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DETERMINATION OF ARSENIC (III) USING GOLD NANOPARTICLES-MODIFIED SCREEN-PRINTED CARBON ELECTRODE IMMOBILIZED WITH ACETYLCHOLINESTERASE ENZYME

by

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Determination of Arsenic (III) using Gold Nanoparticles-Modified Screen-Printed Carbon Electrodes Immobilized with Acetylcholinesterase Enzyme

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ABSTRACT

Acetylcholinesterase (AchE) enzyme immobilized gold nanoparticles-modified screen-printed carbon electrodes (AuNPs/AchE/SPCEs) are used to determine arsenic (III), As^{3+}, using chronoamperometric technique. Gold nanoparticles were first electrochemically deposited on the screen-printed carbon electrodes (SPCEs) followed with modification of the surface with covalently bonded AchE enzyme. The presence of As^{3+} inhibits the AchE enzyme activity decreasing the chronoamperometric oxidation current. The results showed that the sensitivity, limit of detection (LOD), and limit of quantitation (LOQ) of AuNPs/AchE/SPCEs electrodes are better than the AchE immobilized SPCEs (AchE/SPCEs) electrodes with no gold nanoparticles. Good precision results were obtained for studies conducted using sets of different arsenic concentrations and electrodes. Selectivity studies of the possible interfering ions present in the ground water were also performed.

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CHARTER 1

INTRODUCTION

1.1 Background and Significance

Environmental monitoring is a global objective which has been shown to be closely linked to the quality of life as it focuses on disease control, quality of food, air, and water.[1] The chances of finding agents that can have severe short-term to long-term impact having grievous and possibly fatal consequences on human and animal lives is ever increasing. The agents that needs monitoring in the environment can be broadly classified as small organic, inorganic pollutants, pharmaceuticals, personal care products (PPCPs), xenoestrogens, toxins of microbial origins, endocrine disrupting compounds (EDCs), and other pathogens.[2,3] High levels of different chemicals arising from human activities due to industrialization has resulted in the discharge of large amount of treated and untreated wastewater and even solid wastes into rivers and oceans. Water toxicity and safety are a global concern in developed and developing countries. Natural and anthropogenic substances are leached to streams and groundwater from the soil. These substances are classified according to their chemical nature, but physical state (dissolved species or suspended and colloidal matter) is also crucial for sampling design.[2,4] The chemical categories of water pollutants relevant to environmental analysis are shown in Figure 1.1.

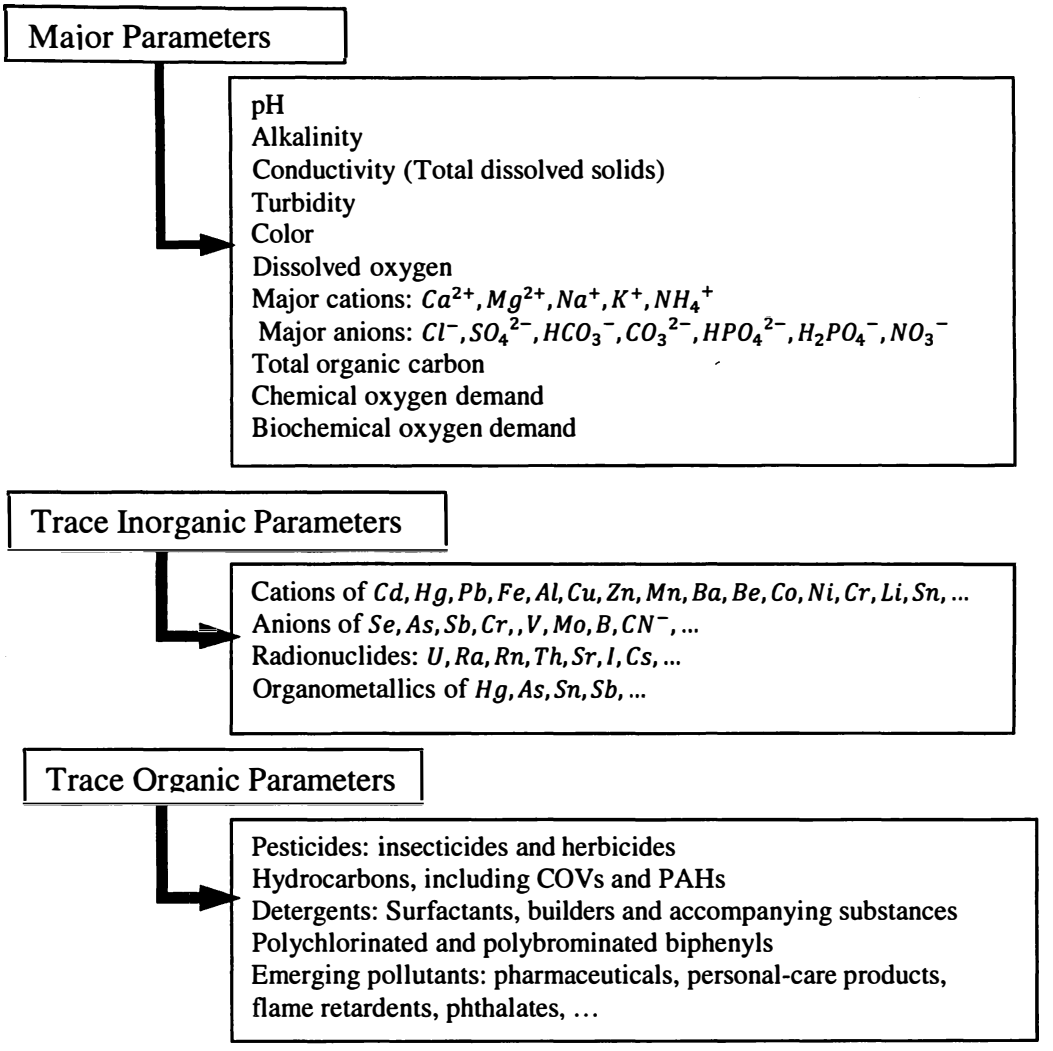

Figure 1.1 Main analytical parameters in environmental water monitoring.[2,4]

Many chemical substances grouped under the trace inorganic and organic parameters are considered to be significant menaces for human and environmental health even at low concentrations. Therefore they are classified as priority pollutants by international organization and environmental agencies establishing protection thresholds at trace or ultra-trace levels. The protection thresholds indicates how dangerous

these pollutants are and how difficult the analytical challenges for an accurate and representative measurements.[4] Some elements, notably arsenic, have attracted much attention since 1983 when the first case of arsenic-induced skin lesions were identified by K. C. Saha then at the Department of Dermatology, School of Tropical Medicine in Calcutta, India.[5] However, in 1990s, the largest poisoning of a population with millions of people exposed was recorded in Bangladesh due to the wide occurrence of arsenic in well water.[5,6] The contamination of groundwater with arsenic has also been reported in several countries of the world having arsenic levels in drinking water above the World Health Organization (WHO) recommended arsenic value of 10 μg/L.[9]

The conventional analytical techniques for determining arsenic (As) in water require expensive instrumentation, high cost of operation, time consuming, requires highly skilled and trained personnel, and are not suitable for field use but only for laboratory conditions. Recently, electrochemical methods had been developed which offers possibilities to determine arsenic at low concentrations. They offer advantages over the conventional analytical techniques because they possess high specificity, sensitivity, low cost, easy to use as no special training is required to operate, field portable, and has the ability to furnish real-time signals for detecting arsenic even at low concentrations are been developed.

1.2 Research Objectives

The objectives of this work are summarized as

- To develop a simple electrochemical procedure using chronoamperometric technique to determine arsenic (III), (As^{3+}) in groundwater using gold nanoparticles modified screen-printed carbon electrodes immobilized with acetylcholinesterase enzyme.
- To investigate the electrode reaction mechanism.
- To demonstrate the effectiveness of the biosensor for field application in term of its sensitivity, and reliability of results without interference from other heavy metals.

CHAPTER 2

LITERATURE REVIEW

2.1 Arsenic

2.1.1 Occurrence of arsenic

Arsenic (As) is an element classified as a metalloid and is one of the abundant elements in the earth's crust. It has atomic number 33 and atomic mass number 74.92. It occurs as a pure elemental crystal, and in many mineral form, usually in the form of sulfide ores, arsenates (As^{5+}), arsenites (As^{3+}), arsenides (As^{3-}), and silicates, and depending on the biogeochemical conditions is released as toxic inorganic species in groundwater.[6-8]

Apart from the natural occurrence, various industrial and agricultural activities such as mining and smelting, leather and wood treatment, refining petroleum, manufacture of metals and alloys, burning fossil fuels and wastes, and pesticides applications have also led to the release of arsenic into the environment.[6-8] Table 2.1 gives a list of major minerals occurring in nature[7]

2.1.2 Arsenic speciation

Arsenic exists in many different forms in the natural environment in the following oxidation states occurring as: arsenates (As^{5+}), arsenites (As^{3+}), As^{0}, and arsenides (As^{3-}). The mobility, the bioavailability and the physiological and toxicological

Table 2.1 Major arsenic minerals occurring in nature[7]

Mineral	Composition	Occurrence
Native arsenic	As	Hydrothermal veins
Proustite	Ag_3AsS_3	Generally one of the late Ag minerals in the sequence of primary deposition
Rammelsbergite	$NiAs_2$	Commonly in mesothermal vein deposit
Safflorite	$(Co,Fe)As_2$	Generally in mesothermal vein deposit
Seligmannite	$PbCuAsS_3$	Occurs in hydrothermal veins
Niccolite	NiAs	Vein deposit and norites
Realgar	AsS	Vein deposit often associated with orpiment, clays and limestones, also deposits from hot springs
Orpiment	As_2S_3	Hydrothermal veins, hot springs, volcanic sublimation product
Cobaltite	CoAsS	High-temperature deposits, metamorphic rocks
Arsenopyrite	FeAsS	The most abundant As mineral, dominantly mineral veins
Tennanite	$(Cu,Fe)_{12}As_4S_{13}$	Hydrothermal veins
Enargite	Cu_3AsS_4	Hydrothermal veins
Arsenolite	As_2O_3	Secondary mineral formed by oxidation of arseopyrite, native arsenic and other As minerals
Claudetite	As_2O_3	Secondary mineral formed by oxidation of realgar, arsenopyrite and other As minerals
Scorodite	$FeAsO_4.2H_2O$	Secondary mineral
Annabergite	$(Ni,Co)_3(AsO_4)_2.8H_2O$	Secondary mineral
Hoernesite	$Mg_3,(AsO_4)_2.8H_2O$	Secondary mineral, smelter water
Haematilite	$(Mn,Mg)_4Al(AsO_4)(OH)_8$	-
Conichalcite	$CaCu(AsO_4)(OH)$	Secondary mineral
Adamite	$Zn_2(OH)(AsO_4)$	Secondary mineral
Domeykite	Cu_3As	Found in vein and replacement deposits formed at moderate temperatures
Loellingite	$FeAs_2$	Found in mesothermal vein deposit
Pharmacosiderite	$Fe_3(AsO_4)_2(OH)_3.5H_2O$	Oxidation product of arsenopyrite and other As minerals

effects of arsenic depend upon its chemical forms as determined by its oxidation state which is important in the speciation of arsenic in natural water.[9,10] In neutral water particularly, in groundwater inorganic arsenics are found as arsenite ions (As^{3+}, AsO^{2-} or H_3AsO_3) and arsenate ions (As^{5+}, $HAsO_4^{2-}$ or H_2AsO_4).[9-13] In water with sufficient dissolved oxygen, As^{5+} species are the thermodynamically stable and predominant form. However, in slightly reducing environment and lower pH , As^{3+} is the more stable form of arsenic.[14] Other arsenic species of organic origin such as monomethylarsenic acid (MMA), dimethylarsenic acid (DMA), trimethylarsine oxide (TMAsO), and trimethylarsine (TMAs) have also been reported.[7,9] These organic forms are products of methylation of the inorganic arsenic compounds produced under oxidizing conditions. Some of the chemical structures of arsenic species found in water are shown in Figure 2.1

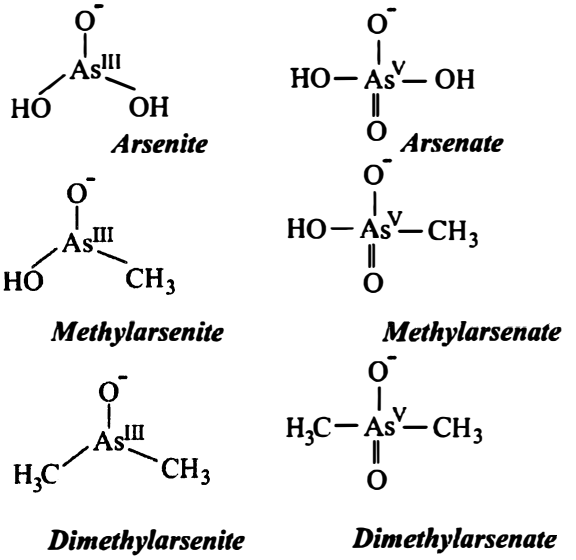

Figure 2.1 Chemical structure of arsenic species in water.[9]

2.1.3 Arsenic toxicity

The chemical forms and oxidation states are very important determinant regarding the toxicity of arsenic. Toxicity also depends on other factors such as physical state, the rate of absorption into cells, the rate of elimination, and the nature of chemical substituents in the toxic compound. Inorganic arsenics in ground water are reported to have higher toxicity than their organic species. The trivalent inorganic forms such as arsenic trichloride ($AsCl_3$), arsenic trioxide (As_2O_3), and arsine (AsH_3) are more mobile and possess a greater potential toxicity of about 50 times that of pentavalent arsenic due to its reactivity with enzymes in human metabolism.[6,13] The usual toxic mode of an element is the inactivation of enzyme systems, which serves as biological catalyst. Although trivalent inorganic arsenic is known to be more toxic than the pentavalent inorganic form, arsenic (V) (As^{5+}) can replace inorganic phosphate during glycolysis because it resembles it. This is achieved by the production of 1-arseno-3-phosphoglycerate instead of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate during glycolysis. The 1-arseno-3-phosphoglycerate formed is unstable and immediately hydrolyzes to 3-phosphoglycerate thereby continuing the glycolysis process. However, the ATP (adenosine triphosphate) molecule generated from 1,3-bisphosphoglycerate is prevented as As^{5+} can uncouple oxidative phosphorylation explaining its toxicity as shown in Figure 2.2.

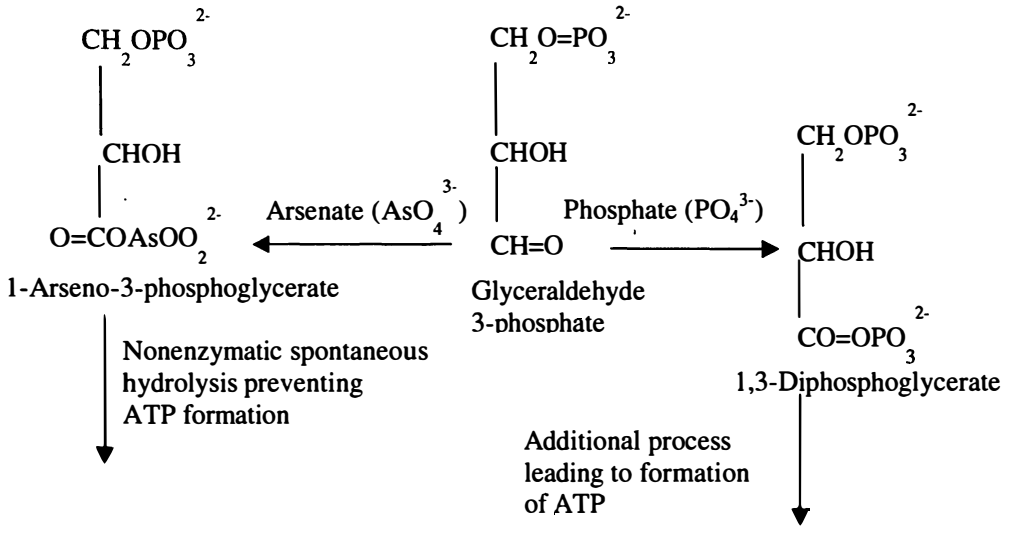

Figure 2.2 Prevention of ATP generation by arsenic (V).[6]

In addition, As^{5+} can also prevent generation of cellular energy by inhibiting the conversion of pyruvate into acetyl-CoA, thereby blocking the citric acid cycle resulting in further loss of ATP.[6] As^{3+} interferes with enzymes by binding to thiol (-SH) groups and hydroxyl (-OH) groups , especially in when there are two adjacent thiol groups in the enzyme. The inhibitory action is based on inactivation of pyruvate dehydrogenase in the pyruvate acetyl CoA reaction preventing ATP generation. The As^{3+} binds to the thiol group of lipoamide, a coenzyme by replacing the two hydrogen atoms from the thiol groups and attaches with a sulfur molecule to form a complex as shown in the reactions in Figure 2.3

2.1.4 Health effects of arsenic

The presence of arsenic in drinking water constitutes a major public health problem with threat to the lives of people in several countries of the world such as

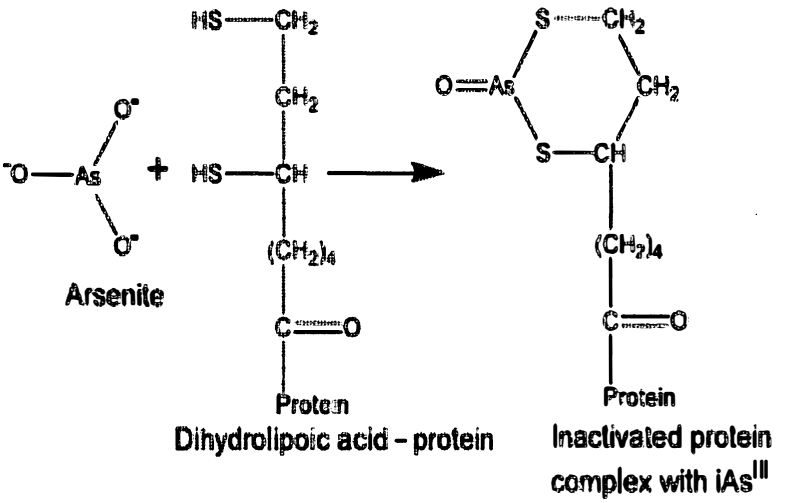

Figure 2.3 Inactivation effect of arsenic (III) (As^{3+}), on thiol groups[6]

Canada, the United States of America, Argentina, Chile, China, India, Taiwan, Mexico, and Bangladesh.[6-9,15] The wide variety of chemical species of arsenic found in the environment and the different levels of toxicity and mobility of these species had impacted the way by which these species are transformed due to biological activity or other changes in the environment, such as changes in oxidation-reduction potential and pH.[7] Arsenic has been reported to cause various human diseases with the following system as targets; respiratory system, circulatory system, and reproductive system.[9,11] The following diseases are evident in affected individuals: skin lesions Figure 2.4, bladder, kidney, liver, and lung cancer, heart disease, keratosis and stillbirth, resulting from the continued consumption of higher levels of arsenic in drinking water.[5-17,20]

2.1.5 Arsenic measurement

The World Health Organization (WHO), and the U.S. Environmental Protection Agency (U.S.EPA), recommended that the maximum permissible contaminant level

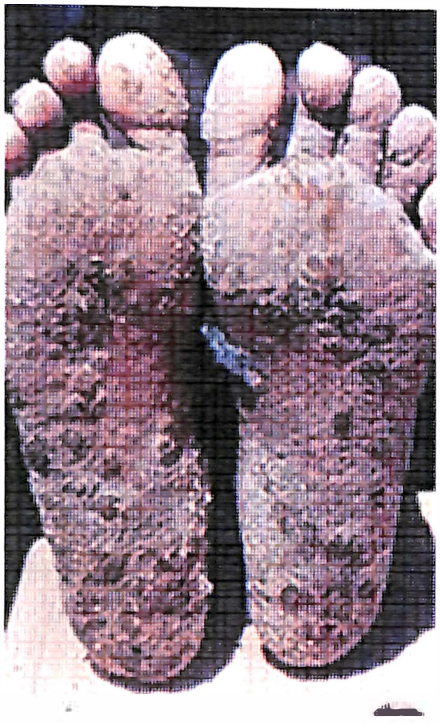

Figure 2.4 Skin lesions due to arsenic poisoning[5]

(MCL) of inorganic arsenic in drinking water is set at 10μg/L (10 ppb) and exposure to higher concentration can result in harmful effects.[5-17,20] Accurate measurement of arsenic in drinking water at levels recommended by WHO and U.S.EPA has before now required laboratory analysis using sophisticated instrumentations that needed trained staff to operate them. Furthermore, these methods are usually bulky, laboratory based, and expensive to operate and maintain. Some of the several methods for the detection of arsenic compounds are Hydride Generation Atomic Absorption Spectroscopy (HGAAS), Atomic Fluorescence Spectroscopy (AFS), Graphite Furnace Atomic Absorption (GFAA), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) which is very sensitive with a

limit of detection (LOD) in the nanomolar range. However, the method is expensive and not suitable for field detection[6,12] Recently however, some low-cost electrochemical methods for detecting arsenic has attracted considerable attention as reported in various scientific literatures because of the potential high sensitivity of the methods, short time for measurements, low cost of operation and maintenance, ease of field application, and portability due to the miniaturize size of the electrodes usually screen-printed electrodes (SPEs). These electrochemical methods make use of several types of electrodes using anodic stripping voltammetry.[12] Forsberg and coworkers[18] reported the findings of their investigation using different electrode materials that includes mercury (Hg), platinum (Pt), and gold (Au) to determine arsenic by anodic stripping voltammetry (ASV) and differential pulse anodic stripping voltammetry (DPASV) and concluded that Au provided a more sensitive response towards arsenic oxidation than the other electrode materials studied. In order to improve the analytical ability of the electrode surface, some modifications were reported to be made on the electrode surfaces which include the use of metal nanoparticles, enzymes, polymers, and complexing agents on the electrode surfaces.[12,16, 24,25]

2.2 Nanomaterials

Nanotechnology has recently been inspiring a great amount of interest in the field of analytical chemistry. It is defined as the creation of functional materials, devices and systems through the control of matter at the 1–100 nm scale. The increasing interest in nanomaterials is driven by their many desirable properties which include physical

(roughening of the conductive sensing interface), electronic, and chemical properties (increased catalytic ability).[11,19-21,24,25,27] There are various methods describing the synthesis of metal nanoparticles in solution and by deposition on solid surfaces. These includes chemical reduction of the metal in presence of different reagents, UV light or electron-beam irradiation, and electrochemical methods which are the easiest and fastest approach to preparing metallic nanoparticles.[19,27] The ability to modify the size and structure and hence the properties of nanomaterials offers excellent possibilities for constructing novel sensors and enhancing the performance of the bioanalytical assay.[18]

2.3 Biosensors

Biosensors are gaining more attention for the detection of contaminants in environmental monitoring (water, food, and air) because they offer potentials for fast, high sensitivity in small volume samples, low-cost of instruments. They also require minimal sample preparation, field portable method, miniaturized on-line and in-situ analysis with minimal waste production. A biosensor is an analytical device which converts a biological response into an electrical or optical signal. It comprises of a bio-receptor or bio-recognition component, which can be an enzyme, natural receptors, bacteria or cells that recognizes the target analyte, a transducer for converting the biochemical response into a measurable electrical or optical signal, whose magnitude is proportional to the concentration of defined analyte in the solution, and the amplifier which responds to the small input signal from the transducer and generate a large output signal containing essential waveform features of an input signal. The amplified signal is

processed by the signal processor for display, analysis, and storage.[3,22,23,28,30] Figure 2.6 shows a representation of the components of a biosensors[23], while in Table 2.2 are listed types of receptors with the biological receptors which are part of electrochemical biosensors indicated in bold character, and the electrochemical techniques used for measurement.[28]

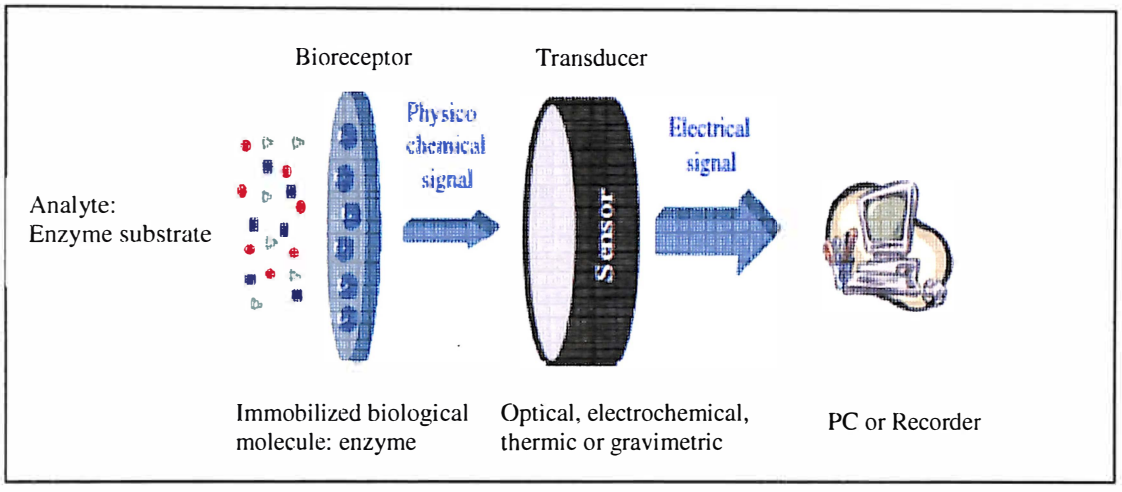

Figure 2.5 Components of a biosensor.[23]

2.4 Immobilization of Enzyme on Electrode

Immobilization of the enzyme on the working electrode surface is an important aspect in the design of enzyme based biosensors. Various immobilization methods have been developed and grouped into four main categories: adsorption, covalent linking, entrapment, and cross-linking or affinity.[23,26,28,29] These immobilization methods are represented in Figure 2.6.

Table 2.2 Types of receptors used in biosensors and the electrochemical measurement techniques [28]

Analytes	Receptor/Chemical recognition system	Measurement technique/ transduction mode
1. Ions	mixed valence metal oxides permselective, non-conductive inorganic crystals trapped mobile synthetic or **biological ionosphores,** ion exchange, glasses, **enzyme(s)**	**potentiometric, voltammetric**
2. Dissolved gases, vapors, odours	bilayer lipid or hydrophobic membrane, inert metal electrode, **enzyme(s),** **antibody, receptor**	in series with 1 **amperometric** **amperometric** or **potentiometric** **amperometric, potentiometric** or **impedance**, piezoelectric, optical
3. Substrates	**enzyme(s)** **whole cells** **membrane receptors** **plant or animal tissue**	**amperometric or potentiometric** in series with 1, or 2, or metal **carbon electrode, conductometric**, piezoelectric, optical calorimetric **as above** **as above** **as above**
4. Antibody/ antigens	**antigen/antibody** **oligonucleotide** **duplex, aptamer** **enzyme labelled** chemiluminescence or fluorescence labelled	**Amperometric, potentionmetric** or **impedimetric**, piezoelectric, optical, surface plasma resonance in series with 3 optical
5. Various proteins and low molecules weight substrates, ions	specific ligands **protein receptors and channels** **enzyme labelled** fluorescent labelled	as 4

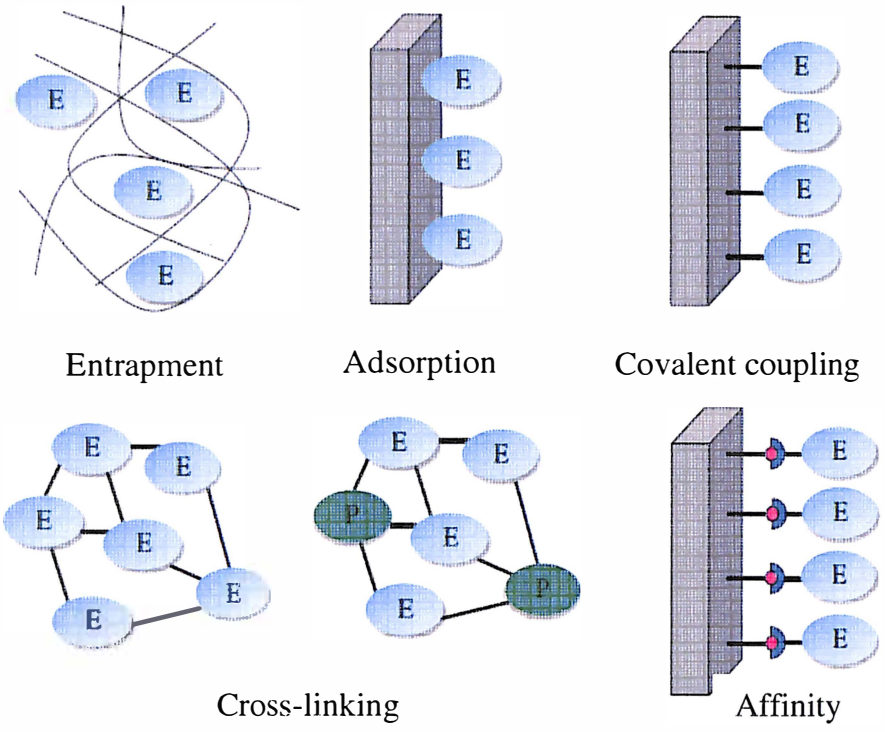

Figure 2.6 Schematic representation of enzyme immobilization methods. E: enzyme, P: inert protein[23]

2.4.1 Entrapment

This immobilization procedure is achieved by simultaneously depositing enzyme, mediator, and additives on a sensing layer usually a three dimensional matrices carbon paste, silica gel, electropolymerized film, and photopolymer.[23,26,28 ,29]

2.4.2 Adsorption

The immobilization process is accomplished by dissolving enzyme in solution and the solid support is placed in contact with the enzyme solution for a fixed period of time. The unadsorbed enzyme is then removed by washing with buffer solution.[23,26,29]

2.4.3 Covalent bonding

This procedure of immobilization involve the use of bifunctional groups such as carbodiimide, glutaraldehyde, used to initially activate the surface of the solid support, followed by enzyme coupling to the activated support, and finally the removal of excess or unbound biomolecules.[23,28,29]

2.4.4 Cross-linking

This method of enzyme immobilization involves cross-linking enzyme with glutaraldehyde or other bifunctional groups, or in the presence of an inert protein such as bovine serum albumin (BSA).[23,26,29]

The various immobilization procedures described have advantages and disadvantages which are summarized in Table 2.3.

2.5 Amperometric Biosensors

Electroanalytical techniques of sensors are based on measuring the applied potential (voltages) and/or current (amperes) in an electrochemical cell containing the analyte. The current density is a function of electrochemically active particles present in the solution, whose oxidation or reduction takes place on the surface of a working electrode, proportional to its concentration. Amperometric biosensors can be grouped into three categories namely first generation, second generation, and third generation biosensors.[22,31]

Table 2.3 Advantages and disadvantages of enzyme immobilization methods.[23]

	Binding nature	Advantages	Drawbacks
Adsorption	Weak bond	1. Simple and easy 2. Limited loss of enzyme activity	1. Desorption 2. Non-specific adsorption
Covalent coupling	Chemical binding between functional groups of the enzyme and those on the support	1. No diffusion barrier 2. Stable 3. Short response time 4. Low enzyme activity loss	1.Matrix not regenerable 2.Coupling with toxic product
Entrapment	Incorporation of the enzyme within the gel or a polymer	1. No chemical reaction between the monomer and the enzyme that could affect the activity. 2. Several types of enzyme can be immobilized within the same polymer	1.Diffusion barrier 2. Enzyme leakage 3. High concentration of monomer and enzyme needed for electropolymerization. 4. High enzyme activity loss
Cross-linking	Bond between enzyme/ cross-linker(e.g. glutaraldehyde)/inert molecule (e.g. BSA)	1. Simple	1. High enzyme activity loss
Affinity	Affinity bonds between a functional group (e.g. avidin) on a support and affinity tag (e.g biotin) on a protein sequence	1. Controlled and oriented immobilization	1. Need of the presence of specific groups on enzyme (e.g. biotin)

- sensors based on measurement of concentration of natural substrates and products of enzyme reaction (amperometric biosensors without mediators (first generation)).
- sensors using mediators as carriers of electrons from active enzyme center to electrode (second generation)
- amperometric biosensors using direct electron transfer between enzyme and electrode (third generation)

During any enzymatic reaction some products are generated and some substrates are consumed. If they are electroactive, their concentration can be measured by amperometric sensors. The enzymes generally catalyzing such reactions are the oxidases and the dehydrogenases, and the sensors are based on measuring concentration of substrates or products of enzyme reaction.[22,23]

The schemes showing the enzyme reactions in which oxidases and dehydrogenases respectively participate are shown as follows:

$$S + E_{FAD} \Longleftrightarrow E_{FAD}S \Longrightarrow E_{FADH2} + P \qquad \text{Eq. (1)}$$

$$E_{FADH2} + O_2 \Longrightarrow E_{FAD} + H_2O_2 \qquad \text{Eq. (2)}$$

Scheme I Enzyme reactions involving oxidases.

(S = substrate, E = enzyme, FAD = Flavin adenine dinucleotide, P = product $FADH_2$ = reduced form of FAD, O_2 = Oxygen, and H_2O_2 = Hydrogen peroxide)

$$S + E + NAD^+ \Longleftrightarrow S^*E^*NAD^+ \Longleftrightarrow E + P + NADH + H^+ \qquad \text{Eq. (3)}$$

$$NADH \Longrightarrow NAD^+ + H^+ 2e^- \qquad \text{Eq. (4)}$$

Scheme II Enzyme reactions involving dehydrogenases.

(NAD^+ = Nicotinamide adenine dinucleotide, NADH = reduced form of NAD^+)

However, if the sensor uses a mediator which is a low-molecular weight particle capable of transferring electrons between the redox center of an enzyme and a working electrode, the mechanism will be as indicated in Figure 2.7, E_{Red}, E_{Ox}: reduced and oxidized enzymes; Med_{Red}, Med_{Ox}: reduced and oxidized mediators

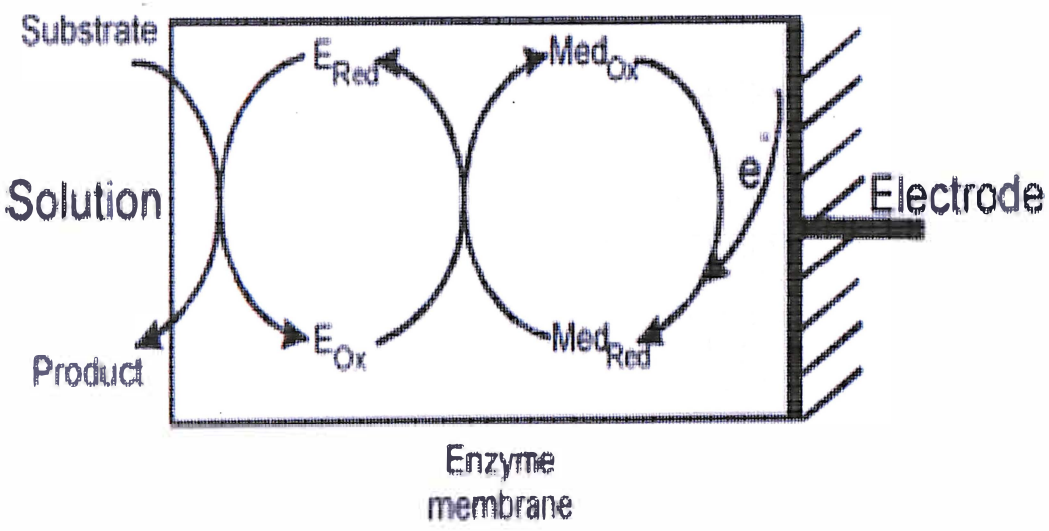

Figure 2.7 Principle of operation of amperometric mediated biosensor.[22]

Then the reaction mechanism in Figure 2.8, reactions (2) and (4) in schemes I and II becomes scheme III

$$E_{FADH2} + MED_{Ox} \Longrightarrow E_{FAD} + MED_{Red} \qquad \text{Eq. (5)}$$

$$NADH + MED_{Ox} \Longrightarrow NAD^{+} + 2H^{+} + MED_{Red} \qquad \text{Eq. (6)}$$

Scheme III Mediated enzyme reactions involving oxidases and dehydrogenases.

2.6 Mechanism of Acetylthiocholine iodide Hydrolysis

Acetylcholinesterase enzyme (AchE) immobilized on the surface of the working electrode is a serine esterase that function as a neurotransmitter and rapidly converts the acetylcholine (Ach) to choline and acetate after transmission of the nerve impulse.[46] The mechanism of its inhibition by some inhibitors such as organophosphorus compounds and carbamates used as herbicide, pesticides, insecticides, and nerve gas agents are well documented.[41,42,46] Electrochemical detection of acetylcholinesterase inhibitors involves the measurement of the oxidation of the substrate acetylthiocholine iodide (ATI) which undergoes hydrolysis and catalyzed by acetylcholinesterase to thiocholine iodide and acetic acid as shown in Figure 2.8.[15,41,42,46]

$$CH_3COSCH_2CH_2N^{+}(CH_3)_3I^{-} + H_2O \xrightarrow{AchE} HS\text{-}CH_2CH_2N^{+}(CH_3)_3I^{-} + CH_3COOH \qquad \text{Eq. (7)}$$

$$2(CH_3)_3N^{+}(CH_2)_2SH + I_3^{-} \longrightarrow 2(CH_3)_3N^{+}(CH_2)_2S + 2H^{+} + 3I^{-} \qquad \text{Eq. (8)}$$

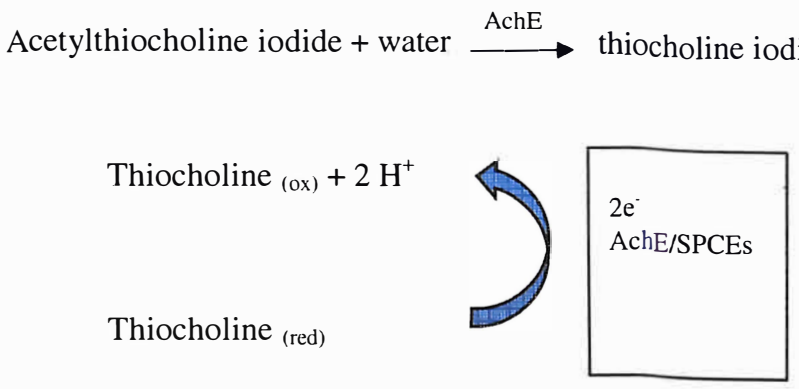

Figure 2.8 Mechanism of oxidation of ATI

The thiocholine iodide generated is electroactive and undergoes an anodic oxidation current that can be measured, thereby providing oxidative current signal that is used to determine arsenic. In the presence of As^{3+} a known inhibitor of acetylcholinesterase, the acetylcholinesterase is inactivated and the amount of thiocholine produced is reduced leading to a decrease in the oxidation current. The extent of inhibition is correlated to the reduction of thiocholine oxidative current relative to the current measured in the absence of an inhibitor (As^{3+}).

2.7 Electrochemical Techniques

2.7.1 Cyclic Voltammetry

Cyclic voltammetry (CV) is the first experiment often performed in an electroanalytical study. It is the most applicable electroanalytical technique used for

mechanistic study of redox systems.[32-34] During a cyclic voltammetry experiment, a cyclic potential is applied between the working and the reference electrode while observing the current flowing between the working and counter electrode.[34]

Cyclic voltammetry consist of a linear potential scan from an initial potential (E_i) where no oxidation or reduction is occurring to a switching potential (E_s) and a linear potential scan back to the final potential (E_f) at constant scan rate. The first scan is referred to as the forward scan (sweep) while the return scan is reverse scan (sweep).[32-34] Figure 2.9 is a representation of a typical potential versus time signal for cyclic voltammetry.

During a potential sweep, the electroanalytical instrument (potentiostat) measures the current resulting from the applied potential and a plot of current versus potential is obtained known as cyclic voltammogram as shown in Figure 2.10. .

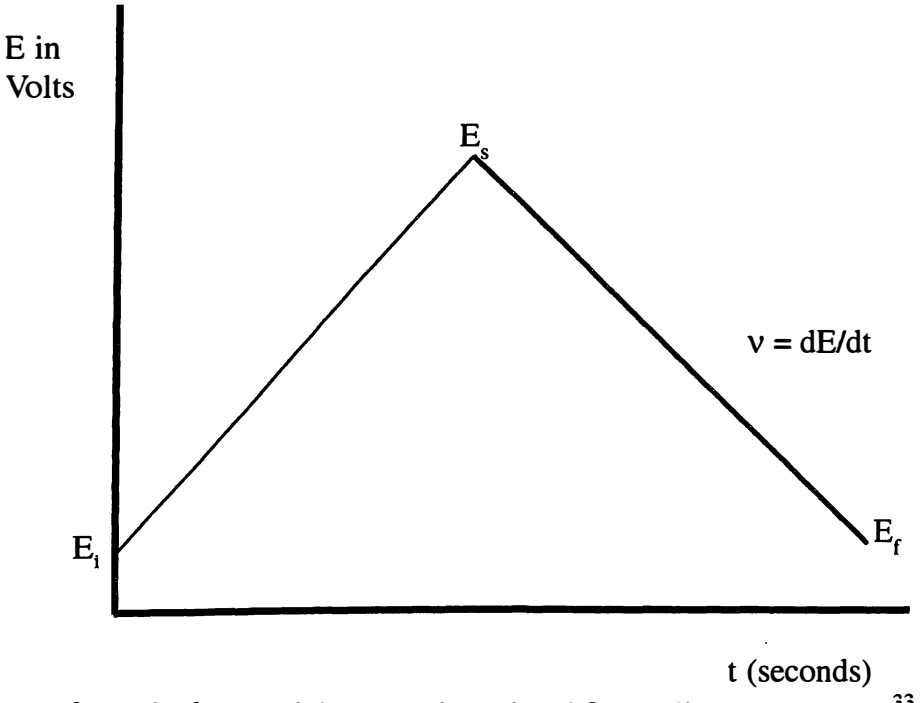

Figure 2.9 Typical potential versus time signal for cyclic voltammetry.[33]

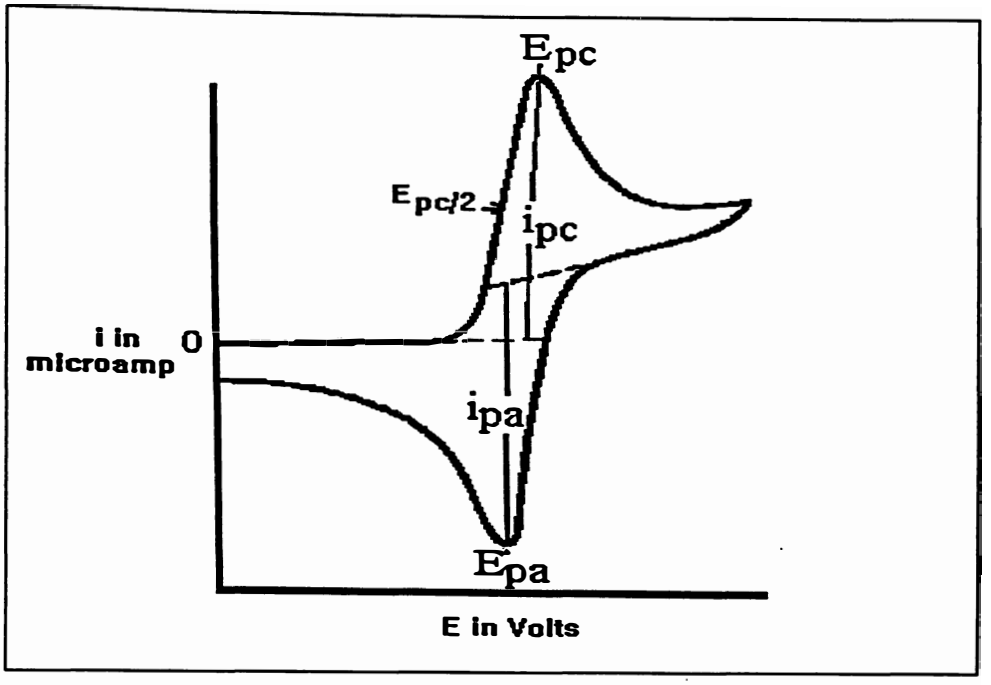

Figure 2.10 Typical cyclic voltammetric output for a reversible redox couple. E_{pc} and E_{pa} represent the cathodic and anodic peak potentials, respectively. The cathodic and anodic peaks currents are represented by i_{pc} and i_{pa}, respectively.[34]

2.7.2 Chronoamperometry

Chronoamperometry is an electrochemical technique in which the potential of the working electrode is stepped and the resulting current from faradaic processes occurring at the working electrode (caused by the potential step) is monitored as a function of time. It is a useful tool for determining diffusion coefficients of electroactive species or the surface area of the working electrode and for investigating kinetics and mechanisms of electrode processes. Figure 2.11a shows a potential-time profile applied to the working electrode. Initial potential E_I is chosen such that no reduction or oxidation of electroactive species, or any other electrode reaction occurs. At time t = 0 the potential is

instantaneously changed to a new value potential E_2, where the reduction or oxidation of the oxidized species (Ox) and reduced species (Red) occurs at a diffusion controlled rate. At E_2 the surface active concentration of the electroactive species Ox and Red is effectively zero. A typical chronoamperogram is shown in Figure 2.11b. The potential step initiates a current as a result of oxidized (Ox) to reduced (Red) species, or vice versa. During the electrolytic process a drop in current (I) at a planar electrode with time is observed.[33,34]

The quantity of charge, Q, passed across the interface is related to the amount of the material that has been converted, and the current is related to the rate at which the conversion occurs. This is in accordance with Faraday's first law:

$$Q = nFN \qquad \text{Eq. 9}$$

where N is the number of moles of the electroactive species converted, F is Faraday's constant, n is the stoichiometric number of electrons involved in the reaction.

The most useful equation in chronoamperometry is the Cottrell equation, which describes the observed current (planar electrode) at any time following a large forward potential step in a reversible redox reaction (or to large overpotential) as a function of $t^{-1/2}$

$$i_t = (nFACD^{1/2})/\pi^{1/2}t^{1/2} = Kt^{-1/2} \qquad \text{Eq. 10}$$

where n = stoichiometric number of electrons involved in the reaction; F = Faraday's constant (96,485 C/equivalent), A = electrode area (cm^2), C = concentration of

electroactive species (mol/cm^3), and D = diffusion constant for electroactive species (cm^2 /s).

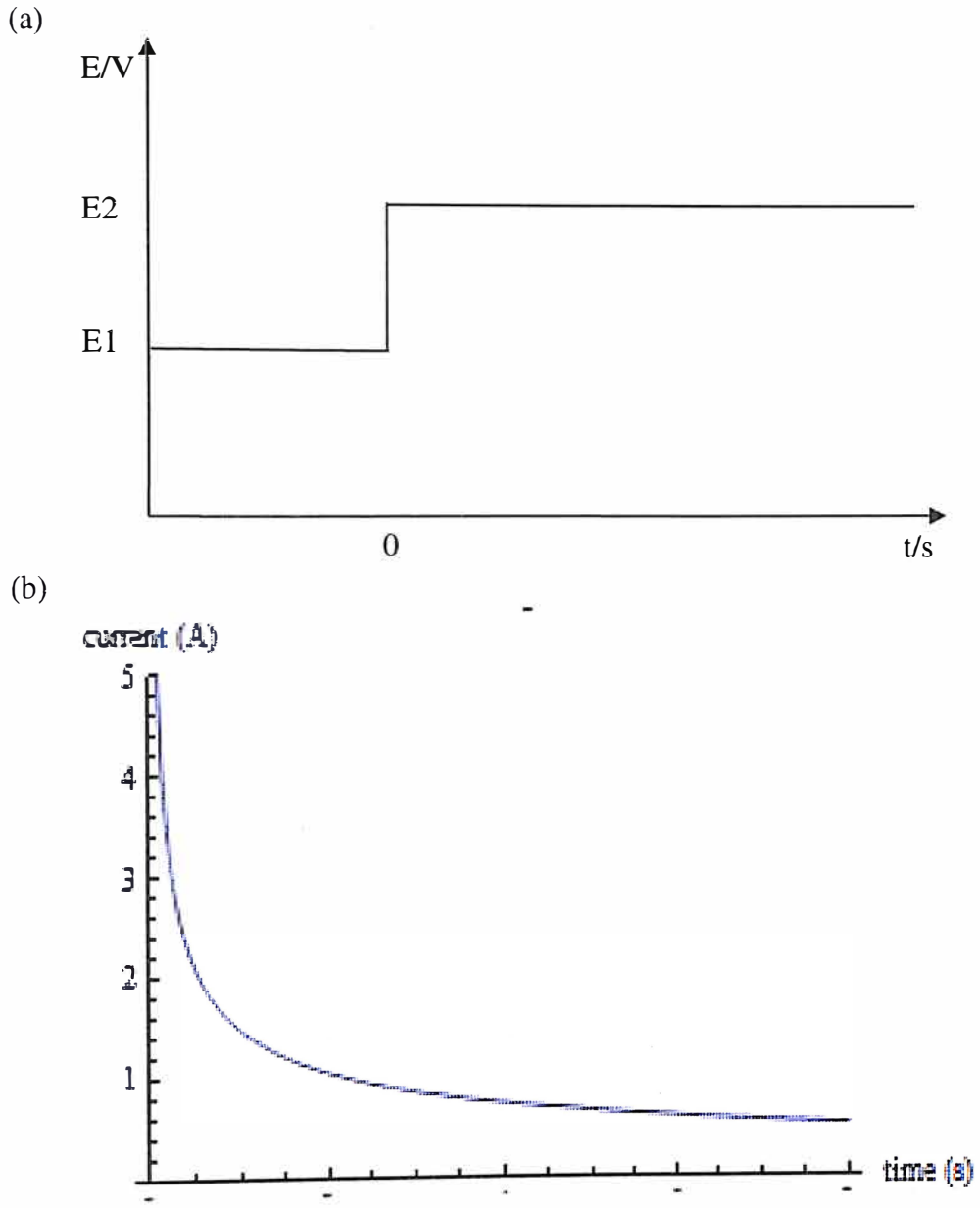

Figure 2.11 (a) The potential-time profile for a single step chronoamperometric experiment. (b) Current vs. time response for a potential step experiment. E2 is chosen such that the reaction is diffusion controlled.

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

N-cyclohexyl-N'-[2-(N-methylmorpholino)ethyl] carbodiimid-4-toluensulfonate and Acetylcholinesterase (E. C. 3.1.1.7, ≥1000 units/mg from electric eel) used for immobilization were from Sigma Aldrich. Acetylthiocholine iodide (ATI) used as the enzyme substrate and potassium chloride (KCl) used for cyclic voltammetric scans and interference studies were from Sigma Aldrich. Sodium (meta) arsenite ($NaAsO_2$, 99%) used for preparing stock solution of As^{3+} was from Sigma Aldrich. Sodium hydroxide (NaOH) and acetic acid used to prepare Britton-Robinson buffer were from Sigma Aldrich. Gold (III) chloride trihydrate ($HAuCl_4$. $3H_2O$, ACS reagent, ≥ 49%) and sulfuric acid (H_2SO_4) used for electrodeposition of Au on electrodes were from Sigma Aldrich. Magnesium chloride ($MgCl_2$) used for interference studies was from Sigma-Aldrich. Boric acid (H_3BO_3) and phosphoric acid (H_3PO_4) used for preparing Britton-Robinson buffer, Calcium chloride ($CaCl_2$), and Sodium chloride (NaCl) used for interference studies were from Fisher. All reagents were used as obtained without purification.

3.2 Reagents preparation

Deionized water from a Barnstead NANOpure Ultrapure water system was used throughout this work to prepare the solutions.

0.1M solution of Britton-Robinson buffer of pH = 7 was prepared by dissolving 2.754 g sodium hydroxide, 2.570 g boric acid, 1.517 mL acetic acid, 1.794 mL phosphoric acid, and 1.804 g potassium chloride in deionized water and diluting to 1.0L.

0.05 M N-Cyclohexyl-N-[2-(N-methylmorpholino)ethyl] carbodiimid-4-toluensulfonate was prepared by dissolving 0.1059 g N-Cyclohexyl-N-[2-(N-methylmorpholino)ethyl] carbodiimid-4-toluensulfonate in 5 mL Britton- Robinson buffer (pH = 7).

Acetylcholinesterase enzyme solution was prepared by dissolving 2.5 mg of the enzyme in 1 mL of Britton-Robinson buffer (pH = 7) solution.

0.01 M acetylthiocholine iodide (ATI) was prepared by dissolving 0.0289 g of ATI in 10 mL Britton-Robinson buffer (pH = 7) in deionized water.

0.01 M As^{3+} stock solution was prepared daily by dissolving 0.013 g of Sodium (meta) arsenite ($NaAsO_2$) in 10 mL Britton-Robinson buffer.

0.1 mM solution of gold (III) chloride trihydrate ($HAuCl_4.\ 3H_2O$) in 0.5 M H_2SO_4 (Sulfuric acid) used for electrodeposition of gold nanoparticles was prepared by dissolving 0.0039 g $HAuCl_4.3H_2O$ in 100 mL deionized water.

0.1 M solution of potassium chloride used for cyclic voltammetric scan during the activation of the electrode was prepared by dissolving 0.7455 g KCl in 100 mL deionized water.

3.3 Experimental

3.3.1 Electrochemical Studies

Electrochemical measurements were conducted using Princeton Applied Research (PAR) Potentiostat/Galvanostat model 263A (PerkinElmer Instruments, TN), Figure 3.1, with screen printed carbon electrodes (SPCE) (Pine Instrument Co, NC) consisting of carbon working (area 20 mm^2), Ag/AgCl reference and counter electrodes, Figure 3.2. All experiments were carried in Britton-Robinson buffer solution at pH = 7 and at room temperature 25 ^{o}C. The set-up for the electrochemical measurements is shown in Figure 3.3.

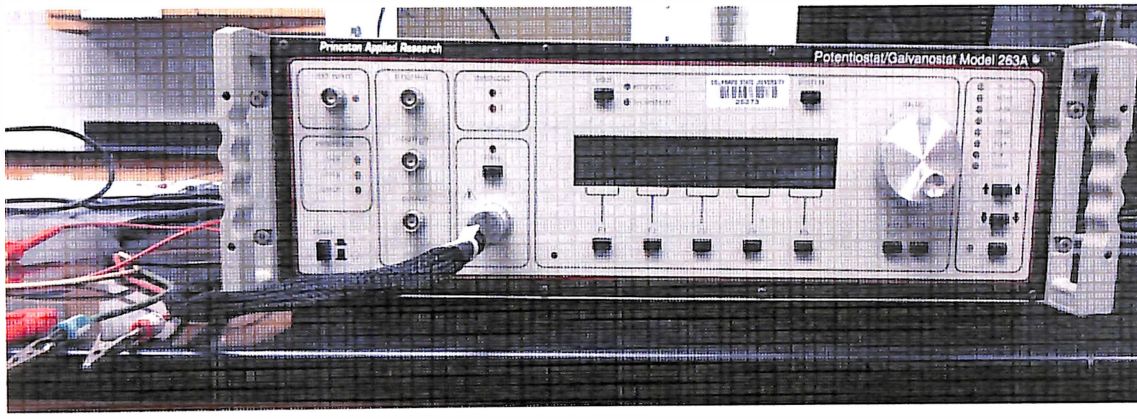

Figure 3.1 Princeton Applied Research (PAR) Potentiostat/Galvanostat model 263A

3.3.2 Pretreatment of Screen-printed Carbon Electrodes (SPCEs)

Prior to electrodeposition, the working and counter electrodes surface were softly polished with a silicon carbon paper P4000 disc (Buehler) and the electrode system was

washed with deionized water. The working electrode surface was activated by running 20 cyclic voltammetric scans between +2.0 and -2.0 V at a scan rate of 100 mVs^{-1} in a 0.1 M KCl solution followed by cleaning with deionized water.[15,35,36]

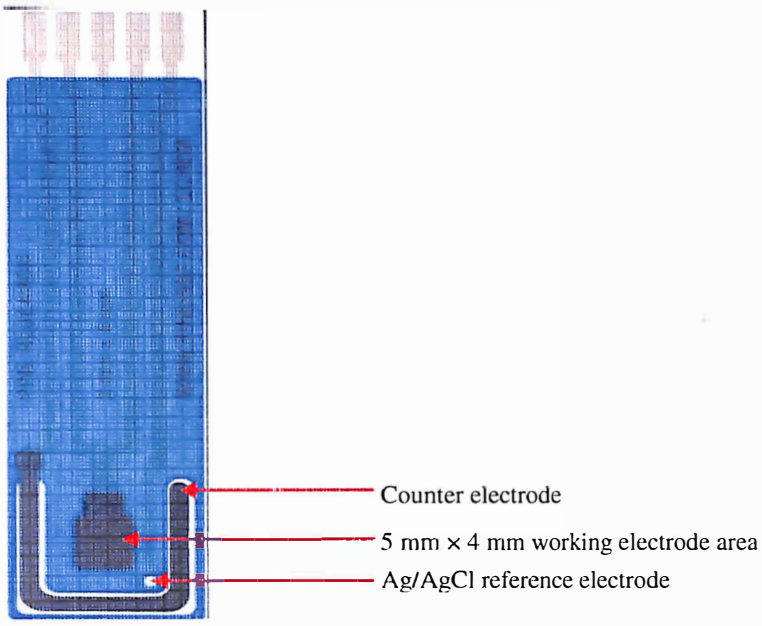

Figure 3.2 screen printed carbon electrodes (SPCE)

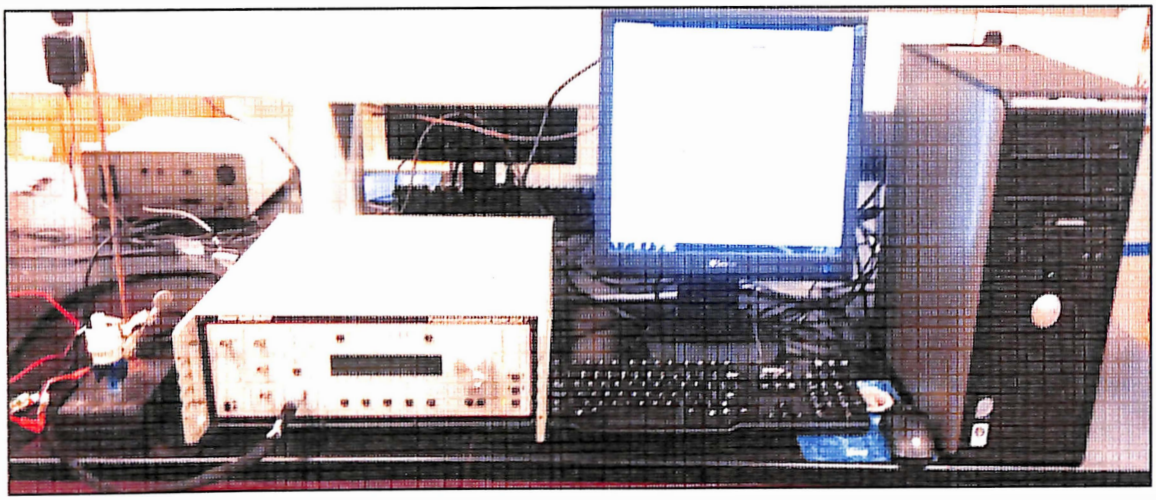

Figure 3.3 Instrumental set-up for electrochemical measurements.

3.3.3 Preparation of gold nanoparticle-modified SPCEs

The gold nanoparticle electrodeposition procedure was similar to that described in previous works.[20,35-40] The electrodeposition of gold nanoparticles on the working electrode of SPCEs was performed by applying a potential of +0.18 V for 15 s from a stirred solution of a 0.1 mM gold (III) chloride trihydrate ($HAuCl_4$. $3H_2O$) in 0.5 M sulfuric acid.

3.3.4 Surface Studies

To investigate the structural properties of the electrodeposited films on the SPCEs electrodes, a JEOL scanning electron microscope (SEM, JSM-6490LV) was used. For elemental analyses, an energy-dispersive X – ray spectrometer (EDS) installed in the SEM was used. For the analyses, gold was deposited for the samples which do not contain gold using a Denton vacuum desk IV sputter, and carbon was deposited for Au containing samples using a carbon coating system (DV-401, Denton vacuum).

3.3.5 Immobilization of Acetylcholinesterase enzyme (AchE) on gold nanoparticle-modified screen-printed carbon electrode (AuNPs/SPCEs)

The acetylcholinesterase was covalently immobilized on the gold nanoparticle-modified SPCEs working electrode surface by adapting the immobilization procedure described in previous works .[15,41-43] 5 μL of a 0.05 M N-cyclohexyl-N'-[2-(N-methylmorpholino)ethyl] carbodiimid-4-toluensulfonate solution prepared in Britton-Robinson (pH = 7) was applied on the working electrode surface and kept at room temperature (25°C) for 80 mins followed by the addition of 5 μL of Britton-Robinson

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buffer (pH = 7) solution containing 2.5 mg/mL of AchE on the surface of the working electrode. The SPCE electrode was then kept at 30°C for 2 hrs for immobilization to be completed, and then rinsed with the buffer solution prior to use for the electrochemical measurements.

3.3.6 Determination of As^{3+}

To determine As^{3+}, chronoamperometric studies were conducted on both the AchE immobilized SPCE (AchE/SPCE) and the gold modified AchE immobilized SPCE (AuNPs/AchE/SPCE) electrodes using similar experimental conditions reported by Méndez and coworkers.[15] A potential of +0.6 V *vs.* Ag/AgCl reference electrode was used. The chronoamperometric study was first carried out in a buffer solution of pH = 7 in the absence of acetylthiocholine iodide ATI and As^{3+}. This is followed by chronoamperometric measurements for 3.64 × 10-4 M ATI and the various concentrations of As^{3+} (1) 1.90×10^{-8} M, (2) 5.60×10^{-8} M, (3) 7.40×10^{-8} M, (4) 9.00×10^{-8} M, (5) 1.07×10^{-7} M, (6) 1.22×10^{-7} M, (7) 1.37×10^{-7} M, and (8) 1.52×10^{-7} M.

3.3.7 Precision studies

Precision studies for the results of both AchE/SPCE and AuNPs/AchE/SPCE electrodes were conducted using sets of chronoamperometric measurements on the same electrode surface and four different electrode surfaces prepared in the same manner using As^{3+} concentrations in the range of 1.9×10^{-8} M to 1.52×10^{-7} M.

3.3.8 Interference studies

Some ions were investigated for possible interference in ground water. These include sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), lead (Pb^{2+}), mercury (Hg^{2+}), cadmium (Cd^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}) . The interference study was carried out by conducting chronoamperometric studies on the solutions of the various ions in the presence of As^{3+} by applying a potential of 0.6 V *vs.* Ag/AgCl reference electrode. The solutions of all ions were prepared in Britton-Robinson buffer of pH = 7 and acetylthiocholine iodide ATI concentration of 3.64×10^{-4} M.

3.3.9 Shelf Life Study

The stability of the gold nanoparticles modified enzyme immobilized electrode AuNPs/AchE/SPCE was determined using the duration of shelf life of the electrode under storage condition in the buffer solution of pH = 7 at a temperature of 4 ^{o}C.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Cyclic voltammetric scan pretreatment of SPCE electrodes

The screen-printed carbon electrodes SPCEs were activated by running 20 cyclic voltammetric (CV) scans in 0.1 M KCl between 2.0 and -2.0 V *vs.* Ag/AgCl reference electrode. In the first CV scan, the redox peaks appearing in the voltammogram of 0.1 M KCl were not well defined, Figure 4.1a. The magnitude of the current of these peaks, however, increased in the consecutive multiple CV scans indicating that the activation of the electrode, Figure 4.1b

4.2 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) Study

The SEM micrographs of the working electrode of the SPCEs before and after electrodeposition of the gold are shown Figures 4.2 (a) and (b), respectively. The electrodeposition of gold nanoparticles on the working electrode of SPCEs was performed by applying a potential of +0.18 V for 15 s from a stirred solution of a 0.1 mM gold (III) chloride trihydrate ($HAuCl_4$. $3H_2O$) in 0.5 M sulfuric acid. The EDS spectrum of the electrodeposited film is shown in Figure 4.2 (c). From the EDS spectrum, Figure 4(c), it can be seen that the deposit consists of Au, Cl, Pd, and Rh. Cl peak in the EDS is most probably due to the electrodeposited side product of $HAuCl_4$. $3H_2O$ entrained in the

film, and the Pd and Rh peaks due to impurities present in $HAuCl_4 . 3H_2O$.

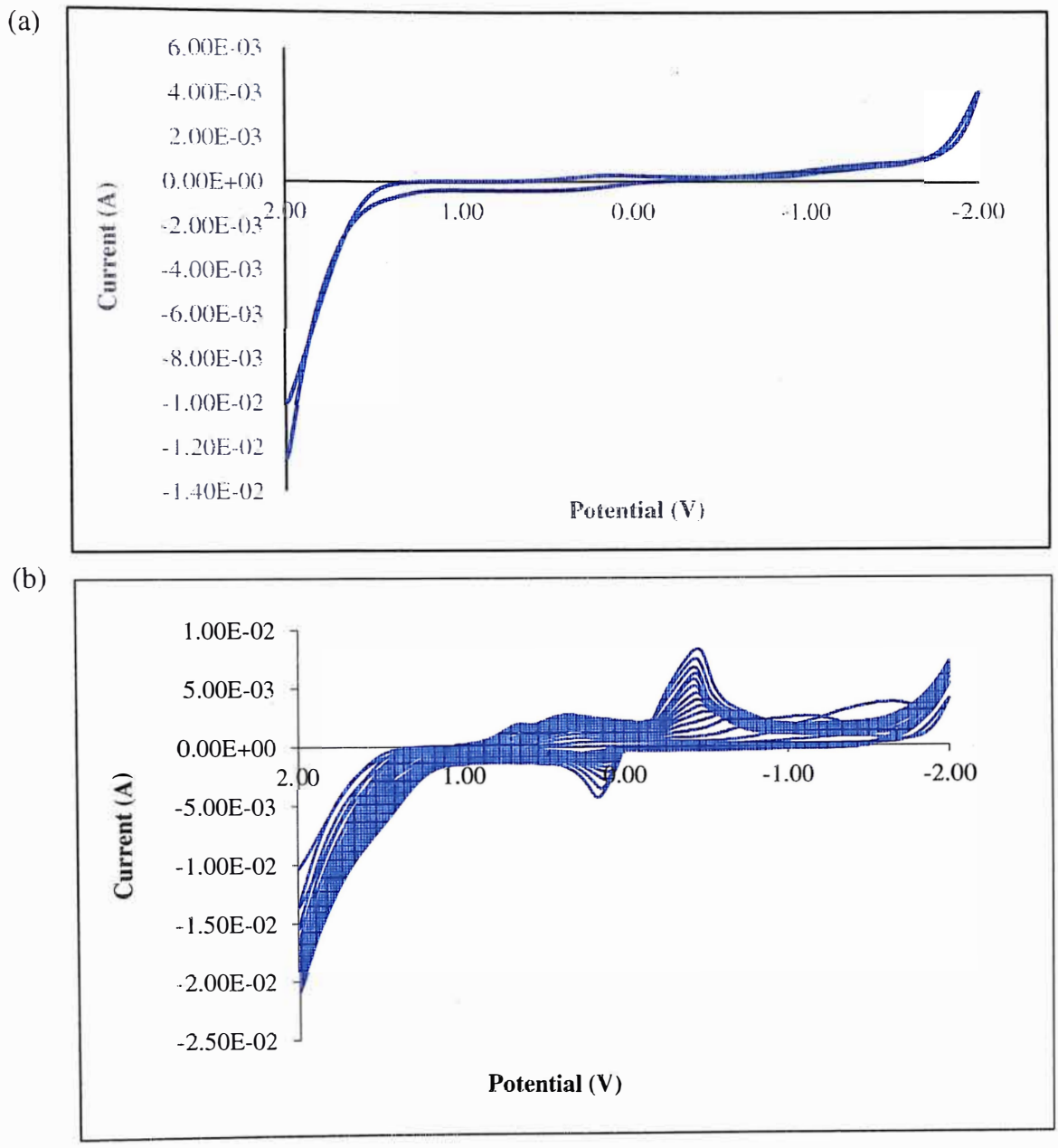
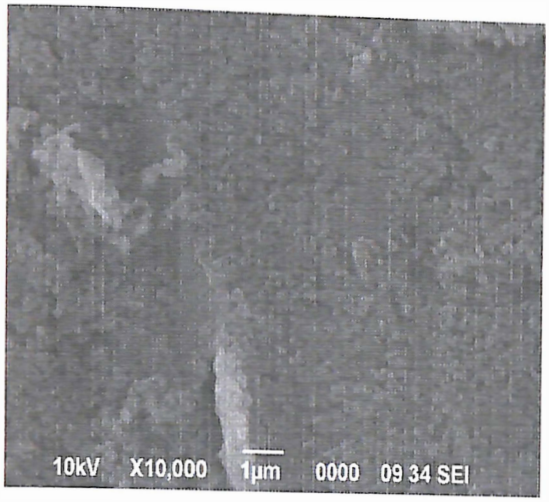
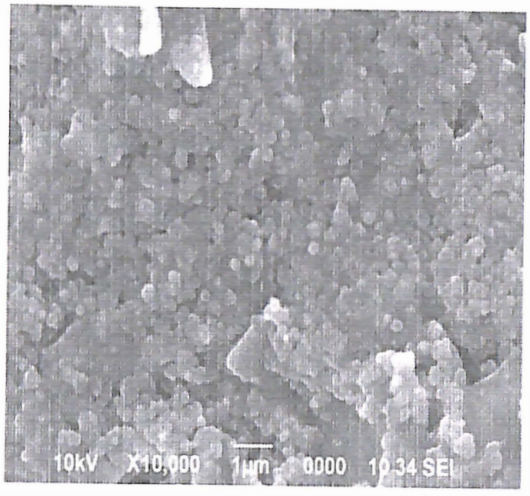


Figure 4.1 Cyclic voltammograms during activation of the SPCE in 0.1 M KCl: (a) for the first scan and (b) for multiple scans. Potential were recorded *vs.* Ag/AgCl reference electrode.

(a)



(b)



(c)

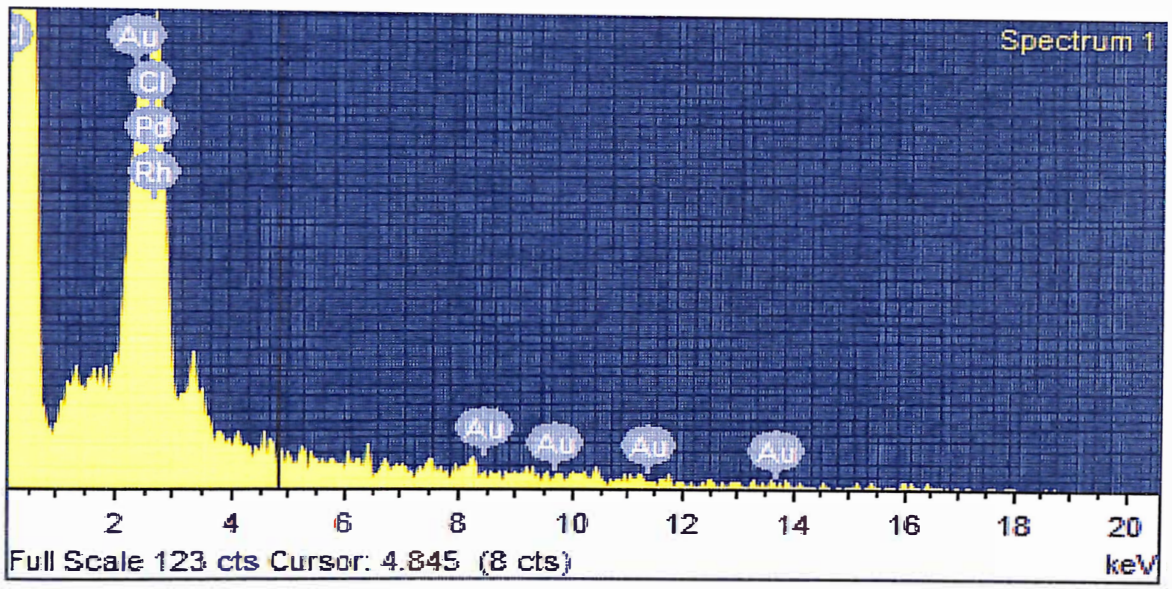


Figure 4.2 (a) SEM micrograph of the working electrode bare SPCE. **(b)** SEM micrograph of the deposited film from a stirred solution of 0.1 mM gold (III) chloride trihydrate ($HAuCl_4.\ 3H_2O$) in 0.5 M sulfuric acid by applying a potential of +0.18 V *vs.* Ag/AgCl for 15 s. (c) The EDS spectrum of the deposit in (b).

4.3 Chronoamperometric Results

Both the AchE/ SPCE and AuNPs/AchE/SPCE electrodes produce chronoamperometric oxidation current for acetylthiocholine iodide (ATI) as shown in Figures 4.3a and 4.3b, respectively. Figures 4.4 and 4.6 exhibit that for both AchE/ SPCE and AuNPs/AchE/SPCE electrodes, ATI solution initially resulted in a large oxidation current with a sharp magnitude decrease in about 3 s. Addition of As^{3+} to the solution of ATI resulted in a decrease of the steady-state oxidation current due to its inhibition of the ATI oxidation, Figures 4.3a and b, following the mechanism depicted in Figure 2.8. Similar trend was observed by Méndez and coworkers.[15]

As shown in Figures 4.3a and b, the chronoamperometric oxidation current produced by the AuNPs/AchE/SPCE was found to be higher than that of AchE/SPCE electrode. This is due to the electrocatalytic properties of gold[44] and larger surface area from the modification of the working electrode surface through the electrochemical deposition of gold nanoparticles.

The chronoamperometric results of the various concentrations of As^{3+} for AchE/SPCE electrode are shown in Figure 4.4. The corresponding value of the steady-state oxidation current obtained with the AchE/SPCE for ATI alone in the absence of As^{3+} (I_0), for the various As^{3+} concentrations (I), and $\Delta I = I_0 - I$ are shown in Table 4.1. The calibration plot of $\Delta I = I_0 - I$ against the concentration of As^{3+} from Table 4.1 shows a linear dependency with a correlation coefficient, $R^2 = 0.9971$, Figure 4.5. This is similar to the results of Méndez and coworkers.[15]

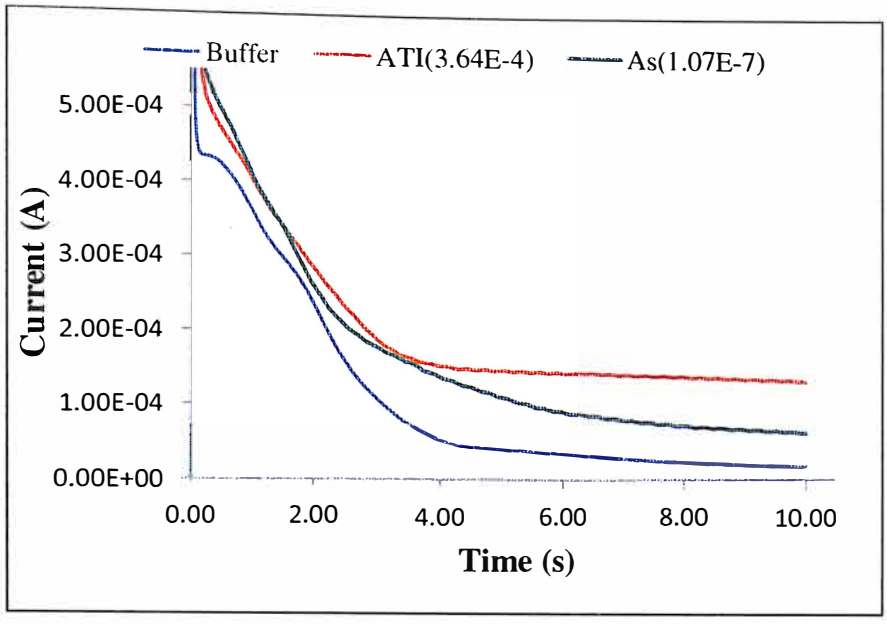


Figure 4.3 (a) Chronoamperogram obtained with AchE/SPCE electrodes at an applied potential of +0.6 V *vs.* Ag/AgCl reference electrode for Britton-Robinson buffer pH = 7, 3.64×10^{-4} M ATI, and 1.07×10^{-7} M As^{3+}

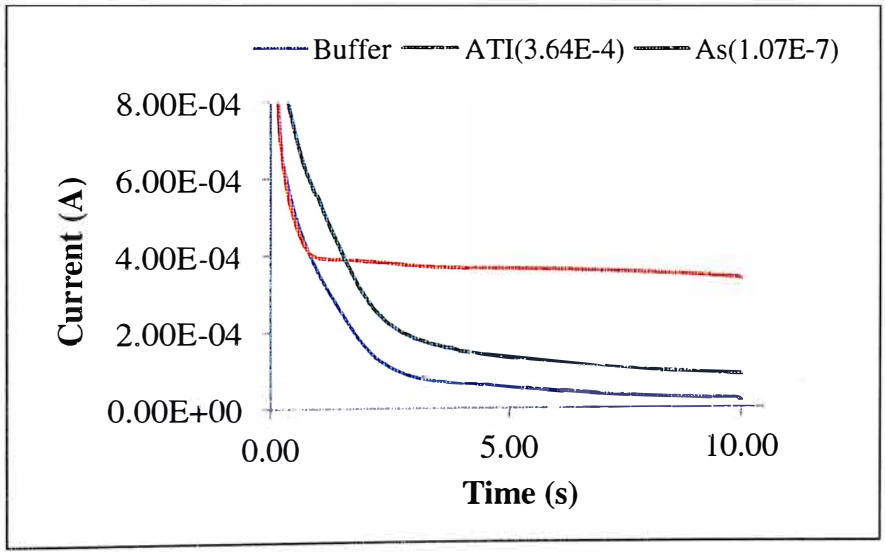


Figure 4.3 (b) Chronoamperogram obtained with AuNPs/AchE/SPCE electrodes at an applied potential of +0.6 V *vs.* Ag/AgCl reference electrode for Britton-Robinson buffer pH = 7, 3.64×10^{-4} M ATI, and 1.07×10^{-7} M As^{3+}

The chronoamperometric results of the AuNPs/AchE/SPCE electrodes are shown in Figure 4.6. Table 4.2 shows the corresponding values of steady-state oxidation currents obtained with the AuNPs/AchE/SPCE electrode for ATI alone in the absence of As^{3+} (I_0) and for the various As^{3+} concentrations (I), $\Delta I = I_0 - I$. The calibration plot of $\Delta I = I_0 - I$ against the concentration of As^{3+} shows a linear dependency with a correlation coefficient ($R^2 = 0.9942$), Figure 4.7.

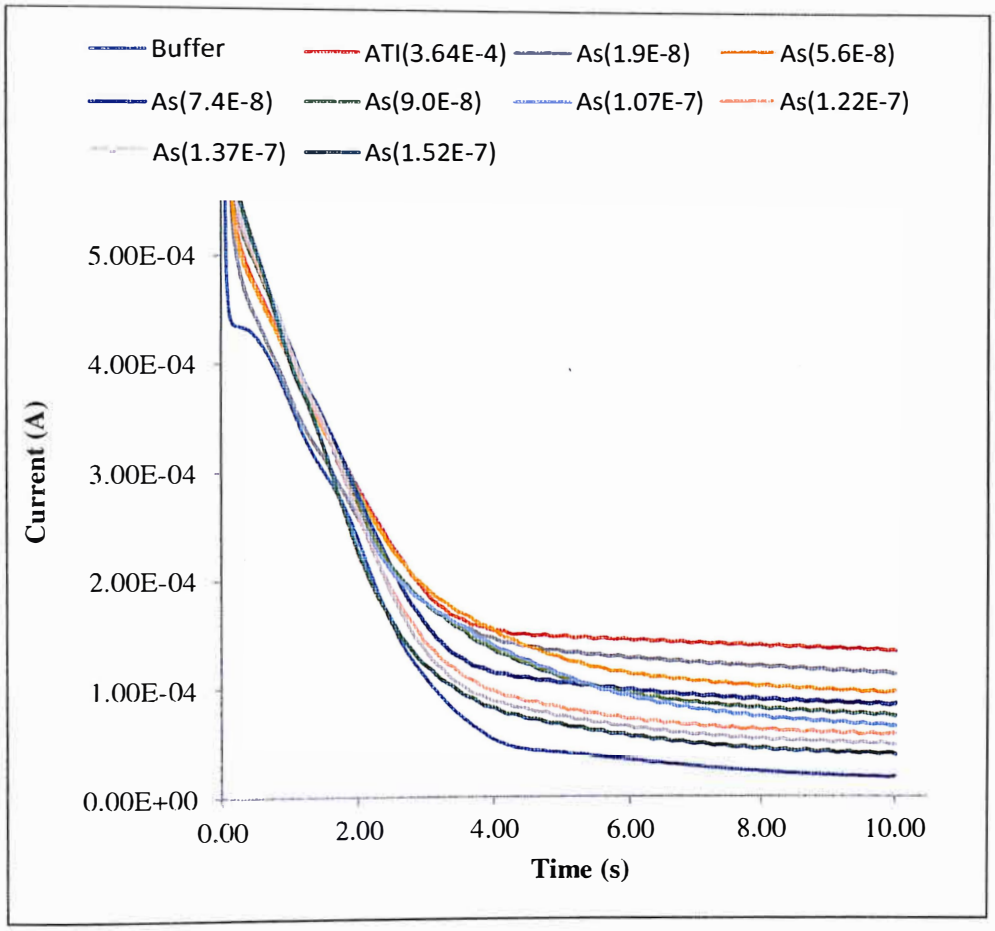


Figure 4.4 Chronoamperograms obtained with AchE/SPCE electrodes at an applied potential of +0.6 V *vs.* Ag/AgCl reference electrode for Britton-Robinson buffer pH = 7, 3.64×10^{-4} M ATI, and various molar concentrations of As^{3+} shown in parenthesis.

Table 4.1 The value of the steady-state oxidation current for 3.64×10^{-4} M ATI solution (I_0), various As^{3+} molar concentrations (I), and the corresponding $\Delta I = I_0 - I$ values using AchE/SPCE.

Sample	Conc. (M)	I (A)	$\Delta I = I_0 - I$ (A)
ATI	3.64×10^{-4}	1.34×10^{-4} (I_0)	
As^{3+}	1.90×10^{-8}	1.14×10^{-4}	2.00×10^{-5}
As^{3+}	5.60×10^{-8}	9.68×10^{-5}	3.72×10^{-5}
As^{3+}	7.40×10^{-8}	8.63×10^{-5}	4.77×10^{-5}
As^{3+}	9.00×10^{-8}	7.48×10^{-5}	5.92×10^{-5}
As^{3+}	1.07×10^{-7}	6.53×10^{-5}	6.87×10^{-5}
As^{3+}	1.22×10^{-7}	5.73×10^{-5}	7.67×10^{-5}
As^{3+}	1.37×10^{-7}	4.74×10^{-5}	8.66×10^{-5}
As^{3+}	1.52×10^{-7}	3.88×10^{-5}	9.52×10^{-5}

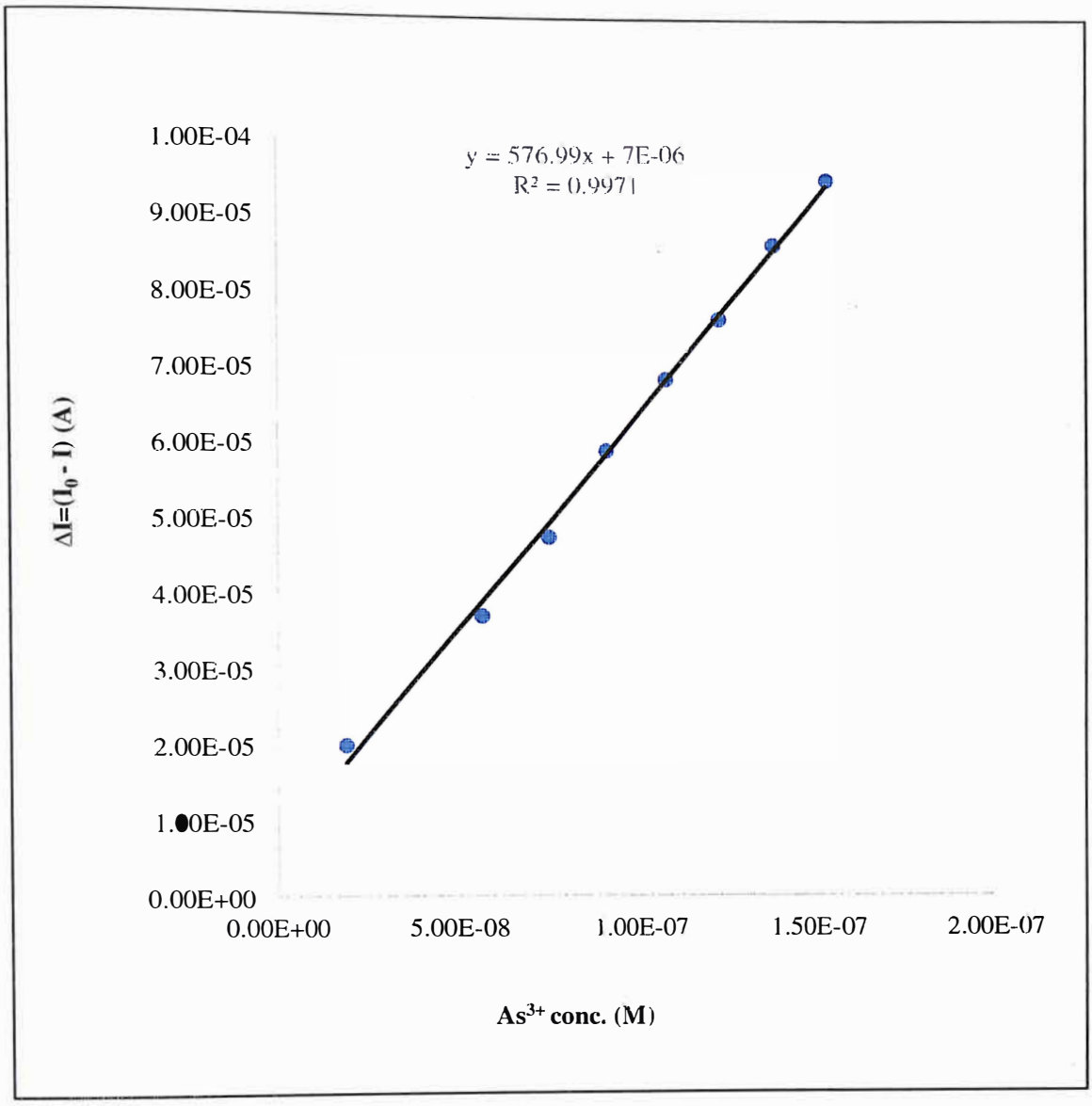


Figure 4.5 Calibration plot of ΔI *vs.* As^{3+} molar concentrations. Potential applied = +0.6 V *vs.* Ag/AgCl reference electrode using AchE/SPCE electrode in Britton-Robinson buffer pH = 7 and ATI concentration of 3.64×10^{-4} M

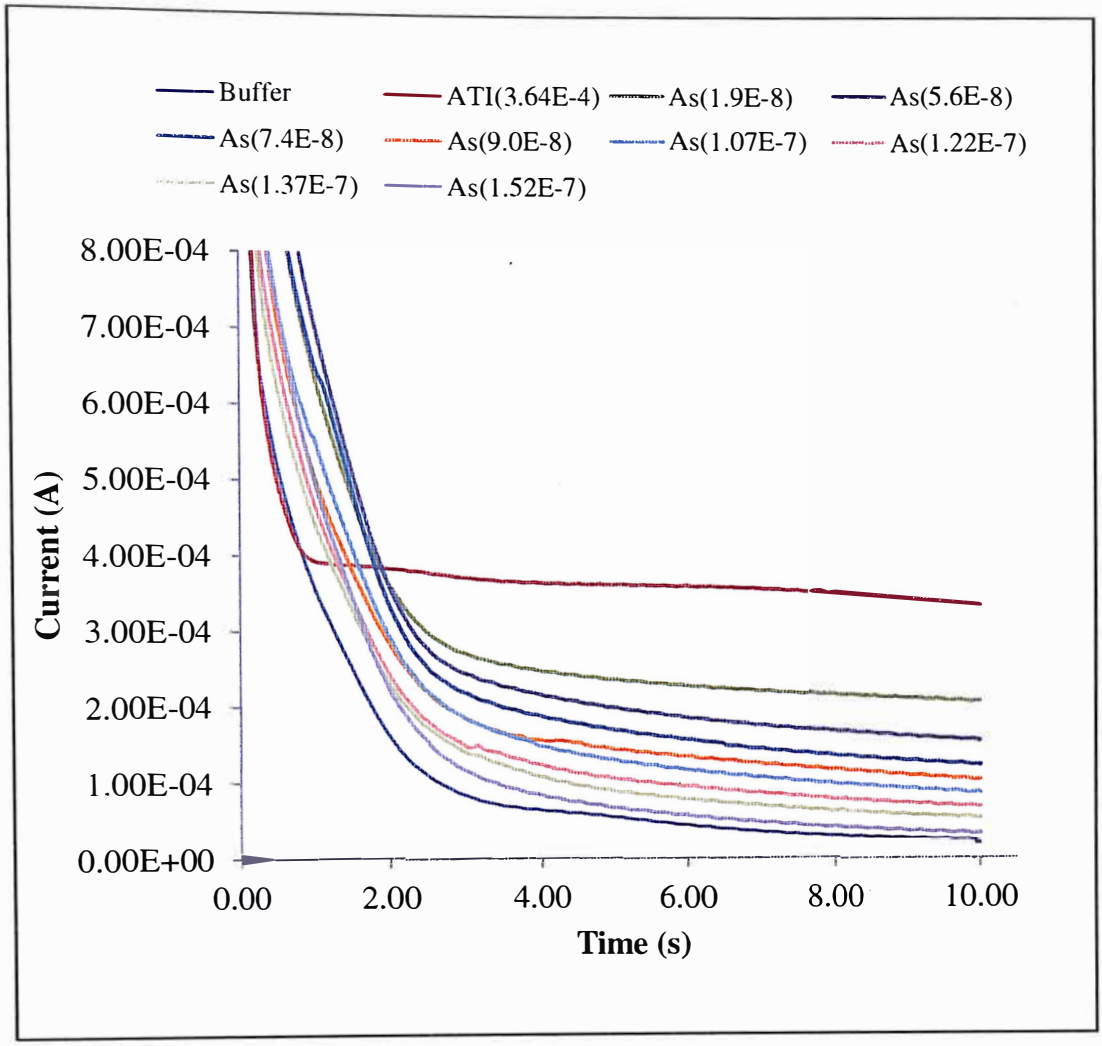


Figure 4.6 Chronoamperogram obtained with AuNPs/AchE/SPCE electrode at an applied potential of +0.6 V *vs.* Ag/AgCl reference electrode for Britton-Robinson buffer pH = 7, 3.64×10^{-4} M ATI, and various molar concentrations of As^{3+} shown in parenthesis.

Table 4.2 The value of the steady-state oxidation current for 3.64×10^{-4} M ATI solution (I_0), various As^{3+} molar concentrations (I), and the corresponding $\Delta I = I_0 - I$ values using AuNPs/AchE/SPCE.

Sample	Conc. (M)	I (A)	$\Delta I = I_0 - I$ (A)
ATI	3.64×10^{-4}	3.37×10^{-4} (I_0)	
As^{3+}	1.90×10^{-8}	2.08×10^{-4}	1.29×10^{-4}
As^{3+}	5.60×10^{-8}	1.56×10^{-4}	1.81×10^{-4}
As^{3+}	7.40×10^{-8}	1.25×10^{-4}	2.12×10^{-4}
As^{3+}	9.00×10^{-8}	1.06×10^{-4}	2.31×10^{-4}
As^{3+}	1.07×10^{-7}	8.73×10^{-5}	2.50×10^{-4}
As^{3+}	1.22×10^{-7}	6.94×10^{-5}	2.68×10^{-4}
As^{3+}	1.37×10^{-7}	5.41×10^{-5}	2.83×10^{-4}
As^{3+}	1.52×10^{-7}	3.35×10^{-5}	3.04×10^{-4}

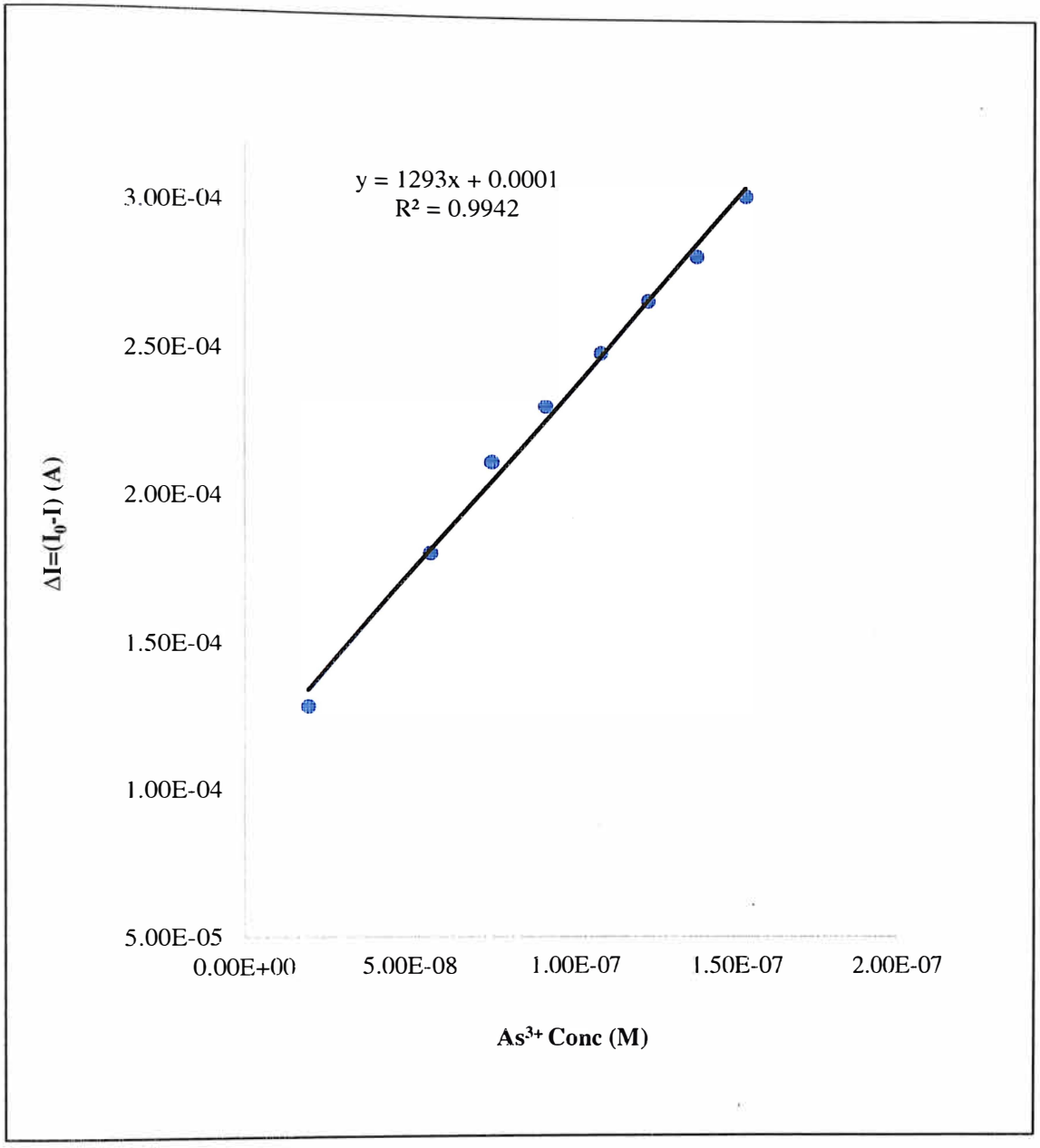


Figure 4.7 Calibration plot of ΔI *vs.* As^{3+} molar concentrations. Potential applied = +0.6 V *vs.* Ag/AgCl reference electrode using AuNPs/AchE/SPCE electrode in Britton-Robinson buffer pH = 7 and ATI concentration of 3.64×10^{-4} M

4.4 Sensitivity

The calibration plots of ΔI *vs.* As^{3+} concentration in Figures 4.5 and 4.7 shows that AuNPs/AchE/SPCE electrode has a higher sensitivity with a slope value of (1293.0 A/M) than that of the AchE/SPCE electrode with a slope value of (577.0 A/M).

4.5 Limit of Detection and Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the AchE/SPCE and the AuNPs/AchE/SPCE electrodes were determined using six sets of concentrations for 1.90×10^{-8} M As^{3+}. The LOD and LOQ were determined using the standard deviation (δ) of the steady state oxidation current and the slopes (S) obtained from Figures 4.5 and 4.7 using the equations 11(a) and 11(b)[45]

$$LOD = \frac{3\delta}{S} \qquad \text{Eq. 11a}$$

$$LOQ = \frac{10\delta}{S} \qquad \text{Eq. 11b}$$

The results shown in Table 4.3 exhibit that the AuNPs/AchE/SPCE has lower limit of detection and lower limit of quantification than the AchE/SPCE.

Table 4.3 Limit of Detection and Limit of Quantitation of As^{3+} for AchE/SPCE and AuNPs/AchE/SPCE electrodes

Electrodes	LOD	LOQ
AchE/SPCE	1.91×10^{-7} M (14.3 ppb)	6.27×10^{-7} M (47.0 ppb)
AuNPs/AchE/SPCE	1.52×10^{-8} M (1.14 ppb)	5.06×10^{-8} M (3.80ppb)

4.6 Precision Studies using Different Sets of Concentrations for AuNPs/AchE/SPCE and AchE/SPCE Electrodes.

For both AuNPs/AchE/SPCE and AchE/SPCE electrodes, precision studies were conducted using six sets of concentrations of 1.90×10^{-8} M, 9.00×10^{-8} M, and 1.52×10^{-7} M As^{3+} solution. The % relative standard deviation (RSD) showed in Table 4.4 exhibit that for all the As^{3+} concentrations, AuNPs/AchE/SPCE has a better precision than AchE/SPCE electrode. The results shown in Table 4.4 also illustrates that the precision of the AuNPs/AchE/SPCE measured are good, RSD ≤ 10%

Table 4.4 RSD values of precision using different concentrations of As^{3+} on same AuNPs/AchE/SPCE and AchE/SPCE electrode surface.

Conc. (M)	AuNPs/AchE/SPCE RSD (%)	AchE/SPCE RSD (%)
1.90×10^{-8}	10.4	27.1
9.00×10^{-8}	5.20	13.5
1.52×10^{-7}	2.60	3.40

4.7 Precision studies using different sets of AuNPs/AchE/SPCEs Electrodes

Precision studies were performed using four different sets of AuNPs/AchE/SPCEs electrode surfaces made using the same way. The studies were conducted for As^{3+} concentration range between 1.90×10^{-8} M to 1.52×10^{-7} M using the % relative standard

deviation % RSD of the slope of the calibration plots of $\Delta I = I_0 - I$ against the concentration of As(III) shown in Figures 4.8 (a – d). I_0 and I are the steady-state oxidation currents for 3.64×10^{-4} M ATI and the various As^{3+} concentrations, respectively.

The % RSD of the results calculated using the standard deviation and the mean of the slopes of the calibration plots shown in Table 4.5 illustrates that the precision of the measurements, % RSD of 8.99 is good.

Table 4.5 Parameters of the calibration plots of the electrodes and RSD

Electrode	Slope (A/M)	R^2	% RSD
1	1947.4	0.9928	8.99
2	1907.2	0.9866	
3	1652.1	0.9953	
4	1648.4	0.9918	

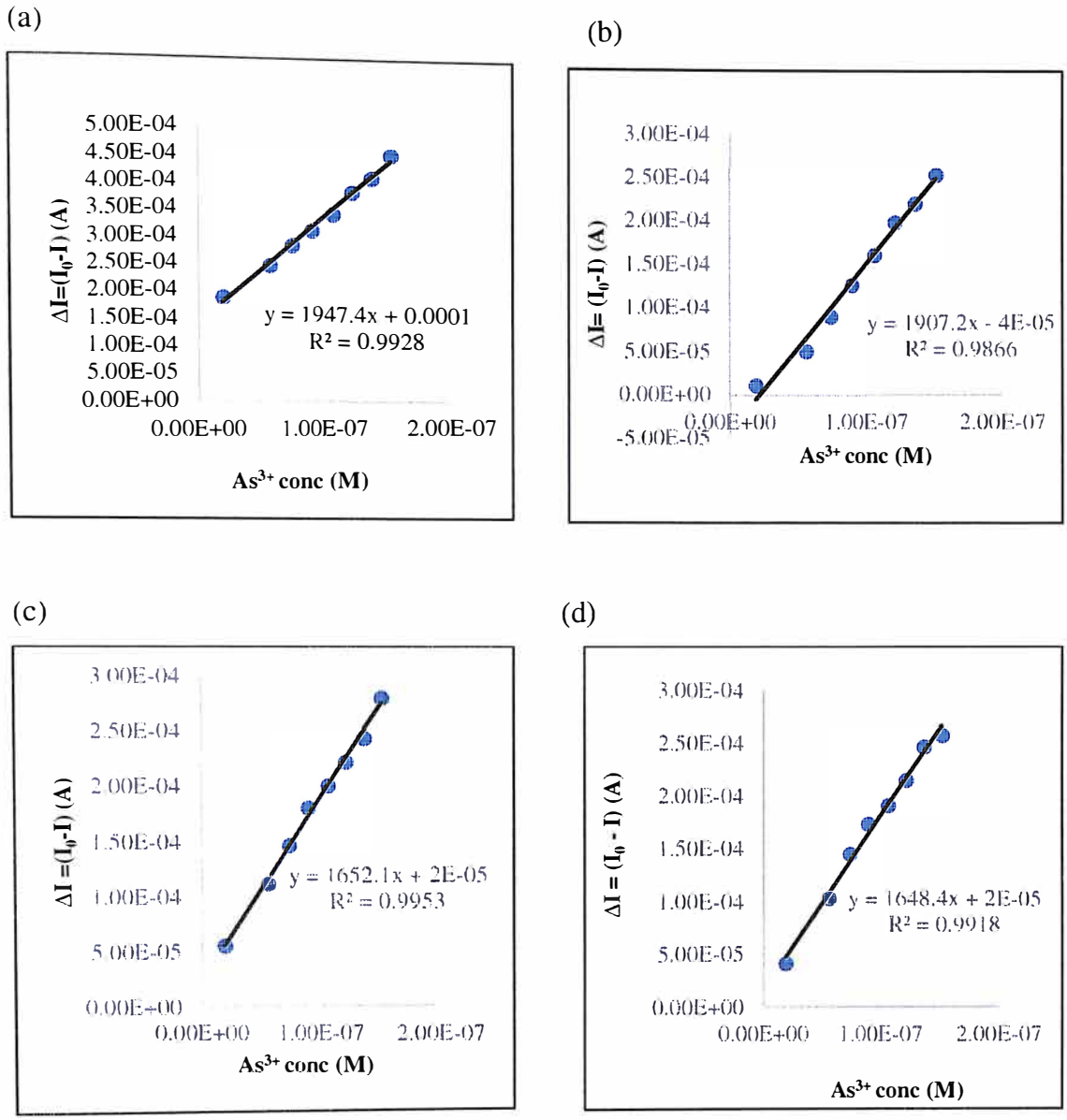


Figure 4.8 (a) – (d) Calibration plots of ΔI *vs.* As^{3+} molar concentrations for the four sets of AuNPs/AchE/SPCEs. Potential applied = +0.6 V *vs* Ag/AgCl reference electrode in Britton-Robinson buffer pH = 7 and ATI concentration of 3.64×10^{-4} M

4.8 Interference Studies

The interference studies were conducted on metal ions like sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), lead (Pb^{2+}) , mercury (Hg^{2+}), cadmium (Cd^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}). Three different concentration levels of the metal ions (9.00×10^{-8} M, 9.00×10^{-7} M, and 9.00×10^{-6} M) each containing 9.00×10^{-8} M of As^{3+} were used. All chronoamperometric studies were carried in 3.64×10^{-4} M acetylthiocholine iodide ATI and Britton-Robinson pH = 7 solution with an applied potential of +0.6 V *vs* Ag/AgCl reference electrode.

The interference effects of the ions were determined using the difference between the steady-state oxidation current (I_0) of the ATI in the presence of As^{3+}, and the steady-state oxidation current (I) in the presence of the metal ions.

The results obtained, Figure 4.9, exhibit that potassium ion has the largest and zinc ion has the lowest interference effect.

4.9 The Shelf Life of AuNPs/AchE/SPCE Electrode

To study the stability or the shelf life of AuNPs/AchE/SPCE electrode, studies were conducted for 7 days by recording chronoamperograms of 3.64×10^{-4} M acetylthiocholine iodide ATI on a daily basis. The AuNPs/AchE/SPCE electrode was stored at 4 °C in Britton-Robinson buffer solution of pH = 7 between each measurement.

The electrode stability for each day is expressed using % activity (A), which is the ratio of the chronoamperometric steady-state oxidation current of each day to the first

day. The results obtained, Figure 4.10, illustrates a decrease in stability of the electrode reaching 50% decline at the 5th day. Similar studies made by Méndez and coworkers[3] showed that a 50% stability decrease of AchE/SPCE electrode occurred in 15 day indicating that AchE/SPCE has a better shelf life than AuNPs/AchE/SPCE electrode.

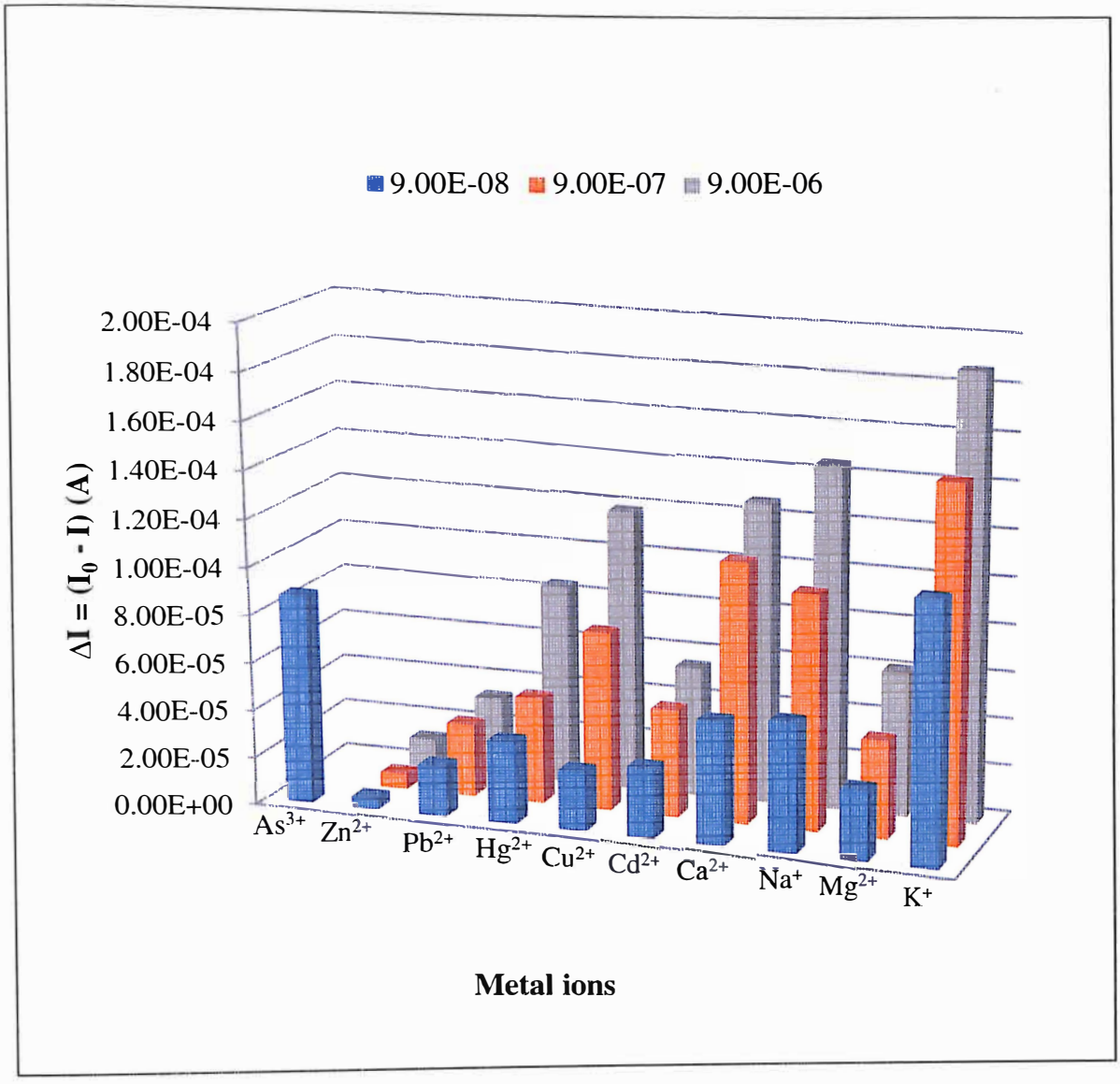


Figure 4.9 Inhibition current of metals ions on AuNPs/AchE/SPCE electrode in presence of As^{3+} solution at 9.00×10^{-8} M.

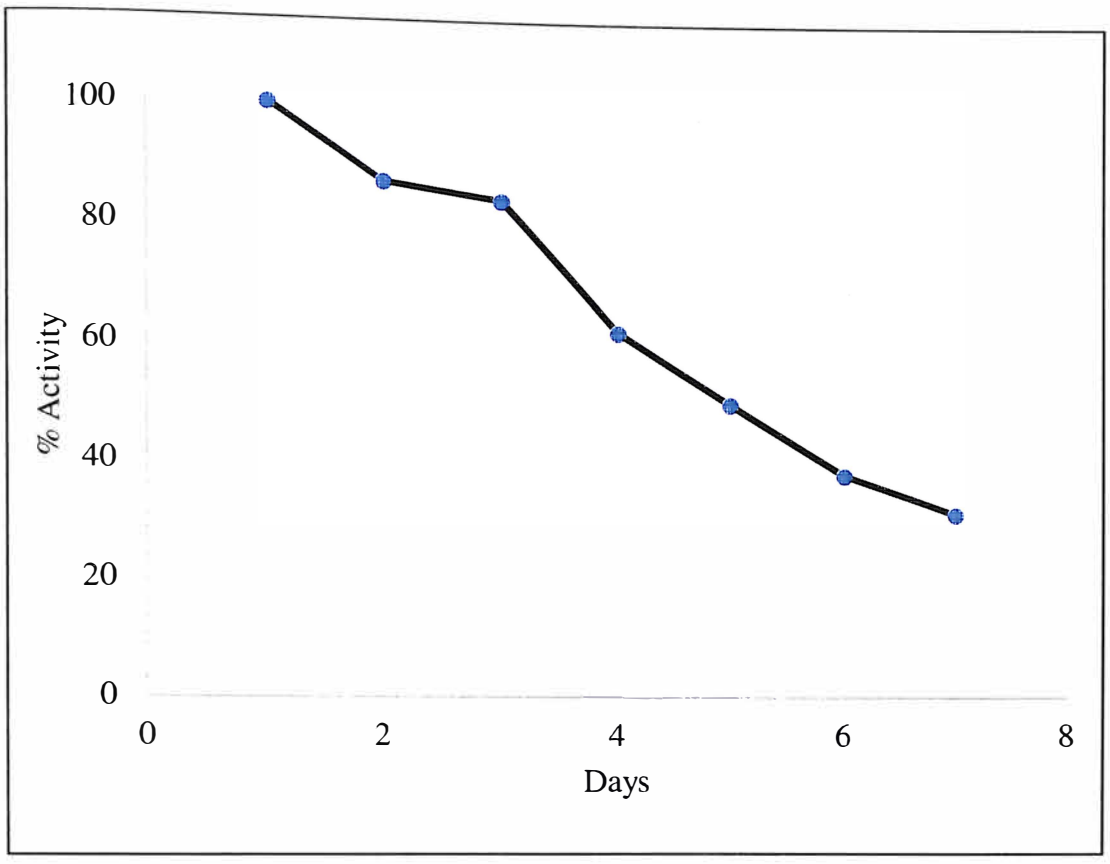


Figure 4.10 % Activity of AuNPs/AchE/SPCE electrode as a function of days showing its shelf life.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The gold nanoparticles-modified screen-printed carbon electrode immobilized with acetylcholinesterase AchE enzyme AuNPs/AchE/SPCE gives a chronoamperometric oxidation current for As^{3+} that can be utilized to determine As^{3+} in ground water with good precision both in terms of As^{3+} concentrations and the electrode surfaces, and with limit of detection about 1 ppb.

Compared with the screen-printed carbon electrode immobilized with acetylcholinesterase enzyme with no gold nanoparticles, AchE/SPCE, the AuNPs/AchE/SPCE electrode gives results with better sensitivity, limit of detection, LOD, limit of quantitation, LOQ, and precision. This attributed to the electrocatalytic properties of the deposited gold and an increase in the surface area of the working electrode.

Ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} at low concentration ranges of 9.00×10^{-6} M, 9.00×10^{-7} M, and 9.00×10^{-8} M exhibit interference effects. The shelf life of the AuNPs/AchE/SPCE electrode decreases by 50% in less than 5 days. However, this cannot be considered as a limitation to the use of the electrode, taking into account that the SPCE electrodes are cheap and disposable.

5.2 Recommendations for future work

Further research works are recommended in the following areas:

(1) Improving the selectivity of the AuNPs/AchE/SPCE electrode by reducing interference of other ions. It is necessary to study the impact of potential chemical and environmental interferences on the sensing performance of the new chemistries. Matrix interference studies representing the range of conditions under which the electrode is expected to function in the field will be established and tested.

(2) Robustness: Improving the shelf life of the AuNPs/AchE/SPCE electrode by studying the effectiveness at different storage temperatures.

(3) Conducting Field test: This will help to develop quality control for sampling, enzyme immobilization, and in field assay procedures. Test will be conducted account for the broad range and complexity of environmental matrixes, the variety of possible co-contaminants, and the wide dynamic range of pollutant concentrations.

(4) Developing a multi-analyte electrodes: Expand the work to develop multiple electrodes that detect a wide range of common and emerging water contaminants

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- Working knowledge of instruments: FTIR, AAS, X-Ray, TGA, and BET
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- Tutored students in college preparatory classes

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