ADDIS ABABA UNIVERSITY

College of Natural and Computational Sciences

Department of Chemistry

Analytical Chemistry Stream

DEVELOPMENT OF CHOLINE CHLORIDE MODIFIED GLASSY CARBON ELECTRODE FOR THE DETERMINATION OF PENTACHLOROPHENOL

BY

MERON BERHE

ADVISOR:

NEGUSSIE NEGASH (Ph.D)

JULY, 2021

ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

DEVELOPMENT OF CHOLINE CHLORIDE MODIFIED GLASSY CARBON ELECTRODE FOR THE DETERMINATION OF PENTACHLOROPHENOL

A Thesis Submitted to the Department of Chemistry in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

BY

MERON BERHE

ADVISOR:

NEGUSSIE NEGASH (Ph.D)

JULY, 2021

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

This is to certify that the thesis prepared by Meron Berhe entitled Development of Choline Chloride Modified Glassy Carbon Electrode (ChCl/GCE) for the Determination of Pentachlorophenol submitted for the fulfillment of the requirements for Degree of Master (Analytical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved by the examining board signature date

Dr. Negussie Negash _____

_____

(Advisor)

Dr. Merid Tessema _____

_____

(Examiner)

Dr. Solomon Mehretie _____

_____

(Examiner) I

Declaration

I declare that this thesis entitled “Development of choline chloride modified glassy carbon electrode for the determination of pentachlorophenol” is my own work done under the supervision of Dr. Negussie Negash, Department of Chemistry, Addis Ababa University. It has not previously submitted in any form for obtaining any qualification at any university, and all the references have been clearly recorded and acknowledged.

Name: Meron Berhe

Signature: _____

This thesis has been submitted for examination with my approval as University advisor.

Advisor: _____

Signature: _____

Acknowledgements

First and for most, I would like to thank the Almighty God for giving me the strength to do the project work and for everything that happens to me.

I would like to express my sincere gratitude to my advisor Dr. Negussie Negash. I am grateful for all his guidance, motivation, and friendly approach during this work. I sincerely thank Ato Tesfu Hailu (Analytical chemistry PhD student) for his sincere guidance, technical aid and assistance in every day laboratory problems facing me in the research direction, and I would like to thank also Gizawu and Endale (PhD students) for helpful discussions and intellectual advice.

I would like to express my thanks to SIDA and Addis Ababa University, Department of Chemistry, for providing me this scholarship, for the financial support and giving me access to laboratory facilities for this study.

I would like to extend my thanks to Ato Hagos Yishak for morally supporting and helping me by giving valuable advice during the difficulty times.

I am grateful to all my classmates and my friends for their support and making my time enjoyable at Addis Ababa University.

Finally, my heartfelt gratitude shall go to my family, my mother Mamu Beyen, my sister Hana Berhe and my uncle Tsige Atsbeha for their understanding and providing me unfailing support during this program.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ABS	Acetate Buffer Solution
A	Ampere
ChCl	Choline Chloride
Ch^+	Choline Cation
CE	Counter Electrode
CME	Chemical Modification of Electrode
CP	Chlorophenol
CV	Cyclic Voltammetry
D	Diffusion Coefficient
DCP	2,4-Dichlorophenol
E_{pa}	Anodic Peak Potential
Epc	Cathodic Peak Potential
$E_{1/2}$	Half Wave Potential
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GCE	Glassy Carbon Electrode
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
i_{pa}	Anodic Peak Current
i_{pc}	Cathodic Peak Current
I	Current
M	Molar
mM	Milli Molar
MWCNT-EP	Multi Wall Carbon Nanotubes-Epoxy
PBS	Phosphate Buffer Solution
PCP	Pentachlorophenol
ppm	Parts per million
RE	Reference Electrode
R	Regression Coefficient

TCP	2,4,6-trichlorophenol
WE	Working Electrode
µA	Microampere
µL	Micro Liter
µM	Micro Molar

Abstract

A simple, sensitive electrochemical sensor was developed for the determination of pentachlorophenol (PCP) using low cost and environmental friendly choline chloride (ChCl) modified glassy carbon electrode (GCE). The structural and electrocatalytic activities of the electrodes were characterized using Fourier transform infrared spectroscopy (FTIR), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The covalent attachment of ChCl on to GCE results in a stable positively charged choline chloride modified glassy carbon electrode (ChCl/GCE) with large effective surface area. The ChCl/GCE also showed a remarkable improvement for the oxidation activity of PCP, which results in enhancement in the current response compared to the bare GCE. Under optimum conditions, the square wave voltammetric current of PCP was proportional to its concentration in the linear range of 9×10^{-9} to 1×10^{-5} mol L^{-1} with a detection limit 4.45×10^{-9} mol L^{-1} (S/N=3). The developed method was more sensitive and simple as compared with the literature reports. Additionally, it is also successfully applied for the determination of PCP in three different water samples collected from the river around the industrial area, the tap water and effluents from the pulp and paper industry.

Keywords: Choline chloride, Pentachlorophenol, Cyclic voltammetry, Square wave voltammetry

1. Introduction

In last decades environmental contamination increased due to discharge of toxic industry wastes, chemicals, herbicides, insecticides, and plasticizers from medical and agricultural activities and causes serious problems in living organisms. Organochlorinated compounds like 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6- trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) are the major groups of environmental pollutants due to their toxicity and extensive usage (Olanirann and Igbinosa , 2011). PCP is the most toxic chlorinated phenol, but it is widely used as bactericides, insecticides, fungicides and wood preservatives in wood treatment, paper production, leather industry, and agriculture. It is highly toxic and persistent in water and soil. The accumulation in living organisms causes health effects including carcinogenicity, acute toxicity, and other adverse effects (Remes *et al.*, 2012). Therefore, it is important to develop sensitive and repaid method to determine the trace concentration of PCP in various environmental samples.

Gas chromatography-mass spectrometry (Mardones *et al*., 2003), high-performance liquid chromatography (Baiocchi et al. 1995; Han *et al*., 2005), enzyme-linked immunosorbent assay (Chuang *et al*., 2006), phosphorescence (Wang *et al*., 2009), electrochemical chemiluminescence (Deng *et al*., 2017) are some of the developed methods to determine PCP. However, their high costs, long analysis times, complicated sample pretreatment and requirement of skilled man power which makes them unsuitable for routine analysis. However, electrochemical methods have certain advantages like high sensitivity, selectivity, quick response, cheap price and simplicity.

Some of the reported electrochemical methods for detection of PCP are multi-wall carbon nanotubes-epoxy composite electrode (Remes *et al.*, 2012), GCE with a film of CuS nanocomposite-chitosan (Zou *et al*., 2013), poly (Rhodamine B)/graphene oxide/multiwalled carbon nanotubes composite film modified electrode (Zhu et al., 2016). However, those methods have problems due to the narrow linear range, low sensitivity, high cost, complex modification, and toxicity of chemicals. So it is important to develop sensors which are inexpensive, environmental friendly chemicals and simple such as choline chloride.

Choline chloride is a quaternary ammonium salt that contains –OH and $–N^{+}(CH_3)_3$) groups. It is non-toxic, biodegradable and relatively inexpensive since it is the waste product of soybean oil

production (Bahrani *et al*., 2018). The –OH group of ChCl makes it easily immobilized on the surface of carbon electrode through covalent linkage and the choline cationic polar head group ($–N^{+}(CH_3)_3$) also enhance the electrostatic attraction (Jin and Lin, 2004). The electrochemical sensor for the determination of PCP in aqueous solutions using choline chloride glassy carbon electrode (ChCl/GCE) was developed in this work. Fourier transform infrared spectroscopy, electrochemical impedance spectroscopy, and cyclic voltammetry was used was used to confirm the covalent attachment of ChCl.

2. Literature Review

2.1. Pentachlorophenol

Pentachlorophenol is an organochlorinated compound which consists of the benzene ring, –OH group and chlorine atom(s) with chemical formula of C_6HCl_5O and its structure formula is shown in Figure 1.

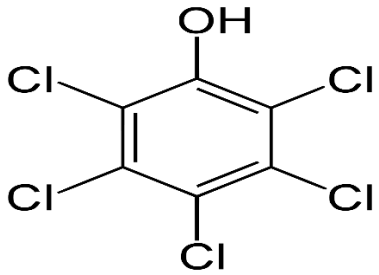

Figure 1 : Chemical structure of pentachlorophenol

2.1.1 Physical and Chemical Properties of Pentachlorophenol

PCP is white crystalline solid and little odor at room temperature but it have phenolic odor when hot. Some of the physical properties of PCP are listed in the Table 1.

Table 1: Physical properties of pentachlorophenol.

Molecular weight	266.35 g/mole
Melting point	190°C
Boiling point	310°C
Density	1.98 g/cm³
Vapor pressure {20°to l00°C.)	0.00011 to 0.12 mm Hg

Solubility in water {20°c.)	14 ppm
pKa	4.7

The aqueous solubility of PCP is low, but the sodium salt is more soluble. PCP is soluble in most organic solvents such as soluble in methanol(1800 g/L), ethanol(1200 g/L), isopropanol (850 g/L), ethyl glycol (110 g/L); acetone (215 g/L), diethyl ether (150 g/L), benzene(150 g/L) and slightly soluble in cold petroleum ether, carbon tetrachloride and paraffin (Lide, 2002; Bevenue and Beckman, 1967).

PCP is a highly stable compound in the pure form and not combustible, but decomposition occurs in the environment under certain conditions releasing irritants and toxic gases. Decompositon by heat produces hydrogen chloride, chlorinated phenols, and carbon monoxide, thermal degradation (at 600°c) products of pentachlorophenol include pentachlorobenzene, hexachlorobenzene, octachlorostyrene, octachloronaphtahalene, decachlorobiphenyl, hexachlorodibenzofuran, octachlorodibenzofuran, and octachlorodibenzodioxin (WHO,1987).

2.1.2. Environmental Distribution of Pentachlorophenol

PCP introduced into the environment as effluent waste from several industrial processes, through its use as biocides or as by-products of other industrial operations, such as pulp bleaching with chlorine, water disinfection or chlorination (King *et al*., 2010), and it may also be released to the atmosphere during its use as a wood preservative. It can also appear as degradation products of other chlorinated xenobiotics. Wood treatment factories contribute significantly to pentachlorophenol load in surface water in the concentration ranges from non-detectable to 10,500 µg/L. The majority of analyzed water samples contained less than 10 µg/L. The pH in water, soil and sediment affects the degree of ionization, fate and transport of PCP. PCP is ionized at pH 6.7 and in easily leachable form, so the groundwater contamination may be a result of soil contamination. The bottom sediments highly accumulated PCP than surrounding waters up to 590 µg/kg (Olanirann and Igbinosa , 2011; WHO, 1987).

2.1.3. Uses of Pentachlorophenol

Pentachlorophenol and its sodium salt was first introduced in the 1930s for use as wood preservative (Lopez-Echartea *et al*., 2016). PCP widely uses as pesticide, molluscicide,

fungicide, bactericide, herbicide, defoliant, anti-sapstain and anti-microbial agent in industry and agriculture (Feng *et al*., 2015). Because of its toxicity to insects, it become one of the nonspecific pesticides used in our environment. PCP has been used to control mold and termite infestation in the construction and lumber industries and in homes, and for the control of powder post beetles and wood-boring insect. Sodium pentachlorophenate has been used as a molluscacide for the destruction of the snail which act as intermediate hosts for the larvae that cause schistosomiasis in human. The snails perpetuating this life-cycle are found in streams, irrigation ditches, swamps, lakes, and contaminate drinking water supplies in areas, where schistosomiasis is prevalent (Bevenue and Beckman, 1967).

PCP have many applications as a fungicide and bactericide. It used to control fungus and bacteria growth in the processing of cellulosic products, starches, adhesives, proteins, leather, oils, and rubber and it has also been used in paints, rug shampoos and textiles to control mildew problems. PCP used as herbicide and defoliant in agriculture to control weeds on pasture land, in pineapple and sugarcane fields and uses as a preplanting herbicide and preharvest defoliant on crops producing cooking oils. Those uses of PCP indicates the extent of its proliferation and contamination of the environment (IARC, 2019; Bevenue and Beckman, 1967).

2.1.4. Effects of Pentachlorophenol

PCP and its metabolites pose a health hazard due to their toxicity to numerous organisms. The primary source of PCP pollution comes from the application of pesticides that are made from chlorophenols and the chlorination of wastewater containing phenol. Due to their potential carcinogenic and mutagenic activity and toxicity it is harmful for human health. PCP absorbed by inhalation in humans have resulted in neurological, blood and liver defects, and eye irritation. If it enter to body, the cells in the body produced heat, causing in body temperature elevation with an extremely rapid pulse rate and increased respiration. The elevated body temperature can increase to dangerous levels like rapid heartbeat, causing injury to various organs, tissues, and even cause death (Olanirann and Igbinosa , 2011).

The PCP poisoning also affects the animals. The long-term exposure to low levels pentachlorophenol cause the damage of endocrine system and immune system of animals. Because of their lipophilicity it can be transported through the cell membrane and bio-

accumulate in aquatic organisms through the uptake from the surrounding water or along the food-chain. Low levels of dissolved oxygen, low pH and high temperature increase the toxic effects of PCP. Most aquatic invertebrates and vertebrates are affected by PCP concentrations below 1 mg/L in acute toxicity tests and it kills the fish species in concentrations above 0.2 ppm. Algal species appear to be the most sensitive aquatic organisms, as 1 μg/L PCP can cause significant inhibition. PCP disrupts the energy-yielding mechanism of the aerobic organism or accelerate the breakdown of ATP in the living organism (Albert, 1978; Lopez-Echartea *et al*., 2016).

2.1.5. Production of Pentachlorophenol

Pentachlorophenol is synthesized via two pathways, either by stepwise chlorination of phenols, known as the Boehringer process in the presence of catalysts (anhydrous aluminium chloride or ferric chloride) or alkaline hydrolysis of hexachlorobenze, the Dow process. In the Boehringer process chlorination of phenol occurs in two stages. In first stage chlorine is bubbled through phenol at 105 °F to yield tri-and tetra chlorophenols (Phenols with three and four chlorine atoms respectively). In second stage, the temperature is gradually increased to 130 °F to keep the reaction mixture molten and to further chlorinate the tri and tetrachlorophenols to form pentachlorophenol. However, the technical grade penta contains from 4 to 12 percent tetrachlorophenols, which are toxic and the high temperatures produce several contaminants including hexachlorobenzene, dioxins, and furans. So, the analytical grade of pentachlorophenol requires in purification to remove the contaminants that were created during the manufacture of pentachlorophenol (Fisher, 1991).

The worldwide production of pentachlorophenol in 1981 was estimated to be 90 000 tonnes per year, but decreased through time. In Poland, Germany, the Netherlands, Switzerland, the United Kingdom, Spain, and France stopped the production in 2003, but China still produces pentachlorophenol with an annual production volume of 5000 tons reported in 2010 (United Nations, 2010).

2.2. Chemically Modified Electrode

Chemically modified electrode (CME) is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic, or polymeric film

of a chemical modifier for different function. They are prepared by immobilizing the electrode with the desired sensing properties, for example chemical, electrochemical and enhanced transport. CMEs uses a minimum quantity of reagents for analysis, accelerate the electron transfer reactions, preferential accumulation, and provide better selectivity by reduction of interference of the analysis are some of the advantages (Durst *et al*., 1997).

CMEs can be subdivided into four categories based on the nature of the modification process. (a) CMEs with monomolecular layers are fabricated by formation of self-assembled monolayers onto the electrode surface by chemical bonding; (b) CMEs also can be modified by covalent attachment of a monolayer via formation of a covalent bond between electrode and electroactive reagent, either by a synthetic route or by controlling the oxidation/reduction potentials in a suitable medium. (c) CMEs also can be modified with films of redox polymers, ion exchange polymers (viz. Nafion^{tm}), electrically conducting polymers, crown ethers, complexing agents, electrostatic modification etc. (d) Modified electrodes can be obtained by coating them with nanomaterials such as metallic nanoparticles, carbon nanotubes, graphene, metal complexes, clay, or with macrocyclic chemical compounds (Murray *et al.*, 1987).

In the 1970s, the modification of electrode surfaces by covalent attachment of monolayers of different species to electrode surfaces arises. The stronger attachment to the electrode surface can be accomplished by covalent linking of the desired component to surface groups present on, or formed on, the electrode. The electrode surface is usually pretreated by an oxidative reaction to form active surface groups. Then the surface is treated with the linking agent and the desired component (Bard and Faulkner, 2001).

All catalytic CMEs have relied on the immobilization of redox center on the electrode surface. The immobilized redox center acts as a fast electron transfer mediator for substrate species, which is oxidized or reduced at the naked electrode. The basic principle involved in CMEs electrocatalysis by a surface confined electron-transfer mediator is illustrated in Figure 2. The analyte diffuses from the bulk solution to the electrode surface, where it is oxidized in a purely chemical reaction with the oxidized form of the mediator (M_{Ox}). The potential of the electrode is maintained at a value sufficiently positive for M_{Ox} to be stable state of the mediator and its reduced form (M_{Red}) to be rapidly re-oxidized to the catalytically active form. Thus the heterogeneous electron transfer takes place between electrode and mediator and not directly

between the electrode and analyte. In essence, then, the mediator can be considered to function simply as an electron shuttle between the electrode and the analyte (Ciucu, 2014).

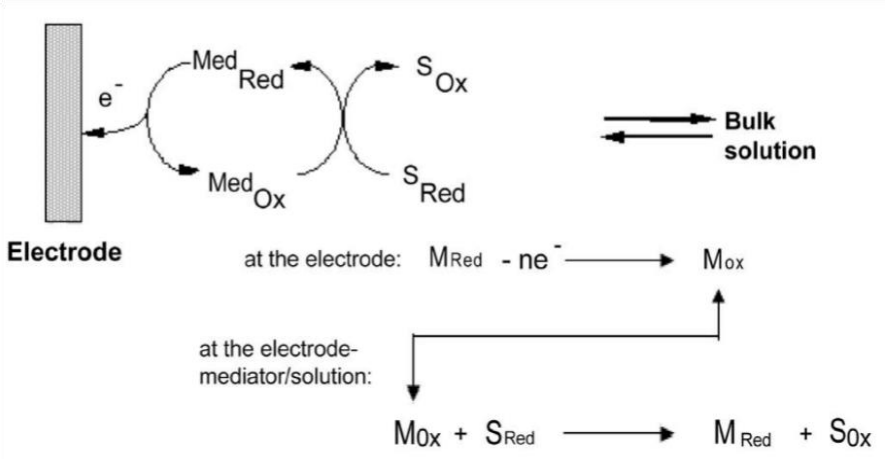

Figure 2: The model of electrocatalytic reaction on CME. Where S_{Ox}/S_{Red} and M_{Ox}/M_{Red} are the oxidized and reduced forms of analyte to be detected and mediator respectively.

2.3. Choline Chloride

Choline chloride is an organic compound which contains quaternary ammonium salt ($-N^{+}(CH_3)_3$) and alcohol (-OH) groups (Figure 3). It is non-toxic, biodegradable and relatively inexpensive since it is a waste product of soybean oil production. It is mainly uses as animal feeds. The choline cation is an essential nutrient belongs to an important class of vitamin B which plays an essential role in chemical reactions of the body by assisting in various metabolic mechanisms. It is also commonly used in deep eutectic solvents (Bahrani *et al*., 2018).

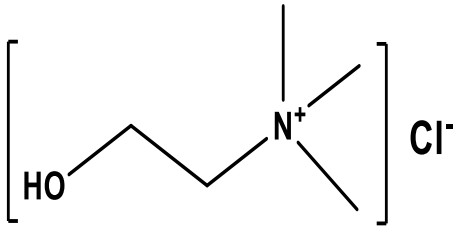

Figure 3: Chemical structure of choline chloride.

ChCl also used for modification of carbon electrodes by electro-chemical oxidation forming covalently bound to the edge plane sites of the carbon surface through the oxygen atom. The hydroxyl group of ChCl makes it easy to be immobilized or facilitate to assembled onto the

surface of carbon electrode through covalent linkage and the cholinium cationic polar head group ($-N^{+}(CH_3)_3$) can decrease the resistance of the electron transfer and enhance the electrostatic attraction (Jin and Lin, 2004).

2.4. Electrochemical Method

Electrochemical methods are analytical methods depend on the electrical properties of a solution of the analyte such as current, potential, or charge related to chemical parameters. They uses relatively inexpensive equipment to produce unique characterization information for molecules and chemical systems, qualitative and quantitative analytical data, thermodynamic and kinetic data. Electrochemical methods are powerful and versatile analytical methods that offer high sensitivity, low detection limits, accuracy and precision as well as a large linear dynamic range. Among the electrochemical methods pulsed voltammetric techniques are a common way of increasing detection sensitivity, with the aim of excluding capacitive contributions from the response and lowering detection limits (Wang, 2006).

2.4.1. Voltammetric techniques

Voltammetry is an electrochemical technique in which the current is measured as a function of applied voltage or electrode potential at a given time from the electrode - solution interface reactions. The current-voltage curve is called voltammogram. Voltammograms identify the target species by comparing potential from the redox potential table and determine concentration from the peak current. Electrodes and electrical instruments that control voltage are the basic equipment in voltammetry. Voltammetric techniques desired three electrodes i.e., working electrode (WE), counter electrode (AE) and reference electrode (RE). The reference electrode control the potential of the working electrode by measuring the voltage between these two electrodes (Simões and Xavier, 2017).

The voltage of the electrode that causes the chemical species to be oxidized or reduced in voltammetry is the controlled parameter. The current is measured as the electron flow on the electrode surface as a function of applied voltage at a given time. The current due to electron transfer that occurs when oxidation and reduction take place on the electrode surface is called Faradaic current. This current is linearly dependent upon the concentration of the electro active species (analyte) involved in a chemical or biological determination process (at a scanned or

fixed potential). The total current is the summation of the faradaic currents for the sample and blank solutions, as well as the non-faradaic charging background current (Bard and Faulkner, 2001).

The electrode reaction involves several steps. But the rate reaction is determined by the slowest step in the sequence called the rate determining step. The rate determining step will be a diffusion of redox species in solution near an electrode, adsorption on an electrode, or charging processes at the double layer. There are three transport mechanisms by which the electroactive species reach the surface, i.e., diffusion in a concentration gradient, migration of ions in a potential gradient, and convection. A concentration gradient is built up at the electrode/electrolyte interface when the compound is oxidized or reduced at the electrode surface. Voltammetry techniques vary depending on a mode of the potential control. Some of the most common techniques are cyclic voltammetry, differential pulse, square wave and stripping voltammetry (Elgrish *et al*., 2018; Wang, 2006).

2.4.2. Cyclic voltammetry

Cyclic voltammetry (CV) is a powerful and frequently used technique for acquiring qualitative information about electrochemical reactions. CV is also used to study electron transfer-initiated chemical reactions including catalysis (Elgrish *et al*., 2018). It is a simple, rapid, and powerful method for characterizing the electrochemical behavior of analytes that can be electrochemically oxidized or reduced. CV consists of scanning linearly the potential of a stationary working electrode using a triangular potential waveform as shown in Figure 4. This triangular potential excitation signal sweeps the potential of an electrode between two values, sometimes called the switching potential. (Wang, 2006).

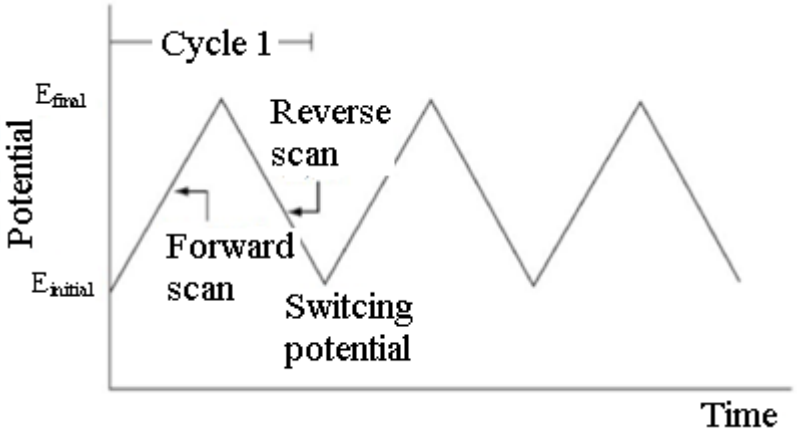

Figure 4: Potential –time excitation signal in cyclic voltammetric experiment.

The sweep rate at which the electrode potential between potential limits E_{inital} and E_{final} is also called scan rate. It indicates that during the experiment the potential was varied linearly at the speed (scan rate) per second. In the forward scan, the potential is swept negatively from the starting potential E_{inital} to the switching potential E_{final}. This is referred to as the cathodic trace. The final potential is called the switching potential, that is the point where the voltage is sufficient enough to have caused an oxidation or reduction of an analyte. The scan direction is then reversed, and the potential is swept positively back to E_{inital}, referred to as the anodic trace (Simões and Xavier, 2017; Elgrish *etal*., 2018).

During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting current–potential plot is called a cyclic voltammogram. The cyclic voltammogram is resulting from a single electron reduction and oxidation shown in Figure 5. The oxidation process occurs when a positive potential ramp is applied and the electro active species loses an electron at the electrode giving rise to an anodic peak current (i_{pa}) which usually gives an oxidation peak at a given potential (E_{pa}). When the potential is applied in the negative direction leading to the reduction process, cathodic currents (i_{pc}) are observed, typically giving a reduction peak at a given potential (E_{pc}).

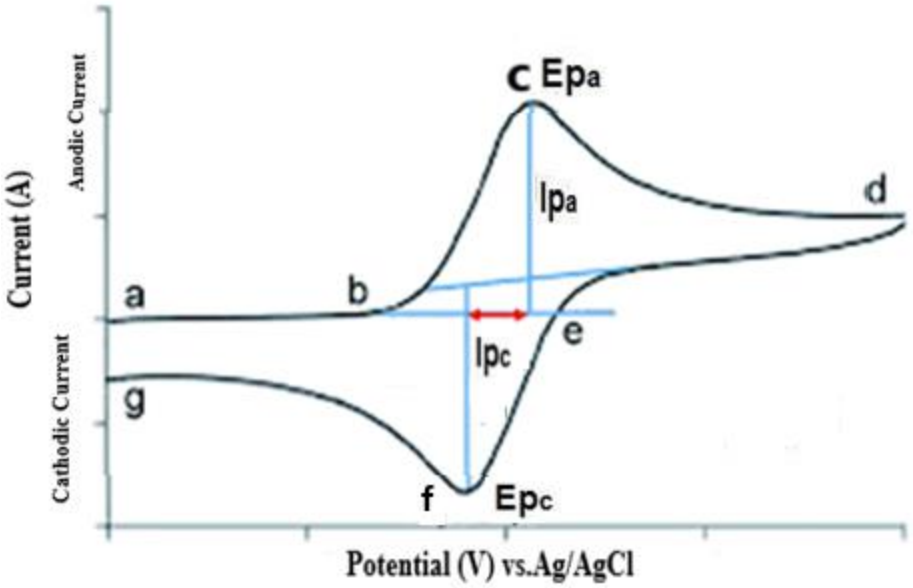

Figure 5: Cyclic voltammogram

In the initial potential there is no net conversion of reduced form (R) in to the oxidized form (O) (point a). As the potential is scanned negatively from point a to point d, O is steadily depleted near the electrode as it is reduced to R. At point c, where the peak anodic current (Ipa) is observed, the current is dictated by the delivery of additional O via diffusion from the bulk solution. The volume of solution at the surface of the electrode containing R, called the diffusion layer, continues to grow throughout the scan. This slows down mass transport of O to the electrode. Thus, upon scanning to more negative potentials, the rate of diffusion of O from the bulk solution to the electrode surface becomes slower, resulting in a decrease in the current as the scan continues (c → d). When the switching potential (d) is reached, the scan direction is reversed, and the potential is scanned in the positive direction. While the concentration of O at the electrode surface was depleted, the concentration of R at the electrode surface increased, satisfying the Nernst equation. The R present at the electrode surface is oxidized back to O as the applied potential becomes more positive. At points b and e, the concentrations of O and R at the electrode surface are equal, following the Nernst equation, $E = E_{1/2}$. This corresponds to the halfway potential between the two observed peaks (c and f) and provides a straightforward way to estimate the E^0 for a reversible electron transfer. The two peaks are separated due to the diffusion of the analyte to and from the electrode (Elgrish *et al*., 2018).

This equilibrium between the oxidized and reduced forms of the electro active compound is described by the Nernst equation that relates the electrochemical cell potential (E) and the relative activities of the O and R analyte in the system at equilibrium.

$$E = E^{o} + \frac{\mathrm{RT}}{\mathrm{nF}} \ln\left(\frac{O}{R}\right) \text{ ---------- (1)}$$

When the electron transfer kinetics between the electrode and the analyte is fast compared to mass transport, the equilibrium is quickly established and the processes follow the Nernst equation which refers to electrochemical reversibility. By contrast, when the electron transfer is slow and the equilibrium cannot be established with a sufficient rate, it will deviate from the Nernst equation, then the electrochemical cell becomes irreversible. The half-wave potential ($E_{1/2}$) for a reversible couple is centered between E_{pa} and E_{pc} (Scholz, 2015).

$$E = \frac{Epa + Epc}{2} \text{ ---------- (2)}$$

The separation between the peak potentials for a reversible couple is given in equation 3

$$\Delta E = Epa - Epc = \frac{0.059}{n} \text{ V ---------- (3)}$$

The peak separation can be used to determine the number of electrons transferred, and as a criterion for a Nernstian behavior. Accordingly, a fast one-electron process exhibits a ΔE_p of about 59 mV. The peak current for a reversible process (at 25°C) is given by the Randles–Sevcik equation (Wang, 2006)

$$I_p = (2.69\times10^5)\, n^{3/2}\, AD^{1/2}\, \nu^{1/2}\, C \text{ ---------- (4)}$$

Where i_p is the peak current in A, n the number of electron in the redox reaction, A- the electrode surface area in cm^2, D- is the diffusion coefficient in $cm^2\ s^{-1}$, C- the concentration in $molcm^{-3}$, and v the scan rate in Vs^{-1}.

For an irreversible process those with slow (sluggish) electron transfer at the electrode surface, the peak current is given by

$$I_p = 2.69\times10^5\, (n_a\alpha)\, AD^{1/2}\, \nu^{1/2}\, C \text{ ---------- (5)}$$

Where α is transfer coefficient, and n_a is the number of electrons in the rate determining steps. All other quantities have the same meaning as equation (4). Totally irreversible reaction only the peaks on the forward scan is observed and characterized by a shift of the peak potential with the scan rate (Wang, 2006).

2.4.3. Square wave Voltammetry

Square-wave voltammetry (SWV) is a large-amplitude differential technique in which a waveform composed of a symmetric square wave is superimposed on a base staircase potential. The currents are measured at the end of the direct square-wave pulses (I_1) and reverse (I_2) pulses, and the signal is the resulting differential current (ΔI). The resulting peak-shaped voltammogram is symmetric about the half-wave potential, and the peak current is proportional to the concentration. The potential current curve shape is the result of potentials of height ΔE (pulse amplitude), which vary according to a potential step E_{step} (in mV) and τ duration (period). On the potential–time curve, the pulse width ($\tau/2$) is denoted by t, and the frequency of pulse application is denoted by f and is given by ($1/t$) (Garay and Lovric, 2002; Wang, 2006). The Square-wave waveform is shown in Figure 6.

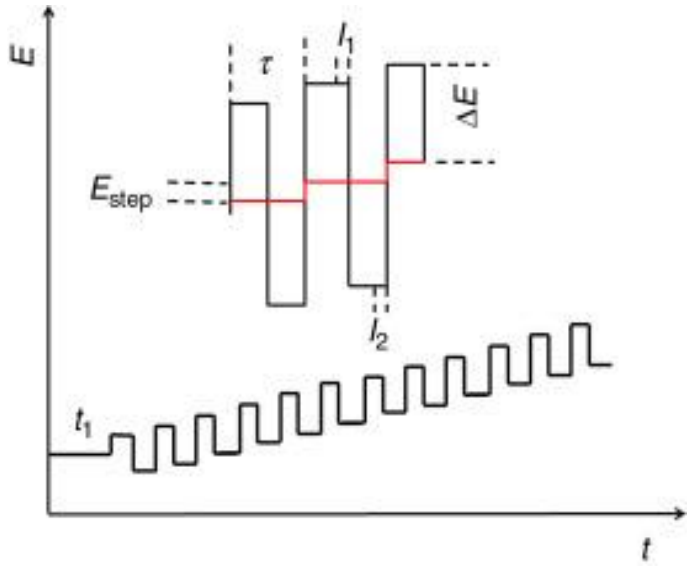

Figure 6: Square-wave waveform.

SWV is one of the most advanced pulse voltammetric techniques, which is a fast and powerful technique that offers background suppression, an enhanced sensitivity, the applicability to a wider range of electrode materials and systems, and simple methods for thermodynamic and

kinetic measurements. The SWV technique has also been used in the development of sensors and biosensors because of its high sensitivity and selectivity. It is currently of great interest to the pharmaceutical industry for the use of biomarkers in the detection of disease, environmental pollutants, such as heavy metals, and other chemical contaminants that are part of the environmental liability in contemporary societies (Simões and Xavier, 2017).

2.5. The Electrochemical Cell

An electrochemical cell, where the voltammetric experiment is carried out, consists of a working electrode at which the reaction of interest occurs, a reference electrode which is a stable potential against the potential of the working electrode, and the current-carrying counter (auxiliary) electrode. An electrode provides the interface across which a charge can be transferred or its effects felt (Bard and Faulkner, 2001). The modern voltammetric analyzer is the potentiostatic control of the working electrode, which minimizes errors due to cell resistance. The potentiostatic control is accomplished with a three-electrode system and a combination of operational amplifiers and feedback loop. The reference electrode is placed as close as possible to the working electrode and connected to the instrument through a high-resistance circuit that draws no current from it. To complete the current path auxiliary electrode is placed in the solution. Symmetry in the placement of these electrodes is important for the assumption that the current paths from all points on the working electrode are equivalent (Simões and Xavier, 2017; Wang, 2006).

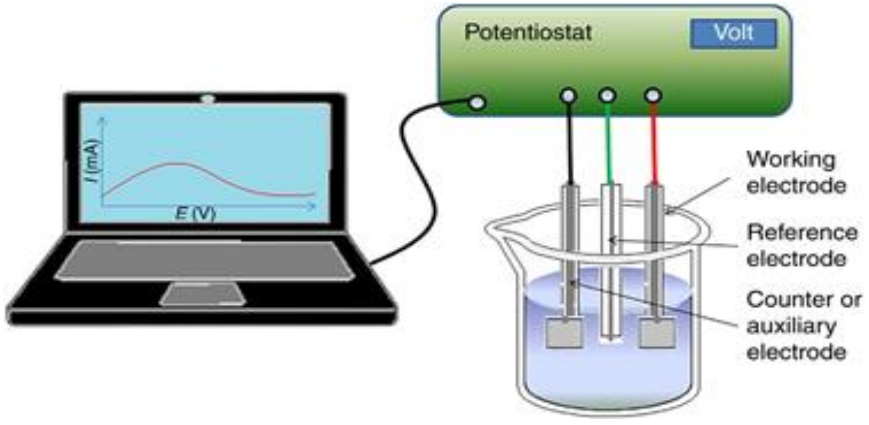

Figure 7: The electrochemical cell with three electrode.

2.5.1. The Working Electrode

The working electrode is where the reaction of interest take place. The reduction or oxidation of a substance occurs at the appropriate applied potential, results in the mass transport of new material to the electrode surface and the generation of a current. Between the working electrode and the reference electrode a fixed potential difference is applied, and this potential drives the electrochemical reaction at the working electrode's surface (Bard and Faulkner, 2001; Royce, 1982).

There are a lot of working electrodes available for use. Platinum, gold, silver, mercury and carbon (graphite, glassy carbon) electrodes are the most popular, but carbon–based working electrode materials include all allotropic forms of carbons graphite, glassy carbon, amorphous carbon, fullerenes, and nanotubes which are the most common working electrode. The selection of working electrode depends on the following factors (Royce, 1982).

1) The material should exhibit favorable redox behavior with the analyte, reproducible, fast electron transfer without electrode fouling.
2) The potential window over which the electrode performs in a given electrolyte solution should be as wide as possible to allow for the greatest degree of analyte characterization.
3) The cost of the material should be cheap.
4) Its ability to be machined or formed into useful geometries.
5) The ease of surface renewal following a measurement, and
6) Toxicity, it should be non-toxic.

2.5.1.1. Glassy Carbon Electrode

Glassy carbon electrodes (GCE) have been widely used in electroanalytical chemistry because of their excellent mechanical and electrical properties such as broad potential window, low back ground current, high temperature resistance, hardness, low density, low electrical resistance, low friction, extreme resistance to chemical attack and impermeability to gases and liquids. The structure of glassy carbon involves thin, tangled ribbons of cross-linked graphite-like sheets. To enhance their analytical performance and to create active and reproducible glassy carbon electrodes, surface pretreatment is usually employed (Wang, 2006). The electron transfer rate or

adsorptive behavior of the GCE is also increase with modification (Bard and Faulkner, 2001). Figure 8 shows an image of common glassy carbon electrodes.

Figure 8: Glassy carbon electrodes.

2.5.2. Reference Electrodes

The reference electrode is an electrode with a known, constant potential and is insensitive to the composition of the solution under study. The potential of a working electrode in a voltammetry experiment is controlled with respect to some standard reference electrode. Some of the commonly used reference electrode that have an electrode potential independent of the electrolyte used in cell are the saturated calomel electrode (SCE), standard hydrogen electrode (SHE), and the saturated silver/silver chloride electrode (Ag/AgCl) electrode. The SHE is the historical and universal reference and can be easily constructed in the laboratory; however, its application is limited to certain applications, as it must be prepared each time it is used. The Ag/AgCl electrode is commonly employed as a reference electrode as it is more robust and can be stored (Simões and Xavier, 2017).

The Ag/AgCl consist of silver wire with internal electrode solution of KCl. The solution of KCl is placed in ionic contact with the test solution via a salt bridge or porous glass frit (Figure 9). The potential assumed by an Ag/AgCl reference electrode depends only on the activity of the chloride anion. The half-reaction for this reference electrode is as follows:

$$\mathbf{AgCl_{(s)} + e^- \rightarrow Ag^+_{(s)} + Cl^-_{(aq)}}$$

A silver wire coated with a layer of silver chloride is immersed in an internal solution saturated with potassium chloride to hold the chloride activity constant. The chloride ion concentration remains fixed at the saturation limit and serves as reference.

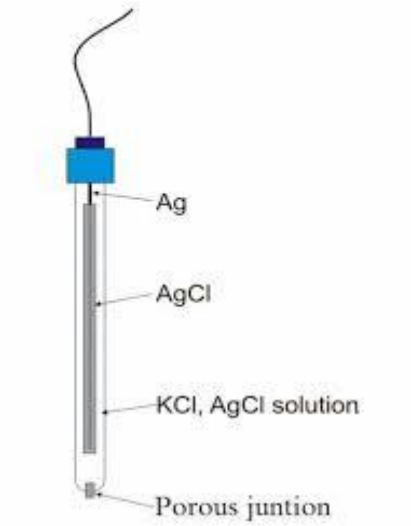

Figure 9: Silver-silver chloride reference electrode.

2.5.3. Counter (Auxiliary) Electrode

The current produced from the electrochemical reaction at the working electrode is balanced by a current flowing in the opposite direction at the counter electrode. The excess current flows through a reference electrode may alter its internal chemical composition. Therefore, a counter electrode used to prevent from this and to complete the electrical circuit, then the current is recorded as electrons flow between the WE and CE. The kinetics of the reaction occurring at the counter electrode do not inhibit those occurring at the working electrode. The surface area of the counter electrode is greater than the surface area of the working electrode and should be chemically inert. A platinum wire is commonly used as a counter electrode, but copper or aluminum wire may work in non-corrosive solutions (Elgrishi *etal*., 2018).

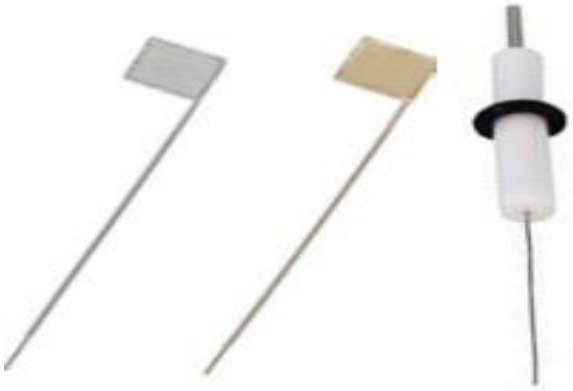

Figure 10: Counter electrodes.

2.5.4. Supporting Electrolyte

An electrochemical experiment required supporting electrolytes to increase the conductivity of the solution, eliminate electromigration effects, maintain a constant ionic strength, and complete the electrical circuit. They are necessary to limit analyte migration through the use of electrolyte in high concentration (Wang, 2006). High electrolyte concentration relative to the analyte concentration ensures the charge balance as migrating to the electrode surface. The supporting electrolyte should be highly soluble in the chosen solvent, chemically and electrochemically inert in the experiment conditions, and they should be prepared from highly purified reagents. Supporting electrolyte may be an inorganic salt, a mineral acid, or a buffer. Buffer systems (such as acetate, phosphate, or citrate) are used when a pH control is essential (Elgrishi *et al*., 2018).

2.6. Analytical Methods for the Determination of Pentachlorophenol

Various analytical methods have been reported for determination of PCP such as gas chromatography, gas chromatography-mass spectrometry, high performance liquid chromatography, electrochemical chemiluminescence, phosphorescence, solid-phase microextration, capillary electrophoresis, photoelectro-chemical immunosensor, spectrophotometry, and voltammetry. Among these, electrochemical methods have certain advantages like high sensitivity, selectivity, quick response, cost-effective and simplicity.

In this work, the electrochemical method for the determination of PCP in aqueous solutions using choline chloride modified glassy carbon electrode (ChCl/GCE) was developed.

Electrochemical methods such as cyclic voltammetry and square wave voltammetry was used to determine PCP.

3. Objective

3.1. General Objective

To develop, characterize and application of a sensor of choline chloride modified glassy carbon electrode for determination of pentachlorophenol.

3.2. Specific Objectives

- To characterize choline chloride modified glassy carbon electrode for determination of pentachlorophenol using cyclic voltammetry.
- To examine the electrochemical behavior of choline chloride in determination of PCP using square wave voltammetry.
- To optimize the basic electroanalytical parameters required for the sensitive determination of PCP at the choline chloride modified electrode using CV and SWV
- To apply the prepared electrodes for the determination of PCP in real sample.

4. Experimental Part

4.1. Reagents and Chemicals

Pentachlorophenol, 4-chlorophenol, acetic acid and sodium acetate from BDH, 2,4,6-trichloro phenol, 2,4- dichlorophenol, and para-nitrophenol from Fluka, orthonitrophenol from Reidle-Dehein , monopotassium phosphate and dipotassium phosphate are from North Ampton, phenol from BDH, catechol from Labmerk chemicals, and distilled water. All the other chemicals and reagents used were analytical grade and were used without any further purification.

4.2. Instruments and Apparatus

A model CHI630A electrochemical analyzer used for all voltammetry measurements including cyclic voltammetry, square wave voltammetry coupled to a Dell computer, the electrochemical

impedance spectroscopy performed using CHI760D model instrument coupled to AOC computer and Fourier Transform Infrared Spectroscopy (PerkinElmer) Spectrum 65 in the region 4000-400 cm^{-1} with resolution 4 cm^{-1} and 4 number of scans using Kbr pellets. The electrochemical cell consisted of a bare glassy carbon working and/or ChCl/GCE modified electrodes, Ag/AgCl reference electrode, platinum counter electrode, ultrasonic cleaner power-yo80W, variable volume single channel manual pipettes, pH meter (senses ion TM+MM150), electronic balance (Model: Scientech: ZSA 120) were also used.

4.3. Preparation of Supporting Electrolytes

The supporting electrolyte of acetate buffer solution was prepared from 0.2 M acetic acid and 0.2 M sodium acetate in distilled water. The pH of the solutions was calculated by using the Henderson–Hasselbalch equation proportion, and adjusted using a pH meter. Phosphate buffer of pH 7 was prepared from 0.1 M monopotassium phosphate and 0.1 M dipotassium phosphate in distilled water, and the pH was adjusted using a pH meter.

4.4. Preparation of Pentachlorophenol

Stock solution of 5 mM PCP was prepared by dissolving 66.5 mg of PCP in methanol and subsequent standard solutions were prepared from the stock solution using ABS pH 5.5. Fresh sample of PCP was prepared from stock solution on daily basis. The prepared stock solution of PCP was kept in amber glass and stored in the refrigerator.

4.5. Preparation of Choline Chloride

The ChCl solution was prepared according to the method reported in the literature (Wang *et al.,* 2009). Briefly, 14 mg of ChCl and 38 mg of KCl was weighed and dissolved in 0.1 M PBS pH 7 to form 2 mM ChCl solution.

4.6. Preparation of Modified Electrode

Before modification, the bare glassy carbon electrode was polished successively with 0.05 μm alumina slurry on polishing pad to obtain a mirror-like electrode surface. Then it is rinsed with distilled water and was sonicated with 50:50 ethanol and distilled water solution in an ultrasonic bath for 3 minutes. The polished GCE was pretreated by scanning the potential between -0.2 and +1.5 V for five cycles at a scan rate of 100 mV s^{-1} using pH 7 of PBS. Then, the ChCl modified glassy carbon electrode (ChCl/GCE) was prepared potentiodynamically by cycling 2 mM ChCl in 10 mM KCl solution in the potential range of -1.7 V to 1.8 V at 25 mVs^{-1} for 9 cycles (Figure 11). Finally, to remove physically adsorbed and unreacted ChCl the modified electrode was rinsed using distilled water and used without any further treatment.

4.7. Analytical Procedure

Cycle voltammetry and square wave voltammetry measurements were carried out in 0.1 mol L^{-1} pH 5.5 ABS as supporting electrolyte at room temperature. In a typical process, 10 μM of PCP prepared by transferring 10 mL of ABS into a clean electrochemical cell then 20 μL of the stock solution of PCP was added using micropipette, and then, three-electrode system was installed into it. After 240 s of accumulation, the SWV was performed at optimized parameters. The electrochemical impedance spectroscopy was performed in 0.1 M $Fe(CN)_6^{3-/4-}$ in the range of 10 kHz to 0.1 Hz at 0.15 V.

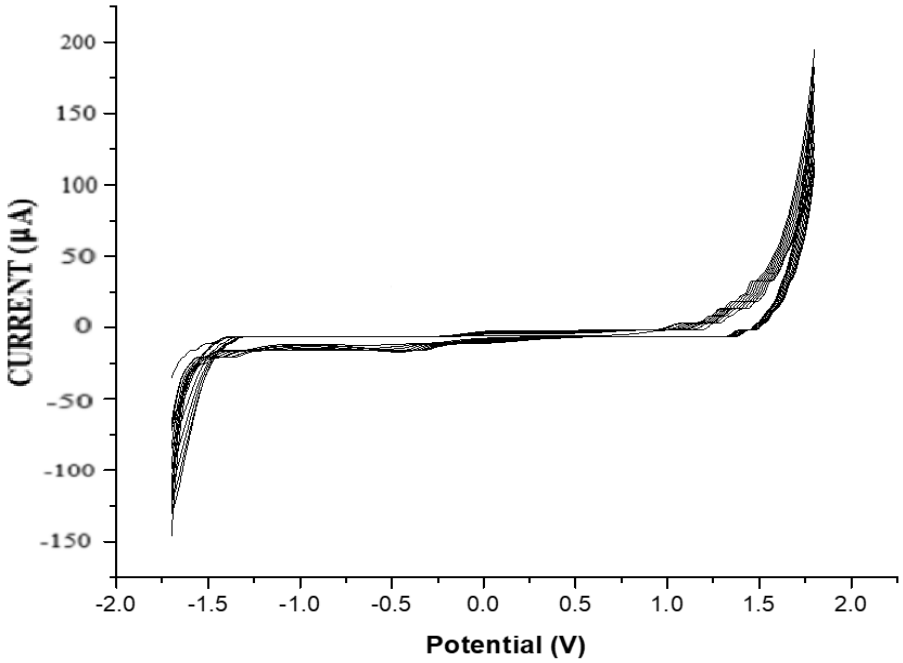

Figure 11: Cyclic voltammograms of covalent attachment of ChCl on GCE at 25 mV s^{-1} scan rate for 9 cycles.

5. Results and Discussion

5.1. Characterization of the Modified Electrode

Figure 12 shows the mechanism of preparation of ChCl/GCE. The bare GCE can be oxidized to produce cation radicals on the surface, then the hydroxyl of ChCl reacts with the cation radicals of the GCE through nucleophilic attack, the covalently linked ChCl with GCE results in stable positively charged ChCl/GCE (Jin and Lin, 2004). The chemical structure and covalent linkages of ChCl on the surface of the electrode clarify using FT-IR spectra. The strong peak at 3300–3500 is due to the stretching vibration of –OH group, the bands at between 3000-2800 cm^{-1}, 1080, 1050 cm^{-1} is for the C–H stretching vibration of the $–CH_3$ and $–CH_2$-groups and C–C–O of the choline chloride, respectively (Parsaee *et al*., 2018). The peaks in the range of 900–1000 cm^{-1} indicates the presence of a specific group of quaternary ammonium compounds and stretching of the C-N group of peaks positioned at the wavelength of 955 and 935 cm^{-1}. In comparison with FT-IR spectrum of ChCl (Figure 13), there is no change at the location of C-C frequency which indicates the presence of Ch^+ in the nanocomposite structure (Bahrani *et al*., 2018).

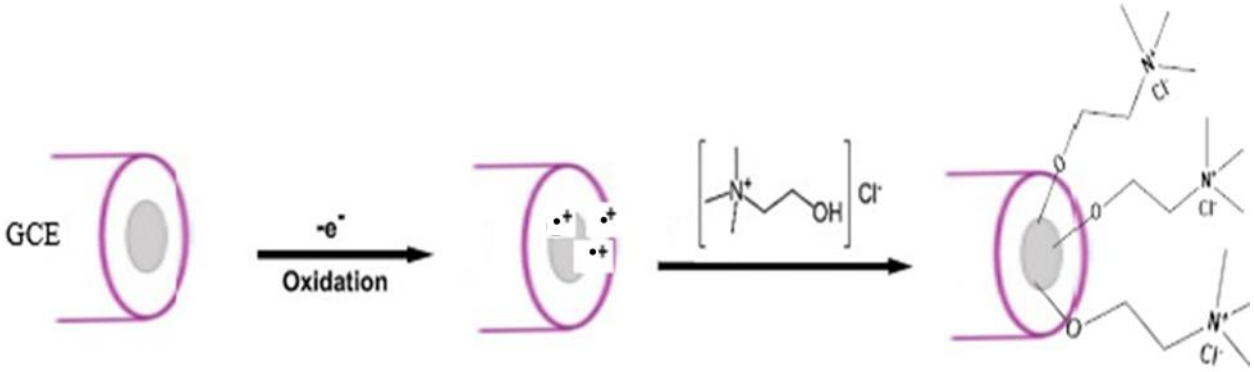

Figure 12: Schematic representation of ChCl/GCE preparation.

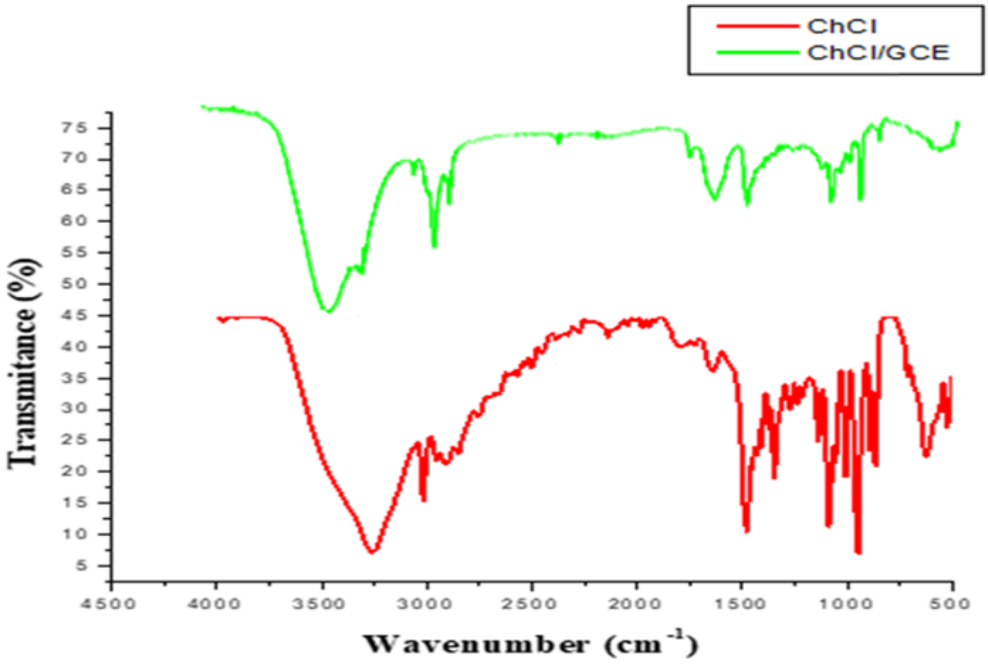

Figure 13: FT-IR spectra of ChCl and the ChCl/GCE.

The electron-transfer properties of the modified surface electrode was studied using electrochemical impedance spectroscopy. The interface properties of the electrode described by the electron-transfer resistance (R_{et}) at the electrode surface which is equal to the semicircle diameter of the Nyquist plot (Feng *et al*., 2015). The semicircle diameter of bare GCE after fitted with equivalent circuit in $Fe(CN)_6^{3-/4-}$ solution is 138.3 Ω , but it reduced to 77.06 Ω at ChCl/GCE. This indicates the ChCl modified electrode improves the electron transfer rate of

$Fe(CN)_6^{3-/4-}$ since it lowers the R_{et} due to the static gravitation between negatively charged $Fe(CN)_6^{3-/4-}$ and positively charged quaternary ammonium [$-N^+(CH_3)_3$] groups of ChCl on the ChCl/GCE surface (Wang *et al*., 2009).

The electrocatalaytic ability of the modified electrode also verified using cyclic voltammetry (Figure 15), as shown the oxidation and reduction peak current of $K_4[Fe(CN)_6]$ at ChCl/GCE increases from bare GCE electrode. The electroactive surface area was calculated from cyclic voltammogramms recorded at different scan rates using Randles-Sevcik equation.

$$Ip = 2.69\times10^5 (n_a\alpha) AD^{1/2} \nu^{1/2} c$$

Where ip- oxidation peak current of $K_4[Fe(CN)_6]$ in A obtained from cyclic voltammogram , A- the surface area of electrode in cm^2 , c- the concentration of analyte 5 mM $K_4[Fe(CN)_6]$ in 0.5 M KCl, D- the diffusion coefficient of $K_4[Fe(CN)_6]$ known as 7.26×10^{-6} cm^2s^{-1} (Konopka and Duffle, 1970), ν- the scan rate and n- the number of electron transferred in the redox reaction. The electroactive surface area of the GCE was calculated to be 0.0158 cm^2. In the same manner, the area for ChCl/GCE electrode was 0.043 cm^2. The higher peak current of $K_4[Fe(CN)_6]$ at hCl/GCE is due to the largest active surface area of the ChCl modified electrode.

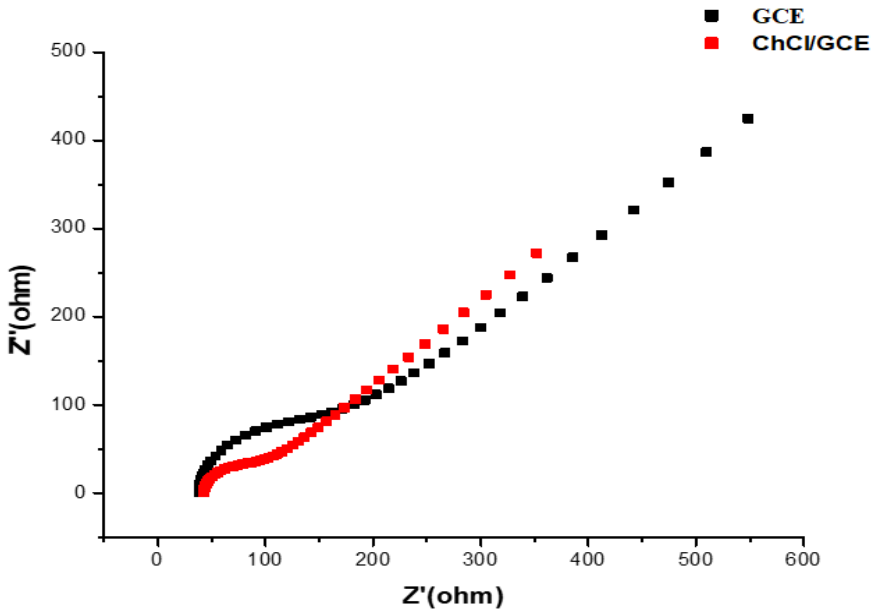

Figure 14: Nyquist plots of EIS of bare GCE and ChCl/GCE at 5 mM $Fe(CN)_6^{3-/4-}$ solution.

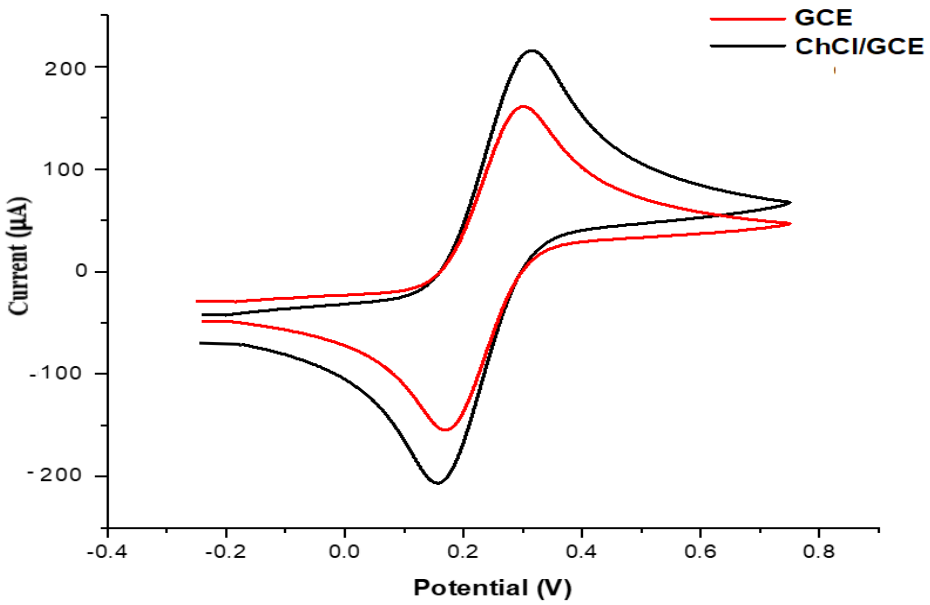

Figure 15: Cyclic voltammogram of 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl at bare GCE and ChCl/GCE at 100 mV s^{-1} scan rate.

The optimized cycle number which responds to a higher current and minimizes potential was investigated by varying the number of cycles using CV. As seen in Figure 16, at nine number of cycles high peak current was obtained, therefore, nine cycles were selected as the optimum number of cycles.

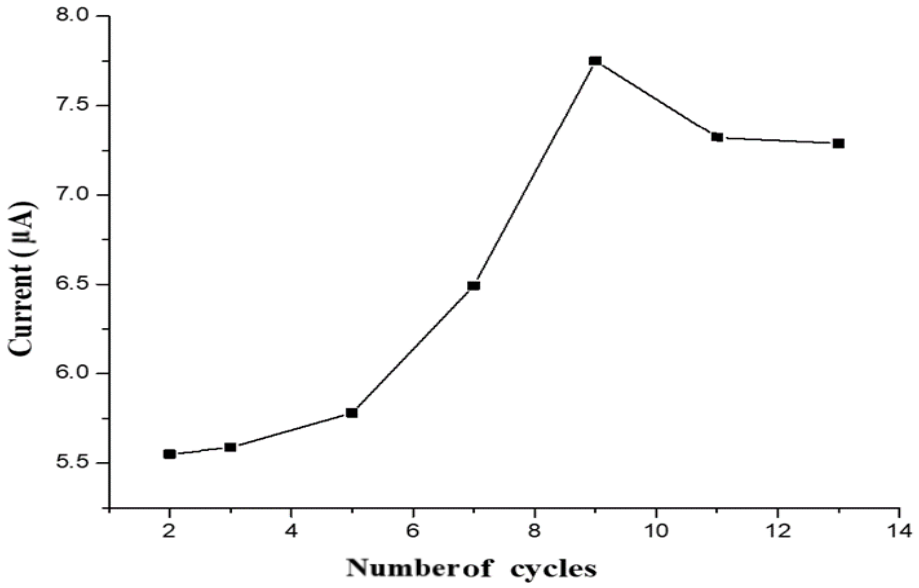

Figure 16: The plot of current versus number of cycles in covalent attachment of ChCl on GCE.

5.2. Electrochemical Behavior of PCP

The electrochemical oxidation of PCP in 0.1 M ABS at 100 mV s^{-1} scan rate was studied at bare and ChCl modified electrode using CV. As shown in figure 17, the modified electrode exhibited a higher peak current with 7.478 µA whereas the bare glassy carbon electrode 4.355 µA. This indicates the ChCl is an excellent catalytic activity to PCP oxidation. The cyclic voltammogram of redox of PCP at bare and modified electrodes shows only oxidation peak, this indicates the electrochemical redox process of PCP is irreversible.

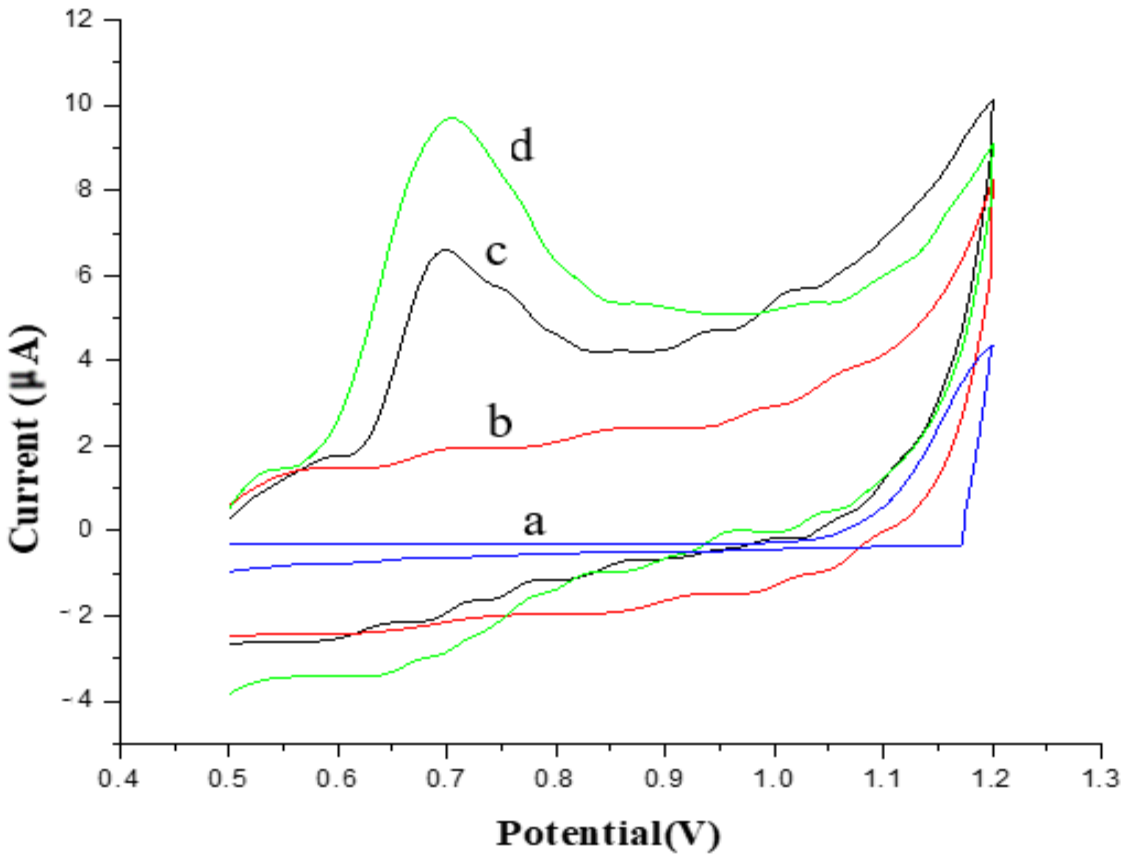

Figure 17: Cyclic voltammograms of bare GCE (a), ChCl/GCE electrode (b) without PCP, bare GCE electrode with in PCP (c), and ChCl/GCE electrode with in PCP (d) in 0.1 M ABS pH = 5.5, 100 mV s^{-1} scan rate.

5.3. Effect of Supporting Electrolyte and pH

The influences of supporting electrolytes and pH of the solution in the voltammetric responses of PCP was investigated. Different supporting electrolyte solutions were studied at ChCl/GCE for the electrochemical oxidation of PCP using CV includes ABS, PBS, CBS, BRB, HCl acid, and KCl solution. The electrochemical oxidation of PCP with ABS gives high peak current and its potential shifts to negative (Figure 18). Therefore, ABS was selected as supporting electrolyte for further experimental works.

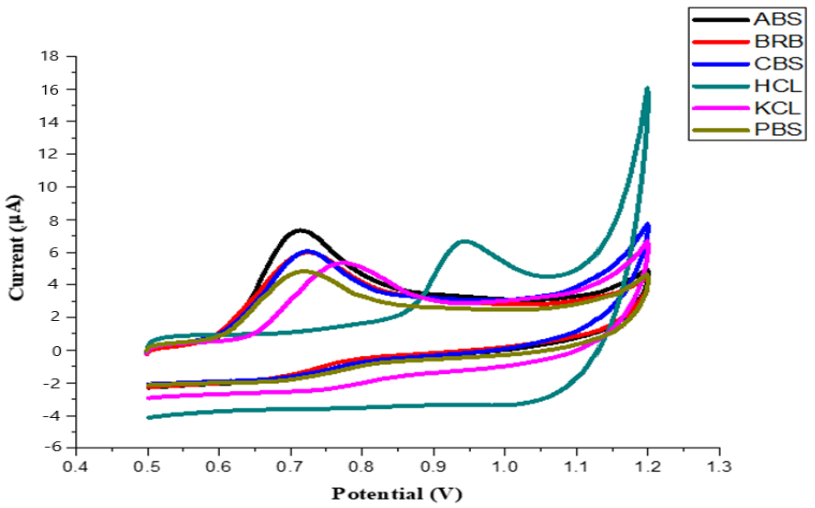

Figure 18: Cyclic voltammograms of 1×10^{-4} M PCP in different supporting electrolytes at scan rate 100 mV s^{-1}.

The pH of the supporting electrolyte solution plays an important role on the electrochemical oxidation of PCP. So, it was studied using CV in the pH range 3.5 to 6.0 by (Figure 19). As shown in Figure 20A, the peak current increases with the pH up to 5.5 and then decreases. The oxidation peak current of PCP is greatly influenced by the pH of the solution. The oxidation peak potential shifted negatively and linearly with increasing pH from 3.5 to 6.0 (Figure 20B) with linear regression equation Epa = -0.0589pH +1.044 (R^2 = 0.9558). The slop -0.0589 indicates that the number of electrons and protons were equally involved in the electrochemical process (Ling *et al*., 2020).

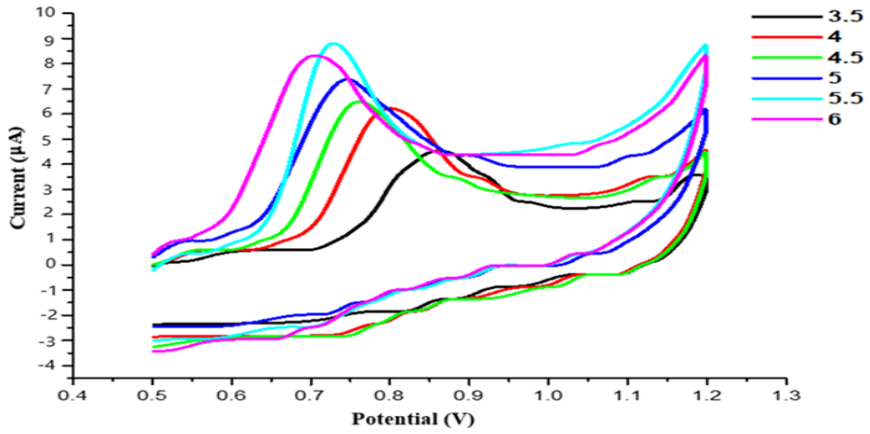

Figure 19: Cyclic voltammograms of the oxidation reaction of 1×10^{-4} M PCP at different pH of 0.1 M of ABS at scan rate 100 mV s^{-1}.

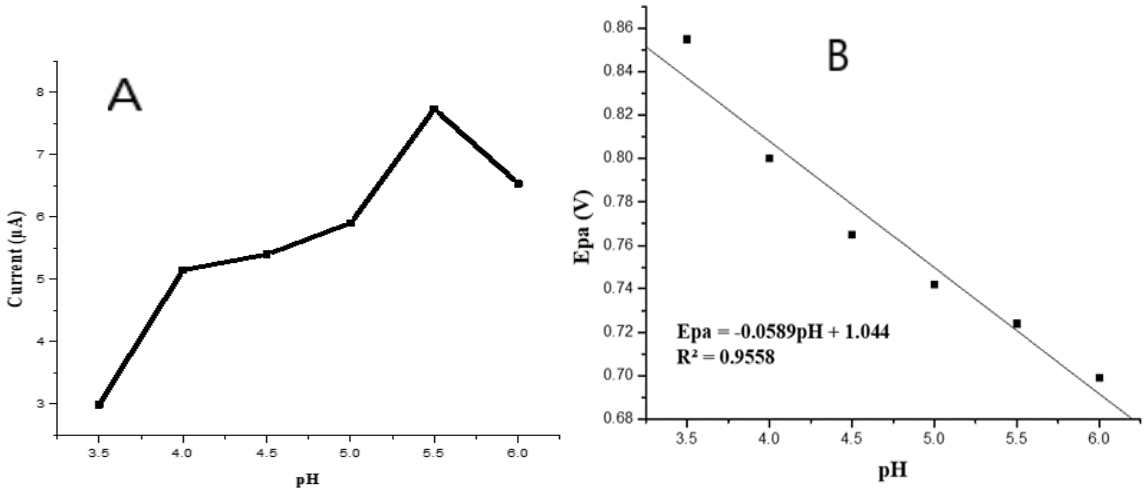

Figure 20: Plot of anodic peak currents of PCP versus pH of 0.1 M ABS (A), and plot of peak potential versus pH (B).

5.4. Effect of Scan Rate

The electrochemical oxidation of PCP also studied at different scan rate (50 - 500 mV s^{-1}) to investigate the kinetics of the reaction. The anodic peak current of PCP increased continuously as the scan rate increases from 50 - 500 mV s^{-1} (Figure 21) with linear regression equations Ipa (µA) = 0.0557 ν (mV s^{-1}) + 1.741 (R^2 =0.992). This relationship indicates that the oxidation of PCP at the modified electrode is adsorption controlled kinetics that is in agreement with reported (Feng *etal.*, 2015).

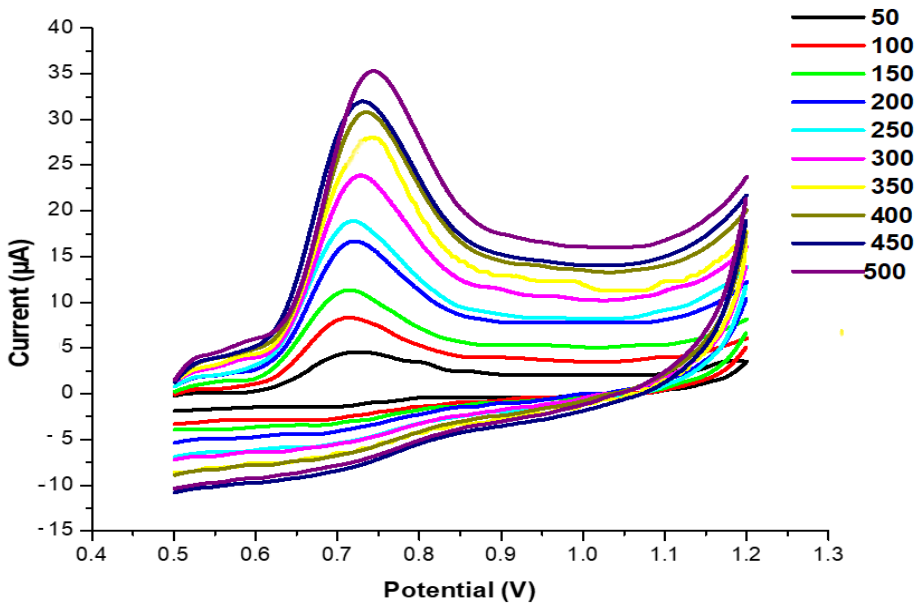

Figure 21: Cyclic voltammograms of oxidation of 1×10^{-4} M PCP in 0.1 M of ABS pH = 5.5 at ChCl/GCE at different scan rates 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mV s^{-1}.

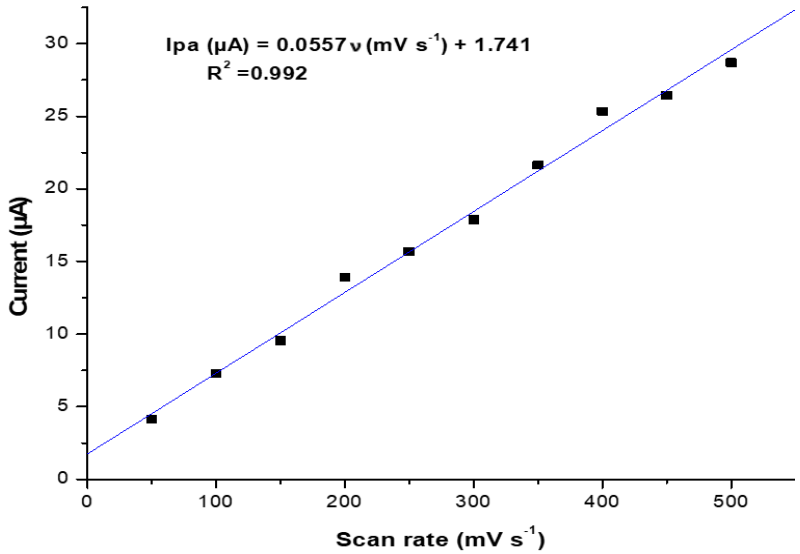

Figure 22: The dependence of peak current on scan rate.

5.5. Optimization of Parameters

The square wave voltammetric parameters such as the pulse amplitude, frequency, and increment were also optimized on the peak current response of ChCl modified glassy carbon electrode at

100 µM in pH 5.5 ABS by measuring the peak current enhancement. The particular variable was investigated under the identified condition by keeping all variables constant except one under study. The optimum pulse amplitude, frequency, and increment uses for the further experiment are 0.045 V, 20 Hz, and 0.004 V, respectively.

5.6. Effect of Accumulation Potential and Time

The effect of accumulation potential and time on the oxidation peak current was studied by SWV. As shown in Figure 22A, the oxidation peak current increases greatly within accumulation time the first 240 s then decreases slowly with longer accumulation time, indicates that the adsorption of PCP on the modified electrode surface was saturated or at stable value. The influences of the accumulation potential also investigated in the range -0.4 to +0.4 V (Figure 22B), the oxidation peak current changes with the potential and highest anodic current observed at + 0.2 V of accumulation potential. Therefore, 240 s accumulation time and +0.2 V accumulation potential was used as for the optimal experiments.

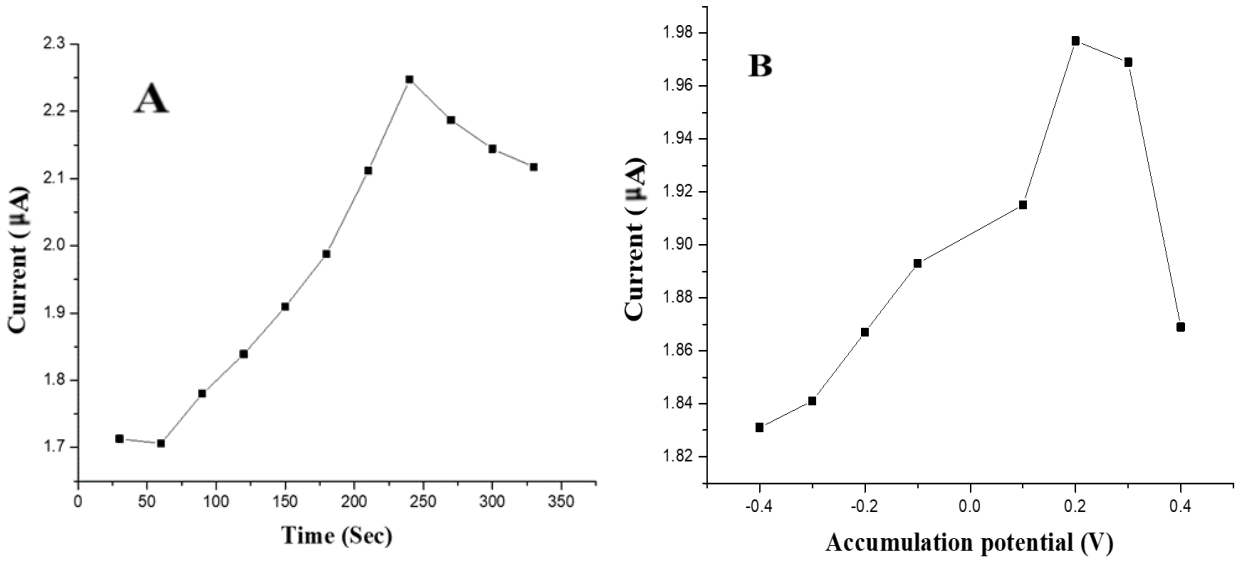

Figure 23: Plot of peak current versus accumulation time (A), and accumulation potential (B).

5.7. Analytical Performance of the Method

Under optimum experimental conditions, the dependence of voltammetric signal on the concentration of PCP and the sensitivity of the method were investigated using square wave voltammetry for different concentration of PCP (Figure 24). The peak current increased linearly

with the concentration of PCP in the range of 9 ×10 $^{-9}$ to 1 ×10 $^{-5}$ mol L^{-1}. The calibration curve for 9 points with linear regression equation of Ip (µA) = 0.4036 c (µmol/L) + 0.0267 (R^2 = 0.998) with limit of detection was 4.45×10 $^{-9}$ mol L^{-1}. Figure 25 shows a calibration curve for square wave voltammograms of PCP at ChCl/GCE modified electrode for the concentration range 9 ×10 $^{-9}$ to 1 ×10 $^{-5}$ mol L^{-1}.

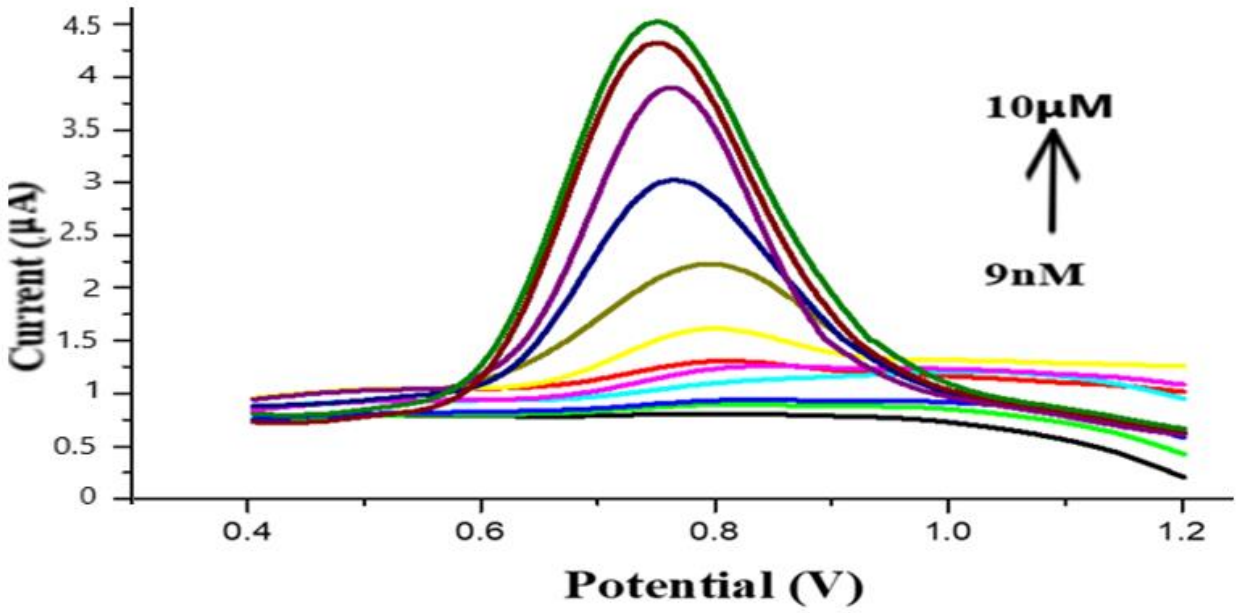

Figure 24: Square wave voltammograms of different concentration of PCP at ChCl/GCE modified electrode in 0.1 M ABS (pH 5.5), pulse amplitude 0.045 V, frequency 20 Hz, and increment 0.004 V.

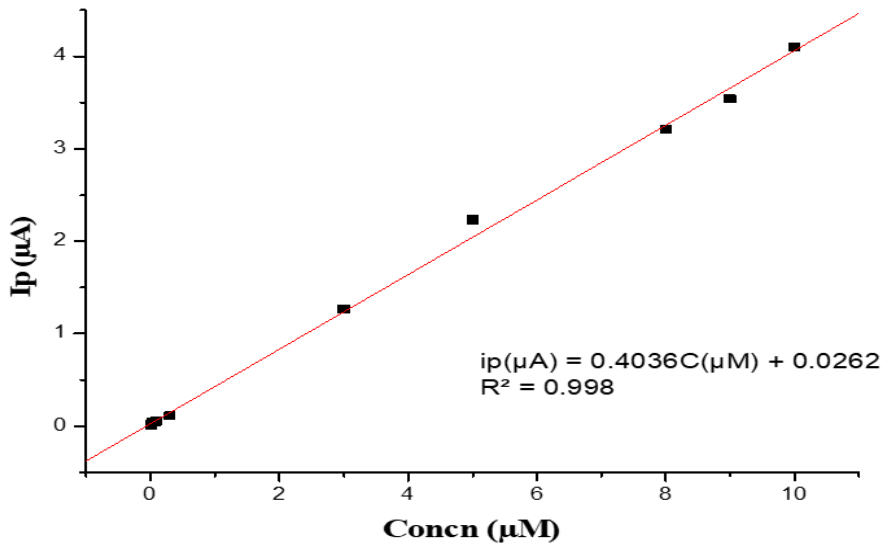

Figure 25: Plot of SWV anodic peaks current versus PCP concentration from 9 ×10 $^{-9}$ to 1×10 $^{-5}$ mol L^{-1}.

The analytical performance of the new developed method was also compared with other methods (Table 2) and shows low detection limit, wide linear range, when compared with the reported literatures for the determination of PCP at different modified electrodes. ChCl/GCE can be seen that it improve the detection performance of PCP with low cost, time saving, and simple operation.

Table 2: Comparison of the developed method for the determination of PCP at ChCl/GCE with other reported methods.

Methods	Linear range (µM)	LOD (µM)	References
CuS/Nanocomposite Chitosan/GCE	1.88-75	0.63	Zou *etal*., 2013
β-CD/CNT/GCE	0.8 –10.4	0.04	Xu *etal*.,2010
CS/CPE	0.1 – 5 and 5 – 100	0.04	Xu *etal*.,2014
MWCNT-EP composite electrode	2.0 –12	0.801	Remes *et al.*, 2012
ZnSe QDs/MWNTs/DMF/GCE	0.08 – 4	0.002	Feng *etal.*, 2015
ChCl/GCE	0.009 – 10	0.004	This work

β-CD beta-cyclodextrin, CNTs carbon nanotubes, GCE glassy carbon electrode, MWCNT multiwall carbons, EP epoxy, ZnSe QDs ZnSe quantum dots, CS chitosan, CPE carbon paste electrode.

5.8. Reproducibility and Stability

The vital characteristics for the modified electrode include reproducibility, repeatability and stability also examined. The ChCl/GCE for determination of PCP was investigated by using three different electrodes modified under the same condition, the relative standard deviation (RSD) was 3.63%. The RSD for intra-day repeatability was 3.28% for 10 successive measurement performed on the same day. The inter-days repeatability RSD that measured the same concentration of PCP at different days was 3.86%, which displayed the proposed electrode having good reproducibility and regeneration. The ChCl/GCE stability with positive charge may be less due to absorption of anions from the experimental solution (Jin and Lin, 2004); therefore, it is stored in HCl solution under 4^0c to keep the stability. After the ChCl/GCE was stored for 3 days and 7 days, the peak current response decreases 3.28% and 9.59% respectively. The peak current response decreases 19.3% after the modified electrode stored for 1 month, this indicating good stability of the sensor.

5.9. Interference Study

The interference of various ions and phenol derivatives for the detection of PCP at the ChCl/GCE was investigated by adding into the ABS (pH 5.5) containing 10 μM PCP using SWV. 10-fold additions of common inorganic ions such as Na^+, Pb^{+2}, Ni^{+2}, Fe^{3+}, Cl^- , NO^{3-}, and SO_4^{2-} and catechol had no effect or below 5% on the response of PCP at the designed electrode. The effects of phenol in the electrochemical determination of PCP at ChCl/GCE were examined (Figure 26). The voltammograms indicates the peak current of PCP increased proportionally with the concentrations of phenol.

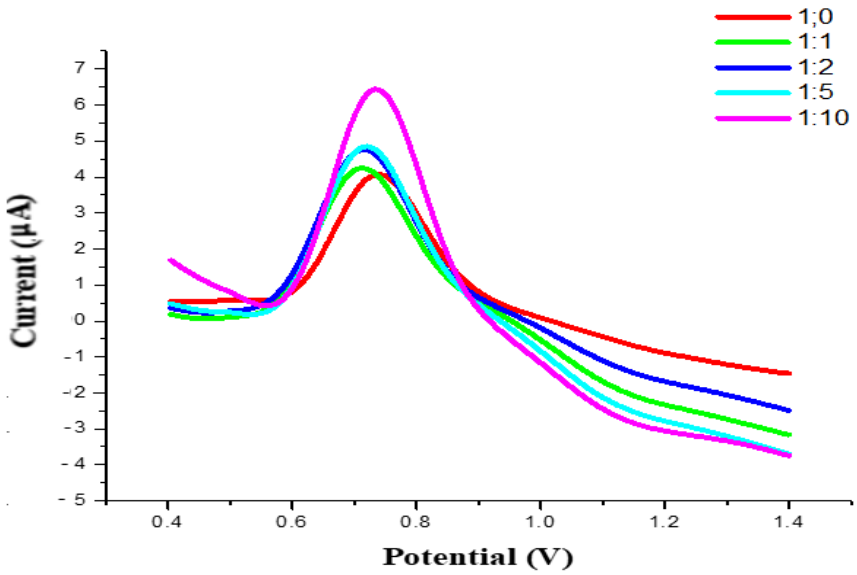

Figure 26: Square wave voltammograms of 1× 10^{-5} M PCP spiked with 2, 5, and 10 fold concentration ratio of phenol in 0.1 M ABS pH = 5.5.

The effect of possible interfering substances including 4-CP, 2,4-DCP, and TCP also studied by addition 1:1, 1:2, 1:5, and 1:10 mixed ratio with PCP concentration. Due to the same active site of the -OH group in their structure (Negussie *et al*., 2014) chlorophenols interfere with the determination of PCP by increasing the response of peak current as it increases concentration. As shown in figure 27A, 4-CP shifts the potential positively and increases the peak current up to five fold concentration of 4-CP, but addition 10-fold of 4-CP decreases the peak current. This is due to the fouling of the electrode. TCP shifts the potential negatively, and the peak current increases with increasing concentration (Figure 27C).

The addition of different ratio 2,4-DCP increases the peak current of PCP with as concentration increases. Figure 27D shows the voltammograms of chlorophenols with the same concentration of 10µM, the oxidation peak current of PCP is higher than from the other chlorophenols which indicates the ChCl/GCE is a selective sensor for determination of PCP.

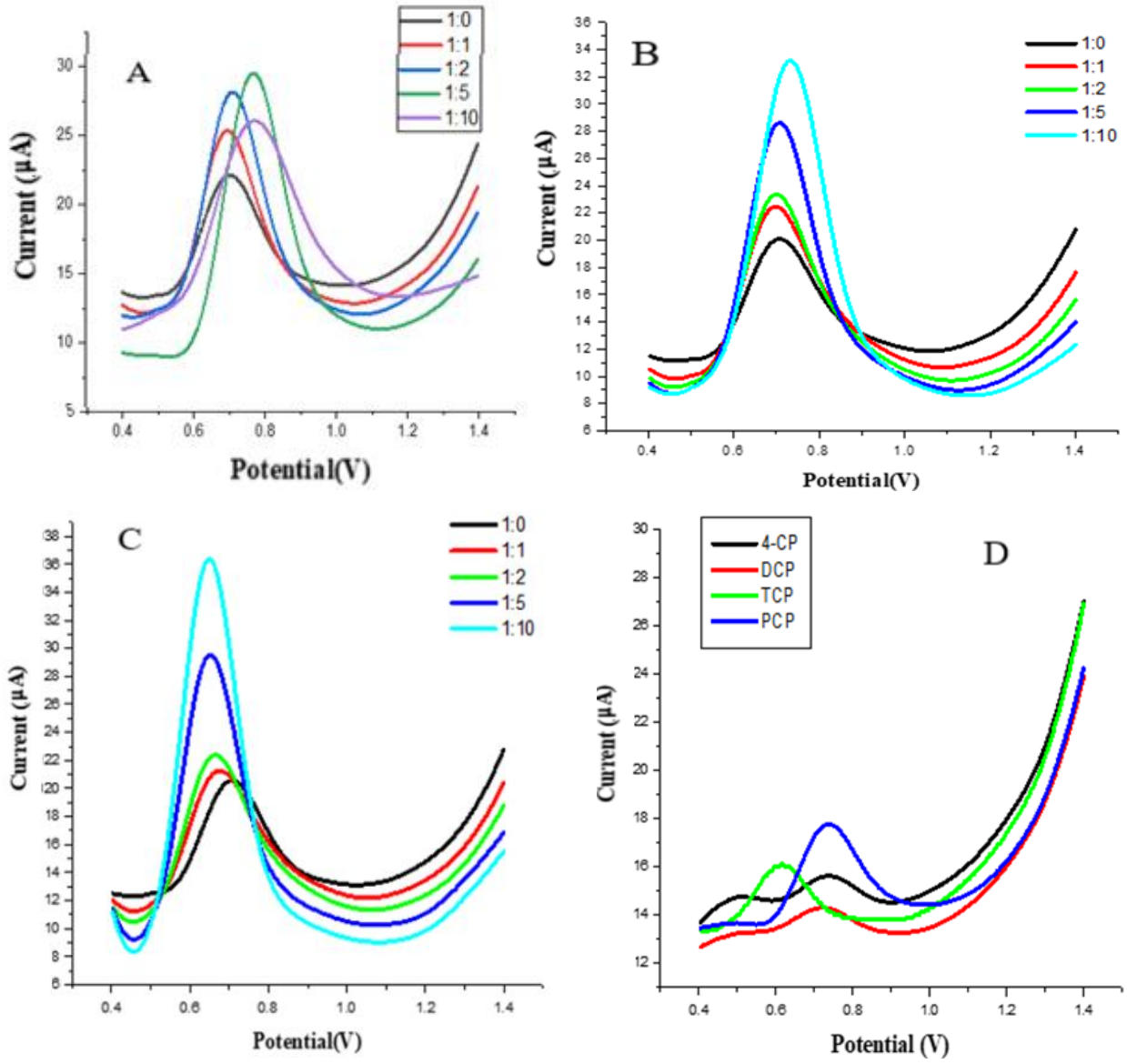

Figure 27: Square wave voltammograms of 1×10^{-5} M PCP spiked with 2, 5, and 10 fold concentration ratio of 4-CP (A), 2,4-DCP (B), TCP (C), and the equimolar 1×10^{-5} M concentration of PCP, 4-CP, 2,4-DCP, and TCP (D) in 0.1 M ABS pH = 5.5.

The effect of ortho-nitrophenol and 4-nitrophenol were examined in the electrochemical determination of PCP in 0.1M ABS at ChCl/GCE. As shown in Figure 28, the oxidation peak current of PCP decreases as addition of respective concentration of 4-nitrophenol, and ortho-nitrophenol increases. Another peak current also observed at other potential in 5 and 10 fold concentration of the nitrophenols.

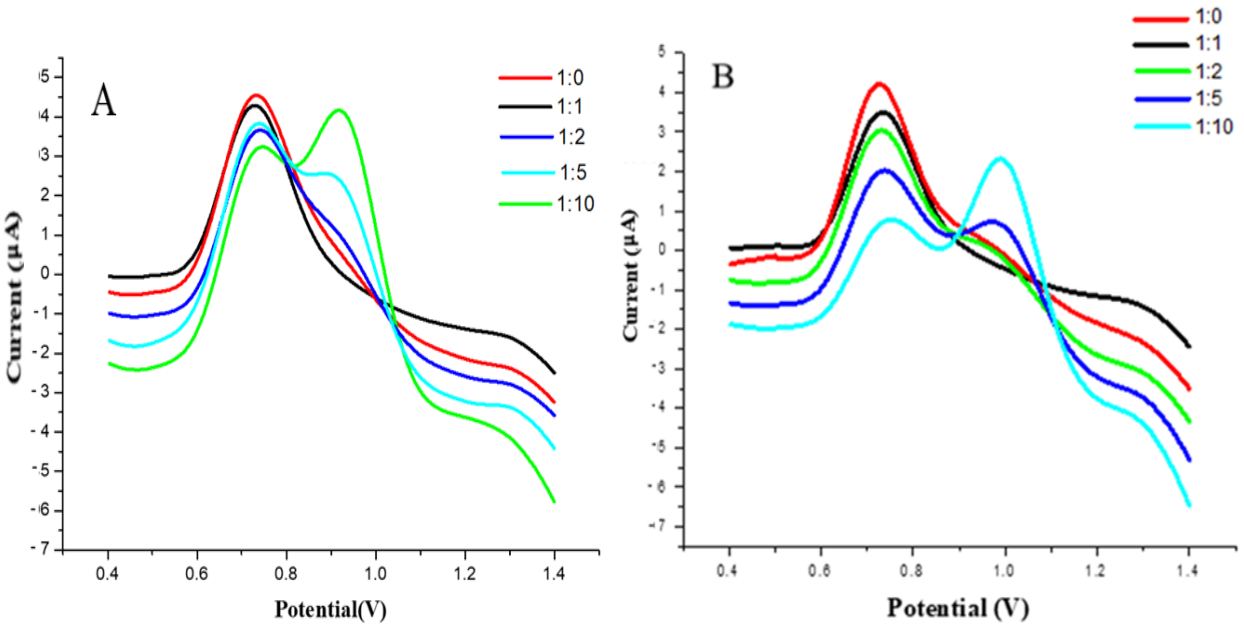

Figure 28: Square wave voltammograms of 1×10^{-5} M PCP spiked with 2, 5, and 10 fold of ortho-nitrophenol (A), and 4- nitrophenol (B) in 0.1 M ABS pH = 5.5.

5.10. Application of the Method in Real Samples

This newly developed electrochemical sensor based on the ChCl/ GCE was used to analyze PCP in a real water sample collected from a different area which was expected near to PCP pollution by SWV under the optimized experimental conditions. The water samples, collected from pulp and paper factory, the Akaki River and tap water were filtered to remove the solid residue and employ without any further treatment. No peak currents were observed for PCP in the real samples from the Akaki River and tap water, but in the samples from the pulp and paper factory have oxidation peak current that is equivalent with 0.68 µM concentration of PCP in the absence of PCP. Different concentrations of standard solution PCP were added to the real water samples using the standard addition method to do the recovery experiments. The square wave voltammetric peak current for each sample was recorded which then was converted to concentration units using the regression equation of the calibration curve. The calculated recovery values for the water sample solutions (5 ml water and 5 ml ABS) are summarized in Tables.

Table 3: Recovery of PCP in water sample from the Akaki River.

Sample No	Spiked PCP (μM)	Found PCP (μM)	Recovery (%)
1	0.2	0.185	93.5
2	0.5	0.547	109.4
3	0.8	0.862	107.75
4	1.1	1.07	97.27
5	1.5	1.42	94.66
6	2	1.91	95.5

Table 4: Recovery of PCP in tap water.

Sample No	Spiked PCP (μM)	Found PCP (μM)	Recovery (%)
1	0.5	0.477	95.4
2	2	1.97	98.5
3	5	5.22	104.4
4	8	8.28	103.5
5	10	10.565	105.6

Table 5: Recovery of PCP in waste water from pulp and paper factory.

Sample No	Spiked PCP (μM)	Found PCP (μM)	Recovery (%)
1	0.05	0.726	92
2	0.2	0.886	103
3	0.4	1.076	99.2
4	0.8	1.439	92.4
5	1	1.619	93.9
6	1.5	2.282	106.8

As can be seen from the tables, the SWV anodic peak current increased with increasing concentration of the spiked standard PCP solution, this indicates the sensitivity of the developed method. The excellent recovery results (92 –107.75%) indicating the applicability of the developed method for the determination of PCP in a real polluted water sample.

6. Conclusions

A simple, sensitive and non-toxic electrochemical sensor was developed using choline chloride by simple covalent attachment for the determination of PCP. The covalent linkage of ChCl on to GCE results in a stable positively charged ChCl/GCE with large effective surface area which exhibits the electron transfer and promotes the electrocatalytic activity of the PCP oxidation. The developed method had good sensitivity and selectivity with a linear range of 9×10^{-9} to 1×10^{-5} mol L^{-1} and detection limit 4.45×10^{-9} mol L^{-1} (S/N=3). It is also more sensitive, wider linear range, and small LOD as compared with the reported methods. The low cost, easy preparation, environmental friendly chemicals and the satisfactory recovery results in real samples are the advantages of this method. However, it is important to develop a better modified electrode for determination of PCP in the presence of chlorophenols and nitrophenols. Furthermore, it is also possible to construct a composite modified electrode containing ChCl, so that it may increase the linear range, selectivity, specially within the chlorophenols.

References

Albert, L. (1978). Direct determination of pentachlorophenol by differential pulse polarography. Virginia Commonwealth University. Doi:10.25772/5J4R-2V30

Baiocchi, C.; Roggero, M.; Giacosa, D.; Marengo, E. (1995). HPLC separation and determination of complex mixtures of phenolic compounds on different stationary phases. An efficient general screening method for the presence of phenols in water. *Journal of Chromatographic Science*, 33:338–346. Doi:10.1093/chromsci/33.6.338

Bahrani, S.; Razmi, Z.; Ghaedi, M.; Asfaram, A.; Javadian, H. (2018). Ultrasound-accelerated synthesis of gold nanoparticles modified choline chloride functionalized graphene oxide as a novel sensitive bioelectrochemical sensor: Optimized meloxicam detection using CCD-RSM design and application for human plasma sample. *Ultrasonics–Sonochemistry*, 42:776-786. Doi:10.1016/j.ultsonch.2017.12.042

Bard, A.J.; Faulkner, L.R. (2001). Electrochemical methods: fundamental and applications, 2nd Ed., New York.

Bevenue, A.; Beckman, H. (1967). Pentachlorophenol: A discussion of its properties and its occurrence as a residue in human and animal tissues. *Residue Reviews*, 19:83-134. Doi:10.1007/978-1-4615-8425-4_5

Ciucu, A. (2014). Chemically modified electrodes in biosensing. *Biosensors and Bioelectronics*, 5: 154. Doi:10.4172/2155-6210.1000154

Chuang, J.; Emon, J.; Finegold, J.K.; Chou, Y.; Rubio, F. (2006). Immunoassay method for the determination of pentachlorophenol in soil and sediment. *Bulletin of Environmental Contamination and Toxicology,* 76:381–388. Doi:10.1007/s00128-006-0932-z

Deng, Y.; Chang, Q.; Yin, K.; Liu, C.; Wang, Y.; (2017). A highly stable electrochemiluminescence sensing system of cadmium sulfide nanowires/graphene hybrid for supersensitive detection of pentachlorophenol. *Chemical Physics Letters*, 685:157–164. Doi:10.1016/j.cplett.2017.07.044

Durst, R.A.; Baumner, A.J.; Murray, R.W.; Buck, R.P.; Andrieux, C.P. (1997). Chemically Modified Electrodes: Recommended Terminology and Definitions. *Pure and Applied Chemistry*, 69:1317-1323. Doi:10.1351/pac199769061317

Elgrishi, N.; Rountree, K.; McCarthy, B.; Rountree, E.; Eisenhart, T.; Dempsey, J. (2018). A practical beginner's guide to cyclic voltammetry. *Journal of Chemical Education*, 95:197-206. Doi:10.1021/acs.jchemed.7b00361

Feng, S.; Yang, R.; Ding, X.; Li, J.; Guo, C.; Qu, L. (2015). Sensitive electrochemical sensor for the determination of pentachlorophenol in fish meat based on ZnSe quantum dots decorated multiwall carbon nanotubes nanocomposite. *Ionics*, 21**:**3257–3266. Doi:10.1007/s11581-015-1512-1

Fisher, B. (1991). Pentachlorophenol (PCP): Toxicology and environmental fate. *Journal of Pesticide Reform*, 117:33-140

Garay, F.; Lovric, M. (2002). Square-wave voltammetry of quasi-reversible electrode processes with coupled homogeneous chemical reactions. *Journal of Electroanalytical Chemistry*, 518: 91-102. Doi: 10.1016/S0022-0728(01)00695-7

Han, D-M.; Fang, G-Z.; Yan, X-P. (2005). Preparation and evaluation of a molecularly imprinted sol–gel material for on-line solid-phase extraction coupled with high performance liquid chromatography for the determination of trace pentachlorophenol in water samples. *Journal of Chromatography A*, 1100:131–136. Doi:10.1016/j.chroma.2005.09.035

IARC Working Group on the Evaluation of Carcinogenic Risks to humans. (2019). Pentachlorophenol and some related compounds. *International Agency for Research on Cancer; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 117: 33-140. Available from: https://www.ncbi.nlm.nih.gov/books/NBK543329/

Jin, G.; Lin, X. (2004). The electrochemical behavior and amperometric determination of tyrosine and tryptophan at a glassy carbon electrode modified with butyrylcholine. *Electrochemistry Communications*, 6:454–460. Doi:10.1016/j.elecom.2004.03.006

Kan, Q.; Yang, L.; Chen, Y.; Luo, S.; Wen, L.; Cai, Q.; Yao, S. (2010). Photoelectrochemical detection of pentachlorophenol with a multiple hybrid CdSexTe1−x/TiO2 nanotube structure-based label-free immunosensor. *Analytical Chemistry*, 82: 9749-9754. Doi:10.1021/ac101798t

Konopka, S.; Duffle, B. (1970). Diffusion coefficients of ferri- and ferrocyanide ions in aqueous media, using twin - electrode thin - layer electrochemistry *Analytical Chemistry,* 42: 1741-1744. Doi: 10.1021/AC50160A042

Lide, D.R. (ed.) (2002). CRC handbook of chemistry and physics. 83rd Edition. *CRC Press*, Boca Raton, FL.

Ling, W.; Xiao, L.; Ran, Y.; Jian-Jun, L.; Ling-Bo, Q. (2020). A highly sensitive and selective electrochemical sensor for pentachlorophenol based on reduced graphite oxide-silver nanocomposites. *Food Analytical Methods*, 13: 11. Doi:10.1007/s12161-020-01823-2

Lopez-Echartea, E.; Macek, T.; Demnerova, K.; Uhlik, O. (2016). Bacterial biotransformation of pentachlorophenol and micropollutants formed during its production process. *International Journal of Environmental Research and Public Health*, 13:1146. Doi:10.3390/ijerph13111146

Mardones, C.; Palma, J.; Sepúlveda, C.; Berg, A.; Baer, D. (2003). Determination of tribromophenol and pentachlorophenol and its metabolite pentachloroanisole in Asparagus officinalis by gas chromatography/mass spectrometry. *Journal of Separation Science,* 26: 923–926. Doi:10.1002/jssc.200301303

Murray, R.W.; Ewing, A.G.; Durst, R.A. **(**1987). Chemically modified electrodes. Molecular design for electroanalysis. *Analytical Chemistry, 59*: 379A-390A. Doi:10.1021/ac00132a001

Negussie, N.; Hailemichael, A.; Merid, T. (2014). Determination of Phenol and Chlorophenols at Single-Wall Carbon Nanotubes/Poly(3,4-ethylenedioxythiophene) Modified Glassy Carbon Electrode Using Flow Injection Amperometry. *International Scholarly Research Notices*, 2014:1-10. Doi:10.1155/2014/926213

Olaniran, A.O.; Igbinosa, EO. (2011). Chlorophenols and other related derivatives of environmental concern: properties, distribution and microbial degradation processes. *Chemosphere*, 83:1297-306. Doi:10.1016/j.chemosphere.2011.04.009

Parsaee, Z.; Karachi, N.; Abrishamifar, S.; Kahkha, M.; Razavi, R. (2018). Silver-choline chloride modified graphene oxide: Novel nano-bioelectrochemical sensor for celecoxib detection and CCD- RSM model. *Ultrasonics sonochemistry*, 45:106-115. Doi:10.1016/j.ultsonch.2018.03.009

Remes, A.; Pop, A.; Manea, F.; Baciu, A.; Picken, S.J.; Schoonman, J. (2012). Electrochemical determination of pentachlorophenol in water on a multi-wall carbon nanotubes-epoxy composite electrode. *Sensors (Basel)*, 12:7033-46. Doi:10.3390/s120607033

Royce, C.E. (1982). Electrochemical pretreatment of glassy carbon electrodes. *Analytical Chemistry*, *54*: 2310-2314. Doi:10.1021/ac00250a038

Scholz, F. (2015). Voltammetric techniques of analysis: the essentials. *Chem Texts Springer International Publishing*, 1:1-24. Doi:10.1007/s40828-015-0016-y

Simões, F.R.; Xavier, M.G. (2017). Electrochemical sensors. *Nanoscience and Its Applications*, 155–178. Doi:10.1016/b978-0-323-49780-0.00006-5

United Nations (2010). Exploration of management options for pentachlorophenol (PCP). Paper for the 8th meeting of the UNECE CLRTAP Task Force on Persistent Organic Pollutants, Montreal, 18–20 May 2010.

Wang, H-F.; He, Y.; Ji, T-R.; Yan, X-P.; (2009). Surface molecular imprinting on Mn-doped ZnS quantum dots for room-temperature phosphorescence optosensing of pentachlorophenol in water. *Analytical Chemistry*, 81:1615–1621. Doi:10.1021/ac802375a

Wang, J. (2006). Analytical electrochemistry. 3rd Ed. Canada.

Wang, P.; Mai, Z.; Dai, Z.; Li, Y.; Zou, X. (2009). Construction of Au nanoparticles on choline chloride modified glassy carbon electrode for sensitive detection of nitrite. *Biosensors and Bioelectronics*, 24:3242-7. Doi:10.1016/j.bios.2009.04.006

WHO. (1987). Pentachlorophenol. World Health Organization, International Programme on Chemical Safety. Environmental Health Criteria 71. Geneva.

Xu, J.; Wang, Y.; Qiu, H.; Qiuc, H.; Zhangc, Y. (2014). The electrochemical oxidation of pentachlorophenol and its sensitive determination at chitosan modified carbon paste electrode. *Russian Journal of Electrochemistry*, 50:531-536. Doi:10.1134/S1023193514060093

Xu, H.; Zhan, X.L.; Zhan, J.H. (2010). Determination of pentachlorophenol at carbon nanotubes modified electrode incorporated with beta-Cyclodextrin. *Journal of Nanoscience and Nanotechnology*, 10:7654-7. Doi:10.1166/jnn.2010.2772

Zhu, X.; Zhang, K.; Lu, N.; Yuan, X. (2016). Simultaneous determination of 2,4,6-trichlorophenol and pentachlorophenol based on poly(Rhodamine B)/graphene oxide/multiwalled carbon nanotubes composite film modified electrode. *Applied Surface Science*, 361:72-79. Doi:10.1016/j.apsusc.2015.11.154

Zou, J.; Ma, J.; Zhang, Y.; Li, L.; Jiang, J.; Chen, J. (2013). Electrochemical determination of pentachlorophenol using a glassy carbon electrode modified with a film of CuS nanocomposite-chitosan. *Analytical Letters*, 46:1108-1116. Doi:10.1080/00032719.2012.749483