIS techniques were used to analyze the theobromine content. Graphical results using PAD and EIS are shown in Figure 2.21 and 2.22 respectively. The theobromine content as determined by PAD was found to be 2.23 ± 0.08 mg/bag. The theobromine content as determined by EIS was found to be 2.37 ± 0.06 mg/bag. Again, using the t-test, there is no statistical difference found between the two results at the 95% confidence level.

E.3. HPLC analysis:

HPLC analysis was performed directly on the filtered extracts of the coffee and tea samples for the purpose of acting as a comparison technique for both PAD and EIS detection schemes. HPLC calibration curves obtained for caffeine and theobromine standards are shown in Figures 2.23 and 2.24 respectively. Representative chromatograms for the coffee and tea extract are shown in Figures 2.25 and 2.26. The caffeine content determined for the coffee sample was found to be 1.23 mg/g coffee. The theobromine content in the analysis of the tea sample was found to be 1.80 mg/bag. The results compiled from all three procedures are seen in Table 2.1. The caffeine, as determined by PAD and EIS, was consistent with the HPLC analysis however, the theobromine content determined by PAD and EIS was significantly different from that determined by HPLC at the 95% confidence level. It is realized however, that only a limited number of samples were taken and therefore to state for certainty the differences seen here, a larger sample data base needs to be taken.

F. Reproducibility:

A reproducibility study was implemented to determine the degree of precision the fabricated MIPs displayed employing the PAD and EIS procedure described. As described in the experimental portion of this thesis, the caffeine and theobromine contents of 6 and 8 mM were analyzed repeatedly six times, both by PAD and EIS, and resultant standard deviations were determined and shown in Table 2.2. The average percent relative standard deviations (%RSD) for the corresponding MIP were found to be less than 3% as can be seen in Table 3.1.

Table 3.1

Average percent relative standard deviations (%RSD)

MIP	PAD Procedure	EIS Procedure
Caffeine	**2.27**	**2.29**
Theobromine	**2.78**	**2.48**

G. Selectivity:

An interference or selectivity test was performed using mixtures of 4, 6, 8, 10 and 12 mM caffeine and theobromine standards and analyzed by both PAD and EIS detection methods using both CIE and TIE.

The % relative errors found for the CIE and TIE electrodes by PAD and EIS analysis are summarized in Tables 2.3 and 2.4. As can be seen from the data, % relative errors are typically less than 5% for equimolar amounts of "interfering" analyte. There does appear to be a trend of increasing error as concentration increases as might be expected. It is seen in the normal response curve of a given mPpy, that saturation is indicated at approximately 10 mM analyte.

The CIE and TIE electrodes were tested for their response to 10 mM caffeine and 10 mM theobromine solutions respectively in differing concentrations of interfering analyte. These selectivity results are graphically shown in the Figures 2.27 and 2.28. Table 3.2 displays the calculated slopes (rates of change), of the corresponding graphical data.

Table 3.2

Selectivity parameters for caffeine and theobromine

MIP	PAD Procedure	EIS Procedure
	Rate of Change (ΔI (amps) /ΔmM)	**Rate of Change (ΔQ (farads)/ΔmM)**
Caffeine	**4.33×10^{-7}**	**5.44×10^{-8}**
Theobromine	**2.28×10^{-7}**	**5.65×10^{-8}**

The relatively low rates of change indicate that over the concentration interval tested (2-15 mM) there is little interference. This change in current would correspond to a change in concentration of < 0.1 mM analyte. In examining the curve closely, there does appear to be a slight increase in the slope of this curve starting at an ~8 mM concentration of interfering analyte. Therefore, interference may become significant as concentration increases.

H. Ruggedness study :

A ruggedness study was designed to determine how long the CIE and TIE electrodes would give useful and reproducible data. As outlined in the experimental section, caffeine and theobromine standards (1-20 mM) were prepared and analyzed repeatedly for 5 days to aid in establishing the ruggedness of both CIE and TIE. The PAD data generated is shown graphically in Figures 2.29 and 2.30. These data do not indicate a deteriorating electrode over the time interval studied as inferred by a relatively constant standard deviation. Over the concentration range and time interval tested (5 days), the average percent relative standard deviation was determined to be less that 7%.

This is a good indication of the ruggedness of the MIP i.e., the electrodes proved to be useful over the 5 day interval with minimal change in response characteristics. It is realized however, that the electrode response should be calibrated for each use.

IV. CONCLUSIONS

From the data generated for this research, the following conclusions are made:

1. MIPs consisting of polypyrrole embedded with caffeine and theobromine appear to be selective to the respective analytes in the concentration range examined 1.0 to 20 mM.
2. Selectivity appears to decrease as the mole ratio of interferent to analyte increases.
3. The MIP response, using PAD and EIS detection schemes, towards caffeine and theobromine in the tested concentration range of 1mM-20 mM, reveals a decrease in ΔI and Q respectively, up to a concentration of 10 mM and then both measurements become relatively steady. This condition would indicate that at concentrations exceeding 10 mM the MIP is saturated.
4. To test for the applicability of the CIE, the caffeine content was determined in a commercial sample of coffee. Both PAD and EIS measurements were made and statistically no difference was found between the two values at the 95% confidence level using a standard t-test.
5. To test for the applicability of the TIE, the theobromine content was determined in a commercial sample of tea. Again both quantitative techniques, PAD and EIS were used to evaluate the TIE response. Statistically, there was no difference found between the two values at the 95% confidence level using a standard t-test.
6. The caffeine, as determined by PAD and EIS, was consistent with results obtained by HPLC analysis however; the theobromine content determined by PAD and EIS was significantly different from that determined by HPLC at the 95% confidence

level. It is realized however, that only a limited number of samples were taken and therefore to state for certainty the differences seen here, a larger sample data base needs to be taken.

7. A reproducibility study was implemented to determine the degree of precision the fabricated MIPs displayed employing the PAD and EIS procedure described. The average percent relative standard deviations (%RSD) for the corresponding MIPs were found to be less than 3%.
8. An interference or selectivity test was performed and the % relative errors determined for the CIE and TIE electrodes by PAD and EIS analysis. These results established percent relative errors typically less than 5% for equimolar amounts of "interfering" analyte. There does appear to be a trend of slightly increasing error as analyte concentration increases.
9. A ruggedness study was designed to determine how long the CIE and TIE electrodes would give useful and reproducible data. Over the concentration range and time interval tested (5 days), the average percent relative standard deviation was determined to be less that 7%. The ruggedness data obtained for the MIP suggests that the electrodes are useful for at least a five-day interval with minimal change in response characteristics.

V. FUTURE WORK

1. To explore how the thickness of the MIP affects the sensitivity. A variation in thickness can be achieved simply by allowing longer deposition times.

2. To explore how the concentration of template molecule affects the sensitivity and selectivity of an MIP. This study used a concentration of 5mM analyte for the preparation of the mPpy. Varying the concentration of the template molecule would undoubtedly have an effect on the response characteristics of the MIP and characterization of this effect would be beneficial.

3. To incorporate chiral functionalities into a MIP to enable selective determination of the optical isomers.

4. Prepare MIP analogs of polypyrrole such as polyaniline and polythiophene, to try and improve upon selectivity and sensitivity characteristics.

VI. BIBLIOGRAPHY

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