Disposable and low-cost free chlorine sensor based on pencil drawn paper electrode

by

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A thesis submitted in partial fulfillment of the requirements for the degree of
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Declaration

This is to certify that the thesis work entitled "Disposable and low-cost free chlorine sensor based on pencil drawn paper electrode" has been carried out by Apu Kumar Dutta in the Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh. The above thesis work has not been submitted anywhere for the award of any degree or diploma.

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Abstract

This is the first alternative analysis of pencil drawn paper electrode for free available chlorine (FAC) detection. It is one of the new technologies for fabricating simple, low lost, portable and disposable analytical device for many application areas including clinical diagnosis, food quality control and environmental monitoring. In this work a complete analysis of physical and electrochemical properties of pencil graphite electrode (PGE) and pencil drawn paper electrode (PDPE) has been performed to detect FAC in surface water. Cyclic voltammetry (CV) and amperometry were used to study the electro-catalytic properties. Excellent catalytic activity has been obtained for free chlorine detection using both PGE and PDPE with a sensitivity of 121 ± 5.38 $\mu AmM^{-1}cm^{-2}$ and 36 ± 3.65 $\mu AmM^{-1}cm^{-2}$ respectively and a linearity range up to 12 mM and 7 mM for both platforms respectively. Also limit of detection PGE and PDPE has been found to be 59.5 and 88.9 μM respectively. There is no response obtained for common interfering ions in surface water such as NO_3^-, SO_4^{2-}, CO_3^{2-}, Cl^- and HCO_3^-. Both the sensors were evaluated for their storage stability and response time and thereby can be applied as a low-cost electrochemical sensor for determining FAC in surface water. The unique properties of this sensor allow the passive liquid transport and compatibility with chemical/biochemical are the main advantage of using this sensing platform. In addition, this sensor could better meet the current objectives of a viable low cost and portable device in addition to offering high sensitivity, selectivity and multiple analyte discrimination.

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INTRODUCTION

CHAPTER I

Introduction

1.1 General

Electrochemistry is the branch of chemistry concerned with the interrelation of electrical and chemical effects. A large part of this field deals with the study of chemical changes caused by the passage of an electric current and the production of electrical energy by chemical reactions. In fact, the field of electrochemistry encompasses a huge array of different phenomena (e.g., electrophoresis and corrosion), devices (electro chromic displays, electro analytical sensors, batteries, and fuel cells), and technologies (the electroplating of metals and the large-scale production of aluminum and chlorine). While the basic principles of electrochemistry discussed in this text apply to all of these, the main emphasis here is on the application of electrochemical methods to the study of chemical systems.

In this chapter, an electrochemical sensor the electrochemical sensor plays an essential analytical role in the fields of environmental conservation and monitoring, disaster and disease prevention, and industrial analysis. A typical chemical sensor is a device that transforms chemical information in a selective and reversible way, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. A huge research effort has taken place over the several years to achieve electrochemical sensors with attractive qualities including rapid response, low cost, miniaturisable, superior sensitivity and selectivity, and appropriate detection limits. In the highly diverse field of chemical (and biochemical) sensing, the sensor is governed by both the aspect of the environment it is measuring and the matrix in which it is in. As well as sensors that use electrochemistry as the type of energy transfer that they detect, optical [1], thermal [2] and mass-based [3] sensors are also well-developed. From an analytical perspective, electrochemistry is appealing as it directly converts chemical information into an electrical signal with remarkable detectability, experimental simplicity and low cost. There is no need for sophisticated instrumentation, e.g., optics. A very attractive feature of electrochemistry is that it depends on a surface phenomenon, not an optical path length, and thus sample volumes can be very small, lending itself to miniaturization. The interest in electrochemical sensors continues unabated today, stimulated by the wide range of potential applications. Their impact is most clearly illustrated in the widespread use of electrochemical sensors seen in daily life, where they continue to meet the expanding need for rapid, simple and economic methods of determination of numerous analytes [4-6].

Chlorine is widely used as a disinfectant in the water treatment industry for inactivation of pathogenic micro-organisms such as *Cryptosporidium* [7, 8] and *Escherichia coli* [9]. Chlorine in aqueous solutions is used for a number of purposes, such as disinfection and bleaching, and also as a powerful oxidizing agent in various manufacturing processes [10].

1.2 Basic concept of chlorine

Chlorine is a chemical element with symbol Cl and atomic number 17. The second-lightest of the halogens, it appears between fluorine and bromine in the periodic table and its properties are mostly intermediate between them. Chlorine is a yellow-green gas at room temperature. It is an extremely reactive element and a strong oxidizing agent, among the elements, it has the highest electron affinity and the third-highest electronegativity, behind only oxygen and fluorine.

The most common compound of chlorine, sodium chloride (common salt), has been known since ancient times. Around 1630, chlorine gas was first synthesized in a chemical reaction, but not recognized as a fundamentally important substance. Carl Wilhelm Scheele wrote a description of chlorine gas in 1774, supposing it to be an oxide of a new element. In 1809, chemists suggested that the gas might be a pure element, and this was confirmed by Sir Humphry Davy in 1810, who named it from Ancient Greek "pale green" based on its color.

Because of its great reactivity, all chlorine in the Earth's crust is in the form of ionic chloride compounds, which includes table salt. It is the second-most abundant halogen (after fluorine) and twenty-first most abundant chemical elements in Earth's crust. These crustal deposits are nevertheless dwarfed by the huge reserves of chloride in seawater. Elemental chlorine is commercially produced from brine by electrolysis. The high oxidizing potential of elemental chlorine led to the development of commercial bleaches and disinfectants, and a reagent for many processes in the chemical industry. Chlorine is used in the manufacture of a wide range of consumer products, about two-thirds of them organic chemicals such as polyvinyl chloride, and many intermediates for production of plastics and other end products which do not contain the element. As a common disinfectant, elemental chlorine and chlorine-generating compounds are used more directly in swimming pools to keep them clean and sanitary. Elemental chlorine at high concentrations is extremely dangerous and poisonous for all living organisms, and was used in World War I as the first gaseous chemical warfare agent.

In the form of chloride ions, chlorine is necessary to all known species of life. Other types of chlorine compounds are rare in living organisms, and artificially produced chlorinated organics range from inert to toxic. In the upper atmosphere, chlorine-containing organic molecules such as chlorofluorocarbons have been implicated in ozone depletion. Small quantities of elemental chlorine are generated by oxidation of chloride to hypochlorite in neutrophils as part of the immune response against bacteria.

In public water supplies chlorine used as disinfectant in many years ago. More than the allowed federal standard amount of (4 ppm) [11] free available chlorine (FAC) in drinking water causes strong respiratory and dermal damage to human. To ensure public health and safety, it is extremely important to accurately and effectively monitor chlorine residuals during the treatment and transport of drinking water. There are few optical sensors are available to detect free chlorine such as HACH DR/4000 Spectrophotometric, DPD (N, N-diethyl-p-phenylenediamine) ferrous titrimetric techniques [12, 13]. These methods are not portable and require expensive instrumentation. Several alternative methods have been suggested. One approach is based on optical detection using differential absorption spectroscopy [14]. This method requires UV or laser light sources and complicated instrumentation. A significant amount of work has been done on FAC detection based on classical volumetric techniques [15]. However, electrochemical sensing techniques, and more specifically amperometry, have been employed for their simple sensor structure and good sensitivity [16, 17, 18]. They have limitations in, sensitivity, lifetime and on-line measurement. There are few miniaturized amperometric flow-through sensor for residual chlorine measurement has been studied [19] but the sensor cell consists of protruded electrodes on silicon and a glass cover, which requires relatively complicated fabrication process. Transparent polymer based microsensor was reported by Trichur et al. [20] offers advantages such as future integration of on-chip optics. For improved linearity over a wide range of chlorine concentrations, three electrode configurations have been selected [21]. Microfluidic packaging in this case allows easy approach to external connections with the sensor. However market demands encouraged the development of electrochemical transducer based sensor systems which are cost-effective, fast, reliable and portable in nature. The majority of these devices in particular FAC sensors are based on expensive macro electrodes (such as Pt and gold electrodes) [22].

Therefore, the aim of this study is to develop a FAC sensor based on disposable and cost effective electrode platform. Sensitivity and stability of the sensors (along with the matrix effect) will be investigated.

1.3 Function of chlorine in water quality

Chlorine is the most common primary disinfectant used in the treatment of surface and the drinking water swimming pool/spa water. Chlorine exists as gas, solid, and liquid. Each has advantages and disadvantages in its use and different ways of introduction to the water, which also plays a role in the management of chlorine and its effectiveness. The chemical reaction that occurs upon introduction of chlorine to pool water is important to understand to ensure proper water management and to reduce risk of recreational water illness. When chlorine is added to surface water, some of the chlorine reacts with organic materials and metals in the water, and will not be available for further disinfection. This is called the chlorine demand of the water. The remaining chlorine concentration is termed total chlorine (TC). Total chlorine is consisting of combine chlorine (CC) and FAC.

$$\mathrm{TC} = \mathrm{CC} + \mathrm{FAC}$$

When chlorine interacts with drinking water, it proceeds through a series of chemical reactions. After addition of chlorine in to the water, a portion of it reacts first with organic materials and metals in the water and is not available for disinfection (this is called the chlorine demand of the water). The remaining chlorine concentration after the chlorine demand is accounted for is called total chlorine. Total chlorine has two main elements:

- The combined chlorine which has reacted with nitrates and is unavailable for disinfection and
- The free chlorine, which is the chlorine available to inactivate disease-causing organisms, and thus a measure to determine whether the water is drinkable.

Generally in complete pure water for example, the free chlorine concentration equal to the chlorine concentration added initially, as the chlorine demand is zero. However, in natural water or surface water, organic material will exert a chlorine demand, and nitrates and other ions will form combined chlorine. Chlorine kills bacteria through following mechanism. For example, if using completing clean water the chlorine demand will be zero, and there will be no nitrates present, so no combined chlorine will be present. Thus, the free chlorine concentration will be equal to the concentration of chlorine initially added. In natural waters, especially surface water supplies such as rivers, organic material will exert a chlorine demand, and nitrates will form combined chlorine. Thus, the free chlorine concentration will be less than the concentration of chlorine initially added.

Chlorine kills bacteria through a simple chemical reaction. When chlorine solution is poured into water, it breaks down into many different chemicals, including hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). Both kill micro-organisms and bacteria by attacking the lipids in the cell walls and destroying the enzymes and structures inside the cell, rendering them oxidized and harmless. The difference between HOCl and OCl^- is the speed at which they oxidize. Hypochlorous acid HOCl is a much better disinfectant compare to OCl^-, and is often referred to as active chlorine. HOCl is able to oxidize the organisms in several seconds, while the hypochlorite ion may take up to 30 minutes. As HOCl / OCl^- equilibrium depends on pH, the disinfecting effect is strongly relative to the pH of water.

1.3.1 Free available chlorine (FAC) chemistry

The different forms of chlorine are named as follows:

Cl_2 = chlorine

HOCl = hypochlorous acid

OCl^- = hypochlorite ion

When chlorine gas is dissolved in water, it hydrolyzes rapidly according to equation (i). This reaction occurs very rapidly, in only a few tenths of a second at 18°C (64.4 °F).

$$\text{(i)}\ Cl_2 + H_2O \longrightarrow HOCl + HCl$$

Since HCl, hydrochloric acid is a strong acid, addition of gaseous chlorine to water results in a lowering of the pH from the acidic HCl byproduct. The important product of reaction (i) is HOCl or hypochlorous acid. Hypochlorous acid is the killing form of chlorine in water. Hypochlorous acid is unstable because the chlorine molecule is lightly bound and therefore will react quickly. Free available chlorine, or free chlorine, is hypochlorous acid and is taste free and aggressive against germs and organic compounds [24, 25]. Chlorine supplied as sodium hypochlorite, calcium hypochlorite, or bleach is in alkaline or basic form. When a base is present, a different reaction sequence occurs:

$$\text{(ii)}\ NaOCl + H_2O \longrightarrow HOCl + Na^+ + OH^-$$

$$\text{(iii)}\ Ca(OCl)_2 + 2H_2O \longrightarrow 2HOCl + Ca^{++} + 2OH^-$$

In any hypochlorite solution, the active ingredient is always hypochlorous acid. Then once HOCl and OH- are formed an additional reaction occurs:

$$\text{(iv)}\ HOCl + OH^- \longrightarrow OCl^- + H_2O$$

The proportion of chlorine, hypochlorous acid, and hypochlorite ion in solution depends primarily on pH and somewhat on temperature.

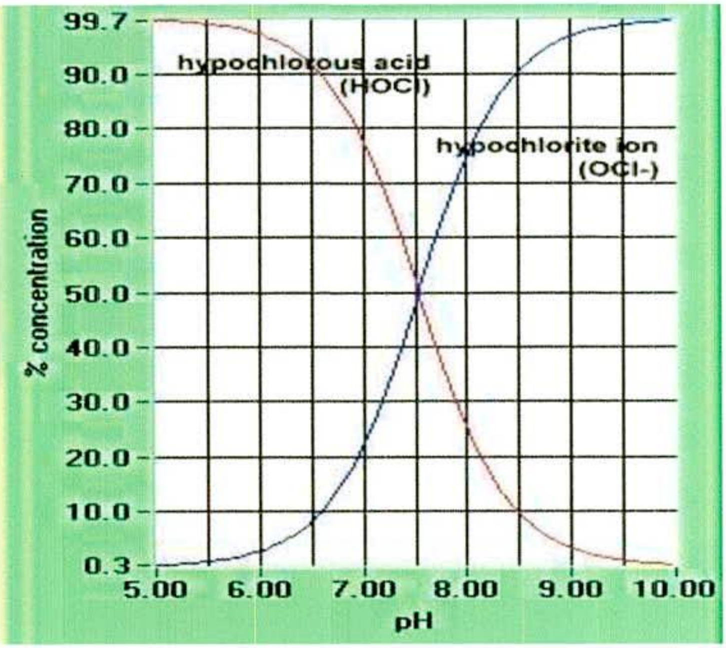

Figure 1.1: Chlorine species change vs. pH.

1.3.2 Determination of FAC in water

Maintain the safe level of chlorine or hypochlorite ion; it is crucial to determine FAC in water. There are few standard methods are widely being used to test FAC in drinking water, that are

i. pool test kits
ii. colour-wheel test kits
iii. digital colorimeters
iv. electronic probes, ORP method and standard off-line chlorine
v. standard amperometric chlorine probe

Pool test kits methods

The first option for testing uses a liquid chemical OTO (othotolidine) that causes a colour change to yellow in the presence of total chlorine. It needs simply fill a tube with water, add 1- 5 drops of the solution, and look for the colour change. These kits are sold in many stores as a way to test the concentration of total chlorine in swimming pool water. This method does not measure free chlorine. Test kits to make basic measurements of free chlorine and pH from a sample of pool water, which are the most important items to control in a swimming pool, are packaged with small dropper bottles of reagents. These reagents are typically OTO for chlorine and phenol red for pH [27]. The kits include vials for mixing a water sample with the test reagents, and colour charts for reading the indicated levels. Besides chlorine and pH, which should be checked frequently, more sophisticated reagent kits provide tests for acid demand and base demand, total alkalinity (TA), calcium hardness and cyanurate ("stabilizer") concentration. These additional tests tend to vary only over weeks or months in a well-maintained pool, and thus need not be checked as frequently as chlorine and pH.

Colour-wheel test kit methods

Colour wheel test kits use a powder or tablet chemical DPD (N,N diethyl-p-phenylene diamine) that causes a colour change to pink in the presence of chlorine. The colour wheels are simpler and less expensive than digital meters because to measure the intensity of the colour change, the field worker uses a colour wheel to visually match the colour to a numerical free or total chlorine reading. The test kit can be used to measure free chlorine and/or total chlorine, with a range of 0 – 3.5 mg/l, equivalent to 0 – 3.5 ppm (parts per million).

Digital colorimeters sensor methods

Digital colorimeters are the most accurate way to measure free chlorine and/or total chlorine residual in the field in developing countries. These colorimeters use the following method:

1. Addition of DPD tablets or powder into a vial of sample water that causes a colour change to pink; and,
2. Insertion of the vial into a meter that reads the intensity of the colour change by emitting a wavelength of light and automatically determining and displaying the colour intensity (the free and/or total chlorine residual) digitally. The range of the meter is 0 - 4 mg/l, equivalent to 0 - 4 ppm (parts per million).

Electronic ORP probes methods

Where the water is sanitized by means of oxidizers, some suppliers of electronic monitoring equipment recommend that the efficacy of the oxidizer be measured by the oxidation-reduction potential of the water, a factor measured in millivolts, where the minimum acceptable ORP level in public pools is 650 millivolts. This is supposed to ensure a 1-second kill rate for microorganisms introduced into the water. Unfortunately, a commonly used non-chlorine supplemental oxidizer, potassium monopersulfate $KHSO_5$, can produce measured 650 mV levels even in the absence of all sanitizing residuals. ORP test cells are available as hand-held instruments, and as probes for mounting permanently in the pool circulation plumbing to control automatic chlorine feeders.

Standard amperometric direct chlorine sensor probes with electrical signal

Standard amperometric cells with two/three electrode chlorine probes are mainly use in process water or drinking water. These probes use an electrochemical amperometric principle with a dedicated liquid reagent behind a physical membrane. These probe measure free chlorine and are precise [28]. However, the reagent requires being controlled often, so it reduces the time without maintenance of the probe. Moreover, the presence of this membrane and the close volume for the reagent reduce the range of pressure and temperature [29]. This kind of probe needs to be use in special low pressure measuring chamber. These tests are widely being used to ensure that water does not have unpleasant taste or odour by maintaining the level of free chlorine residue below ~4 mg/l. Also it ensures that the minimum level of free chlorine residue is 0.2 mg/l, so that the stored water stays as microbiologically clean. Too much chlorine can cause eczema and rashes. However, there are few advantages and disadvantages on these methods that are tabulated in Table 1.1

Table 1.1: Advantages and disadvantages of standard FAC test methods

Tests	Advantages	Disadvantages
Pool test kits	Low cost Very easy to use	Degradation of the othotolidine (OTO) solution that causes inaccurate readings over time Generally not reliable quantitative results Lack of calibration and standardization
Colour-wheel test kits	Accurate readings if used properly Low cost	Potential for user error Lack of calibration and standardization
Digital colorimeters	Highly accurate readings Fast results	Expense Necessity of calibration with standards
Electronic probes	In-line reading and feedback device for controller Low cost probe	Does not really measure Chlorine, measure mean oxydo-reduction potential Need calibration and probe maintenance
Amperometric probe	True free chlorine measurement	Not really in-line measurement Reagent capable of maintenance Limited pressure, i.e. need of special measuring cell chamber

1.3.3 Effect of pH in chlorine sensor

Chlorine dissolved in liquid, is one of the most effective and economical germ-killers for the treatment of water to make it potable or safe to drink. Chlorine's powerful disinfectant qualities come from its ability to bond with and destroy the outer surfaces of bacteria and viruses. Drinking water chlorination is one of the most widely used methods to safeguard drinking water supplies. In addition to water disinfection, chlorine and its derivatives are used to treat sewage and industrial effluent, and as household and industrial bleaches (e.g., to control biological agent growth in water filled industrial systems) to maintain and improve hygiene standards [30].

In general, free chlorine sensors needed to be paired with some form of pH compensation. Compensation of the pH was necessary because only the hypochlorous acid (HOCl) form of free chlorine is reducible at the cathode of an amperometric device. As set forth above, free chlorine is effectively 100% HOCl, while above pH 10; free chlorine is effectively 100% hypochlorite ion (OCl^-).The relative concentrations of hypochlorite ion and hypochlorous acid varying with pH as Illustrated in Figure 1.2 [31]. Detection of pH is integral part of the chlorine determination. Current state of the art mostly reported that pH must be sensed before sensing chlorine for the accurate final output reading. However, attempt has been made to control the pH to avoid the effect of varying pH on chlorine measurements. For example, buffers have been used in order to attempt to maintain the internal electrolyte solution at a selected pH. Further, William [32] has reported a passive pH adjustment for analytical systems. However, attempts to date involved complicated pH maintenance systems or independent pH compensation for the chlorine measurement [33-36].

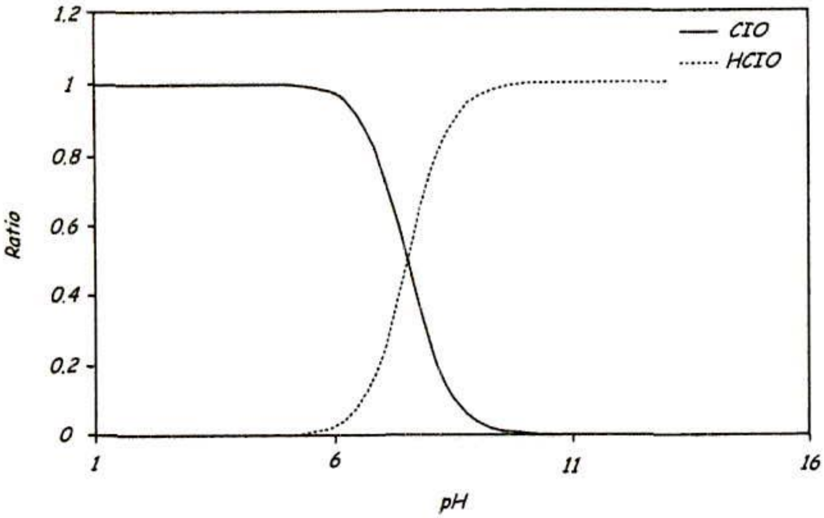

Figure 1.2: Chart of the ratio of free chlorine species as a function of the pH.

Terminology:

In the industry, there are a number of terms used to indicate the various forms of chlorine that are of interest. These terms tend to be used rather loosely and not necessarily consistently. For that reason, the following terms are defined:

Total free chlorine refers to the sum of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The hypochlorite ion is not effective for disinfection, but it is in a free form. All of the total free chlorine would be in the form of hypochlorous acid if the pH was low enough [37].

Total residual chlorine refers to the sum of total free chlorine and combined chlorine. The concentration of chlorine species present in water after the oxidant demand has been satisfied. Low total residual chlorine is of particular interest to ensure there are no downstream consequences for aquatic life.

Combined chlorine rationale

Combined chlorine is the sum of the species resulting from the reaction of free chlorine with ammonia (NH_3), including monochloramine (NH_2Cl), dichloramine($NHCl_2$), and trichlor amine (nitrogen trichloride, NCl_3). Combined Chlorine forms when chlorine chemically bonds with materials containing nitrogen including, but not limited to urine (ammonia), dead algae, and skin. This reduces the chlorine available for disinfection, which reduces disinfection power. CC can be determined using an appropriate test kit and utilizing this formula [38].

$$\mathbf{CC = TC - FAC}$$

High CC levels in surface water

Levels above 0.4 mg/L (ppm) can indicate that the bathing load or contaminant load (from swimmers) is high. High CC can cause complaints of a strong chlorine smell, eye and possible breathing irritation after exposure to pool water and air, particularly for indoor pools. These complaints are further exacerbated when water becomes agitated from patron activity, pool features (wave pools, spray features), or hot tubs as all of these by nature aerosolize the water and corresponding CC into the aquatic venue's air [39]. High levels of CC, poorly positioned air vents, and inadequately designed and maintained ventilation systems increase potential health effects and equipment corrosion [40].

Primary disinfection: the application of a disinfectant at the drinking water treatment plant, with a primary objective to achieve the necessary microbial inactivation.

Secondary disinfection: the subsequent application o f a disinfectant, either at the exit of the treatment plant or in the distribution system, with the objective of ensuring that a disinfectant residual is present throughout the distribution system [37, 41].

1.4 Effect of chlorine in surface water

Chlorine is so effective and relatively easy to use. It has become one of the most widely used disinfectants found in homes, industry and health care facilities. One other important benefit of using chlorine in drinking water is that once treated a small residual amount of chlorine remains in the water distribution system to provide continuing protection from microbiological contamination while the water is delivered to your tap. Chlorine based products are routinely used to sterilize baby feeding bottles, treat domestic and commercial swimming pools, spas and hydrotherapy pools. Chlorine is a simple, reliable, effective, relatively inexpensive and, above all else, safe, way to protect drinking water supplies against contamination by microbiological organisms. As it has been used for so many years the methods and equipment needed to use it safely are well designed, and its effectiveness is well understood.

1.5 The amount of water in chlorine level

The Bangladesh Drinking Water Guidelines state that the level of chlorine in scheme (piped) drinking water should be no more than 5mg/L. Normal chlorine concentrations in scheme drinking water pipeline systems will range from 0.5mg/L to 1.5 mg/L. It is quite normal for chlorine in southern Bangladesh drinking water to be at or below these levels. Chlorine levels may vary due to the flow rate of the water in the system, your proximity to the water treatment plant and during maintenance periods [42].

Even taking a long bath or shower increases a person's risk for chlorine exposure because chlorine can enter the body through skin absorption or through the eyes, nose, and ears. Chlorine has long been used to disinfect our drinking water because it controls the growth of such unwelcome bacteria as Ecoli and Giardia. You have to be careful also, to take precautions even when showering or drinking tap water.

Common exposures to chorine:

- Absorption through skin from water and from the air
- Drinking & Eating
- Breathing in the fumes that chlorine can create

It should be noted that the dangers of chlorine exposure also exist for people who do not normally swim, but instead choose to sit by the pool sunbathing or socializing. In some instances, non-swimmers have tested positive for high levels of trichloramine. What all of this means is that people need to be more aware of some of the seemingly innocent dangers that they are commonly exposed to. Chlorine, by many schools of thought, has become a dinosaur for swimming pool sanitation [39]. There are alternative methods that can be used to keep pools disinfected, including silver-copper ion generators.

1.5.1 Chlorine, cancer, and heart disease

The addition of chlorine to our drinking water began in the late 1800s and by 1904 was the standard in water treatment, and for the most part remains so today. We don't use chlorine because it's the safest or even the most effective means of disinfection, we use it because it is the cheapest. In spite of all our technological advances, we essentially still pour bleach in our water before we drink it. The long term effects of chlorinated drinking water have just recently being recognized.

According to the U.S. Council of environmental quality, "cancer risk among people drinking chlorinated water is 93% higher than among those whose water does not contain chlorine." Dr. Joseph Price wrote a highly controversial book in the late sixties titled Coronaries/Cholesterol/Chlorine and concluded that nothing can negate the incontrovertible fact, the basic cause of atherosclerosis and resulting entities such as heart attacks and stroke, is chlorine." Dr. Price later headed up a study using chickens as test subjects, where two groups of several hundred birds were observed throughout their span to maturity. One group was given water with chlorine and the other without. The group raised with chlorine, when autopsied, showed some level of heart or circulatory disease in every specimen, the group without had no incidence of disease. The group with chlorine under winter conditions, showed outward signs of poor circulation, shivering, drooped feathers and a reduced level of activity. The group without chlorine grew faster, larger and displayed vigorous health. This study was well received in the poultry industry and is still used as a reference today. As a result, most large poultry producers use dechlorinated water. It would be a common sense conclusion that if regular chlorinated tap water is not good enough for the chickens, then it probably is not good enough for us humans!

Research has shown, however, that long-term exposure to chlorine leads to the production of free radicals within the body. Free radicals are carcinogenic, and cause tremendous damage to our cells.

According to a Belgian study that was released in 2003, certain irritants called trichloramines are released any time chlorinated water reacts with organic materials (such as sweat or urine) from people [43]. Trichloramines are believed to initiate a biological process that effectively destroys the cellular barriers surrounding the lungs. Children exposed to large amounts of chlorine could potentially suffer asthmatic attacks. In one research study, rats exposed to chlorine and chloramines developed tumours in their kidneys and intestines. Chlorine can irritate severely, even burn, skin exposed directly to it. It can irritate and burn the eyes and throat as well.

Breast cancer, which now affects one in every eight women in North America, has recently been linked to the accumulation of chlorine compounds in the breast tissue. A study carried out in Hartford Connecticut, the first of its kind in North America, found that, "women with breast cancer have 50% to 60% higher levels of organo chlorines (chlorination by-products) in their breast tissue than women without breast cancer."

One of the most shocking components to all of these studies is that up to 2/3s of our harmful exposure to chlorine is due to inhalation of steam and skin absorption while showering. A warm shower opens up the pores of the skin and allows for accelerated absorption of chlorine and other chemicals in water. The steam we inhale while showering can contain up to 50 times the level of chemicals than tap water due to the fact that chlorine and most other contaminants vaporize much faster and at a lower temperature than water. Inhalation is a much more harmful means of exposure since the chlorine gas (chloroform) we inhale goes directly into our blood stream. When we drink contaminated water the toxins are partially filtered out by our kidneys and digestive system. Chlorine vapours are known to be a strong irritant to the sensitive tissue and bronchial passages inside our lungs; it was used as a chemical weapon in World War II. The inhalation of chlorine is a suspected cause of asthma and bronchitis, especially in children which has increased 300% in the last two decades. "Showering is suspected as the primary cause of elevated levels of chloroform in nearly every home because of chlorine in the water."

Chlorine in shower water also has a very negative cosmetic effect, robbing our skin and hair of moisture and elasticity, resulting in a less vibrant and youthful appearance. Anyone who has ever swum in a chlorinated pool can relate to the harsh effects that chlorine has on the skin and hair. What's surprising is that we commonly find higher levels of chlorine in our tap water than is recommended safe for swimming pools [44].

1.6 Nano-micro technology in FAC determination

Nano scale or micro scale devices based on nanowires or microwares have been realized for applications in electronics, optics, gas, and biomedical sensing [45, 46]. Three-dimensional structures such as free standing nano/micro wires are particularly compelling for electronic interconnects and sensing applications due to their suitability for high-density integration and high sensitivity to surface interactions. Currently amperometric or potentiometric FAC sensor probes are based on planar electrode. Although nano or micro structured electrode have been fabricated for various applications, a simple fabrication technique is highly desired, which can maintain reasonable costs for practical applications.

Diffusion layer overlap is a common problem in the array electrode. The micro-lithographically fabricated iridium-based mercury ultra-micro (UME) electrode array showed a response with chronoamperometry and square wave anodic stripping voltammetry, equivalent to that expected for a single UME with the multiplication of number of UME in the array [47]. That explains that there were no diffusion layer overlap, and solution can utilise all the surface of the array electrode. Moreover, this type of electrode can be integrated on-board connected with digital potentiostat and reference electrode. Such a sensor could be made part of a hand-held device for on-site and/or in situ analytical measurement system.

Sonochemical method has been used to fabricate micro-electrode array reported by Myler et al. [48]. Sensors of this type were fabricated by first depositing an insulating polydiaminobenzene film on supporting gold electrodes. Sonication and subsequent ablation exposed discrete areas of the underlying conducting electrode, which collectively act as a microelectrode array. Polydiaminobenzene coated membranes were found to be capable of allowing the analytes and rejecting electrochemical interferences such as ascorbate in this case. Moreover, it shows high interfacial adhesion, mechanical strength and thermal stability, which is crucial for FAC sensor. Another group also used Sonochemically fabricated microelectrode by first depositing an insulating film on commercial screen printed electrodes, which was subsequently sonicated to form cavities (micro pores) of regular sizes in film. Few cases, chemical deposition of polysiloxane from dichlorodimethysilane was used to deposit a thin protective and diffusion mass transport controlling coating over the electrodes and applied for sensor applications in the presence of interferences [49]. An ultra-thin poly (*o*-phenylenediamine) film composite membrane has also been used as the outer covering barrier on gold sputter-coated porous polycarbonate membrane. Homogeneous poly (*o*-phenylenediamine) films of approximately 30 nm thickness acted as an effective diffusional barrier particularly for acerbate [50].

To improve the sensitivity, few works have been done using immobilisation of carbon nanotube on the electrode surface. In addition to improving the sensitivity, CNT modified electrode reduce the applied potential and found to be stable in amperometric response [51, 52]. Monolayers of 3-mercaptopropionic acid (MPA) and 11-mercaptoundecanoic acid (MUA) self-assembled on a gold disk electrode showed micro-electrode behaviour, with pinholes acting as the microelectrodes [53].

Hemispherical diffusion of redox active solutes to a layer of independent molecule based nanoelectrode ensembles have been described by Creager et al. Analytical expressions are derived for the current–voltage relation corresponding to catalyzed electron transfer at an ensemble of redox-molecule-based nanoelectrode sites, and the expressions are used to interpret preliminary data for ultrasensitive electrochemical detection in flow streams via an electrochemical amplification process. This is thought to involve redox mediation by individual analyte molecules adsorbed onto monolayer-coated electrodes [54]. A one step approach to Pt nanopore ensembles has been developed using an amphiphilic block copolymer [polystyrene-block-poly (acrylic acid)] self-assembly. At lower scan rates, it remains the features of a single nanoelectrode, while at high scan rates the nanoelectrodes act independently. This is an important feature which can be followed for FAC sensor fabrication and other electroanalytical applications [55].

1.6.1 Challenges and future trend

FAC sensor is one of the important sensors with a high environmental impact which will facilitate sourcing pure water; however, its improvement associates few challenges. Majority FAC sensors are pH sensitive, therefore, need to control the pH of the sample during the measurement. Few strategies are currently being used to control pH or to correct pH in the sample solution, such as chemical corrections or using pH probe. Ultimately it makes the FAC sensor fabrication more complicated and expensive.

The signal measured by current FAC probes on the market varies with flow rate as the electrodes are macroscopic in size. Micro or nano cylindrical or disc size electrode may reduce the effect of flow rate due to their spherical and semi-spherical diffusion orientation.

Response time of the current FAC sensor need to be improved, as current available FAC sensors has a large variation of response time start from 60 s. Current ATi membrane probes show response time of 60s, whereas Emerson probes show 4/5 mins. In addition to response time, controlling temperature of the sample is also an issue. The reaction rate varies with the change in temperature; therefore, detection of free available chlorine using the sensor technology can be changed with the change in temperature. Appropriate algorithm must be designed to account temperature corrections. Frequency of calibration of the FAC sensor found to be another issue where current commercial sensors require weekly calibration.

1.7 Electrochemical sensors

Electrochemical sensors are the devices, which are composed of an active sensing material with a signal transducer. The role of these two important components in sensors is to transmit the signal without any amplification from a selective compound or from a change in a reaction. These devices produce any one of the signals as electrical, thermal or optical output signals which could be converted into digital signals for further processing. One of the ways of classifying sensors is done based on these output signals. Among these, electrochemical sensors have more advantage over the others because; in these, the electrodes can sense the materials which are present within the host without doing any damage to the host system. On the other hand, sensors can be broadly classified into two categories as chemical sensors and biosensors. The biosensors can be defined in terms of sensing aspects, where these sensors can sense biochemical compounds such as biological proteins, nucleotides and even tissues [56].

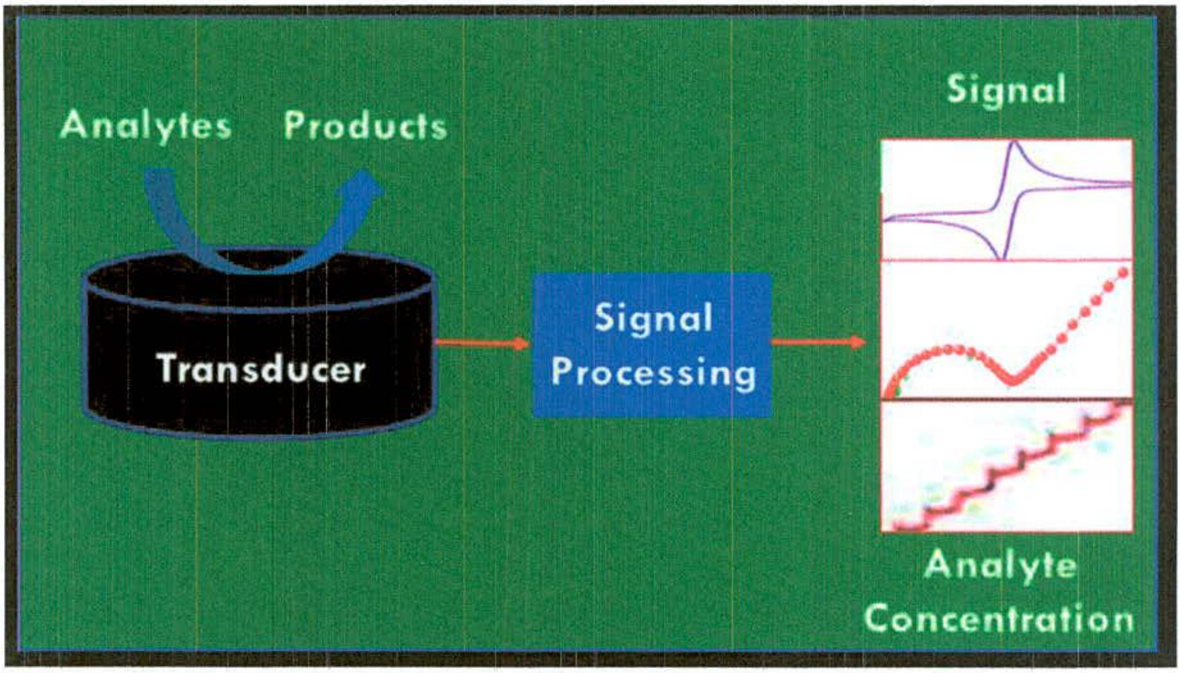

Figure 1.3: Mechanism of electrochemical sensor.

Depending on the exact mode of signal transduction, electrochemical sensors can use a range of modes of detection such as potentiometric, voltammetric and conductimetric. Each principle requires a specific design of the electrochemical cell. Potentiometric sensors are very attractive for field operations because of their high selectivity, simplicity and low cost. They are, however, less sensitive and often slower than their voltammetric counterparts. Examples of transduction techniques include:

- Potentiometric – The measurement of the potential at zero current. The potential is proportional to the logarithm of the concentration of the substance being determined.

- Voltammetric – Increasing or decrease the potential that is applied to a cell until the oxidation or reduction of the analyte occurs. This generates a rise in current that is proportional to the concentration of the electroactive potential. Once the desired stable oxidation/reduction potential is known, stepping the potential directly to that value and observing the current is known as amperometry.

- Conductiometric – Observing changes in electrical conductivity of the solution [57].

The selection and development of an active material is a challenge. The active sensing materials may be of any kind as whichever acts as a catalyst for sensing a particular analyte or a set of analytes. The recent development in the nanotechnology has paved the way for large number of new materials and devices of desirable properties which have useful functions for numerous electrochemical sensor and biosensor applications [5].

Basically by creating nanostructure, it is possible to control the fundamental properties of materials even without changing their chemical composition. In this way the attractive world of low dimensional systems, together with the current tendencies on the fabrication of functional nanostructured arrays could play a key role in the new trends of nanotechnology [58, 59, 60].

Further, the nanostructures can be used for both efficient transport of electrons and optical excitation, and these two factors make them critical to the function and integration of nanoscale devices [61-63]. In fact, nano systems are the smallest dimension structures that can be used for efficient transport of electrons and are thus critical to the function and integration of these nanoscale devices.

1.8 Chemically modified electrodes

Chemically modified electrodes (CMEs) comprise a relatively modem approach to electrode systems a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers, and the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses. Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner [64].

The range of electrode surface properties includes, but is more diverse than, that of ion-selective electrodes (ISEs) which also involve, in their highest forms, rational design of the phase-boundary, partition and transport properties of membranes on or between electrodes. While CMEs can operate both amperometrically (or voltammetrically) and potentiometrically, they are generally used amperometrically, a faradaic (charge transfer) reaction being the basis of experimental measurement or study, whereas ISEs are generally used in potentiometric formats where a phase-boundary potential (interfacial potential difference) is the measured quantity [65]. Gas-sensing electrodes (e.g., for CO_2, NH_3, NOx) are also potentiometrically based [66] although the oxygen electrode, which functions amperometri -cally, is an exception [67]. Chemically sensitive field effect transistors (CHEMFETs) are basically non-faradaic electrode systems in which electric field variations in the semiconductor gate region control the magnitude of the source drain current [68]. Enzyme-based electrodes detect the product(s) of a reaction between an immobilized enzyme layer and a reaction substrate in many ways, including both amperometric and potentiometric means. The distinction between CMEs and amperometric enzyme-modified electrodes is thus very narrow, the latter being based on a natural biological catalyst, but also with a rational (bio) molecular electrode design goal in mind.

1.8.1 General methods of modification of electrodes

The concept of chemically modified electrodes (CMEs) is one of the exciting developments in the field of electro analytical chemistry. Many different strategies have been employed for the modification of the electrode surface. The motivations behind the modifications of the electrode surface are: (i) improved electro catalysis, (ii) freedom from surface fouling and (iii) prevention of undesirable reactions competing kinetically with the desired electrode process [69]. The increasing demand for it has led to the development of a rapid, simple and non-separation method for the simultaneous determination of isomers where the CMEs have emerged as an efficient and versatile approach, and have attracted considerable attention over the past decades due to its advantages in terms of reduced costs, automatic and fast analysis, high sensitivity and selectivity [70-74]. There are numerous techniques that may be used to modify electrode surfaces. Amongvarious CMEs, polymer-modified electrodes (PMEs) are promising approach to determination. Some modification processes are-

Covalent Bonding: This method employs a linking agent (e.g. an organosilane) to covalently attach one of several monomolecular layers of the chemical modifier to the electrode surface [72].

Drop-Dry Coating (or solvent evaporation): A few drops of the polymer, modifier or catalyst solution are dropped onto the electrode surface and left to stand to allow the solvent to dry out [73].

Dry-Dip Coating: The electrode is immersed in a solution of the polymer, modifier or catalyst for a period sufficient for spontaneous film formation to occur by adsorption. The electrode is then removed from solution and the solvent is allowed to dry out [70].

Composite: The chemical modifier is simply mixed with an electrode matrix material, as in the case of an electron-transfer mediator (electro catalyst) combined with the carbon particles (plus binder) of a carbon paste electrode. Alternatively, intercalation matrices such as certain Langmuir-Blodgett films, zeolites, clays and molecular sieves can be used to contain the modifier [74].

Spin-Coating (or Spin-Casting): it is also called spin casting; a droplet of a dilute solution of the polymer is applied to the surface of a rotating electrode. Excess solution is spun off the surface and the remaining thin polymer film is allowed to dry. Multiple layers are applied in the same way until the desired thickness is obtained. This procedure typically produces pinhole-free thin films for example; oxide xerogel film electrodes prepared by spin-coating a viscous gel on an indium oxide substrate [70].

Electro deposition: In this technique the electrode is immersed in a concentrated solution ($\sim 10^{-3}$ $molL^{-1}$) of the polymer, modifier or catalyst followed by repetitive voltammetry scans. The first and second scans are similar, subsequent scans decrease with the peak current. For example, electrochemical deposition of poly (o-toluidine) on activated carbon fiber [74].

Electropolymerisation: A solution of monomer is oxidized or reduced to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinhole) sites at the electrode surface. Unless the polymer film itself is redox active, electrode passivation occurs and further film growth is prevented.

In this technique the electrode is immersed in a polymer, modifier or catalyst solution and layers of the electropolimerized material builds on the electrode surface. Generally, the peak current increases with each voltammetry scan such that there is a noticeable difference between the first and final scans indicating the presence of the polymerized material. For example, electro polymerization of aniline on platinum electrode perturbations.

Chitosan: Chitosan is a derivative of chitin, which is one of the world's most plentiful organic resources and is derived from the shells of crustaceans. It is a linear polyaminosaccharaide composed of randomly distributed β-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine groups. Due to its structure, chitosan possesses good adhesion and cheap properties; therefore it has been used as an immobilization matrix. Although it has poor electrical conductivity, but it usually has been combined with carbon nanotubes, redox mediator and metal nanoparticles [75].

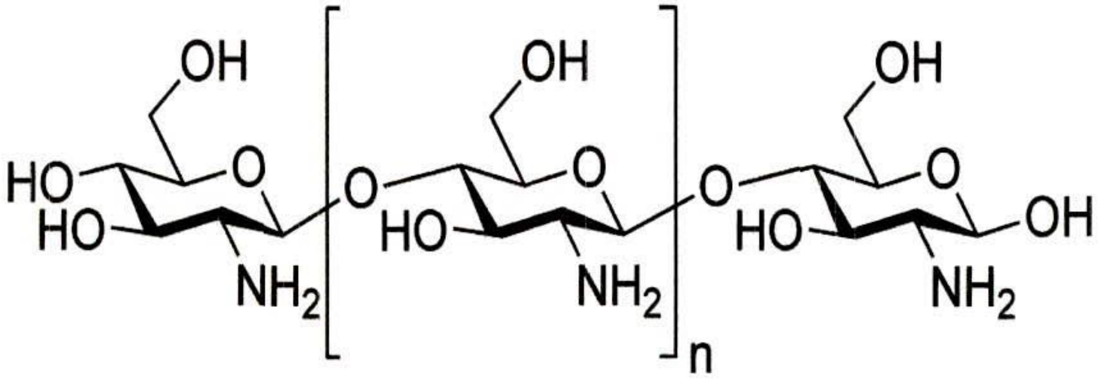

Figure 1.4: Structure of chitosan.

Chitosan is soluble in aqueous acidic media at pH < 6.5. When dissolved, it bears a high positive charge on its amino groups. Chitosan has gel-forming properties as a result of its ability to adhere to negatively charged surfaces and aggregate polyanionic compounds.

1.9 Theoretical backgrounds

1.9.1 Fundamentals of electrochemistry and various electro-analytical techniques

Faradaic currents

The Faradaic current is the current that flows through an electrochemical cell that is generated by the change in oxidation state of the electroactive species occurring at the electrode surface, combined with the current contribution due to the charge transfer between the electrode and the background analyte present in solution. The faradaic current obeys Faraday's law.

Charging currents and the electrical double layer

The application of a potential to the electrode surface causes ions near the electrode surface to migrate towards or away from the electrode depending on the respective charge of the electrode and the ions. This forms an electrical double layer, comprised of the electrical charge at the surface of the electrode and the charge of the ions in the solution near the electrode. This double layer leads to the generation of a non-faradaic charging current.

The electrical double layer is an array of charged particles and orientated dipoles. It is composed of two layers; the layer closest to the electrode is known as the inner Helmholtz plane (IHP) and the outer Helmholtz plane (OHP) (Figure 1.5). The planes were discovered by Hermann von Helmholtz in 1853. The IHP is composed of solvent molecules and specifically adsorbed ions, whilst the OHP represents the imagined outer layer closest to the electrode that passes through the center of solvated ions, but is separated by the molecules at the IHP. These layers are both held at the surface of the electrode. The behavior of the interface between the electrode and the solution is similar to that of a capacitor.

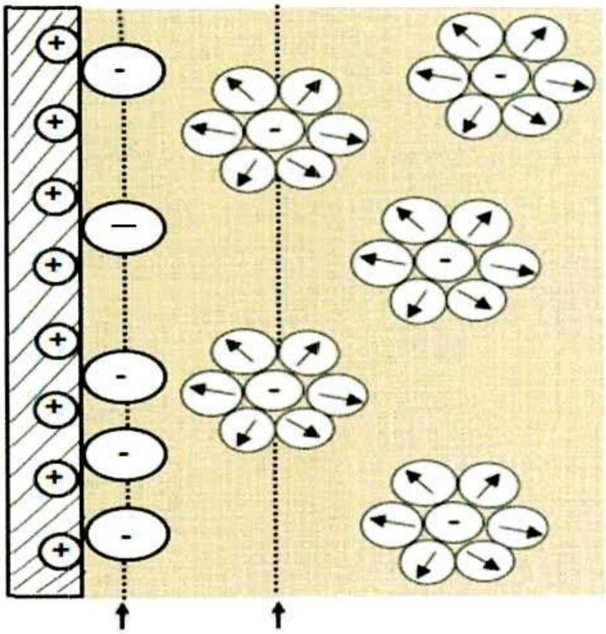

Figure 1.5: Schematic representation of the electrical double layer [76].

Beyond the double layer, is a diffuse layer of scattered ions that extent into the bulk solution. These ions are ordered relative to the columbic forces acting upon them and the random motion of the solution by thermal motion. The balance of the electrostatic forces on ions at the surface of the electrode, which are repelled or attracted dependent on their charge, is counterbalanced by the random motion of the diffuse layer. This causes a non-uniform distribution of ions near the electrode surface. As a result, the field strength of the potential applied to the electrode diminishes rapidly, thereby causing the double layer to be extremely thin at 10-20 nanometers in thickness. It is also essential to use a high electrolyte concentration, typically a 100 fold greater than that of the analyte, as this concentrates the charge at the Helmholtz planes, therefore ensuring that diffusion is the dominant mechanism for mass transport [77, 78].

Mass transfer process in voltammetry

Mass transfer is the movement of material from one location to another in solution. In electrochemical systems, three modes of mass transport are generally considered which a substance may be carried to the electrode surface from bulk solution including diffusion, convection and migration. Any of these or more than one might be operating in a given experiment which is depended on the experimental conditions.

In general, there are three types of mass transfer processes:

- Migration
- Diffusion
- Convection

Migration

Migration is the movement of ions through a solution as a result of electrostatic attraction between the ions and the electrodes. It is the primary cause of mass transfer in the bulk of the solution in a cell. This motion of charged particle through solution, induced by the charges on the electrodes is called migration. This charge movement constitutes a current. This current is called migration current. The larger the number of different kinds of ions in a given solution, the smaller is the fraction of the total charge that is carried by a particular species. Electrolysis is carried out with a large excess of inert electrolyte in the solution so the current of electrons through the external circuit can be balanced by the passage of ions through the solution between the electrodes, and a minimal amount of the electro active species will be transported by migration. Migration is the movement of charged species due to a potential gradient. In voltammetric experiments, migration is undesirable but can be eliminated by the addition of a large excess of supporting electrolytes in the electrolysis solution. The effect of migration is applied zero by a factor of fifty to hundred ions excess of an inert supporting electrolyte.

Diffusion

Diffusion refers to the process by which molecules intermingle as a result of their kinetic energy of random motion. Whereas a concentration difference between two regions of a solution, ions or molecules move from the more concentrated region to the dilute and leads to a disappearance of the concentration difference.

The one kind of mode of mass transfer is diffusion to an electrode surface in an electrochemical cell. The rate of diffusion is directly proportional to the concentration difference. When the potential is applied, the cations are reduced at the electrode surface and the concentration is decreased at the surface film. Hence a concentration gradient is produced. Finally, the result is that the rates of diffusion current become larger.

Convection

By mechanical way reactants can also be transferred to or from an electrode. Thus forced convection is the movement of a substance through solution by stirring or agitation. This will tend to decrease the thickness of the diffuse layer at an electrode surface and thus decrease concentration polarization. Natural convection resulting from temperature or density differences also contributes to the transport of species to and from the electrode. At the same time a type of current is produced. This current is called convection current. Removing the stirring and heating can eliminate this current. Convection is a far more efficient means of mass transport than diffusion.

Electrochemical setup

Electrochemical cell

Typically electrochemical reactions take place in an electrochemical cell (Figure 1.6) which is made from quartz or glass, into which the sample solution is added. The sample solution must be of sufficient depth to cover the two or three electrodes utilised. Variables such as temperature can be controlled by utilising a water jacket cell, which is connected to a thermostatically controlled water bath. Experiments such as amperometry require stirring, thus a magnetic stirrer bar can sit at the bottom of the solution, which is controlled by a magnetic stirrer that sits underneath the electrochemical cell. The stirrer geometry should allow for consistent stirring.

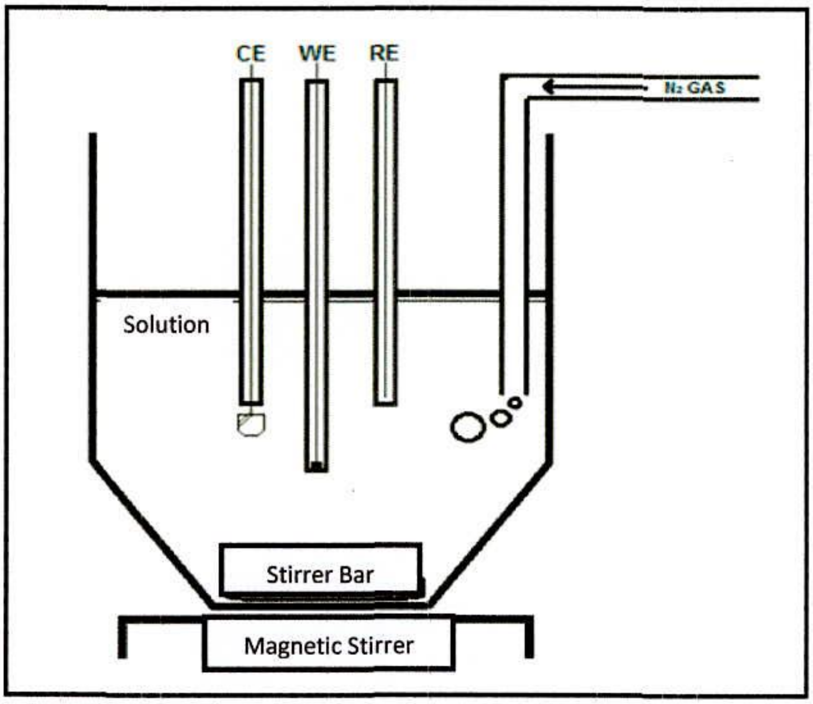

Figure 1.6: Schematic diagram of a voltammetric cell based on a three electrode system. CE: counter electrode, WE: working electrode, RE: reference electrode.

1.9.2 Electranalytical techniques

An electrode is an electrical conductor used to make contact with a nonmetallic part of a circuit (e.g. a semiconductor, an electrolyte or a vacuum). Types of electrodes

- Working electrode
- Reference electrode
- Counter electrode

Working electrode

The Working Electrode is the electrode where the potential is controlled and where the current is measured. The working electrode makes contact with the analyte and transfer charge to and from the analyte. The Working Electrode is an "inert" material such as gold, platinum, or glassy carbon.

Advantages of working electrode:

- Available wire, flat plate & tube, large range of sizes.
- Larger cathodic potential range.
- Wide potential range low background current inexpensive

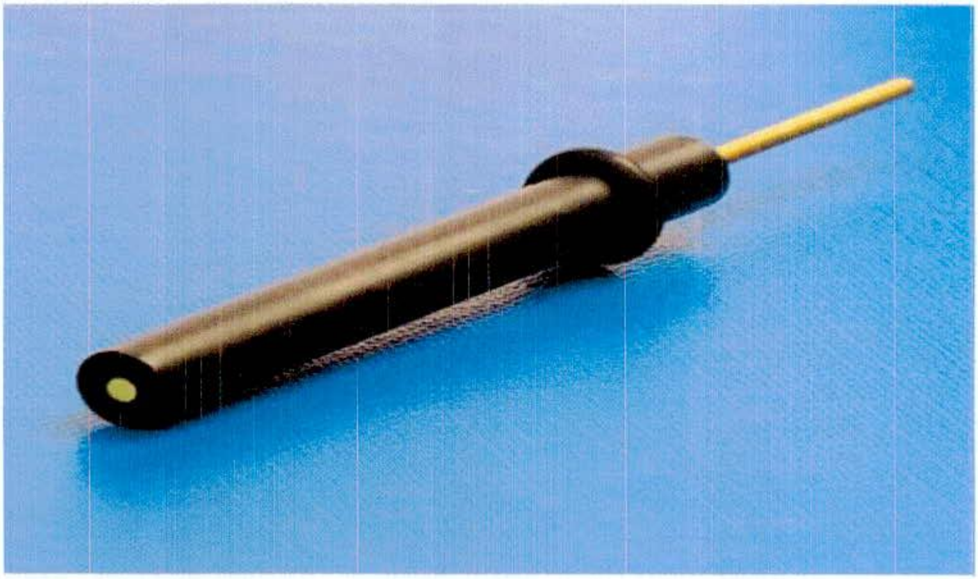

Figure 1.7: GC disk working electrode.

Function of W.E: It serves as a surface on which the electrochemical reaction takes place.

Reference Electrode:

A reference electrode is an electrode which has a stable and well-known electrode potential. The high stability of the electrode potential is usually reached by employing a redox system with constant (buffered or saturated) concentrations of each participants of the redox reaction. A reference electrode is used in measuring the working electrode potential. A Reference Electrode should have a constant electrochemical potential as long as no current flows through it. The reference electrode acts as reference in measuring and controlling the working electrode's potential.

Figure 1.8: Ag/Agcl reference electrode.

Function of R.F: The most common lab Reference Electrodes are the Saturated Calomel Electrode (SCE) and the Silver/Silver Chloride (Ag/AgCl) electrodes.

Counter electrode:

The Counter, or Auxiliary, Electrode is a conductor that completes the cell circuit. The Counter Electrode in lab cells is generally an inert conductor like platinum or graphite. In field probes, it's generally another piece of the Working Electrode material. The current that flows into the solution via the Working Electrode leaves the solution via the Counter Electrode. The auxiliary electrode passes all the current needed to balance the current observed at the working electrode. Auxiliary electrodes are often fabricated from electrochemically inert materials such as gold, platinum, or carbon.

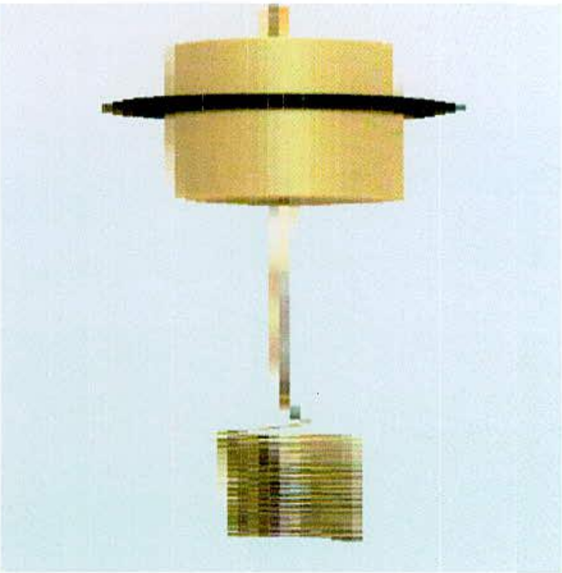

Figure 1.9: Platinum electrode.

Function of C.E: Remove the solution resistance and measure only the electrode potential

Potentiostat

The instrument used to control the potential difference applied across the electrochemical cell is called a potentiostat. A potentiostat adjusts the voltage difference between the anode and the cathode in order to maintain a constant working electrode potential [79]. A potential is applied to the working electrode, resulting in a flow of charge towards the counter electrode. A potential drop (iR) is caused by the electrolyte conductivity, the distance between the electrodes, the magnitude of the current and resistance across the electrode material. If the iR drop is uncompensated, the reaction will no longer operate at the desired potential, and the reaction may cease. The reference electrode monitors the potential at the working electrode and feeds the value back to the opamp. If a difference in potential is observed between the RE and WE, the potential applied to the CE is altered to compensate. A second op-amp is used as a current-voltage converter to measure the flow of current, with a resistor used to output the voltage per unit current.

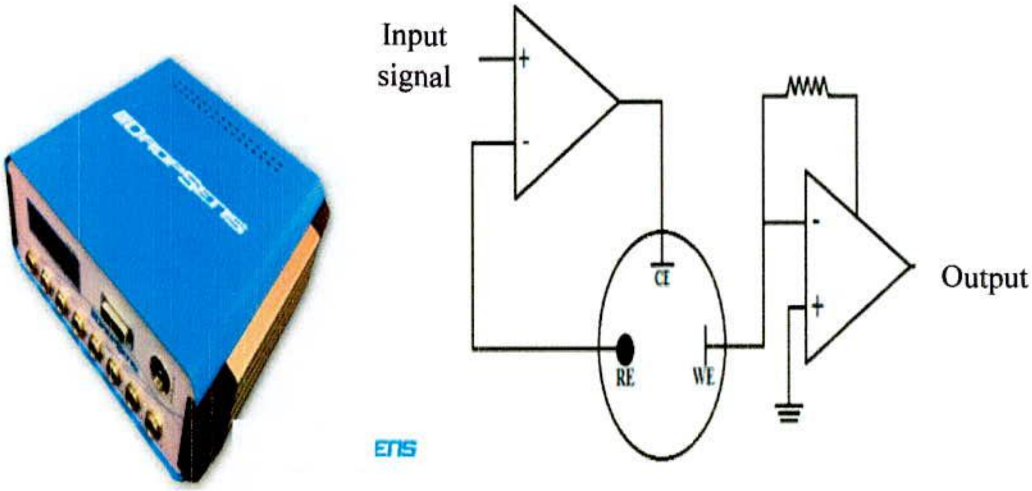

Figure 1.10: A potentiostat with circuit diagram of a three-electrode system.

1.9.3 Cyclic voltammetry (CV)

Cyclic voltammetry is a commonly used and versatile potential dynamic electro analytical technique used to study redox systems, the reversibility of the reaction, the stoichiometry of a system and the diffusion coefficient of an analyte. These can be used to determine the electrochemical characteristics and identity of an unknown compound.

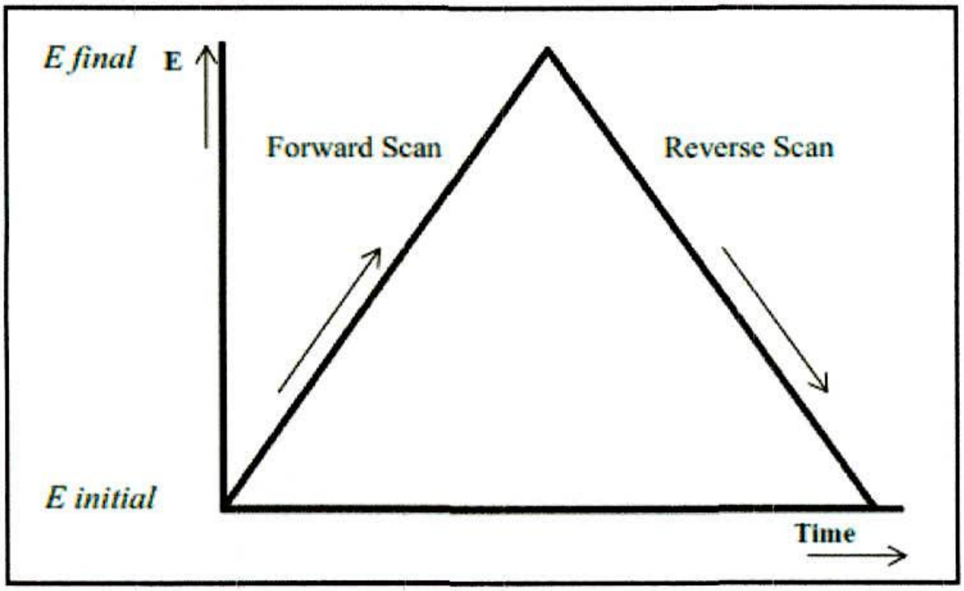

Figure 1.11: Cyclic voltammetry input waveform.

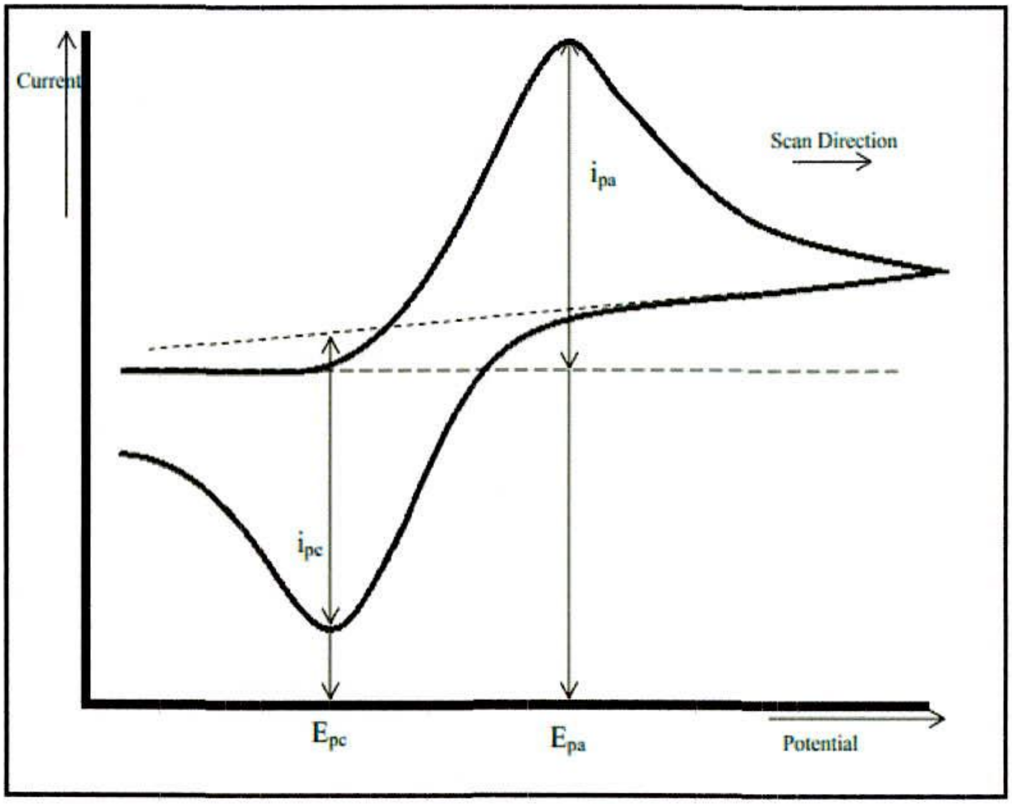

Figure 1.12: Typical Cyclic voltammogram response for a reversible redox couple.

Cyclic voltammograms are characterized by six important parameters.

- The cathodic (E_{pc}) and anodic (E_{pa}) peak potentials
- The cathodic (i_{pc}) and anodic (i_{pa}) peak currents
- The cathodic half-peak potential ($E_{p/2}$)
- The half wave potential ($E_{1/2}$)

Cyclic voltammetry linearly applies a triangular potential ramp to the working electrode at a defined scan rate until it has reached a set switching potential as shown in Figure 1.11. Once the switching potential on the triangular excitation potential ramp is reached, it begins a scan in the reverse direction. During the potential sweep, the current is measured resulting from the potential applied. The resulting plot of current vs. potential is known as a cyclic voltammogram, as illustrated in Figure 1.12.

The two peak currents (i_{pc}/i_{pa}) and two peak potentials (E_{pc}/E_{pa}) form the basis for the analysis of the cyclic voltammetric response to the analyte. The shape of the voltammogram is due to the concentration of the reactant (R) or product (P) at the electrode surface during the scan. Ideally, the scan begins at a potential of negligible current flow whereby the analyte is neither oxidized nor reduced. As the potential is ramped linearly, electron transfer between the electrode and the analyte in the solution begins to occur; this leads to an accumulation of product and a depletion of the reactant. The ramp increases in accordance to the Nernst Equation.

$$E = E^{0} + \frac{0.059}{n} log\frac{[OX]}{[Red]}$$

Where E is the applied potential and $E^{0'}$the formal potential; [OX] and [Red] represent surface concentrations at the electrode solution interface, not bulk solution concentrations. Note that the Nernst equation may or may not be obeyed depending on the system or on the experimental conditions.

At the peak of the anodic wave the reaction becomes diffusion controlled, as the diffusion layer has grown sufficiently from the electrode that the flux of the product to the electrode is too slow to satisfy the Nernst equation. As a result, the concentrate ion of the reactant at the surface reaches zero. Subsequently, the rate of diffusion then decreases, reducing the current flow, in accordance with the Cottrell equation. Once the potential ramp has reached the switching potential, the potential is ramped in the opposite direction resulting in a cathodic potential being applied.

The peak current for a reversible system is described by the Randles-Sevcik equation [80]. The current is directly proportional to the concentration and increases in respect to the square root of the scan rate. This dependence on scan rate implies the reaction at the electrode is controlled by mass transport. The equation applies at standard temperatures. (25°C, n = number of electrons involved, A = electrode area, D = diffusion coefficient, C_B= bulk electrode concentration and v = scan rate).

$$i_p = (2.69 \cdot 10^5)\, n^{\frac{3}{2}}\, AD^{\frac{3}{2}}\, C_B v^{\frac{1}{2}}$$

The reversibility of a electrochemically reversible couple can be identified by the measurement of the potential difference the two peak potentials. An electrochemically reversible system based on a one electron transfer process is denoted in equation A fast one electron transfer exhibits a ΔEp of 59 mV.

$$\Delta E_p = E_{pa} - E_{pc} = \frac{59}{n}\ \mathrm{mV}$$

1.9.4 Amperometry

A fixed potential is applied to an electrode against a reference electrode (Figure 1.13) until a steady state current is generated. This is achieved more readily in a stirred solution due to the greater efficiency of mass transport. Stirring also ensures that the concentration gradient at the working electrode is constant. Once steady state is achieved, standard additions of the analyte of interest are added into the voltammetric cell. The additions result in increases in current, with each addition occurring after steady state has been achieved, the magnitude of the current is proportional to the concentration of the analyte, which in turn is proportional to the rate of the redox reaction at the working electrode surface. An example of a typical amperometric plot is shown in Figure 1.13 (b).

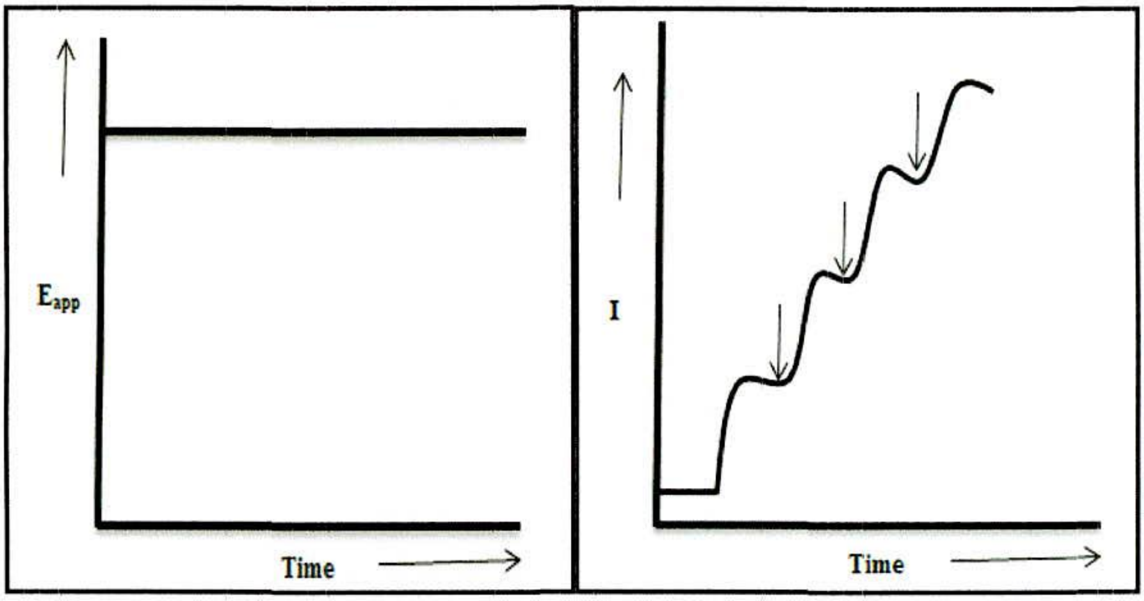

Figure 1.13: (a) Current waveform for amperometric experiments. (b) A typical amperometric plot in stirred solution. Arrows indicate additions of the target analyte.

1.9.5 Chronoamperometry

Chronoamperometry differs from amperometry by being conducted in a quiescent, unstirred solution. A sufficient E_{app} is applied to drive a redox reaction at the surface (Figure 1.16 a); this generates a large current which decays rapidly as the concentration of the analyte is depleted at the electrode surface due to the diffusion Figure 1.14 (b).

The variation in the magnitude of current with time for a planar electrode is described by the Cottrell equation [81], which is derived from Fick's second law. The equation is described as follows; represents the number of electrons, F is the Faraday constant, A is the electrode area (cm^2), C^o is the bulk electrolyte concentration (mol/cm^3), t is time (seconds) and D is the diffusion coefficient (cm^2/s).

$$i_t = \frac{nFAC^0D^{\frac{1}{2}}}{\pi^{\frac{1}{2}}\, t^{\frac{1}{2}}}$$

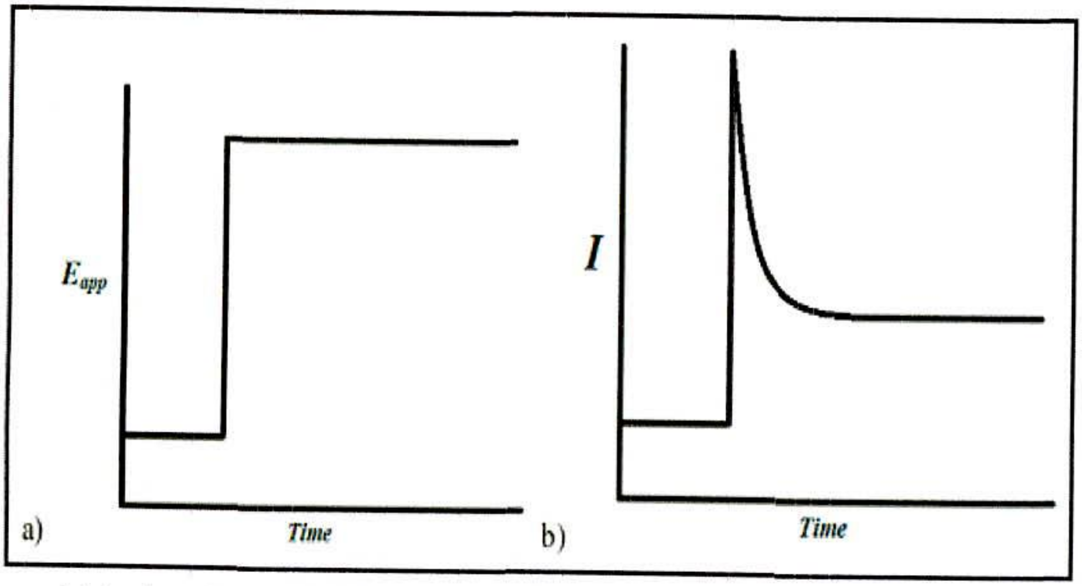

Figure 1.14: a) excitation waveform b) current response output generated from the excitation waveform.

1.9.6 Sensor characterization

1.9.6.1 Scanning electron microscopy (SEM)

The scanning electron microscope (SEM) is a powerful and frequently used instrument, in both academia and industry, to study, for example, surface topography, composition, crystallography and properties on a local scale. The spatial resolution is better than that of the optical microscope although not quite as good as for the transmission electron microscope (TEM). The SEM has an extremely large depth of focus and is therefore well suited for topographic imaging.

Besides surface topographic studies the SEM can also be used for determining the chemical composition of a material, its fluorescent properties and the formation of magnetic domains and so on. The specimen is bombarded by a convergent electron beam, which is scanned across the surface. This electron beam generates a number of different types of signals, which are emitted from the area of the specimen where the electron beam is impinging Figure 1.15.

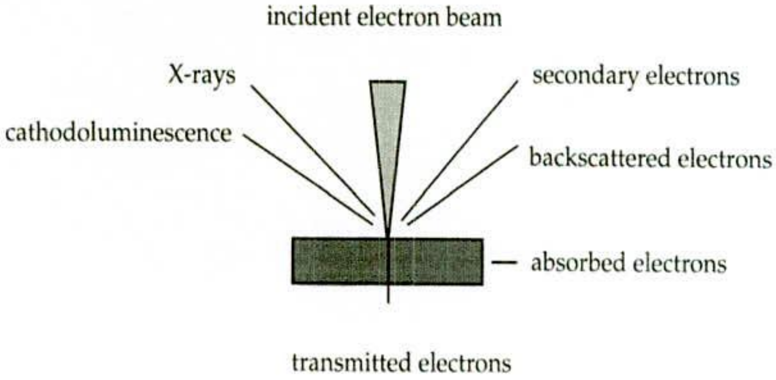

Figure 1.15: Example of some of the different types of signals produced when high-energy electron impinges on a material.

The induced signals are detected and the intensity of one of the signals (at a time) is amplified and used to as the intensity of a pixel on the image on the computer screen. The electron beam then moves to next position on the sample and the detected intensity gives the intensity in the second pixel and so on.

The working principle of the SEM is shown in Figure 1.16. For improved signal-to-noise ratio in the image, one can use a slower scan speed. This means that the electron beam stays a longer time at one position on the sample surface before moving to the next. This gives a higher detected signal and increased signal-to-noise ratio [81].

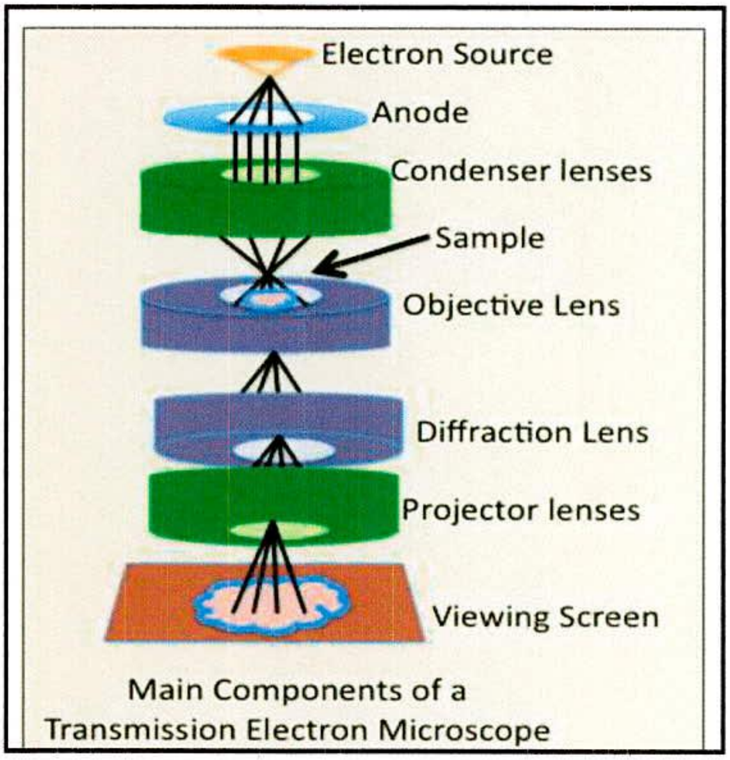

Figure 1.16: Schematic diagram of a SEM.

1.9.6.2 X-ray diffraction (XRD)

X-ray diffraction (XRD) relies on the dual wave/particle nature of X-rays to obtain information about the structure of crystalline materials. A primary use of the technique is the identification and characterization of compounds based on their diffraction pattern. The dominant effect that occurs when an incident beam of monochromatic X-rays interacts with a target material is scattering of those X-rays from atoms within the target material. In materials with regular structure (i.e. crystalline), the scattered X-rays undergo constructive and destructive interference. This is the process of diffraction. The diffraction of X-rays by crystals is described by Bragg's Law, n(lambda) = 2d sin(theta). The directions of possible diffractions depend on the size and shape of the unit cell of the material. The intensities of the diffracted waves depend on the kind and arrangement of atoms in the crystal structure.

However, most materials are not single crystals, but are composed of many tiny crystallites in all possible orientations called a polycrystalline aggregate or powder. When a powder with randomly oriented crystallites is placed in an X-ray beam, the beam will see all possible interatomic planes. If the experimental angle is systematically changed, all possible diffraction peaks from the powder will be detected. The parafocusing (or Bragg-Brentano) diffractometer is the most common geometry for diffraction instruments. This geometry offers the advantages of high resolution and high beam intensity analysis at the cost of very precise alignment requirements and carefully prepared samples. Additionally, this geometry requires that the source-to-sample distance be constant and equal to the sample-to-detector distance. Alignment errors often lead to difficulties in phase identification and improper quantification.

1.13 Objectives of the present work

The objectives of this research are to develop a chlorine sensor and also to study the sensitivity, selectivity, stability of this sensor using various electrochemical techniques.

The specific aims are:

i) Optimization of different grade pencil electrodes for the best electrochemical response,

ii) Fabrication of pencil drawn paper electrode,

iii) Synthesis of Zn, Cu nanoparticles using hydrothermal method and Ni nanoparticles using sol- gel method,

iv) Characterization of pencil electrode using SEM-EDX,

v) Perform detail analytical studies on sensitivity, selectivity, linearity and stability of the sensor platform,

CHAPTER II

LITERATURE REVIEW

CHAPTER II

Literature review

2.1 Introduction

A sensor is a device that detects and responds to some type of input from the physical environment. The specific input could be light, heat, motion, moisture, pressure, or any one of a great number of other environmental phenomena. The output is generally a signal that is converted to human-readable display at the sensor location or transmitted electronically over a network for reading or further processing. A sensor is a device that detects and responds to some type of input from the physical environment. The specific input could be light, heat, motion, moisture, pressure, or any one of a great number of other environmental phenomena. The output is generally a signal that is converted to human-readable display at the sensor location or transmitted electronically over a network for reading or further processing.

The aim of this present study was to investigate the possibility of using pencil electrode and pencil drawn electrode for the anodic cyclic voltammetric and ampromatery determination of trace concentrations of chlorine. The voltammetric behavior of chlorine at the electrode was first investigated by cyclic voltammetry. The optimum conditions required for the cyclic voltammetric and ampromatery determination of chlorine were then identified and finally the possibility of determining chlorine in environmental surface waters was explored. This allows for an extremely facile, green, low-cost, rapid method to fabricate electrode prototypes, as illustrated in Table 2.1 and Table 2.3.

A number of different carbon based materials and technologies have been commonly utilized such as glassy carbon [82], carbon paste [83] or screen-printed [84]. All of these approaches require some degree of expertise, preparation and care. As a result, a number of alternative sources for the manufacture of carbon electrodes have been investigated, such as the utilization of pencil leads as electrodes [85-92]. The pencil "lead" is actually a mixture of graphite, wax and clay, the proportions of which impart different properties to the pencil with increasing amounts of clay making the pencil harder, hence the designation 'H'. Increasing the levels of graphite make the pencil softer, and their marks darker or black and so the designation 'B'. For example, one of the darkest generally available pencils, the 6B is 84 % graphite, 10 % clay and 5 % wax, compared to the common, lighter HB, or No. 2 pencil which has a composition of 68 % graphite, 26 % clay and 5 % wax [93]. Commonly, pencils have been used directly as the working electrode itself [85-92], only recently has the possibility of using pencils to draw electrodes and other electronic components onto a suitable substrate been reported [93]. This latter approach has a number of advantages, as designs and geometries can be easily altered as they are simply drawn by hand on to a suitable substrate. This latter approach has a number of advantages, as designs and geometries can be easily altered as they are simply drawn by hand on to a suitable substrate.

A number of different electrochemical applications including resistors, capacitors, UV and piezoresistive sensors, microfluidic devices, chemiresistive sensors, biosensors, batteries and field effect transistors have been reported [94]. However, there have only been a small number of reports on the application of these devices using voltammetry and until now its application has been generally only in the characterization of the electrode [95].

Hence, some of these pollutants have been regulated by national and international environment protection agencies and their allowable limits have been defined. No doubt, last few decades have witnessed great progress in the instrumentation for analysis of a variety of inorganic and organic pollutants but the high cost of these sophisticated instruments itself a big challenge in low resource setups. In addition to cost, these instruments are not suitable for in field applications. Simple, low-cost, efficient, portable and commercially available instruments or electrochemical sensors are desired to fulfill the demand of worldwide monitoring of pollutants in different matrices [96]. Because of their simplicity, low-cost, large-scale availability, GPE based electrochemical sensors can be a good choice for environmental analysis in limited resource setups. In the recent years, there has been observed an increasing trend for the applications of GPE based electrodes in the environmental analysis. To the best of our knowledge, this is the first review article that covers environmental analytical applications of GPE.

Hence, this review gives brief description of

(i) highly toxic pollutants from inorganic and organic origin.

(ii) applications of GPE based electrochemical sensors for detection of these pollutants in environmental matrices.

(ii) properties of the GPE and modified GPEs which enhance the sensitivity and selectivity of the electrode toward particular target species.

2.2 Electrochemical analysis of inorganic pollutants at graphite pencil electrode

The presence of metals, metalloids and other inorganic pollutants in environmental matrices is the most important environmental problem of the modern age. Both natural and anthropogenic sources contribute significant amounts of inorganic pollutants in the environment. In the coming sections, we enlist and highlight the importance of studying inorganic pollutants in the environment. The applications and potential of GPE based sensors for detection of inorganic pollutants are critically discussed in each section.

2.2.1 Metallic pollutants

Heavy metal pollution is a global environmental challenge. Heavy metals are distributed in the environment as a result of their increased applications in domestic, industrial and agricultural processes. The heavy metals such as mercury, lead, cadmium, chromium and arsenic are considered priority pollutants and have been regulated by a number of national and international environmental agencies. Metallic pollutants are recognized as "systematic pollutants" and they cause various ailments in the human body including damage to different organs. Some metals have been classified as carcinogens (well known or suspected) by USEPA [97]. Metal toxicity to an individual depends on upon many factors such as kind of exposure (environmental or occupational), dose and period of exposure and nature of the metal itself. However, it is obvious that continuous exposure to low-level concentrations of these pollutants may lead to serious health implications. From the polluted environment, these metals may enter to food chain and thus to the bodies of humans, other animals, and aquatic organisms. Therefore, sensitive analytical methods are needed to measure the levels of heavy metals in the environment. In order to deal with a global environmental challenge posed by heavy metal pollution, simple, efficient, portable and cost effective analytical methods are always desired. The last few decades have witnessed some major advancement in analytical instrumentation for analysis of heavy metals. The instruments like atomic absorption spectroscopy (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS) have been widely used for determination of metals in different matrices. Although, these instruments are highly sensitive and selective, but some major issues are associated with them. They are very expensive, require a number of consumables, and their portability for in field applications is a big challenge. Before the analysis, extended procedures of sample preparation are needed. Moreover, analysis is destructive, time-consuming and expert operators are required. Electrochemical methods for detection of heavy metals are simple, efficient, cost-effective, non-destructive and do not require extended sample preparation procedures for common environmental matrices such as water and soil. Electrochemical methods can provide adequate sensitivity and selectivity for detection of metals. In addition, these methods can be used for speciation and multi-elemental analysis. GPE based electrodes are very cheap and easily available and can be used to perform cost-effective analysis in limited resources setups. Hence, in the following paragraphs, we described some applications of GPE and modified-GPE for sensing of metals in environmental samples. GPE based electrodes for detection of inorganic analytes in different environmental matrices.

Wee Ling et al. [98] was studied different anthropogenic activities result in accumulation of cadmium in soil. This cadmium is then transferred to the human body through consumption of contaminated plants and food. The other sources may include smoking and occupational exposure. The fever and muscle ache that leads to respiratory problems and damage to kidneys. Exposure to cadmium has also been linked with lung and prostate cancer. So its environmental monitoring with simple, cheap and efficient methods is desired. An ISE was fabricated by electro polymerization of 4-vinyl pyridine on 2B pencil graphite as ionosphere for Cd^{2+}. The electrode gave good limit of detection (LOD) down to 2.5×10^{-8} M and relatively a broad pH range 4.0–7.5 for cadmium measurement. The major advantages of this modification are cheap material, easy modification and potential to apply in in situ measurements of cadmium in real samples. The developed electrode was used to determine Cd^{2+} by using the potentiometric method in different environmental (soil), food (tea, vegetables) and biological (cow liver, kidney, heart and chicken liver and heart) samples.

Pokpas et al. [99] also suggest zinc and different compounds derived from it are extensively used in industrial processes such as alloy formation, wood treatment, electroplating, rubber processing, dye, pharmaceutical products, and paints. In medical, zinc is used in mouthwashes, disinfectants, antiseptics and mineral–vitamin formations because of its biocidal activity. It is a micronutrient whose small quantiles are essential to the human body, but its high concentrations can cause different ailments including vomiting, fever, pulmonary problems, nausea, and renal disorders. Therefore, highly efficient, cheap and simple methods are needed to monitor the zinc contents in the environmental and biological samples. A potentiometric ion-selective electrode for zinc was fabricated by modification of GPE with polypyrrole nano-film. Polypyrrole was electrochemically deposited on GPE in the presence of tartrazine as a dopant. This electrode showed analytical features such as wide linear range, high selectivity and good stability and reasonably low limit of detection of 8.0 mM at optimum pH. Applications of the modified electrodes were extended for testing of Zn^{2+} in barley flakes, rice and dry milk. There are only few reports which describe simultaneous detection of more than one metal ions on modified GPEs. The nafion grapheme Nano composite pencil graphite bismuth film electrode (NG-PG BiE) was used for simultaneous detection of $Pb^{2+,}$ Cd^{2+} and Zn^{2+} ions in tap water using square wave anodic stripping voltammetry.

2.2.2 Comparative analysis of different technique

Here it will be suitable to compare the GPE based electrochemical methods and other instrumental methods which are used for detection of metallic impurities in environmental samples. Flame atomic absorption spectrometry (FAAS) is one of the major technique used for determination of metals such as cadmium, lead, zinc and copper in different matrices. The disadvantages that are associated with FAAS are mainly related to matrix interference on the analyte signal and higher limit of detections than the normal concentration of metals present in real samples. Thus, removal of interferences and improvement in LOD is achieved through separation and preconcentration of the metals prior to analysis [100]. This kind of sample preparation itself is a tedious job because multistep processes are involved that may comprise reaction (derivatization), extraction, separation, preconcentration and determination. Moreover, the accuracy and reliability of sample preparation assisted FAAS depends upon the number of steps involved. It is matter of fact that these sample pretreatment methods have made trace element assays more convenient, but more often they require large sample or reagent volumes (several tens to hundreds of milliliters) to get high enrichment factors or preconcentration factors. However, when it is difficult to obtain such large sample volumes, these techniques led to low enrichment factors and high detection limits [101]. When dealing with trace elements, total content analyses as well as speciation analysis both are very important. Trace metals exist in different oxidation states and some metal species have been reported to show varying toxicities in their different oxidation states, for example, hexavalent chromium is much more toxic than trivalent chromium [102]. In order to find out the role of trace element species in different diseases, recent research in area of trace element analysis is shifting from total content to speciation analysis. Hence, very efficient extraction methods that would be capable of selective speciation and then highly selective detectors are desired in analytical instruments [103]. In general, no specialized sample preparation is necessary for electrochemical methods. In addition to that electrochemical methods are cost and time effective. For the trace elemental analysis, the speciation can be easily performed by simply altering the detection potential. Moreover, very low LODs can be achieved by electrochemical methods without performing any sample preconcentration. When particularly talking about graphite pencil electrodes, they are easily available in the market and cost much lesser than other carbon or metal-based electrodes. However, the automation of GPE based electrochemical methods presents a challenge that need to be addressed for successful commercialization of such analytical tools.

Table 2.1: Comparison of GPE based sensors with other instrumental methods

Metal	**Matrix**	**Sample preparation**	**Detection method**	**LOD**	**Ref.**
Cadmium	Water	Pyrolysis vapor generation	Spectroscopic	2.2 ng mL^{-1}	103
Lead	Water and human body	Magnetic ion-imprinted Polymer(MIIP) nanoparticles	Spectroscopic	2.4 ng L^{-1}	104
Copper	Water, food and hair samples	Switchable solvent-based liquid phase microextraction	Spectroscopic	1.8 ng mL^{-1}	105
Zinc	Water samples Temperature-assisted	dispersive liquid–liquid microextraction	Spectroscopic	0.89 ng L^{-1}	106

2.3 Inorganic pollutants

In addition to the metals, other substances such as hydrazine, hydrogen peroxide, sulfides, cyanide, perchlorate, oxides of nitrogen and sulfur have a great environmental impact as inorganic pollutants. Few of these have been electrochemically detected in different environmental matrices using GP based electrodes. A brief account of these pollutants is provided in coming sections.

Abdul Aziz el al. [107] examined hydrazine is an inorganic molecule with molecular weight 38 g mol^{-1} Hydrazine is used in many industrial applications such as pesticide in agriculture, starting material for many chemicals and plastics, intermediate in pharmaceutical and corrosion control agent in the treatment of water boilers. As it is lightweight, volatile and water-soluble colorless liquid, it has the ability to be absorbed by oral, dermal and inhalation pathways in the living organisms. Hydrazine has been identified as a highly toxic substance to the living organisms because its exposure may lead to serious damage to kidney, liver, lungs and central nervous systems. It also affects negatively the reproduction system and its carcinogenic effects have also been reported.

Hence, simple, low cost and reliable analytical methods are desired to monitor the environmental levels of hydrazine. Recently, AuNPs modified GPE was prepared by immersing the GPE in a test tube containing a solution of ascorbic acid and gold(III) chloride and heating it up to 75°C for 15 minutes in water bath. This modification procedure does not require any cross-linking molecules and describes direct attachment of AuNPs on GPE. This modified electrode was used for detection of N_2H_4 in drinking water, and excellent limit of quantification down to 100 nM and limit of detection 42 nM was achieved using square wave voltammetry as a mode of detection.

Erler et al. [108] Nitrite ion is an intermediate in the nitrogen cycle. It results from oxidation of ammonia or reduction of nitrate. It is used as a food preservative in the food industry. It is hazardous because it combines with blood pigments to produce meta-hemoglobin, which in turn, depletes oxygen in tissues. It forms carcinogenic N-nitrosamines upon reaction with secondary amines. A polymer nanocomposite modified GPE was formed by single step electro polymerization of poly (vinyl ferrocene) (PVF) in the presence of MWCNTs. The resulting modified electrode was termed as poly(vinyl ferrocenium) (PVF+)/MWCNTS/GPE. The electrochemical detection of nitrite was carried out in commercial mineral water using DPV, and a LOD of 0.1 mM was achieved.33 Similarly, in another work, pyronin y modified GPE was utilized for amperometric detection of nitrite and LOD of 0.5 mM was achieved.

2.4 Electrochemical analysis of organic pollutants at GPE

The rapid industrialization in the modern era resulted in the release of the toxic organic pollutants into the environment. Although most of the industries try to detoxify or degrade these organic pollutants by physical, chemical or biological means but still significant amounts of these pollutants are released into the environment. Organic pollutants are famous by different names including "emerging organic pollutants", "priority pollutants", "endocrine disrupting compounds" [109]. A continuous monitoring of these hazardous chemicals is necessary to take precautionary measures for sustainability of the healthy environment. In the coming section, we discuss the applications of GPE based sensors for detection of different classes of organic pollutants in environmental matrices.

2.4.1 Phenolic compounds

Phenolic compounds are very common and abundant environmental pollutants. Most of the phenolic compounds induce very severe health problems, and they have been classified as hazardous wastes and virulent pollutants by the United State Environmental Protection Agency. They adversely affect human health by accelerating weight loss and weariness. Phenols are reported to cause respiratory cancer, cardiac and immune system disorders in case of severe exposures. The applications of GP based electrodes for high-sensitivity detections of different phenols are given in following sections.

Rana et al. [110] was recently studied, a very simple method for the determination of phenol was developed. In this method, GPE surface was charged by pretreating in NaOH solution. The charged GPE showed the capability of self-electro polymerization of phenol on its surface, and this led to the sensitive detection of phenol. The self-electro polymerized phenol was detected using square wave voltammetry. Very low limit of detection (4.17 nM) was achieved using this pre-charged sensor.

Yang et al. [111] the simplest phenol derivative, 4-nitophenol, is a major threat to the environment. 4-Nitrophenol is widely used in the fungicides, pesticides, and organic dyes and also in some pharmaceutical products. Eventually, as a result of excessive use of these products, the 4-nitrophenol is released into the environment. It has good solubility in water, and it does not degrade easily into other components, which in turn make it more persistent in the ground water. In addition, it is considered as carcinogenic and poses a serious threat to the human health.

Kawde et al. [112] introduced a single step modification of GPE by electrochemical reduction of the Cu(II) on its surface. The Cu is more cost effective compared to Au, Ag, and Pt. The amperometric based electrochemical method was developed for the determination of 4-nitophenol and the wide linear range was obtained from 50 to 850 mM with LOD of 1.91 mM. The presence of potential interferences like phenol, 4-amino phenol and 3,4-dichlorophenol showed no effect on the detection of 4-nitopheneol. In another method, the pencil graphite electrode was modified by the bismuth film and was used for the simultaneous analysis of 2-nitrophenol and 4-nitrophenol. Differential pulse voltammetry was used for the electrochemical analysis of 2-nitrophenol and 4-nitrophenol. Bismuth film electrodes are considered as alternate of the mercury film electrode due to environmental friendliness.

Alkyl phenols added into the environment due to the degradation of alkyl phenol polyethoxylates which is used in the detergent formation, fuel additive, fire retardant, antioxidant, thermoplastic elastomers, and phenolic resins.

2.4.2 Organic dyes

A lot of dyes are released into the water as a residue during the coloring process in the textile industry and severely pollutes the environment. In dying process, almost 10 to 15% and in some conditions, up to 50% dyes are wasted and released to the aquatic bodies. Azo dyes are the commonly used dyes and contain azo group (–N=N–). The presence of azo dyes in the water has adverse effect on the aquatic life and the human health [113].

One of the azo dye is chrysoidine that has brownish red color. Chrysoidine is used in the dying of leather, plastic, natural and synthetic fiber, cosmetics, foodstuff, waxes, and papers. In the case of inhalation, dermal exposure, and oral ingestion, it induces acute and chronic toxicity. Hence its use in foodstuff has been banned. Moreover, it is also considered as carcinogenic. In some countries, it is being used to disinfect the fish skin. Hence, monitoring of its environmental concentrations is highly important from health and safety perspective.

Sulfanilamide is used in the synthesis of azo dye. Sulfanilamide is the first chemically synthesized antimicrobial agent, and it is also utilized in some drugs for the treatment of throat, urinary tract, and vaginal infections. Sulfanilamide is also found in the animal products and at high concentrations, it has been reported to induce allergic effects. Moreover, it is also toxic to the aquatic animals. Molecularly imprinted polymer (MIP) based electrochemical methods are highly selective and sensitive for the determination of the relevant analyte. In molecularly imprinted polymer based electrochemical methods, the complementary cavities are formed for the analyte, which makes it highly selective towards the target analyte. These cavities are formed by initiating the polymerization in the presence of the analyte and afterward the analyte is extracted out. Molecular imprinted polymer based graphite pencil electrode was prepared from the electro polymerization of the pyrrole for determination of sulfanilamide modified GPE [143].

2.4.3 Pesticides

Uygun et al. [115] represent a broad class of chemicals which is further divided into insecticides, herbicides, fungicides, nematicides, molluscicides, and rodenticides. No doubt, pesticides have played a major role in increasing food production worldwide but at the same time, their excessive use has led to serious health threats to the human and wildlife. The excessive use of pesticides results in their accumulation in food, soil, and water. It is reported that almost one million people die or suffer per year from chronic diseases resulted from exposure to pesticides. Chlorpyrifos is the commonly used organophosphate pesticide, and its exposure can initiate disorders in neurological and the autoimmune system. A method has been developed for the trace level determination of the chlorpyrifos by fabrication the molecular imprinted polymer modified pencil graphite electrode. The impedance technique has been used for the measurement of the chlorpyrifos in the soil, tape water and corn leaves. The impedance increases as the concentration of the chlorpyrifos increases in the sample and wide linear range was observed from 20 to 300 mg L^{-1} I detection limit of 4.5 mg L^{-1}.

2.4.4 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants. PAHs have been added into the environment by human activities and also by natural incidents like volcanic eruption, forest fires, burning of fossils and the petroleum fuel. These compounds are considered highly toxic due to their mutagenic and carcinogenic behavior. The PAHs carcinogenicity has been related to the number of benzene rings in their structures and their transformation into the reactive electrophilic intermediates metabolites. The mammalian converts these compounds into the detoxification products in the liver and these metabolized products when excreted are more genotoxic and reactive. The PAH metabolites are responsible for carcinogenic process by covalently binding to the cellular DNA. These aromatic compounds become activated in the presence of sunlight and cellular destruction may start if skin comes into contact with these compounds in the presence of light.

7,12-Dimethylbenz[a]anthracene.

One of the PAHs is 7,12-dimethylbenze[a]anthracene (DMBA) that has been widely investigated for its biological activity. Because of its potential toxic and carcinogenic effects, many methods have been developed for its trace level quantitation. Yardım et al. [116] developed a highly sensitive method based on the disposable pencil graphite electrode and also studied the interaction of DNA and DMBA. The adsorptive stripping voltammetry was used for the low level determination of DMBA and an LOD of 0.194 nM was achieved.

Benzo[a]pyrene. One of the potent carcinogenic

polyaromatic compounds is benzo[a]pyrene (BaP). The BaP is found in coke plants, steel foundries, and aluminum plants. The places where the coal fires is used for heating or cooking normally contains a higher concentration of BaP. The human exposure to the BaP occurs through water, foodstuff, and the air. Like other PAHs, BaP is hydrophobic compound and accumulate in the liver, kidney and the fat tissue. The mammalian metabolizes BaP with the help of epoxide hydrolase and cytochrome P450-dependent monooxygenase to primary and secondary metabolites like phenols, diols, quinones, water soluble conjugates and arene oxide. BaP concentration in the environment is correlated to the total content of the PAHs. The adsorptive stripping square wave voltammetric based method was established by Keskin et al. [117] using pretreated pencil graphite electrode for the determination of BaP. A small linear range has been observed from 0.25 to 1.25 mM with low detection limit 0.027 mM. The developed method was applied to find out the spiked BaP from the urine and satisfactory values for recoveries were obtained.

2.5 A brief overview of materials used for modification of GPE

In order to enhance their electrocatalytic activity toward certain inorganic or organic analytes, GP based electrodes were modified with different materials. These modify cation materials include metal nanoparticles, conducting polymers and carbon based materials. In the following sections, we have discussed modify cation materials and their potential to increase electrocatalytic activity.

2.5.1 Nanoparticles

Nanoparticles (NPs) are characterized by their better physical, chemical, and electronic features than their bulk counterparts. It is the nature of the material used for synthesis that determines properties of NPs. Commonly; NPs are synthesized by chemical reduction of metal salts in the presence of a stabilizer. Stabilizers are attached to the surface of NPs and play a major role in defining their charge, solubility, and stability. NPs are extremely sensitive to the changes occurring on their surface. NPs modified electrodes may result in enhanced electron kinetics due to following

(i) high surface area.

(ii) enhanced mass transport rates.

(iii) better control of NP surface.

(iv) functionalization of NPs with selective groups.

Recently, metal NPs (MNPs) have drawn significant attention in electrocatalysis because of their unique chemical and electronic conduct. The analytes that show sluggish electron kinetics at bare electrodes were reported to have reasonably enhanced electrocatalytic activity at MNP's modified electrodes. In addition, MNPs modified electrodes showed good peak separations for compounds having very close oxidation potentials. Recently, the very simple procedure was adopted for immobilization of Au NPs on GPE for detection of hydrazine. The beauty of this modification is in the process that involves direct attachment of Au NPs on the surface of GPE without the use of any cross-linkers. Similarly, in another report, platinum modified GPE was synthesized by one-step reaction that involves reduction of metal salt while dipping the GPE in reaction media [118]. There are some examples where MNPs are used in combination with other materials such as CNTs. Such combinations are thought to contribute to sensing properties of electrodes through synergistic effect.

2.5.2 Conducting polymers

Different types of polymers are used in electrochemical sensing applications. Overall, these polymers can be categorized into four classes i.e. electroactive, polyelectrolyte, coordinating and biological polymers. The selection of a certain polymer for electrode modification is mainly dictated by the nature of the target analytes. The electrodes can be modified with polymers using different strategies. The frequently used methods include spin coating, dip coating, and electropolymerization. Conducting polymers allow the incorporation of counter ions or other functional moieties into the structure of polymers. In this way, modified electrodes can be made selective against target analytes. Their electronic properties have similarity with metals. The polymer coated electrodes can help in avoiding interferences because selective coating materials will distinguish between the analyte and other species via hydrophilic and hydrophobic interactions, electrostatic affinities and ion exchange capabilities. The use of Ponceau 4R azo dye doped polypyrrole modified GPE for selective potentiometric determination of Cu^{2+} in water is an example of the polymer based ion selective electrode. In this work, the pyrrole monomers were electrochemically polymerized on GPE in the presence of Ponceau 4R azo dye as a dopant. The application of dopant in electropolymerization generates selective recognition sites in the coating which interact selectively with Cu^{2+} ions. Similarly, there are numerous other examples where polymer modified GPEs were employed as ISE for analysis of different metals [98, 119]. MIPs and polymer nanocomposites are also used for modification of GPE for different sensing applications.

2.6 Pretreatment

This is a very common method to enhance the sensitivity of the carbon-based electrodes. This method is widely being used for increasing the sensitivity of the graphite pencil electrode. During electrochemical pretreatment, the electrode surface was cleaned, and some oxygen-containing groups also formed on the surface of GPE. The enhancement of the current could be attributed to the formation of oxygen containing functional groups like carboxylic acid, phenolic, and carbonyl. In this process might be some graphite oxide films are also formed on the GPR surface which is also responsible for the current enhancement for the target analytes. Electrochemical pretreatment is attractive choice due to the following reasons [120].

Very simple,

- less time-consuming.
- no complex material required.
- more applicable compared other modification materials.

Table 2.2: Some recent applications of GPE electrodes

Analyte	**Fabrication**	**Analytical Technique**	**Reference**
4-Nitrophenol	pCu/GPE	Amperometry	[112]
Sulfanilamide	MIP/PPy/GPE	DPV	[114]
4-Nonylphenol, 4-octylphenol, 4-tert-octylphenol	PGPE	DPV	[121]
Benzo[a]pyrene	PGPE	ASWV	[117]
Thiourea	GPE	SWV	[122]
Chrysoidine	DNA/MWCNTs-PDDA/GPE	DPV	[123]
Bisphenol A	ETGPE	ASDPV	[124]
Sudan II	Ds-DNA/GPE	DPV	[119]
Bisphenol A	PANI/MWCNTs/GPE	Amperometry	[125]
Chlorine	PGE	CV, Amperometry	This work

For pretreatment, the number of electrolytes is being used like NaOH, the pretreated electrode was used for the determination of Sudan. Keskin et al. [119] pretreated the pencil electrode by dipping the electrode in DMSO/0.1M $LiClO_4$ mixture at +1.6 V for sensing of benzo[a]pyrene [117]. Recently, the surface of the graphite pencil electrode was charged by pretreating the GPE in NaOH solution. The charged surface was used for the self-electro polymerization of the phenol on the electrode surface for the sensitive sensing of phenol. It is a very simple method for the determination of phenol [110].

2.7 Comparison of GPE with other electrodes

Graphite pencil electrode is still an emerging electrode. There are few characteristics which make it unique over other carbon and metallic based electrodes and a good candidate for electrochemical sensing. This single use electrode has the advantage to make the surface foul free [112, 120]. Any electrode could be a single use electrode, but the cost is an issue. Glassy carbon electrode (GCE), Au and Pt disc electrode could not be employed as disposable electrodes due to high cost and their manufacturing style. Due to this reason, GCE, Au, and Pt disc electrodes are being used for multiple times are polishing the surface by number of different ways to get rid of adsorbed materials and to improve the electrochemical behavior [126–128]. GPE could be single used electrodes as the new surface is readily available are the measurement. However, screen printed electrode (SPE) is well established single use electrode, but it is costly compared to GPE. SPE surface cleaning is usually not required, but polishing could have some positive effect [129]. For carbon paste electrode (CPE) fabrication, a typical preparation is needed which is time consuming [130]. Control of sensing area is another attractive characteristic which is associated with GPE [120] and it is not much feasible for GCE, CPE and the SPEs where the active surface is provided by the supplier. In case of GPE the length of the extruded graphite pencil could be adjusted according to the requirement. Recently, the GPE was used for the sensing of a-naphthol. The response of the bare GPE towards a naphthol was compared with GCE, CPE, Au and Pt disc electrode and it was more sensitive [131]. Casting modification of GPE is difficult and challenging due to its elongated shape and small diameter compared to GCE, CPE, SPE, Au and Pt disc electrode.

Table 2.3: Comparison of different characteristics of GPE with other electrodes

Sr#	Characteristic	GPE	GCE	CPE	SPE	Au disc E	Pt disc E
1	Availability	Easily available	Easily available	Need preparation	Easily available	Easily available	Easily available
2	Cost	Very cheap	Costly	Average cost	Costly (due to single use)	Costly	Costly
3	Disposable	Yes	No	No	Yes	No	No
4	Control of sensing area	Yes	No	No	No	No	No
5	Sensitivity (a-naphthol)	Very good [131]	Good [131]	Good[131]	_	Low [131]	Low [131]
6	Casting modification	Difficult	Easy [126]	Easy [132]	Easy [133]	Easy [134]	Easy [134]

2.8 A brief overview of hand drawn pencil electrode

Michael et al. [135] was investigated comprehensive analysis of paper-based supercapacitors, P-SCs, that are produced utilizing commercially available pencils to draw an interdigitated design upon common household printing paper, developing cheap, green and reliable low profile electrical conductors and electrodes. The P-SCs are optimised in terms of the composition of pencil used, the number of layers and the analysis of single or double side interdigitated electrode designs; such a comprehensive study is seldom explored in previous literature. A full analysis of the physical and electrochemical properties of the pencil drawn electrodes has been performed, including the application of a new capacitive testing/evaluation circuit applied to charge/discharge measurements/analysis, which provides a revolutionary and unambiguous analysis of the capacitance of the fabricated electrodes; an easy to use experiment guide is presented.

Christopher et al. [136] was inspired by recent reports upon the utilisation of hand drawn pencil (PDEs) macroelectrodes we report the fabrication, characterization (physiochemical and electrochemical) and implementation (electrochemical sensing) of various PDEs drawn upon a flexible polyester substrate. Electrochemical characterisation reveals that there are no quantifiable electrochemical responses upon utilizing these PDEs with an electroactive analyte that requires an electrochemical oxidation step first, therefore the PDEs have been examined towards the electroactive redox probes hexaammineruthenium (III) chloride, potassium ferricyanide and ammonium iron (II) sulfate.

Table 2.4: Recent applications of pencil drawn electrodes

Analyte	Fabrication	Analytical Technique	Ref.
Glucose	Pencil drawn working electrode with Ag ink pseudo reference/counter electrode.	Glucose biosensor. Working electrode modified with the mediator; 4-aminophenylboronic acid and glucose oxidase. Voltammetric determination of enzymatically generated 4-aminophenol	[137]
Ascorbic acid, dopamine and paracetamol	Pencil drawn dual electrode	Thin-layer chromatography used to separate compound mixtures. Co-eluting species could be identified using dual electrode redox system	[138]
p-nitrophenol	A 6B pencil drawn counter electrode	Counter electrode as part of a three electrode system with pencil working electrode	[139]
Toluene, THF, ethyl acetate, methanol, hexane to acetone	Chemiresistors hand-drawn on paper	Solvent vapour measured at pencil drawn Chemiresistor	[140]
Mechanical strain	Chemiresistors hand-drawn on paper	Output voltage for an HB pencil was found to be more sensitive than that for a 9B pencil	[141]
Cysteine and hydrogen	Doped pencil leads for drawing modified electrodes	Cysteine and hydrogen peroxide	[142]

2.9 Attractive features and challenges of graphite pencil electrodes

GPE possesses many attractive and unique features which make it an excellent choice for electrochemical sensing. These features are summarized below.

- A unique feature is the provision of the renewable surface after each measurement just like the dropping mercury electrode. This feature makes the electrode surface foul free. But in the case of most of other electrodes like GCE, after each globe due to the industrial revolution. This fact has already been indicated by a number of published reports [86]. Similarly, thousands of publications talk about analytical method development for analysis of inorganic and organic pollutants in water, food and biological samples. However, POC like devices that can readily analyze water or other environmental matrices for the presence of inorganics or organics is still not available to the public. Hence, there is very high stress on existing analytical technologies to meet the demands of low cost, availability, sensitivity and applicability in on field analysis. GPE based electrochemical devices can have the potential to serve in POC applications. Their low cost, easy availability and trait of disposability make them quite fit for POC based devices. Despite low cost and commercial availability of GP based electrodes, the commercialization of readily available GPE sensors for real life samples is still at some distance from the reality. Every lab prepared prototype cannot be brought to the market without considering its suitability for the desired assay, study of consumables and acceptability by the consumers. Although electrochemical methods involving modified GPE demonstrate a grade of maturity good enough to implement them in real analytical applications but up till now, their applications are limited to proof of concepts. For a proof of concept, different materials such as nanoparticles, conductive polymers, and carbon-based materials have been used to modify the surface of GPE. However, which of them can be employed for mass production of modified GPE for certain analysis will be dictated by cost, ease of synthesis and stability of the material over the electrode surface. Moreover, the integration of such modified electrodes into miniaturized systems is nothing less than a challenge as commercialization needs careful designing of the product that should be compatible with market demands. Thus, these challenges need to be addressed in future.

CHAPTER III

EXPERIMENTAL

CHAPTER III

Experimental Section

3.1 Materials

All chemicals and solvents are used in the synthetic and analytical works were of analytical grade obtained from E. Merck, Germany, British Drug House (BDH) of England and Sigma-Aldrich, India. The used chemicals were- Sodium thiosulfate, Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$), Sodium nitrate ($NaNO_3$), Sodium sulfate (Na_2SO_4) Sodium bicarbonate ($NaHCO_3$), Disodium carbonate (Na_2CO_3) and Sodium chloride (NaCl) were purchased from Sigma-Aldrich, India. Sodium hypochlorite (NaOCl) was obtained from MERCK, Germany. Ammonium carbamate and Potassium iodide were obtained from British Drug House (BDH), England. 99.9% dry nitrogen (BOC, Bangladesh) was used for purging purpose. For cleaning and all other purposes double distilled water was used.

3.2 Equipments

Voltammetric and amperometric measurements were performed with a potentiostat / galvanostat (model: μStat 8400, Drop Sens) obtained from Spain. The pencil lead (H, HB, 2H, 2B, 3B, 4B) were purchased from Safe New market, Khulna. The Ag/AgCl reference electrode and the platinum wire counter electrode were purchased from CH Instruments, Inc. (Austin, TX). The reference electrode was filled with 1 M KCl solution. Pencil graphite and pencil drawn paper electrode employed as a working electrode with Ag/AgCl and Pt wire as reference and counter electrodes, respectively. Other instruments that were used (i) an AGE (Glassgo, India) magnetic stirrer with teflon coated magnetic bar. (ii) pH meter (ORION 2 STAR made by Thermo Electron Corporation) was employed for maintaining the pH of the solutions. Preparation of the solutions was done by ordinary laboratory glassware. (iii) An electronic balance (Model: HR-200, japan). Scanning

Electron Microscopy-EDX (manufacture by jeol, USA, work done in DU) was used for the electrode surface characterization.

3.3 Preparation of pencil graphite electrodes (PGEs)

Various grade (H, HB, 2H, 2B, 3B, 4B) pencil graphite was used as the electrode materials. To compare the results glassy carbon electrode has also been used in this study. All the electrodes were tested in 1 mM ferricyanide solution to understand the electrochemistry of PGEs. A cutter was used to make the PGEs from the pencil graphite. The lead was then rinsed with distilled water to remove any traces of adhesive. This exposed surface served as the point of attachment of the PGEs to the potentiostat.

Different grade pencils were used as the base material for the developed PGEs. 2B PGE were chosen as previous research because graphite to possess electron transfer rates more similar to GC than any other graphite hardness when tested with the redox benchmark reagents of Ferricyanide. The eraser was detached and the wood casing removed using a knife until the pencil could be easily passed through a 5 mm in diameter hole.

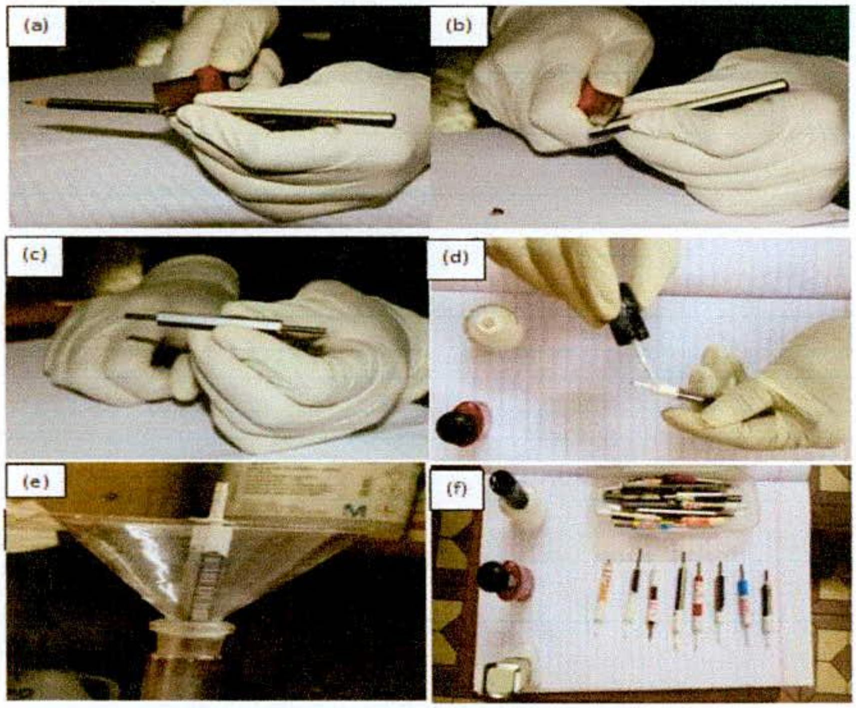

Figure 3.1: Schematic diagram of pencil graphite electrodes fabrication.

Figure 3.1 (a) Collected PGEs from local market (b) Cutting the PGEs with ante-Cutter. (c) Preparation of PGEs (d) Polished PGEs with nail-polish (e) Dried of PGEs after nail-polish (f) Prepared all PGEs with insulating dying.

Finally, the wood from the top about 1.5 cm of the pencil was carefully removed using a small razor blade, until the pencil lead was fully exposed. The lead was then rinsed successively with acetone and distilled water to remove any traces of adhesive. This exposed surface served as the point of attachment of the PGEs to the potentiostat.

3.4 PGEs polishing

This electrode preparation includes the polishing and conditioning of the electrode. Prior to the analysis of each solution, end section of the PGEs was removed using a razor blade, and the exposed PGEs tip polished on white cellulose paper until a substantial amount of graphite was removed. PGEs were then polished on this surface by softly pressing the electrode against the polishing surface in the end for 5-10 minutes. The PGEs was then rinsed with anhydrous ethanol, followed by distilled water, before being immediately tested. Before each run in the same solution, the PGEs were re-polished. Before being successively rinsed with anhydrous ethanol and distilled water. At this point the electrode surface would look like a shiny black mirror.

3.5 Preparation of pencil drawn paper electrode (PDPE)

The pencil drawn electrodes were fabricated by hand drawing a 4 mm diameter circle onto cellulose paper by 2B pencil. A scissor was used to make the pencil hand drawn electrode. At first paper was cut 5 cm long. Upon referring to draw within this paper, this stipulates that we have moved the pencil whilst in contact with the substrate such that the complete area within the 4 mm diameter circles (to be defined as the working area) is drawn. After defining the surface area a connecting strip from the top of the circle allows for a crocodile clip connection to be employed to the potentiostat [144]. Nail polish was applied to each individual electrode to cover the conducting carbon connections. In all experiments utilizing the paper electrode, a platinum wire electrode and the Ag/AgCl reference electrode were used as the counter and reference respectively for comparative purpose.

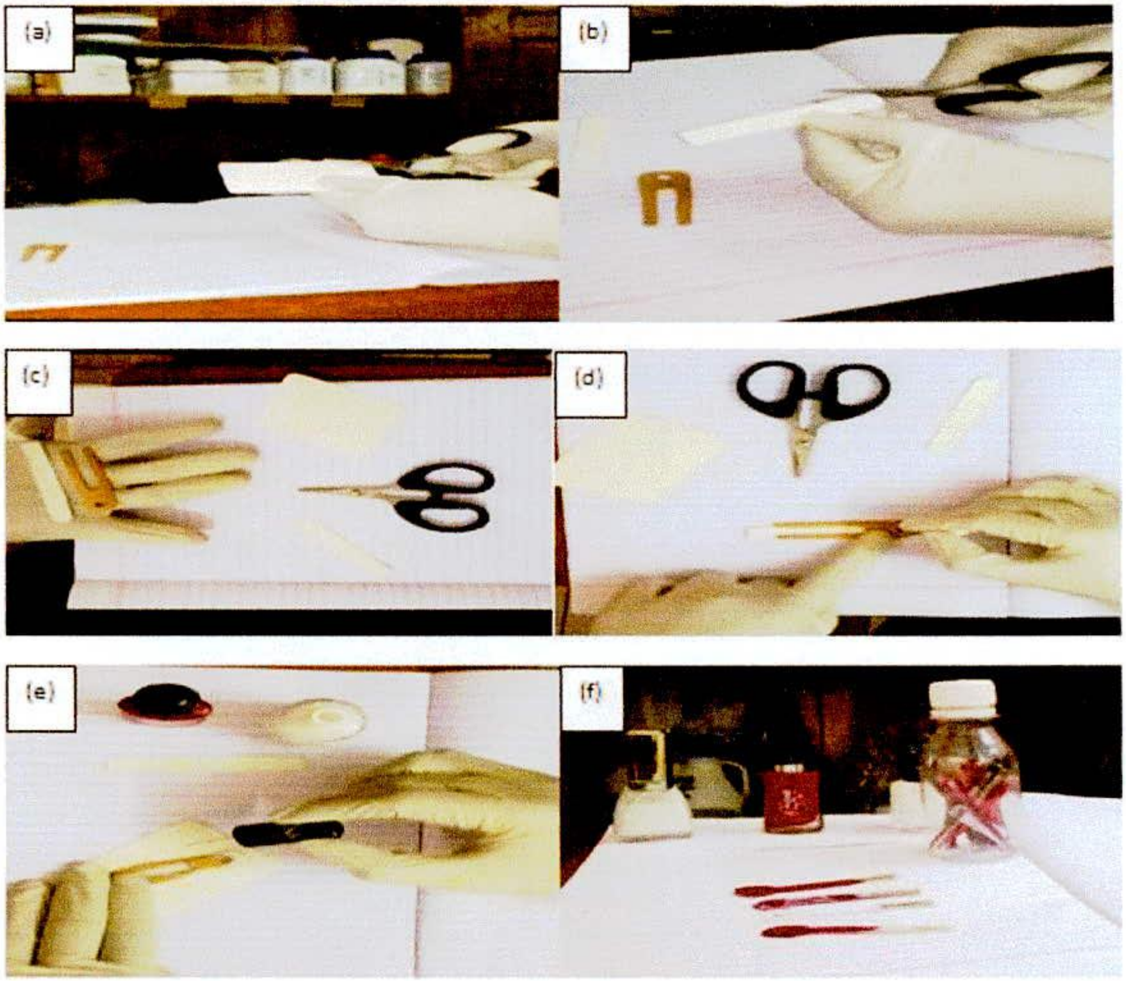

Figure 3.2: Schematic diagram of pencil drawn paper electrodes fabrication.

Figure 3.2: (a) Collected hard cellulose paper (b) Cutting the paper by scissor (c) Preparation of paper electrode (d) Polished paper electrode by 2B pencil (e) Polished hand drown pencil electrode by nail - polish (f) Finally prepared PDPE and dried.

3.6 Synthesis of ZnO nanoparticles

0.070 g of $Zn(CH_3COO)_2{\cdot}2H_2O$ and 0.400 g of NaOH were dissolved into 40 mL of distilled water. After the mixture was magnetically stirred for 20 min and this solution was transferred into a 50 mL Teflon-lined stainless steel autoclave. It was then sealed and maintained at 180 °C for 2 h. After slowly being cooled to room temperature, obtained powders were collected by centrifugation and washed with distilled water and absolute ethanol. The powders were dried at 60 °C for 12 h [145].

3.7 Synthesis of CuO nanoparticles

In a typical synthesis, 0.7345 g $Cu(NO_3)_2{\cdot}3H_2O$ and 0.8230 g hexamethylenetetramine (HMT) were dissolved into 25 ml distilled water under magnetic stirring. After 5.0 ml $NH_3.H_2O$ (5%) was introduced into the mixture under stirring, the clear solution was transferred into a Teflon-lined steel-stainless autoclave of 40 ml. The autoclave was allowed to cool down to room temperature naturally after the system had been hydrothermally treated at 160°C for 6 h. Black precipitates were collected, washed with distilled water and ethanol several times to remove impurities. Finally, the precipitates were dried in air at 50°C for 6 h [146].

3.8 Preparation of NiO nanoparticle

In the sol-gel process [175], $NiCl_2.2H_2O$ (1.5g) was transferred into to a 250 mL round bottom flask and dissolved in 70 mL of absolute ethanol at room temperature, leading to a clear green colored solution. In another beaker, NaOH (0.5g, 0.0125 mol) was dissolved in 100 mL absolute ethanol and the NaOH solution was added to the nickel chloride solution drop wise. The mixture was stirred at room temperature for 2 h. during this time, the reaction mixture was found to form a light green colored gel. After 2h, the gel was filtered, washed thoroughly with distilled water and then finally with ethanol. The precipitate was air- dried, yielding a light green colored powder. The precursor powder was taken in a porcelain crucible and calcined for 30 min at 290 °C in a muffle furnace leading to a dark green color nanocrystal. The reactions involved in the preparation are shown below.

$$NiCl_2 + 2NaOH \xrightarrow{\triangle} Ni(OH)_2 + NaCl + 6H_2O$$

$$Ni(OH)_2 \xrightarrow{\hspace{2em}} NiO + H_2O$$

3.9 Modification of working electrode

PGE is used in this study for electrode modification using ZnO, CuO and NiO nanoparticle obtained using above synthesis process. The modified electrode was prepared by a simple casting method (Figure 3.3).

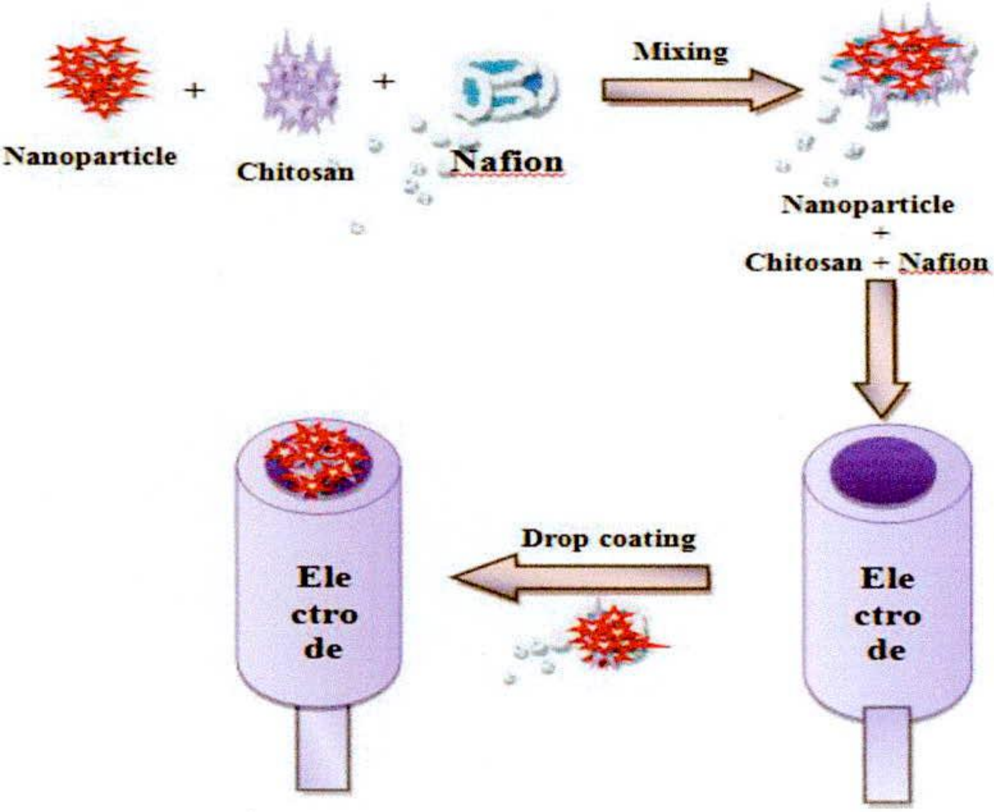

Figure 3.3: A step-by-step diagram of modification of working electrode.

Prior to the surface coating, the PGE was polished in white cellulose paper respectively, and it was rinsed with water. Then, the electrode allowed drying in room temperature. After that, 10 μL of ZnO, CuO and NiO nanoparticle, 5 μL of 1% chitosan and 10% nafion was dropped on the clean surface of PGE, and it was dried at room temperature for overnight.

3.10 Standardization of the electrochemical System

The whole electrochemical set-up was tested using a standard experiment. In the standard experiment we have studied the following redox couple at a glassy carbon (GC) electrode.

$$[Fe(III)(CN)_6]^{3-} + e^- \rightleftharpoons [Fe(II)(CN)_6]^{4-}$$

The reaction above was studied electrochemically by pumping electrons into the system from a GC electrode and by measuring the change in the flow of current during the reaction. This is done most conveniently by scanning the potential of the electrode at a constant rate.

In general, the peak current of diffusion controlled reversible or quasi-reversible electro - chemical reaction follows Randles–Sevcik equation;

$$Ip = 0.4463nF\sqrt{\frac{nFD}{RT}}AC\sqrt{v}$$

Where ip: the peak current, n: the number of electrons, F: Faraday constant, T: the temperature in Kelvin, R: the gas constant, A: the surface area of the working electrode, D: the diffusion coefficient of the electro active species, C: the bulk concentration of the electro active species and *v*: the scan rate of voltammograms.

3.11 Sensor fabrication

Pencil lead was cleaned using lab tissue and rinsed with distilled water. The electrochemical reaction of the pencil graphite electrode surface was carried out at various potential to optimize the effective potential for FAC sensing. In this system Ag/AgCl as reference electrode is used with an initial electrolyte solution of 0.1 M sodium phosphate buffer (pH 7.0), working electrode is a PGEs, and Pt wire as counter electrode.

The working electrode is an electrode where the redox reactions of the substances take place. The reference electrode provides the current required to sustain in electrolysis at the working electrode so that is behavior remains essentially constant with the passage of small current. The counter electrode in the three-electrode system is made of an inert metal.

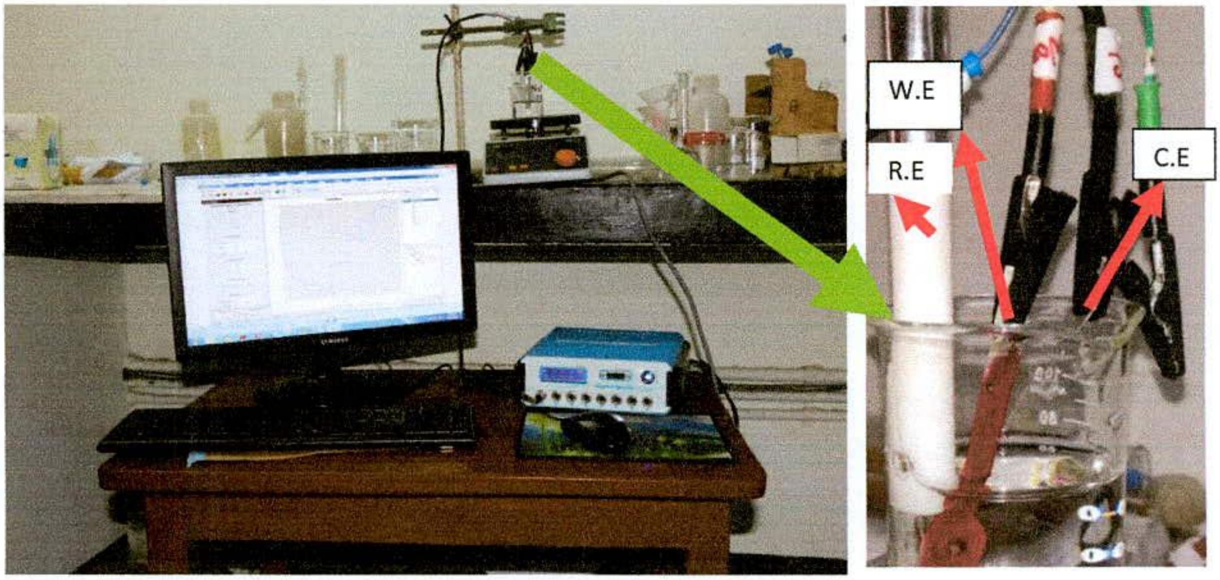

Figure 3.4: Electrochemical experimental setup.

3.12 Free Chlorine Sensing

Free chlorine was sensed by chronoamperometry at 1.5 V versus the Ag/AgCl reference electrode. The experiments were started with 20 mL of 100 mM sodium phosphate buffer (pH 7.0) in the beaker. Different volumes of sodium hypochlorite stock solution were added to the beaker to simulate an increase in free chlorine concentration. Decrease in free chlorine was simulated by removing 1 mL of liquid from the beaker and replacing it with 1 mL of 100 mM sodium phosphate buffer (pH 7.0).

CV was performed over the definite potential range vs. Ag/AgCl in a cell containing 20.0 mL of 0.1 M PBS solution. The cell was assembled and filled with 20.0 mL of supporting electrolyte solution. The surface of the electrodes is completely immersed. The cell is dearated for 15 minutes with high purity nitrogen gas. The solution has been kept quiet for 10 seconds. To determine the potential window, scanning is initially carried out with the supporting electrolyte solution to obtain the background voltammogram. The voltammogram containing the analyte in supporting electrolyte is taken under two different modes, at i) various scan rates and ii) various concentrations.

For amperometric experiments, the voltage was switched directly from open circuit to the appropriate applied potential (vs. Ag/AgCl). The experimental setup is shown in Figure 3.4. Solutions were agitated with a stirrer at a fixed speed for both PBS solution and cell media studies. This was in order to facilitate diffusion and improve the homogeneity of the solution.

3.13 Interference studies

A three electrode system was employed where pencil graphite electrodes were employed as a working electrode with Ag/AgCl and Pt wire as reference and counter electrodes, respectively. 1mM sodium hypochlorite (NaOCl) was added and current response measured at room temperature. E_{app}= 1.5V vs. Ag/AgCl in the presence of 0.1M PBS solution. 0.0073 mM sodium nitrate, 0.0135 mM sodium sulfate, 0.0113 mM sodium carbonate, 0.0145 sodium bicarbonate, 0.0018 mM sodium chloride were added in solution. Again Sodium hypochlorite 1 mM NaOCl was added in 0.1M PBS solution [148].

3.14 Stability studies

Electrode stability was evaluated using amperometry at potential 1.5 V vs. Ag/AgCl by addition of 1mM sodium hypochlorite (NaOCl) at room temperature (298K). Testing was performed every day for a period of 1 week uses pencil drawn paper electrode. In between measurements the electrodes were stored at room temperature.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

Results and Discussion

4.1 Optimization of different grades pencil

The PGEs were electrochemically characterized using 1 mM Ferricyanide in 0.1 KCl solution via CVs. A comparison were made between the pencil grades of 4B, 3B, 2B HB, H, 2H and GC to analyze for obtaining the optimized grade as electrode material. This is usually discounted in the academic literature and little reasoning is given why certain pencils are chosen. Figure 4.1 shows CVs recorded using the redox probe Ferricyanide solution on PGEs at a scan rate of 50 mV/s.

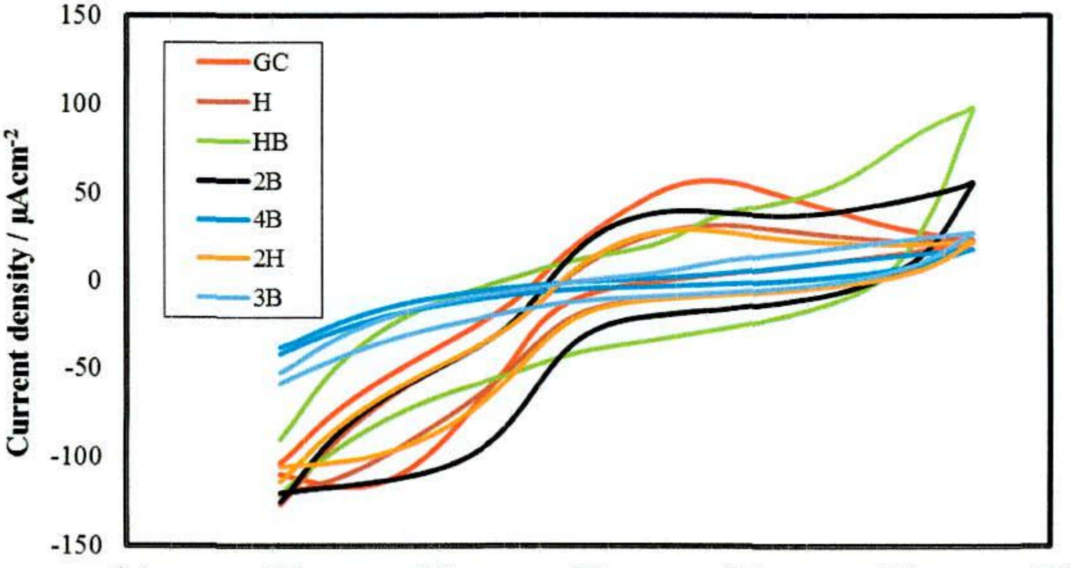

Figure 4.1: CVs of PGEs of 1 mM in Ferricyanide (0.1 M KCl), at scan rate: 0. 05 V/s.

Generally, the observed responses are typical of that expected for graphite-based electrochemical sensors where useful voltammetric signatures are observed, with the 2B found to exhibit the relatively largest peak current. However, 4B graphite electrode also shows better electrochemical reversibility. It is to note that 2B PGE provides a clear decrease in the peak-to-peak separation and an increase of the voltammetric peak current over that of the other PGEs (see Figure 4.1), indicating a more beneficial electrode surface with relatively improved/faster electrochemical reversibility. Therefore, 2B along with 4B has been used in this work for further electrochemical analysis. It is clear that an oxidation and reduction peak for 2B pencil electrode was observed at +0.28 and -0.02V respectively.

4.2 Physical characterization

Characterization of the bare 2B pencil "graphite" (later used for the fabrication of the PGEs) using SEM image is shown in Figure 4.2 (a).

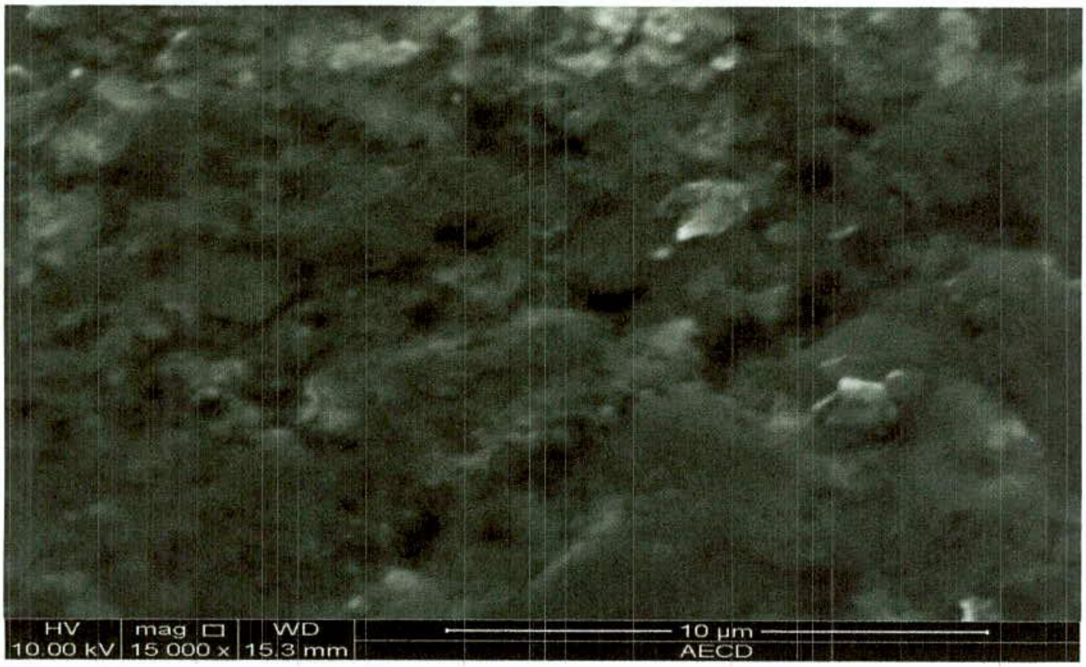

Figure 4.2(a): SEM image of bare 2B PGE.

It is clear that the sheets of graphite within the pencil are folded and contain many defects, grooves which could potentially provide useful electrochemical properties. Greyish-black color corresponds to graphite on the surface. Surface is not smooth as well. It also indicates that the graphite rod of the pencil is not pure crystalline [149].

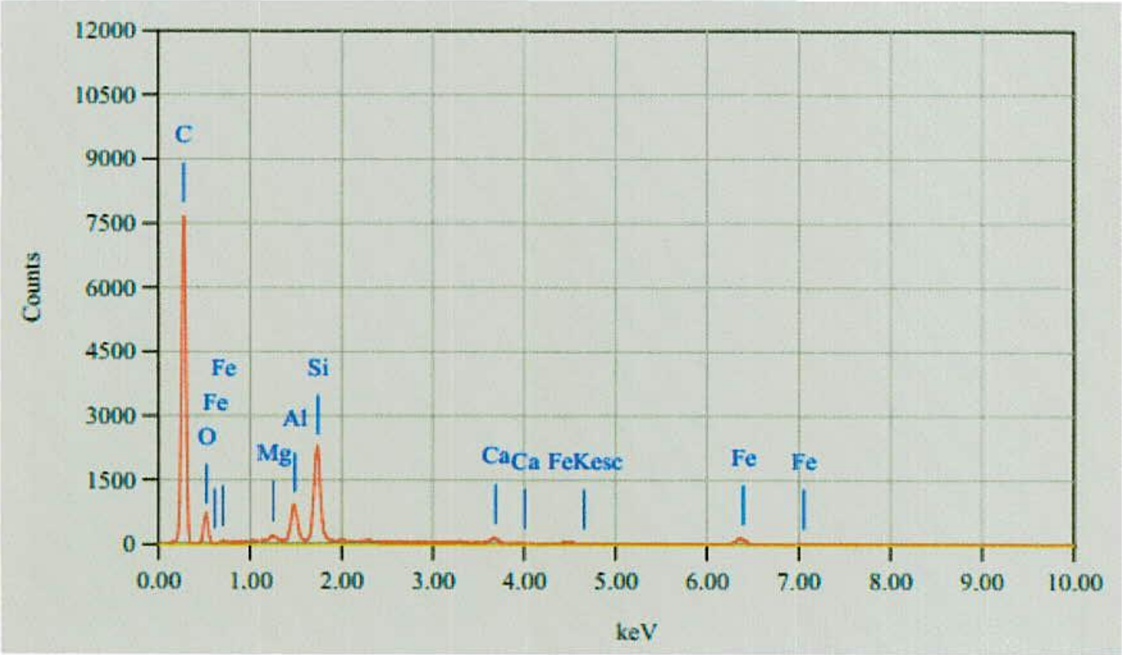

Figure 4.2(b): EDX of bare 2B PGE [151].

Figure 4.2(b) shows the EDX of bare PGE. It is seen from the graph that PGE surface are impure as guessed by SEM. From the Figure it can be seen that 2B pencil composed of 70 % carbon, 10.3 % silicon, 3.68 % Iron, 3.06 % oxygen, 5.50 % calcium, 3.49 % aluminum and trace amount of magnesium and calcium gluing agent co-exist with graphite. In bare GCE, there are 100 % carbon and without having any impurities [149, 150]. So it can be concluded that PGE is mainly carbon composite and also contain many impurities. Table 4.1 represents the various types of component that 2B PGE contain.

Table 4.1: Percentage of different materials in 2B PGE

Element	KeV	Mass %	Counts	Error %	Atom%
C	0.277	79.39	42944	0.00	89.61
O	0.525	3.06	4350	0.03	2.59
Mg	1.253	0.40	786	0.39	0.22
Al	1.486	3.49	6562	0.05	1.77
Si	1.739	10.03	18574	0.02	4.84
Ca	3.690	5.5	1133	0.38	0.32
Fe	6.398	3.68	1617	0.27	0.65
Total		100.00			

4.3 Electrochemical setup of 2B PGE

CVs of Ferricyanide at PGE (Figure 4.3a) were performed at concentration of 2 mM of Ferricyanide in 0.1 M KNO_3, and was scanned at different scan rates of 20, 40, 60, 80, 100, 120, 140, 160 mV/s.

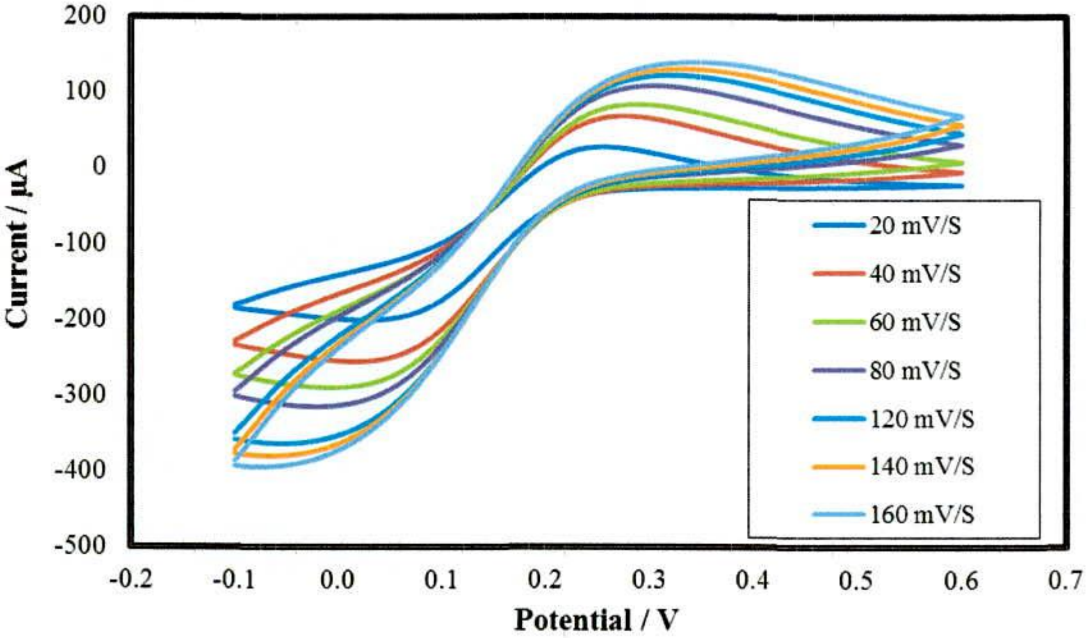

Figure 4.3(a): CVs of Ferricyanide at 2B PGE were performed at concentration of 2 mM of Ferricyanide 0.1 M KNO_3 as supporting electrolyte, different scan rates 20, 40, 60, 80, 100, 120, 140, 160 mV/s.

When the scan rate increases, current is also increases. From these results experimental surface area has been calculated and found to be higher compare to the theoretical value. It could be due to the presence of defects, pores presence within the PGE surface. The physical properties of pencil graphite electrodes that are described the look or feel of a substance. Electrode was made from locally available and cheap pencil graphite instead of conventional costly platinum, gold or glassy carbon electrodes.

Chemical properties that are relate to the ability of a substance to react with other substances or to transform from one substance to another. The chemical properties of a substance relate to its chemical composition and the way the atoms in the molecules are chemically bonded together.

Table 4.2: Electrochemical parameters obtained from voltammograms of Figure 4.3(a)

v Vs^{-1}	$v^{1/2}$	E_{pa} V (+)	E_{pc} V (+)	i_{pa} μA (+)	i_{pc} μA (-)	i_{pa}/i_{pc}
0.02	0.141421	0.26	0.06	61.370	-62.417	0.96
0.04	0.200000	0.24	0.07	86.834	-86.834	1.00
0.06	0.244949	0.26	0.03	98.208	-78.368	1.25
0.08	0.282843	0.25	0.01	109.075	-100.791	1.08
0.1	0.316228	0.29	0.02	124.330	-126.00	0.98
0.12	0.34641	0.29	0.02	130.875	-135.959	0.96
0.14	0.374166	0.31	0.01	131.250	-139.25	0.94
0.16	0.400000	0.31	0.01	144.417	-150.333	0.96

v= scan rate; $v^{1/2}$= SQRT of scan rate; E_{pa}= anodic peak potential; E_{pc}= cathodic peak potential ;i_{pa}= anodic peak current; i_{pc}= cathodic peak current.

In general, the peak current of diffusion controlled reversible or quasi-reversible electro - chemical reaction follows Randles–Sevcik equation

$$I_p = 0.4463nF\sqrt{\frac{nFD}{RT}}AC\sqrt{v}$$

Where i_p: the peak current, n: the number of electrons, F: Faraday constant, T: the temperature in Kelvin, R: the gas constant, A: the surface area of the working electrode, D: the diffusion coefficient of the electroactive species, C: the bulk concentration of the electroactive species and v: the scan rate.

Thus, if we know the value of diffusion coefficient of Ferricyanide at 298K the surface areas for Ferricyanide can be calculated from the slope of the plot of Ip versus √v (Figure 4.3b).

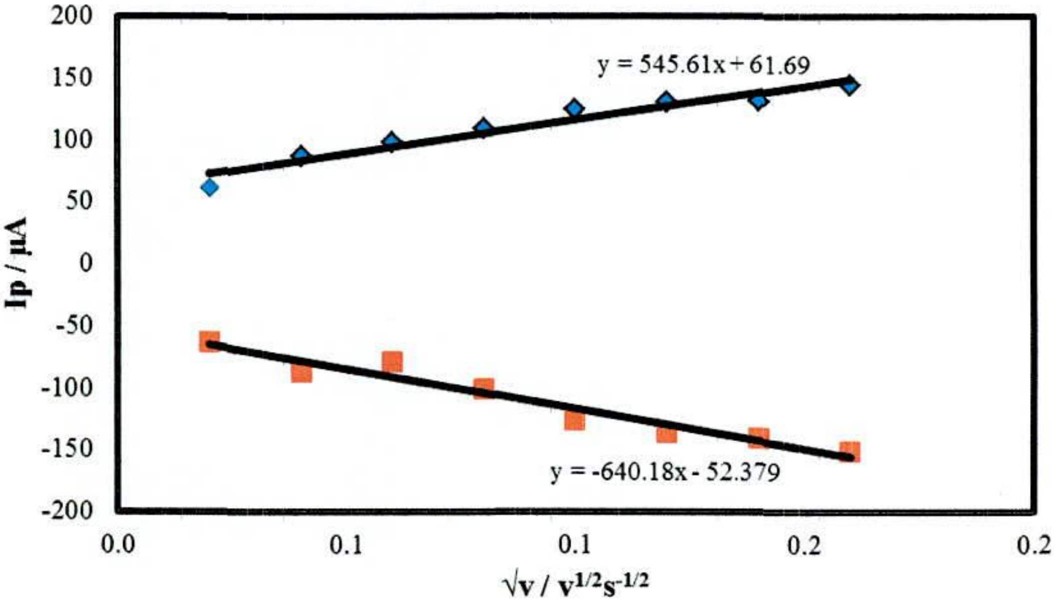

Figure 4.3(b): The anodic and the cathodic peak heights as function of the square root of the scanning rate for PGE.

From Equation (1) we get,

$$\text{Slope} = 0.4463\text{nF}\sqrt{\frac{nFD}{RT}}\,\text{AC}$$

$$\text{A} = \frac{Slope}{0.4463\text{nF}\sqrt{\frac{nFD}{RT}}\,\text{C}}$$

From the curve (Fig 4.4) the value of slope is ~545.6×10^{-6} and the standard value of diffusion coefficient for Ferricyanide in PGEs is 1.52×10^{-6} cm^2/s. where concentration $\text{C} = 2\ 10^{-4}\ \text{mol}/\text{cm}^3$ so we get,

$$\text{A} = \frac{9\times10^{-6}}{0.4463\times1\times96500\sqrt{\frac{1\times96500\times1.52\times10^{-6}}{8.314\times298}}\times2\times10^{-6}}$$

$$\text{A} = 0.013\ \text{cm}^2$$

As can be seen from Figure 4.3b, the scan rate dependence on peak current. The peak potential shifted to a more positive potential with increasing scan rate, confirming that the oxidation reaction is irreversible. However, the peak current for Ferricyanide in 0.1M KNO_3 is linearly proportional to the square root of the scan rate in the range of 20-160 mV/s with a correlation of coefficient of 0.944. The reasonable good linearity and close to zero intercept indicate the reaction is dominated by diffusion control in the interfacial area of the electrode. We must say that a little adsorption behavior could happen in PGE [152] but the behaviors may provide good interaction between the reagent and electrode substrate. However, due to the adsorption behavior of PGE, many cases fast fouling due to strong adsorption could poison the electrode surface [152,153]. Therefore, before using the electrode surface, it is crucial to refresh the PGE surface by polishing it on the paper.

4.4 Electroanalytical activity of bare GC electrode

CV is a commonly used as versatile potential dynamic electro analytical technique to study redox systems, the reversibility of the reaction, the stoichiometry of a system and the diffusion coefficient of an analyte. These can be used to determine the electrochemical characteristics and identity of unknown compounds. In this work, we have investigated the electrocatalytic activity of these electrodes towards chlorine. Figure 4.4 presents the CV responses of bare GC electrodes in 0.1 M PBS without (a) and with (b) free chlorine of 10 mM. In the absence of chlorine, at around + 0.7 V the oxidation of H_2O can be observed. Upon the addition of NaOCl decrease of the anodic current density can be observed at the

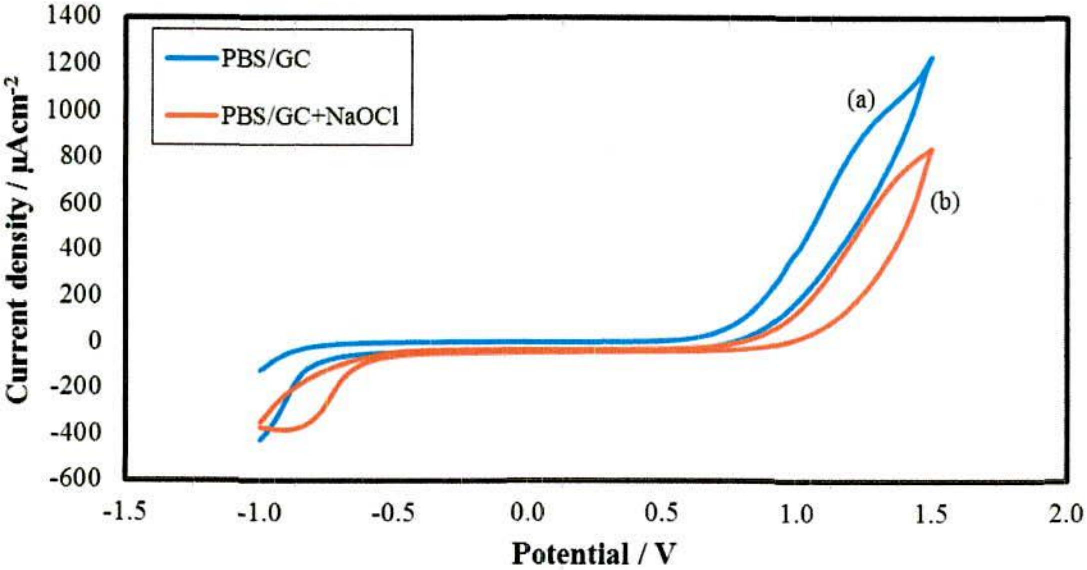

Figure 4.4: CVs in bare GC electrode (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl; in 0.1M PBS at E_{app}=1.5V (scan rate: 0.05 V/s).

same potential for GC electrode. This may indicate that GC may poison in the presence of NaOCl in 0.1M PBS at pH 7. Therefore, we have replaced GC electrode with different grade pencil graphite as electrode material for the free available chlorine detection.

4.5 Electroanalytical activity of 2B and 4B PGE

In order to address the analytical applicability of the PGE, in this work we have investigated the electrocatalytic activity of this electrodes to determine free chlorine. Figure 4.5(a) presents the CV responses of PGE in 0.1 M PBS without (a) and with (b) 10 mM NaOCl. In the absence of NaOCl, anodic current can be seen at around 1.2 V. Upon the addition of NaOCl, a significant increase of the anodic current can be observed on PGE. It also can be seen that with the addition of NaOCl the anodic peak shifts to the lower potential. This behavior may attributes to the oxidation of graphite in the presence of NaOCl [154]. The instability of graphite at oxidizing potential leads to carbon decomposition into CO_2, HCO_3^- and CO_3^{2-}. The oxidation can be greatly accelerated in the presence of hypochlorite (free chlorine), producing salt and CO_2. Therefore, degradation of PGE electrode and its lifetime has to be taken into consideration before it applied as free chlorine sensor.

$$C + 2\,NaOCl \longrightarrow CO_2 + 2NaCl$$

In addition, Entwisle [154] also proposed two mechanisms that may involved with the oxidation of graphite.

Mechanism I:

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e^- \qquad E^o = +\,1.23\ V$$

$$2H_2O + C \longrightarrow CO_2 + 4H^+ + 4e^- \qquad E^o = +\,0.144\ V$$

Mechanism II:

$$NaOCl + 3H_2O \longrightarrow ClO_3^- + Cl^- + O_2 + H^+ + 6e^-$$

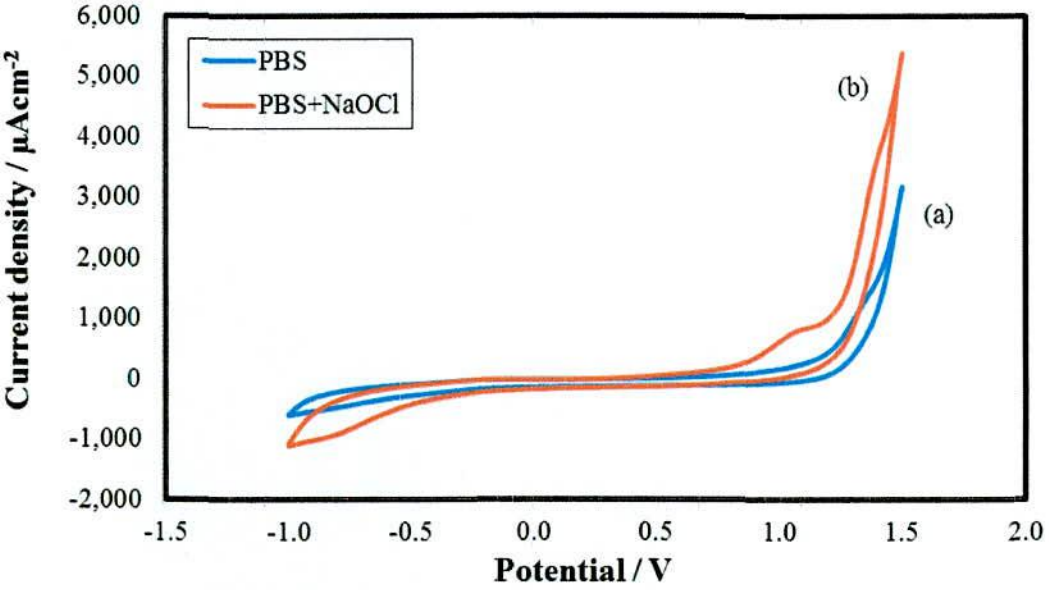

Figure 4.5(a): CVs in 2B PGE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS at E_{app}=1.5V (scan rate: 0.05 V/s).

However, mechanism II mainly happens in the defects and pores (Figure 4.2a) that are available on the PGE. Similar behavior was obtained in boron doped diamond electrode for free chlorine detection, where the reaction occurs on the surface of the electrode [180]. Therefore, based on the literature, we can postulate that mechanism II can occur both on the surface of the PGE as well as in the defects and pores that are available in PGE. Due to this combined effect anodic current increased significantly on the addition of NaOCl while PGE is the working electrode. This behavior cannot be seen when GC, gold or platinum electrode is used as working electrode (voltammogram is not shown here). It is to note that Si Pan [148] modified PGE using ammonium carbamate and observed increased cathodic current due to the addition of NaOCl, which authors claim that ammonium carbamate reacts cathodically with NaOCl and increased cathodic current. In this case PGE does not need any modification; only by the implementation of 1.5 V, a significant increase of anodic current can be seen.

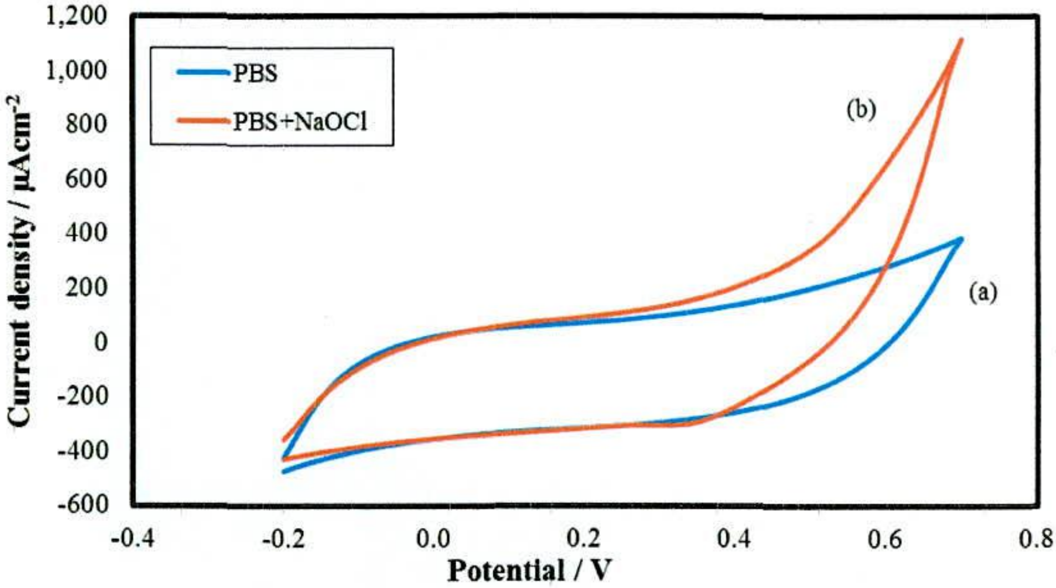

Figure 4.5(b): CVs in 2B PGE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS at E_{app}= 0.7V (scan rate: 0.05 V/s).

In Figure 4.5(b) we try to reduce the potential window if we can see the increase anodic current on addition of NaOCl at lower potential. However, we have found a significant reduction of sensitivity on free chlorine detection at lower than 1.5 V.

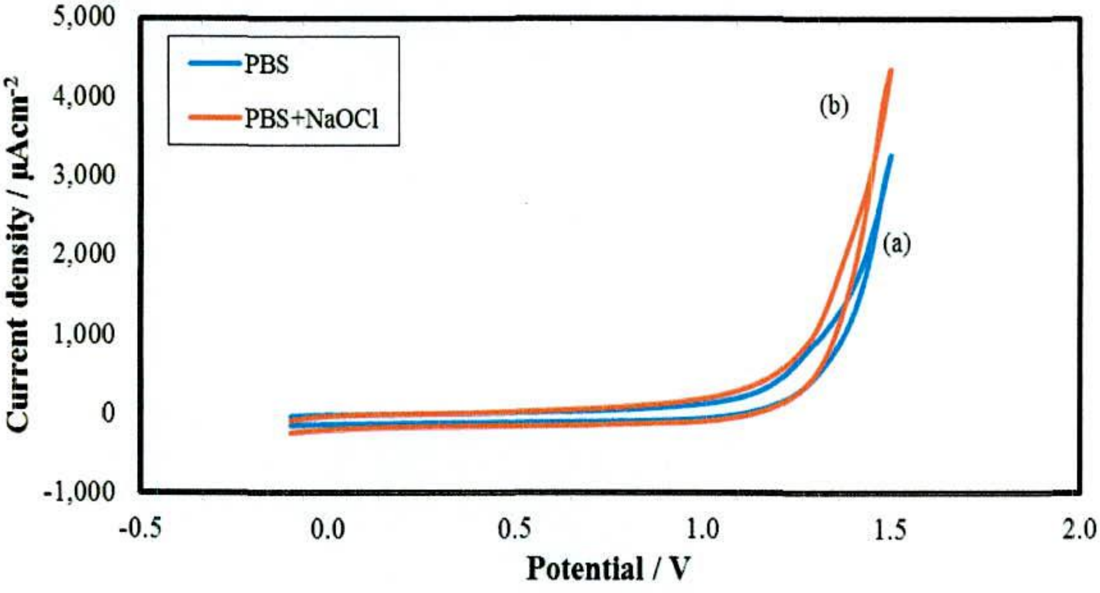

Figure 4.6(a): CVs in 4B PGE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS at E_{app}= 1.5V (scan rate: 0.05 V/s).

Figure 4.6a showed the behavior of 4B PGE however, sensitivity greatly reduced compare to 2B PGE. This may be due to the lower in porosity or defects on the 4B PGE surface.

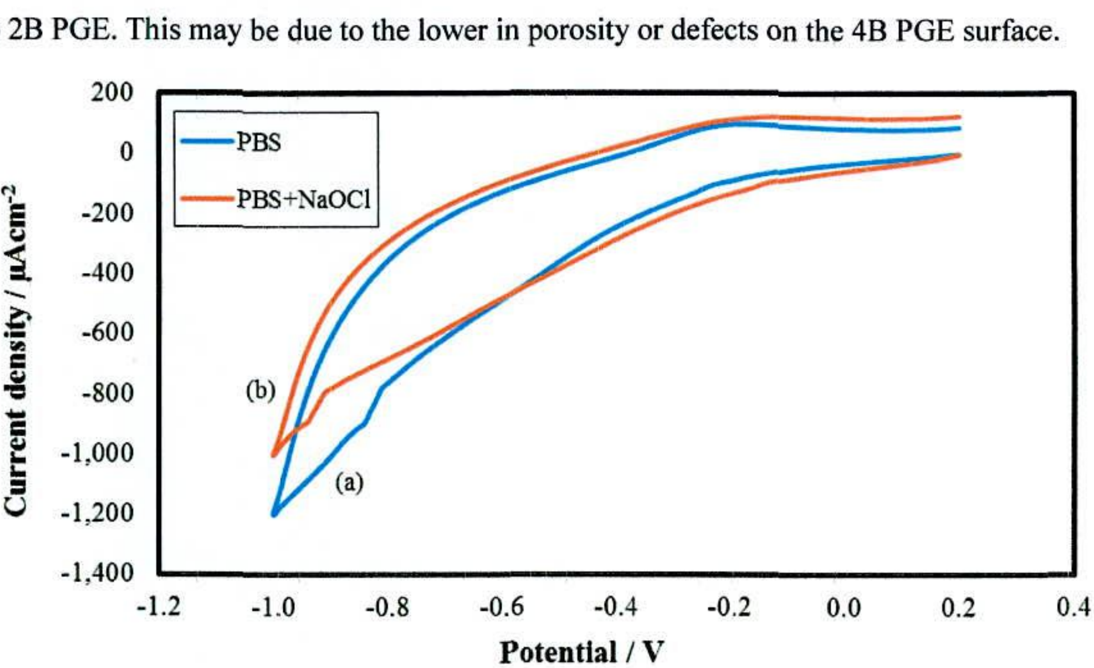

Figure 4.6(b): CVs in 2B PGE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS at E_{app}= 0.2 to -1 V (scan rate: 0.05 V/s).

We have continued examined the behavior of 2B PGE in the negative scan to understand whether any other mechanisms involved on the surface on addition of NaOCl. No significant behavior is observed on the negative potential. As part of our continuous investigation of PGE as free chlorine sensor and with the intention of reducing applied potential for the free chlorine detection, we have further our work by modifying 2B PGE with CuO, ZnO and NiO nanoparticle that have been synthesized using both hydrothermal and sol-gel methods. Electrochemical characterization of copper oxide nanoparticle modified PGE (CuO/PGE), zinc oxide nanoparticle modified PGE (ZnO/PGE) and nickel oxide nanoparticle modified PGE (NiO/PGE) is described in next section.

4.6 Electrochemical characterization of CuO/PGE, ZnO/PGE and NiO/PGE

To establish the analytical application of the CuO/PGE, ZnO/PGE and NiO/PGE the surface of the electrode was constructed by deposition of the aqueous dispersion of CuO, ZnO and NiO nanoparticle in the presence of chitosan and Nafion. Figure 4.7, 4.8, 4.9 represents the CVs of CuO/PGE, ZnO/PGE and NiO/PGE in 0.1 M PBS at a scan rate of 0.05 V/s.

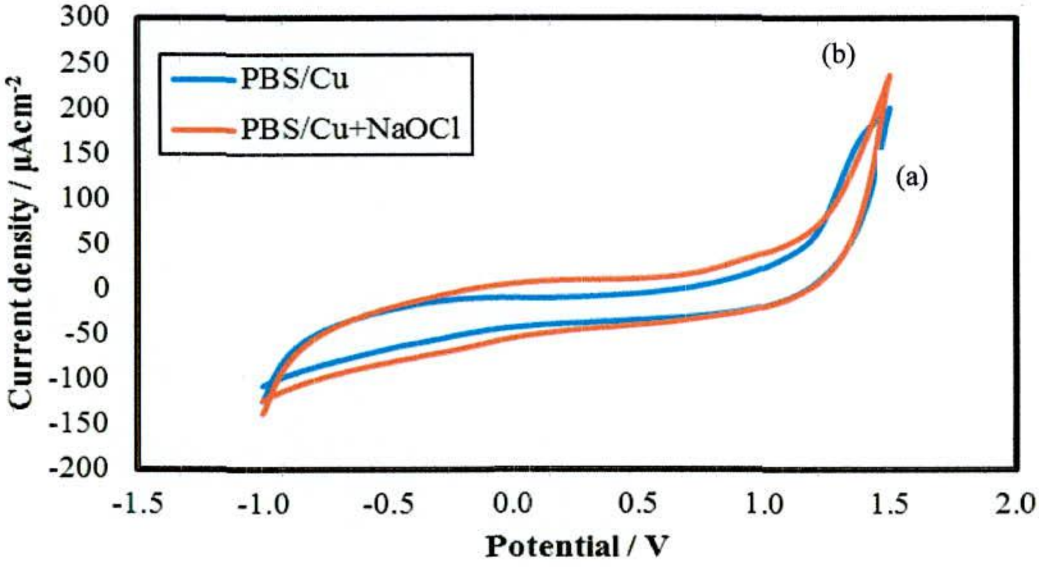

Figure 4.7: CVs of 0.1 M PBS CuO/PGE (a) without NaOCl (b) in the presence of NaOCl at E_{app}= 1.5 V (scan rate: 0.05 V/s).

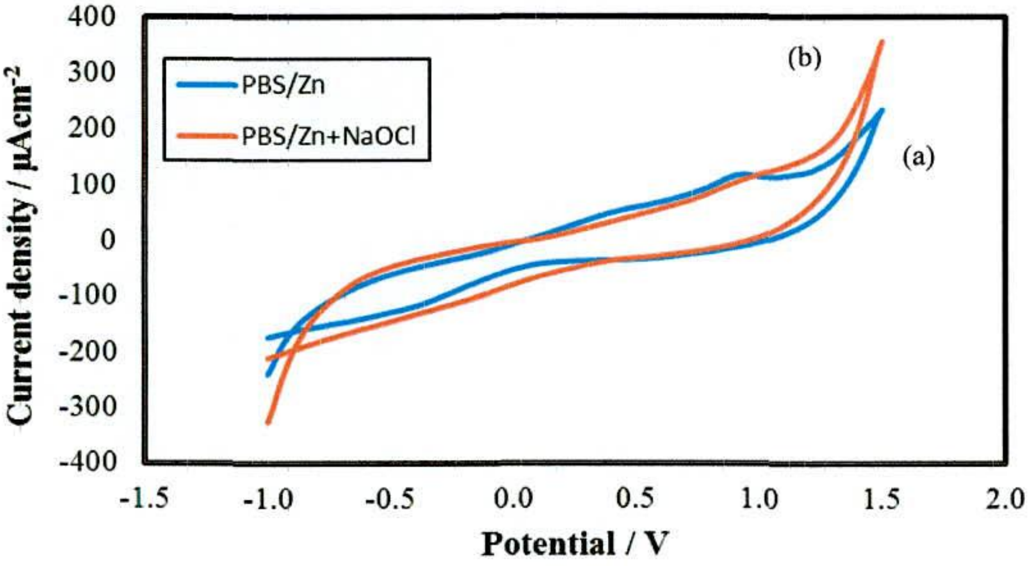

Figure 4.8: CVs of 0.1 M PBS ZnO/PGE (a) without NaOCl (b) in the presence of NaOCl at E_{app}= 1.5 V (scan rate: 0.05 V/s).

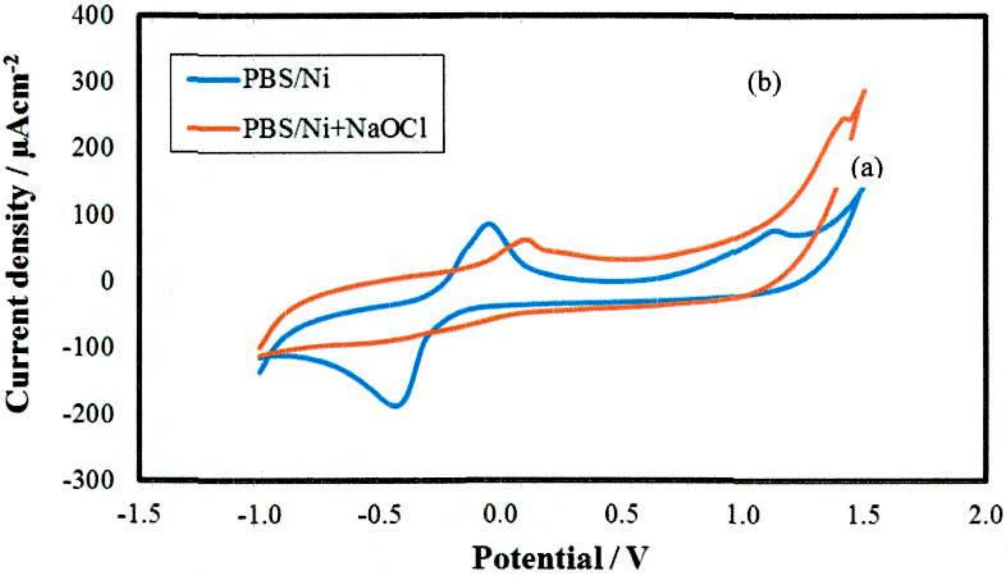

Figure 4.9: CVs of 0.1 M PBS NiO/PGE (a) without NaOCl (b) in the presence of NaOCl at E_{app}= 1.5 V (scan rate: 0.05 V/s).

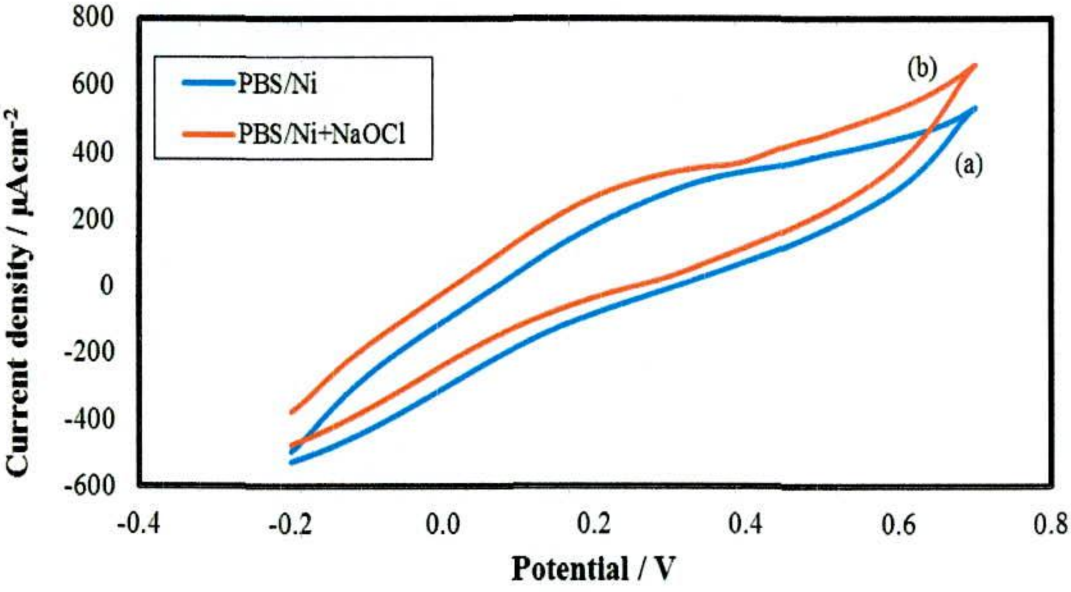

Figure 4.10: CVs of 0.1 M PBS ZnO/PGE (a) without NaOCl (b) in the presence of NaOCl at E_{app}= 0.7 V (scan rate: 0.05 V/s).

In Figure 4.7 it is seen that CuO/PGE exhibits no obvious oxidation and reduction peak in 0.1 M PBS at 1.5 V (scan rate 0.05 V/s). In contrast CuO/PGE we investigated the electrocatalytic activity of these electrodes towards chlorine. Figure 4.7 presents the CV responses of CuO/PGE in 0.1 M PBS without (a) and with (b) 10 mM NaOCl. In the presence of free chlorine, no peak was observed. So CuO/PGE didn't give any response to free chlorine. However, anodic current has been increased for ZnO/PGE (Figure 4.8) and NiO/PGE (Figure 4.9). However, sensitivity is very low compare to black 2B PGE. Therefore, we have further our work on 2B PGE as free chlorine sensor.

We investigated the electrocatalytic activity in Figure 4.10 CVs of the ZnO/PGE electrode in 0.1 M PBS at pH 7 for the potential range between -0.2 V to 0.7 V (at scan rate 0.05 V/s). The CVs responses without (a) and with (b) 10 mM NaOCl. After the addition of free chlorine ZnO/PGE did not give increase anodic current that's we compare in Figure 4.8. This specifies that at lower potential ZnO/PGE exhibit no catalytic activity towards free chlorine sensing.

In this study, we investigate CuO/PGE and NiO/PGE has no catalytic activity towards chlorine; only ZnO/PGE has poor catalytic activity towards chlorine. So nanoparticl modified PGE is not the choice for free available chlorine sensing; rather simple unmodified PGE will be cost effective and disposable. We also try to reduce potential using ZnO/PGE but at low potential ZnO/PGE has no catalytic activity towards chlorine and previously we showed only bare pencil graphite electrodes provide good catalytic activity for free available chlorine. So we try to prepare which is cheap, low lost easy to handle pencil drawn paper based free available chlorine sensor preparation that has been discussed in later.

4.7 Free chlorine sensing by amperometric techniques

Figure 4.11 shows a typical amperometric responses of PGE upon the successive addition 1 mM free chlorine in 0.1 M PBS at the applied potential of 1.5V with continuous stirring. PGE electrode could successfully detect free available chlorine at the applied potential of 1.5V. From the slope (Figure 4.11) the sensitivity of free chlorine on PGE found to be 121 ± 5.38 $\mu AmM^{-1}cm^{-2}$ with the limit of detect of 59.5 μM and linearity up to 12 mM for free available chlorine. The response time t_{90} for the free available chlorine on PGE found to be less than five seconds. Standard deviation has been calculated for each measurement and has been shown in Figure 4.12.

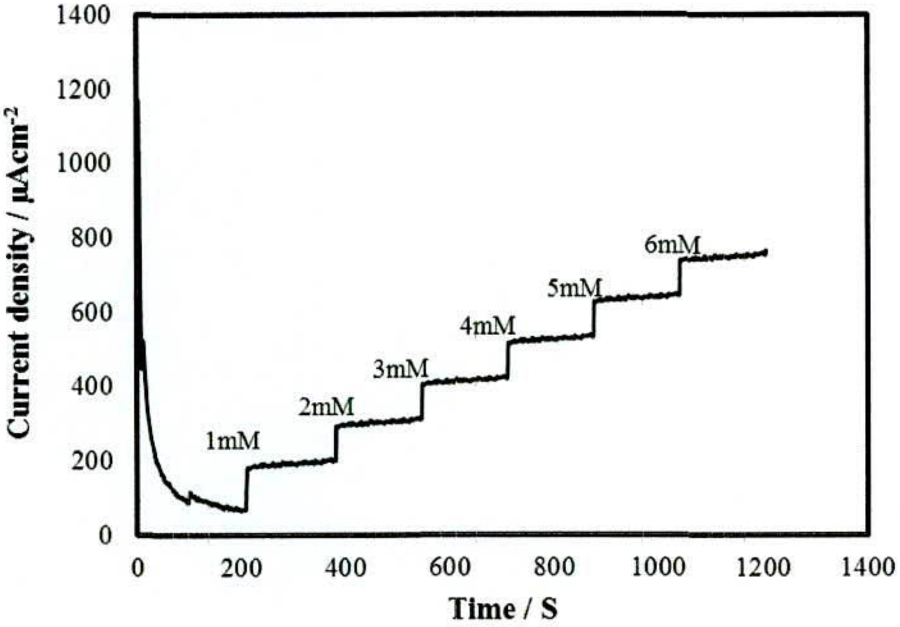

Figure 4.11: Typical amperometric current response of the 2B PGE upon the successive injection of NaOCl with concentrations from 1 mM to 6 mM into a stirred at E_{app}=1.5V in 0.1 M PBS pH 7.

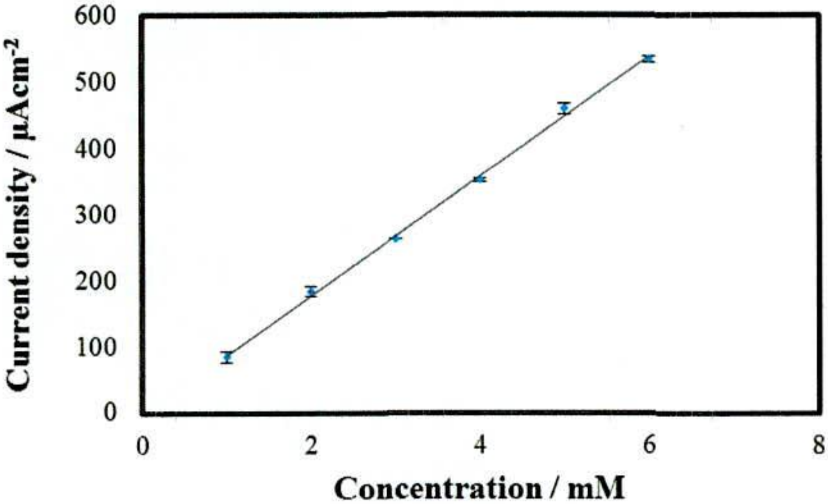

Figure 4.12: The plot of concentration effect current density vs the corresponding concentrations of NaOCl in PGE.

4.8 Sensing effect at deoxygenated environment

CV was initially recorded with 0.1 M PBS solution and same solution containing 10 mM NaOCl. The sample is degassed for 15 minutes with high purity nitrogen gas. Then the solution is kept quit for 10 seconds. Then the surface of the electrode is completely immersed o.1 M PBS solution and scanned potential from -0.1 V to 1.5 V (at a scan rate 50 mV/s).

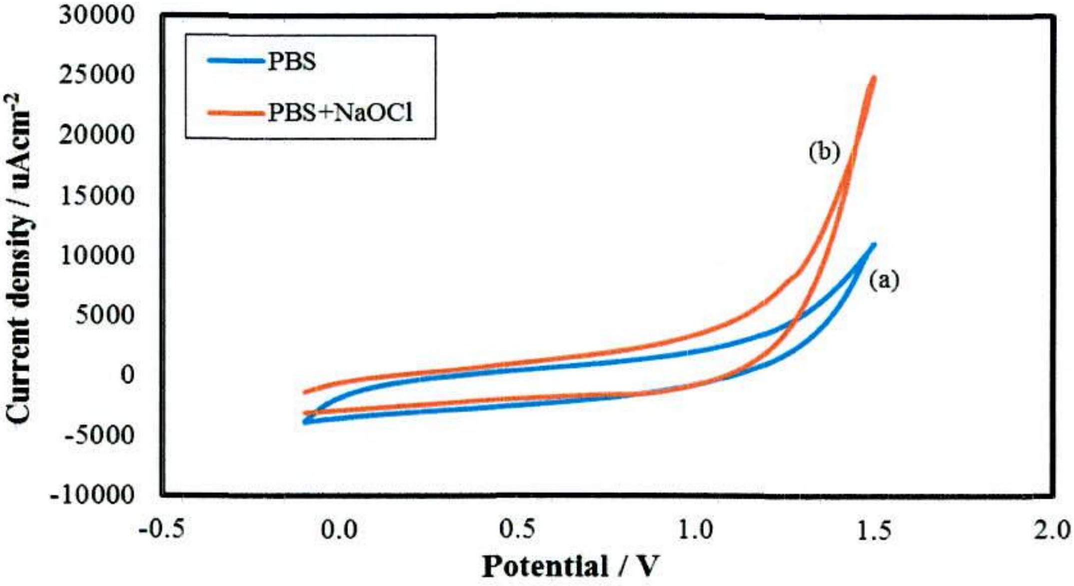

Figure 4.13: CVs in 2B PGE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS at E_{app}=1.5V (scan rate: 0.05 V/s).

These studies suggested anodic current increases more in compare to without degassed solution. In Figure 4.13 CV in PGE with (a) and without (b) of 10 mM NaOCl. In absence of dissolve oxygen PGE has provided better anodic current to free available chlorine [180].

4.9 Effect of iron in presence of dissolve oxygen

From the elementary analysis it is seen that pencil is not contain pure graphite. It has also been found that some other materials present in the PGE, such as Al, Ca, Mg, Si, gluing agent etc co-exist with graphite (Figure 4.2(b)) [151]. In our opinion this species may interfere the reactivity of graphite and inhibit the sensitivity towards chlorine. The formation of monolayers of graphite by the surface corrosion of metal can't ensure successful detection using graphite electrode. An inconsistency may be found during the chlorine measurement using PGE. Therefore, a detail study on iron on the free available chlorine measurement needs to be investigated.

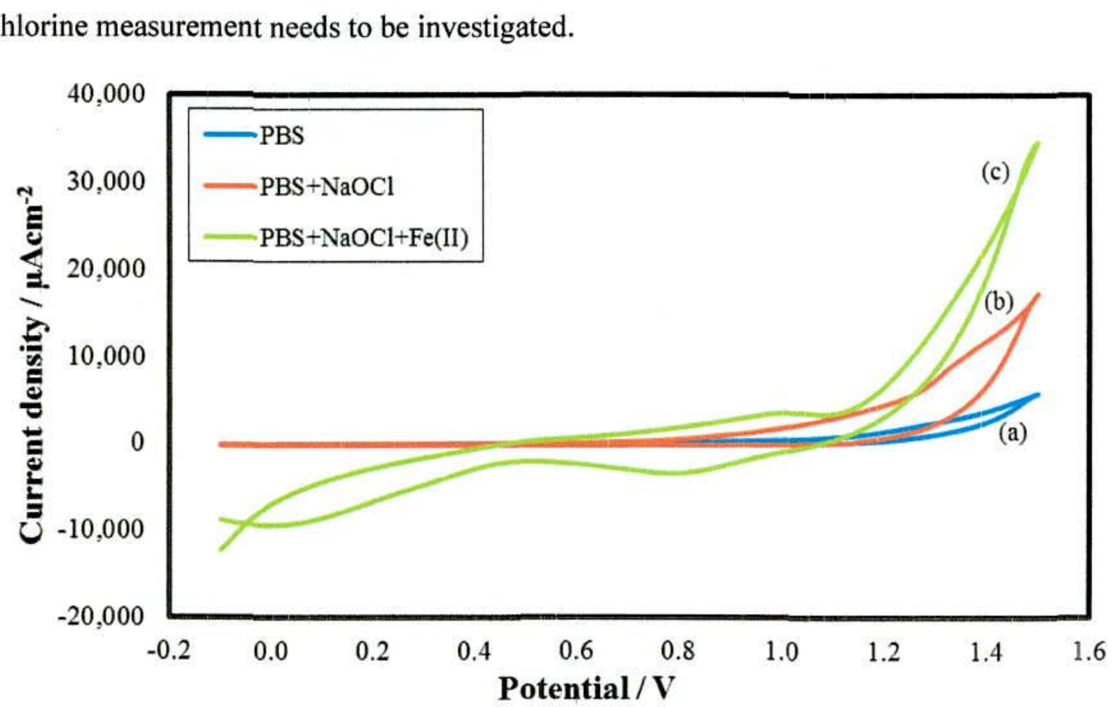

Figure 4.14: CVs in 2B PGE (a) in the absence of NaOCl (b) in the presence of 10mM NaOCl (c) added $FeCl_2$ in 0.1M PBS containing at E_{app}= -0.1 to 1.5V (scan rate: 0.05 V/s).

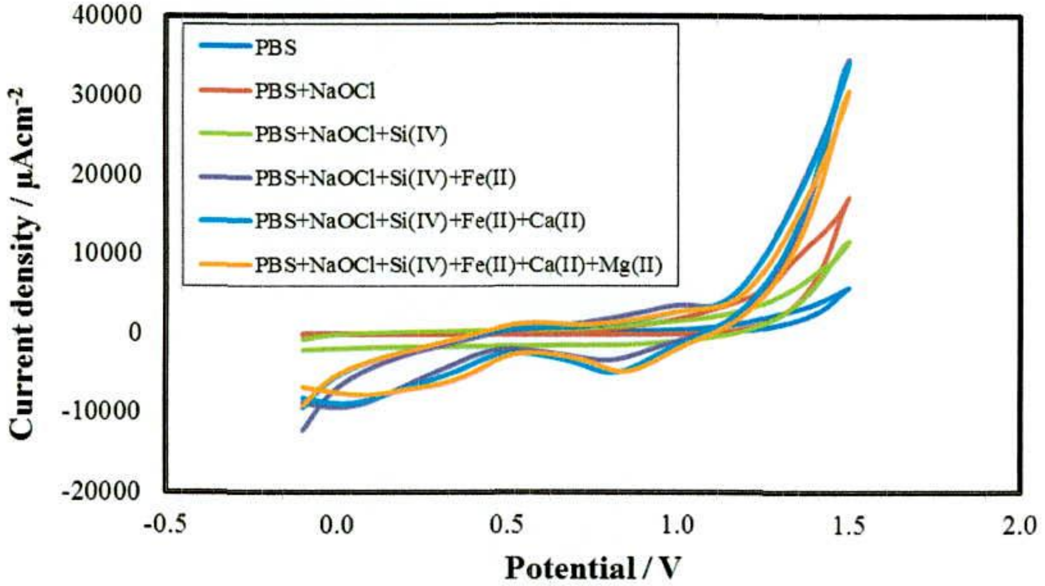

Figure 4.15: CVs in 2B PGE (a) in the absence of NaOCl (b) in the presence of 10mM NaOCl (c) added SiO_2, $FeCl_2$, $Ca(NO_3)_2$ and $Mg(NO_3)_2$ in 0.1M PBS containing at E_{app}= -0.1 to 1.5V (scan rate: 0.05 V/s).

Figure 4.14 represent the catalytic activity of PGEs without (a) and with (b) 10 mM NaOCl in 0.1M PBS at E_{app}= -0.1 to 1.5V (scan rate: 0.05 V/s). After addition iron with same solution the oxidation and reduction peak was observed in (c) but after the addition of Si(IV), Ca(II), and Mg(II) current response is same compare to iron effect. In Figure 4.15 no change in CVs can be seen after the addition of Si(IV), Ca(II), and Mg(II). There by, we can conclude that iron may provide both anodic and cathodic peaks in the presence of dissolve oxygen.

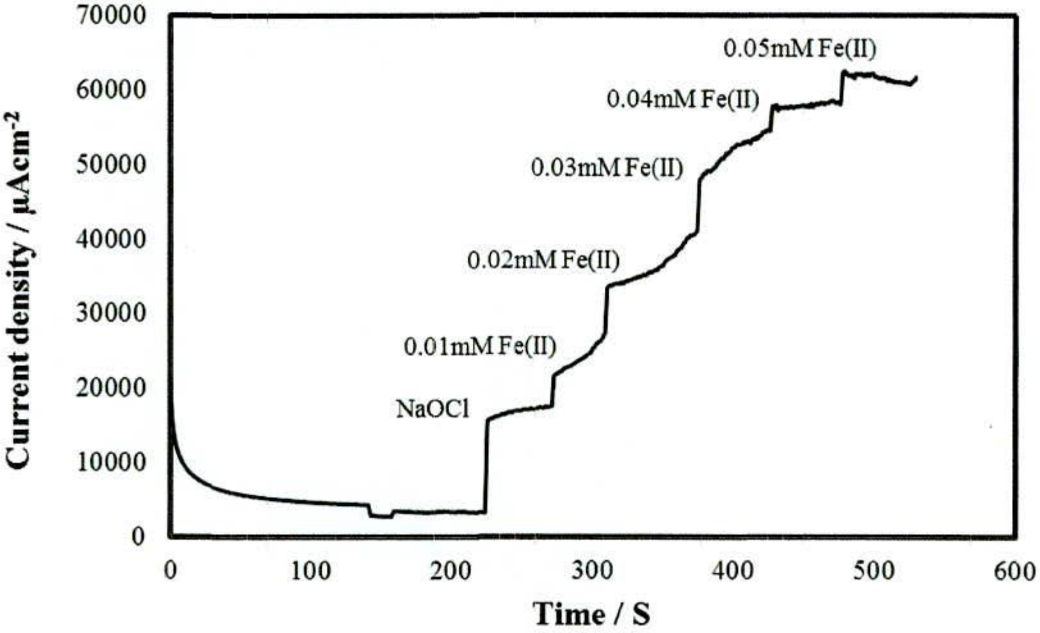

Figure 4.16: Typical amperometric current response of the 2B PGE upon the successive injection of 1st NaOCl and other step $FeCl_2$ into at E_{app}=1.5V in 0.1 M PBS pH 7.

Figure 4.16 typical amprometric current responses of free chlorine and iron in presence of dissolve oxygen. At applied potential of 1.5 V, we added NaOCl and current found to be increases in 0.1 M PBS solution. After that Fe (II) has been added and oxidation current found to be increased. The total current response is irregular after the addition of iron in every step.

4.10 Effect of iron in absence of dissolve oxygen

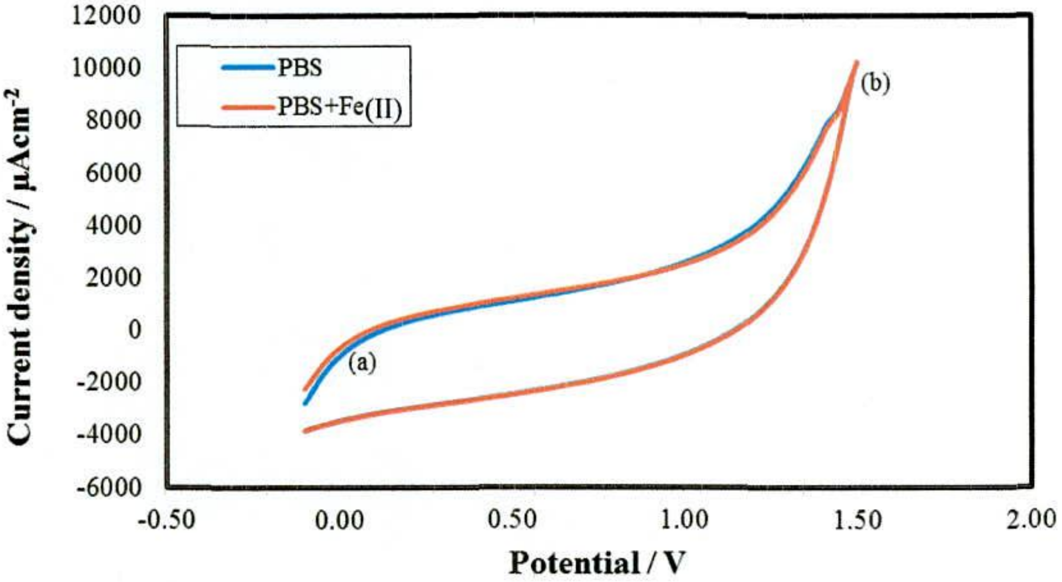

Figure 4.17: CVs in 2B PGE (a) in the absence of $FeCl_2$ (b) in the presence of Fe in 0.1M PBS containing at E_{app}= -0.1 to 1.5V (scan rate: 0.05 V/s).

Figure 4.17 show that the effect of iron in 0.1 M PBS in absence of dissolves oxygen. Dissolve oxygen are removed by passing nitrogen gas for 15 minutes. CV response after elimination of oxygen from buffer solution has been investigated at a scan rate of 50 mV/s. Here, without (a) and with (b) of iron(II) but no peak was observed. So, we can conclude that after removing dissolve oxygen iron did not give any oxidation and reduction peak in PBS solution.

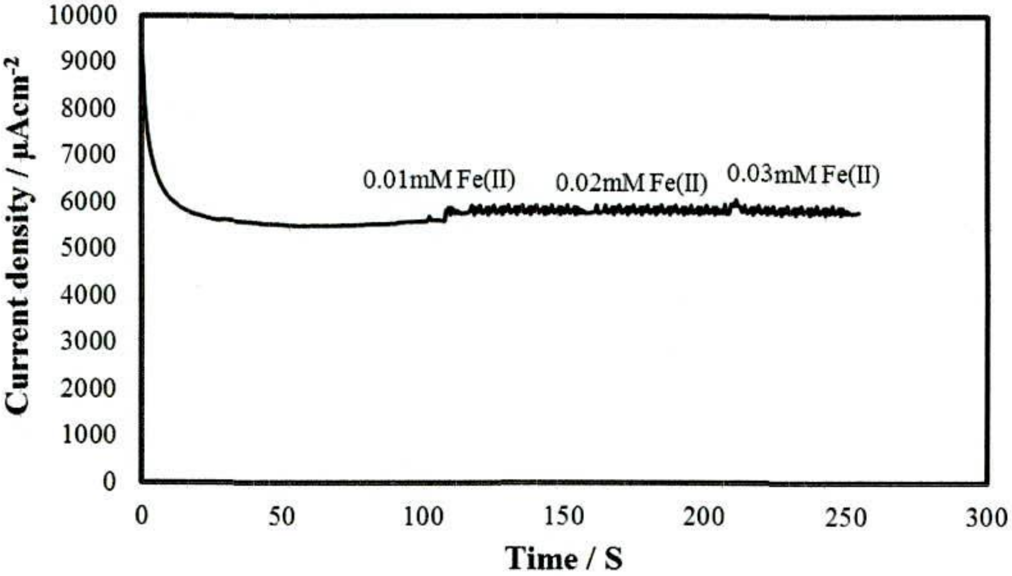

Figure 4.18: Typical amperometric current response of the 2B PGE upon the successive injection of $FeCl_2$ at E_{app}=1.5 V 0.1 M PBS pH 7.

Figure 4.18 shows that the interference studies of iron in absence of dissolve oxygen at applied potential 1.5 V in 0.1 M PBS. Then step wise added Fe(II) solution but no interference in absence of dissolves oxygen. So, we conclude that iron have only effect in presence of dissolve oxygen in PBS.

As we have investigated the sensing of free available chlorine using 2B PGE as sensing platform. However, to reduce further the fabrication cost, as well as improve the size and shape of the sensing platform, we have investigated paper as electrode platform. Therefore, in our next work pencil drawn paper electrode (PDPE) has been used instead of 2B PGE as sensing platform for free chlorine determination.

4.11 Electroanalytical activity of PDPE

CVs of the pencil drawn paper electrodes were carried over the range of -1.0 V to 1.5 V, with a fixed scan rate of 50 mV/s. It is well known that a high concentration of PBS can corrode paper; however a dilute solution of 0.1 M was used throughout to overcome this issue. The porosity of the paper substrate is likely to promote the penetration of the solution into the structure and as such the time submerged is considered a contributing variable to the analyses of the electrochemical properties of the electrodes. To investigate this effect of the time submerged within the electrolyte on the CVs is also investigated. The analysis of the electrodes is carried out for both single sided, where the interdigitated design is drawn onto one side, and double sided, where the design is on both sides. It has been shown that having double sided, or 'back-to-back' electrodes can improve the reliability, and signal strength as a result of the increased surface area [135].

This effect is even more important in terms of paper-based electrodes, where the electrolyte can penetrate into the paper, it is expected that it can then interact with the graphite electrode on the paper surface from both the exterior surface and the interior of the paper. CVs are performed from the moment that the electrodes are submerged. The change in the voltammogram before and after the addition of NaOCl will provide information on the nature of the electrodes, and whether the electrolyte can penetrate into the cellulose network of the paper substrate, and if so how long the system takes to settle to equilibrium [135].

In this work we have investigated the electroanalitical activity of PDPE to determine free chlorine. Figure 4.19 presents the CV responses of PDPE in 0.1 M PBS without (a) and with (b) 10 mM NaOCl. In the absence of NaOCl, anodic current can be seen at around 0.7 V. Upon the addition of NaOCl, a significant increase of the anodic current can be observed on PDPE. It also can be seen that with the addition of NaOCl the anodic peak shifts to the lower potential. This behavior may attributes to the oxidation of graphite in the presence of NaOCl [154]. The instability of graphite at oxidizing potential leads to carbon decomposition into CO_2, HCO_3^- and CO_3^{2-}. The oxidation can be greatly accelerated in the presence of hypochlorite (free chlorine), producing salt and CO_2. Therefore, degradation of PDPE electrode and its lifetime has to be taken into consideration before it applied as free chlorine sensor.

$$C + 2\,NaOCl \longrightarrow CO_2 + 2NaCl$$

In addition, Entwisle [154] also proposed two mechanisms that may involve with the oxidation of graphite.

Mechanism I:

$$H_2O \longrightarrow O_2 + H^+ + e^- \qquad E^o = +1.23\ V$$

$$H_2O + C \longrightarrow CO_2 + 4H^+ + 4e^- \qquad E^o = +0.144\ V$$

Mechanism II:

$$NaOCl + 3H_2O \longrightarrow ClO_3^- + Cl^- + O_2 + H^+ + 6e^-$$

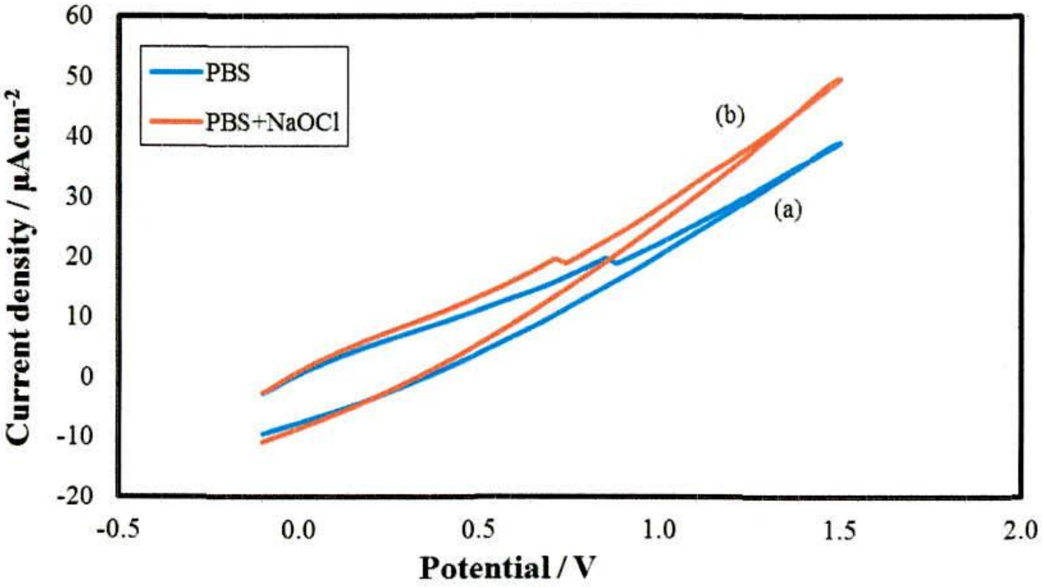

Figure 4.19: CVs in 2B PDPE (a) in the absence of NaOCl and (b) in the presence of 10 mM NaOCl in 0.1M PBS containing at E_{app}= -0.1 to +1.5V (scan rate: 0.05 V/s).

However, mechanism II mainly happens in the defects and pores (Figure 4.2a) that are available on the PDPE. Similar behavior was obtained in boron doped diamond electrode for free chlorine detection, where the reaction occurs on the surface of the electrode [155]. Therefore, based on the literature, we can postulate that mechanism II can occur both on the surface of the PDPE as well as in the defects and pores that are available in PDPE. Due to this combined effect anodic current increased significantly on the addition of NaOCl while PDPE is the working electrode. This behavior cannot be seen when GC, gold or platinum electrode is used as working electrode (voltammogram is not shown here). It is to note that Si Pan [148] modified PGE using ammonium carbamate and observed increased cathodic current due to the addition of NaOCl, which authors claim that ammonium carbamate reacts cathodically with NaOCl and increased cathodic current. In this case PDPE does not need any modification; only by the implementation of 1.5 V, a significant increase of anodic current can be seen.

4.12 Free chlorine sensing by PDPE

Figure 4.20 shows a typical amperometric responses of PDPE upon the successive addition 1 mM free chlorine in 0.1 M PBS at the applied potential of 1.5V with continuous stirring. PDPE electrode could successfully detect free available chlorine at the applied potential of 1.5V. From the slope (Figure 4.20) the sensitivity of free chlorine on PDPE found to be 36 ± 3.65 $\mu AmM^{-1}cm^{-2}$ with the limit of detect of 88.9 μM and linearity up to 12 mM for free available chlorine. The response time t_{90} for the free available chlorine on PDPE found to be less than five seconds. Standard deviation has been calculated for each measurement and has been shown in Figure 4.21.

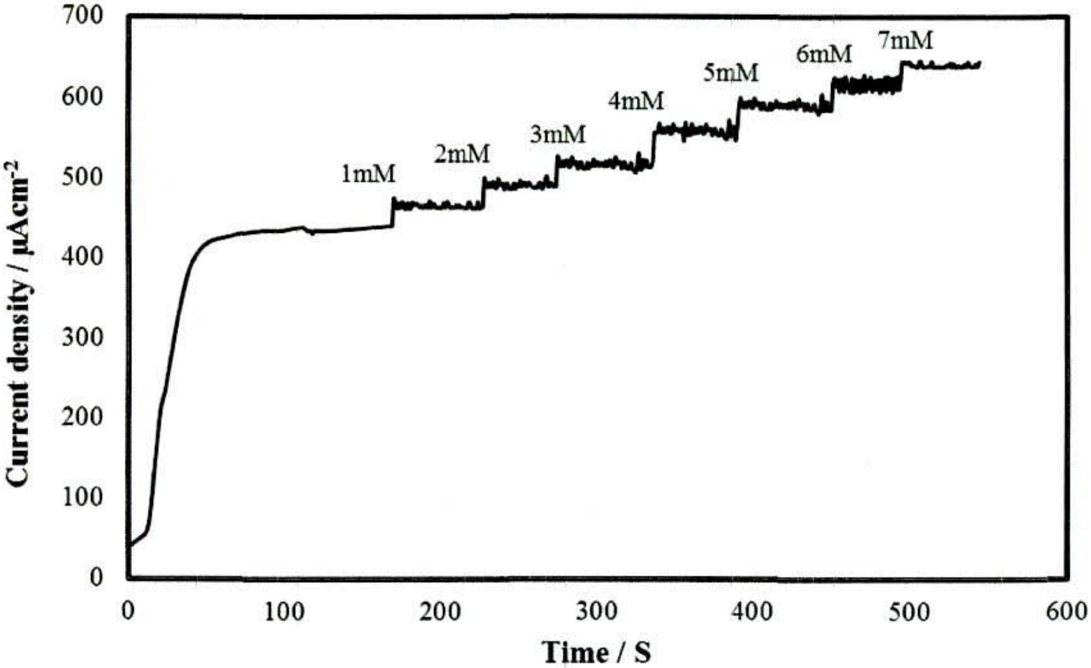

Figure 4.20: Typical amperometric current response of the PDPE upon the successive injection of NaOCl with concentrations from 1 mM to 7 mM into stirred at E_{app}= 1.5V 0.1 M PBS pH 7.

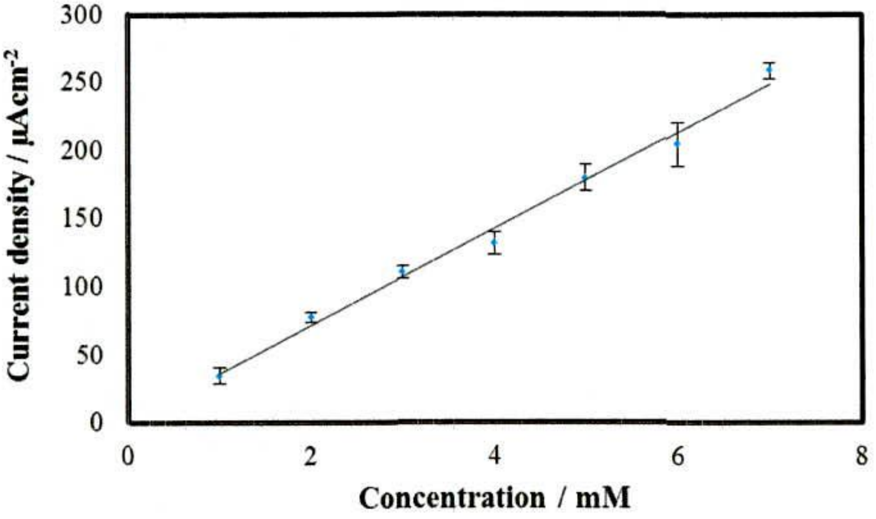

Figure 4.21: The plot of concentration effect current density vs. the corresponding concentrations of NaOCl in PDPE.

4.13 Interferences studies of PDPE

Some interfering agents such as chloride, sulfate, bicarbonate, carbonate, and nitrate ion are usually presence along with free chlorine in surface water. One of the major challenges are sample matrix effect, where these interfering species may affect the detection of free chlorine while using PGE or PDPE. Therefore, we have examined the amperometric responses of the PDPE at an applied potential of 1.5 V in 0.1 M PBS solution at a scan rate 50 mV/s with continuous additions of 0.0018, 0.0135, 0.0113, 0.0145 and 0.0073 mM chloride, sulfate, bicarbonate, carbonate, and nitrate ion respectively, along with 1 mM free available chlorine (Figure 4.22). These concentrations have been selected for matrix effect study in this work based on the reference [148], which is the usual presence in surface water.

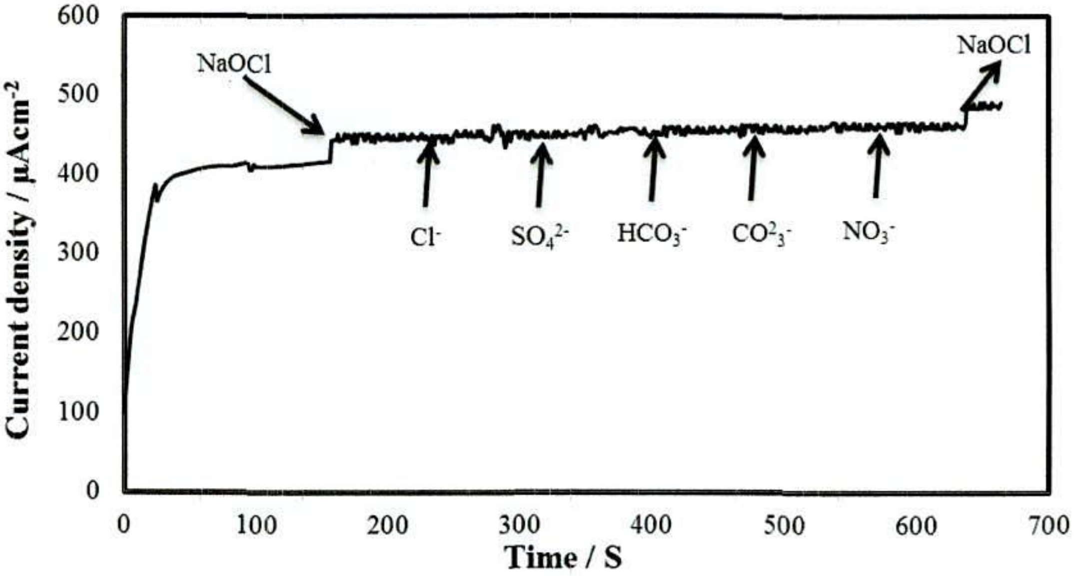

Figure 4.22: Interference test of the sensor in 0.1 M PBS at 1.5V with NaOCl and other interferents including 0.0018, 0.0135, 0.0113, 0.0145 and 0.0073 mM chloride ion, sulfate ion, bicarbonate ion, carbonate ion, and nitrate ion.

Figure 4.22 showed that there is no obvious current signal obtained for the addition of sulfate, bicarbonate, carbonate, and nitrate ion, whereas, high signal can be seen for the free chlorine addition on PDPE. It indicates that PDPE showed good selectivity toward free chlorine and could be used as a noble sensing platform for highly selective and sensitive detection of free chlorine in the surface water.

4.14 Optimization of pH

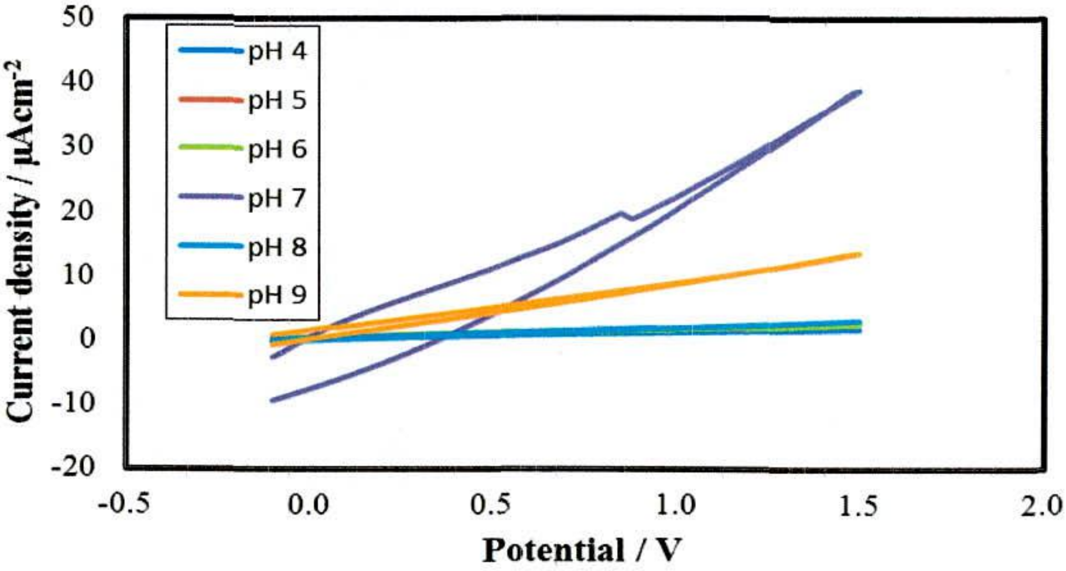

Figure 4.23: CVs of 0.1M PBS 0.05 V/s scan rate at different pH were performed at 2B PDPE.

The effect of pH was determined by scanning the voltammograms over the pH range of 4-9 at room temperature. The response of current density on PDPE for different pH is shown in Figure 4.24. The maximum current can be obtained at pH 7, which aligned with the chlorination of water, where most of the chlorination occurs at around pH 7. Also the normal pH of the surface water is around 7.

4.15 Stability of PDPE

The stability of the PDPE was determined by using it 7 times, with analysis carried out in different times in a day by the addition of 10 mM NaOCl (Fig 4.25). In between the testing, the electrode was stored in air at ambient conditions. It seems electrode retain lower activity after first time usages although we investigate the amprometry of PDPE when we added different times NaOCl in several times (normally keep in solution 3 min.) because we fabricate normal cellulose paper. If could be improved the quality of cellulose paper then it can be used several times at a glance. Figure 4.25, it exposes that the stability of PDPE is 100% at first time (no of usages) and 42% after 4 times (no of usages) of its initial activity and 22 % activity was retained after 7 times usages.

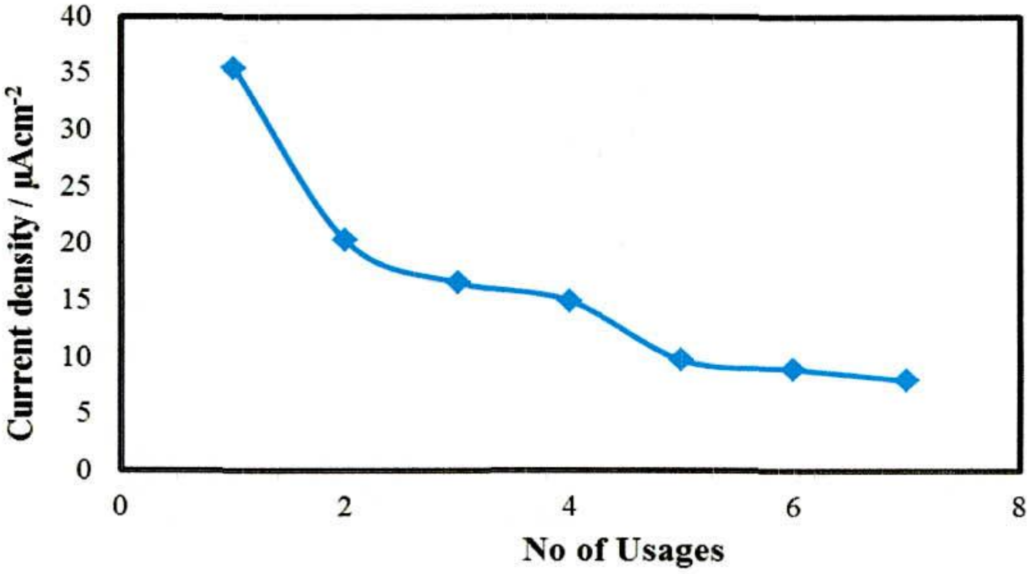

Figure 4.24: Stability of the PDPE over a no. of usages.

4.16 Summary

In present study, we have developed a sensitive and selective free available chlorine sensor using novel PDPE. The results of electrochemical detection of free chlorine indicate PDPE exhibit high selectivity, high sensitivity and low detection limit toward the oxidation of NaOCl. Our present study is also important because it provides us a novel method of fabricating paper based sensor platform to detect free available chlorine, which would be cost effective, facile, easy to handle. A comparative study has been established with other similar type of electrode platform (Table 4.3). The parameters that are selected for the Table are electrode sensing materials, key performance parameters, and corresponding fabrication processes. It seems from the Table that a good number of works has been focused on the optimization of working electrodes than the development of active sensing materials. Although fabricated free chlorine sensors can be developed for cost-effective applications, their sensitivity varies widely and they are less accurate and reliable than the conventional sensors. It indicates that the fabricated electrochemical free chlorine sensor is a challenging area of research that requires further intensive investigations [151].

Table 4.3: A comparison of the performance of some sensor platform for chlorine detection

Electrochemical technique	Electrode / Sensing material	Detection range [mM]	Sensitivity $\mu AmM^{-1}cm^{-2}$	Response time [s]	Stability	Cost	Ref.
Amperometry	Au	1.5-8	69.20	20	7 days	$300	[156]
	Au	2-5	14.20	120	10 days	$300	[157]
	Pt	4-400	–	–	–	$350	[158]
	Pt	1.5	8.00	–	5h	$350	[159]
	PGEs modified	1-6	30.20	3	7 weeks	$160	[148]
	PGEs	1-8	50.00	3	14 days	5 taka only	[151]
	PDPE	**1-7**	**36.00**	**5**	**7 days**	**0.2 taka only**	**This work**

Table 4.3 shows Comparison of analytical performance of our proposed chlorine sensor with other published chlorine sensors where we show that a number of works have been reported on various strategy of employing modified & unmodified electrode surface to improve the sensitivity. However, majority of them involved complicated processing steps, which are not efficient to improve the sensitivity and overall reliability of the system. In this work, we fabricated a novel paper based unmodified electrode and evaluated them in free chlorine detection. Moreover, we have developed a new, cost effective; sensitive (36 $\mu AmM^{-1}cm^{-2}$) PDPE based electrode platform. From the present study a sensitive and selective chlorine sensor has been developed based on PDPE. This study has shown PDPE to be green, low cost, simple and easy to prepare. These electrodes are easy to use in any electrical device. Excellent electro-analytical behavior has been found for free chlorine detection using PDPE. Sensitivity of PDPE has been found to be 36 ± 3.65 $\mu AmM^{-1}cm^{-2}$

and exhibited linear behavior for chlorine detection in the concentration range up to 7 mM with a limit of detection of 88.9 μM.

In addition to PDPE, in this work we also have shown sensing behavior of 2B PGE as electrochemical free chlorine sensor [151]. Where we have taken guidance from a thesis based on our laboratory. The sensitivity has been found to be 121 ± 5.38 $\mu AmM^{-1}cm^{-2}$ and exhibited linear behavior for chlorine detection in the concentration range up to 12 mM for PGE with a limit of detection of 59.5 μM. We also have tried using nanoparticle modified PGE electrode, with a nano particle of ZnO, CuO and NiO. However, the sensitivity and response we have found using nanoparticle modified PGE is not satisfactory in comparison to unmodified PGE. As PGE contain various additives and materials with the graphite. Therefore, the effect of these additives and other materials has been examined, such as the effect of iron, silicon, calcium and magnesium of the sensor response. In fine we have found that unmodified PDPE based electrode platform as one of the lucrative sensing platform for the detection of free available chlorine in water or buffer sample. However, further study is being going on to improve the paper based sensing platform so that it can be used many times at wet condition.

CHAPTER V

CONCLUSIONS

&

RECOMMENDATIONS

CHAPTER V

Conclusions

In summary, a novel chlorine sensor has been successfully fabricated using pencil drawn paper based electrode as sensing platform where no modification of the electrode is needed for free available chlorine detection. Initially different grade pencil graphite electrodes have been investigated and 2B PGE and 4B PGE has been found to have the most suitable and sensitive for free available chlorine detection in aqueous medium. Afterward, pencil drawn paper electrode (PDPE) has been fabricated using 2B PGE and tested them in details whether the paper based platform is suitable for this study. PDPE showed the sensitivity of 36 ± 3.65 $\mu AmM^{-1}cm^{-2}$ with a limit of detection 88.9 μM. Linearity range of FAC detection has been obtained up to 7 mM with RSD value ± 3.65 $\mu AmM^{-1}cm^{-2}$. PDPE electrode showed stable response with excellent selectivity towards free chlorine in the presence of other relevant matrix. Also the response time for the detection has been calculated for both PGE and PDPE around 5s. In addition to PDPE, we have also examined ZnO/PGE, CuO/PGE and NiO/PGE as sensing platform for the detection of FAC. Sensitivity found to have weak for ZnO/PGE and NiO/PGE and no response is obtained for CuO/PGE. However, nanoparticle modified result compare to blank PGE and blank PDPE, the response of nanoparticle modified electrode was weak and did not proceed for further study for the free chlorine detection.

Recommendations

Further the work will extend the system

- Development of advanced sensing materials that will reduce the applied potential.
- Improving the quality of paper for the PDPE so that the sensor can be used many times.
- To enhance the stability and sensitivity.
- Extensive fundamental electrochemical properties need to be investigated.
- Feasibility study of its commercialization.
- To enhance the cross reactivity of chlorine in presence of interferences.

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